Concordance of hemoglobin A1c and reproductive hormone levels in menstrual and venous blood

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Objective: To explore whether menstrual blood collected via a modified menstrual pad is a surrogate for venous blood drawn in analyzing hemoglobin A1c (HbA1c) and fertility-associated hormones.
Design: Cross-sectional study.
Setting: Clinical testing laboratory.
Patients: This study included 152 female participants who have regular menses, aged 19–50 years old.
Interventions: Participants collected menstrual effluent using a menstrual pad modified with a removable dried blood spot (DBS) strip. Peripheral blood samples were collected via venipuncture within 60 hours of menstrual pad use.
Main Outcome Measures: Menstrual pad and venous blood drawn samples were analyzed for levels of HbA1c, thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), anti-müllerian hormone (AMH), and luteinizing hormone (LH). Correlation between menstrual pad and venipuncture samples was performed using Deming linear regression, and r coefficients were measured using Pearson correlation.
Results: The interassay variability of menstrual pad DBS sample measurements was <6%. Menstrual HbA1c values were stabilized in the DBS strips through 53 days, and menstrual hormone levels remained stable through 15 days. Menstrual HbA1c levels were highly correlated with venipuncture samples ($r = 0.96$). The levels of TSH ($r = 0.94$), AMH ($r = 0.94$), FSH ($r = 0.91$), and LH ($r = 0.91$) also showed a high correlation between menstrual strip and venipuncture samples.
Conclusions: The levels of HbA1c, TSH, AMH, FSH, and LH measurements in menstrual effluent showed a high correlation to venous blood samples, supporting the use of menstrual effluent as a surrogate sample for hormone testing. (Fertil Steril Rep® 2024;5:33–9. ©2023 by American Society for Reproductive Medicine.)
Key Words: Menstruation, HbA1C, hormones, menstrual blood, venipuncture

Routine blood examinations monitor metabolic markers such as hemoglobin A1c (HbA1c), thyroid hormones, and a variety of essential nutrients to help identify early signs of disease risk, aid in prescribing preventive care, and track how treatments impact systemic health. In the context of women's reproductive healthcare, clinicians measure blood levels of key hormones associated with fertility when screening for conditions such as polycystic ovary syndrome, ovarian insufficiency, and thyroid disorders (1, 2).

Intrapopulation differences in serum hormone levels are affected by a variety of factors, including age, caloric intake, and physical activity levels (3). Standard practice for hormone measurements relies on blood specimens acquired through venipuncture, a procedure that requires assistance from medical personnel and may cause physical and emotional discomfort for the patient. Venipuncture procedures induce physiological stress responses associated with the anticipation of pain, even with repeated exposures (4). Although at-home testing can increase healthcare access, the collection of venipuncture blood samples at home commonly requires special processing, storage, and temperature-controlled, expedited transportation (5). Similar storage and transport limitations affect reproductive hormone analysis in noninvasive alternative samples, such as saliva and urine (3). Consequently, women frequently experience

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significant obstacles to receiving fertility specialist consultations and reproductive hormone testing, highlighting the need for expanding access to these tests (6).

Recently, fingerstick sampling via dried blood spot (DBS) has been evaluated as a more convenient and less expensive alternative to a venous blood draw for monitoring of HbA1c and hormone levels (7). Dried blood spot sampling preserves blood samples, enabling patients to self-collect specimens without needing to travel to a doctor's office (5, 8). Blood is collected on filter paper, and the samples are dried and shipped to a clinical laboratory for analysis. However, although fingerstick blood sampling with DBS resolves some of the logistical challenges associated with venipuncture, many individuals are still uncomfortable with the self-administration of a fingerstick test because of the potential pain and soreness. Evaluation of physiological and psychological stress responses challenges the characterization of fingerstick as necessarily "less invasive" than venipuncture, as participants' stress responses to fingerstick were equivalent to (and for some measures greater than) their response to venipuncture (9).

Unlike venipuncture or fingerstick blood sampling, menstrual effluent presents a fully noninvasive, passively collected biological sample for biomarker monitoring. Menstrual effluent consists of whole blood, vaginal fluids, and tissues shed from the endometrial lining in response to hormonal signaling (10). Despite its complex composition and proinflammatory molecular signature (11, 12), recent studies have shown a close correlation between menstrual and peripheral blood measurements for inflammatory biomarkers (13), HbA1c (14), cholesterol, follicle-stimulating hormone (FSH), lipoproteins, and high-sensitivity C-reactive protein (15).

Despite the potential advantages of using menstrual effluent for clinical analysis, this approach has not yet been widely applied to women's health. One historical limitation in this field is the challenge posed by blood collection via menstrual cups and the subsequent logistical obstacles of transport, storage, and analysis of liquid blood samples (16). Because the modified menstrual pad may help overcome many of these drawbacks (14, 17), we measured a panel of common clinical analytes with the objective of exploring the concordances between traditional venipuncture and menstrual samples obtained via the modified menstrual pad.

MATERIALS AND METHODS

This was a prospective, observational study of 152 eumenorrheic females (Table 1). Participants were eligible for the study when they were >18 years old, had a regular menstrual cycle, lived in the San Francisco Bay Area, and did not have a hormonal intrauterine contraceptive device. Hormonal medications were not exclusionary, as long as subjects had regular menstrual cycles. The sponsor, Qurasense Inc., funded this study, and study participation was approved by an institutional review board (WCG Clinical, Inc., Princeton, NJ). Participants were recruited via online advertising, which linked to a survey on inclusion and exclusion criteria. Interested participants were subsequently screened using a phone call or in-person visit to confirm eligibility and provide informed consent for participation in the study, which was conducted between November 2021 and March 2022. Enrolled participants were provided with a study kit containing two Q-Pads (Qvin, Inc., Palo Alto, CA) and instructions for menstrual blood self-collection. The Q-Pad is a modified menstrual pad designed to collect and stabilize menstrual fluid for subsequent analysis using standard laboratory tests (Supplemental Fig. 1, available online). Each Q-Pad contains an embedded DBS strip, which becomes saturated with menstrual effluent when a participant wears the pad during their cycle (typically for 4–6 hours). The DBS strip can then be removed from the pad by pulling on an accessible tab.

TABLE 1

Participant demographics.

	Mean (± SD)	Min	Max
Age, y	33.0 (7.9)	19	50
d of Period	d	N	%
	d 1	47	30.9
	d 2	81	53.3
	d 3	19	12.5
	d 4	2	1.3
	d 5	3	2.0
	Total	152	100.0
Interval between Q-Pad and venous blood draw, h	Mean (± SD)	Min	Max
	21.1 (10.1)	0.7	51.2
Interval between blood draw and analysis, d	Mean (± SD)	Min	Max
	1.4 (1.5)	0.02	8.2
Interval between Q-Pad collection and analysis, d	Mean (± SD)	Min	Max
	3.7 (1.6)	0.69	9.1

Table shows breakdown of participant ages, day of period for Q-Pad sample collection, and time intervals between Q-Pad and venipuncture sample collection and analysis.
Max = maximum; Min = minimum.

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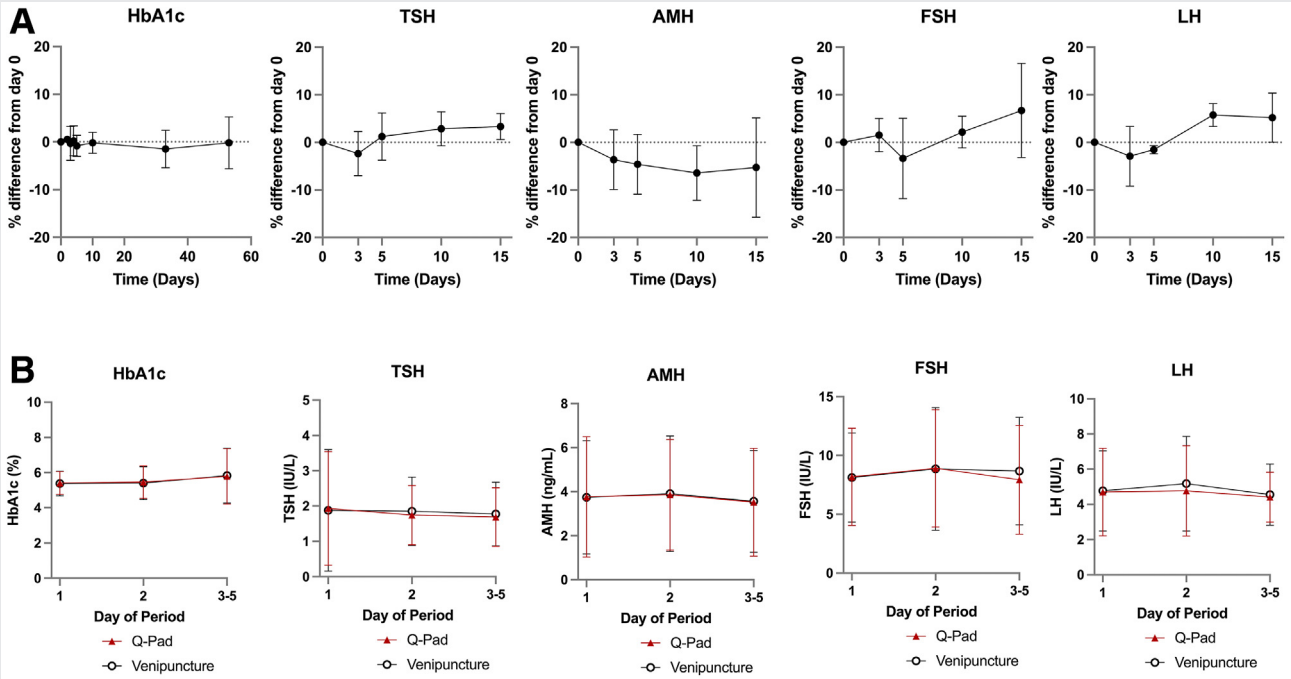
Participants were instructed to use the pads on the days of their heaviest flow, contact the study sponsor to schedule a venous blood draw within 48 hours, and return the saturated DBS strip from their used pads to the study team on the day of the blood draw. A total of 201 participants were enrolled in the study, with the target of reaching a sample size of at least 120 on the basis of Clinical Laboratory Improvement Amendments (CLIA) guidelines for establishing laboratory reference standards (18, 19). We received and analyzed samples from 152 participants; another 49 subjects did not complete participation in the study.

A phlebotomist collected venipuncture samples, which were processed either as whole blood (for HbA1c measurement) or allowed to clot and centrifuged to extract the serum fraction for thyroid stimulating hormone (TSH), FSH, antimüllerian hormone (AMH), and luteinizing hormone (LH) level measurements. Menstrual samples were punched out of the DBS strip and reconstituted in assay buffer. Assays were run according to manufacturer specifications, in a CLIA-certified laboratory (Qvin Labs, Menlo Park, CA).

Hemoglobin A1c levels were analyzed using the Beckman AU480 Analyzer (HbA1c Reagent—Reference No. B00389). Hormone levels were analyzed using the Beckman DXI-600

FIGURE 1



(**A**) Stability of menstrual dried blood spot (DBS) samples was assayed by testing Q-Pad samples from three participants over a time course of 0–53 days for hemoglobin A1c (HbA1c) analysis and 0–15 days for hormone analysis. Graphs depict the percent difference from the DBS sample measurement on day 0. (**B**) Mean HbA1c and hormone levels in Q-Pad and matching venipuncture samples were compared for participants who collected menstrual samples on days 1, 2, or 3–5 of their period. Data are presented as the mean ± SD. Differences between timepoints were not significant (two-way ANOVA, $P>.05$). AMH = anti-müllerian hormone; HbA1c = hemoglobin A1c; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid stimulating hormone.

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Analyzer (TSH Reference No. B63284, FSH Reference No. 33520, LH Reference No. 33510, and AMH Reference No. B13127). The same instruments were used for both venipuncture and menstrual sample analysis.

The data were analyzed using GraphPad Prism and Microsoft Excel. The correlation between the venous blood draw and Q-Pad samples was analyzed using linear regression to generate a Pearson's r coefficient and the Deming linear regression formula. Statistical differences between groups were analyzed using ANOVA, with Tukey's multiple comparisons testing between groups.

RESULTS

Among the 152 submitted DBS samples, 123–136 (80.9%–89.5%) were analyzed for concordance with the venous blood draw (Supplemental Table 1, available online). Follicle-stimulating hormone and LH samples were excluded from concordance analysis when the interval between Q-Pad collection and venipuncture exceeded 48 hours. The most common reasons for sample exclusion included errors associated with Q-Pad sample collection, such as undersaturated or damaged DBS samples. An interference study was conducted on 14 different potential endogenous and exogenous substances; feces were the only substance that interfered with sample quantification (Supplemental Table 2, available online). Suspected fecal contamination affected 3 DBS samples (2%), which were excluded from analysis. Additional technical reasons for exclusion were samples where the instrument was unable to analyze the reconstituted Q-Pad specimen, and samples where the serum values were below a measurable range.

Stability testing was performed on DBS menstrual samples to determine whether storage at room temperature affected analyte measurements. For HbA1c analysis, DBS samples were evaluated through day 53, and for hormone analysis, samples were evaluated through 15 days, supporting the 1–9-day intervals between Q-Pad collection and analysis in the correlations study (Fig. 1A).

Interassay variability for DBS analysis, estimated by measuring the coefficient of variation (%CV) of replicate measurements from three different subjects, was 1.62% for HbA1c, 2.92% for TSH, 4.42% for AMH, 5.29% for FSH, and 4.41% for LH.

To determine whether period day affected hormone measurements, mean values of HbA1c and reproductive hormones were compared for menstrual samples collected from period days 1, 2, or 3–5 (Fig. 1B). We did not observe any significant differences in analyte measurements from Q-Pad or from matching venipuncture samples during the first 5 days of the menstrual cycle.

TABLE 2

Concordance between Q-Pad and venipuncture analyte measurements.

Analyte, units	Venipuncture mean (±SD)	Q-Pad mean (±SD)	Deming regression equation	Pearson *P* value	Pearson r	Q-Pad mean bias vs. venipuncture, % (95% CI)
HbA1c, %	5.46 (0.99)	5.50 (0.97)	y =0.9833*x + 0.1313	<.0001	0.96	0.90 (0.01–1.80)
TSH, IU/L	1.85 (1.22)	1.79 (1.11)	y = 0.9076*x + 0.1155	<.0001	0.94	2.10 (−2.05 to 6.24)
AMH, ng/mL	3.79 (2.52)	3.78 (2.56)	y = 1.0150*x - 0.0635	<.0001	0.94	5.70 (−0.21 to 11.62)
FSH, IU/L	8.60 (4.70)	8.53 (4.67)	y = 0.9937*x - 0.0164	<.0001	0.91	0.78 (−2.84 to 4.40)
LH, IU/L	4.94 (2.41)	4.70 (2.38)	y = 0.9855*x - 0.1763	<.0001	0.91	−3.23 (−7.11 to 0.65)

Table shows mean analyte values and concordance analyses for menstrual Q-Pad and venipuncture samples.
AMH = anti-müllerian hormone; CI = confidence interval; HbA1c = hemoglobin A1c; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid stimulating hormone.

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Summary statistics comparing HbA1c and hormone measurements in venous blood draws and Q-Pad samples are presented in Table 2. Our linearity and bias data indicate that Q-Pad and venipuncture samples may be used interchangeably for the analysis of HbA1c, TSH, AMH, FSH, and LH levels at or near the physiological reference range. The mean bias for Q-Pad measurements of each analyte was below 6% (Table 2). Correlation graphs with Deming linear regression for each analyte are shown in Figure 2A.

Correlation against subject age found age-dependent increases in HbA1c ($P<.0001$) and FSH ($P<.0001$) for both Q-Pad and blood draw samples (Fig. 2B), consistent with previously reported observations for these analytes in healthy adults (20, 21). Anti-müllerian hormone, a hormone frequently used for predicting ovarian reserve and response to ovarian stimulation for fertility treatments, was significantly negatively correlated with subject age ($P<.0001$, Fig. 2B).

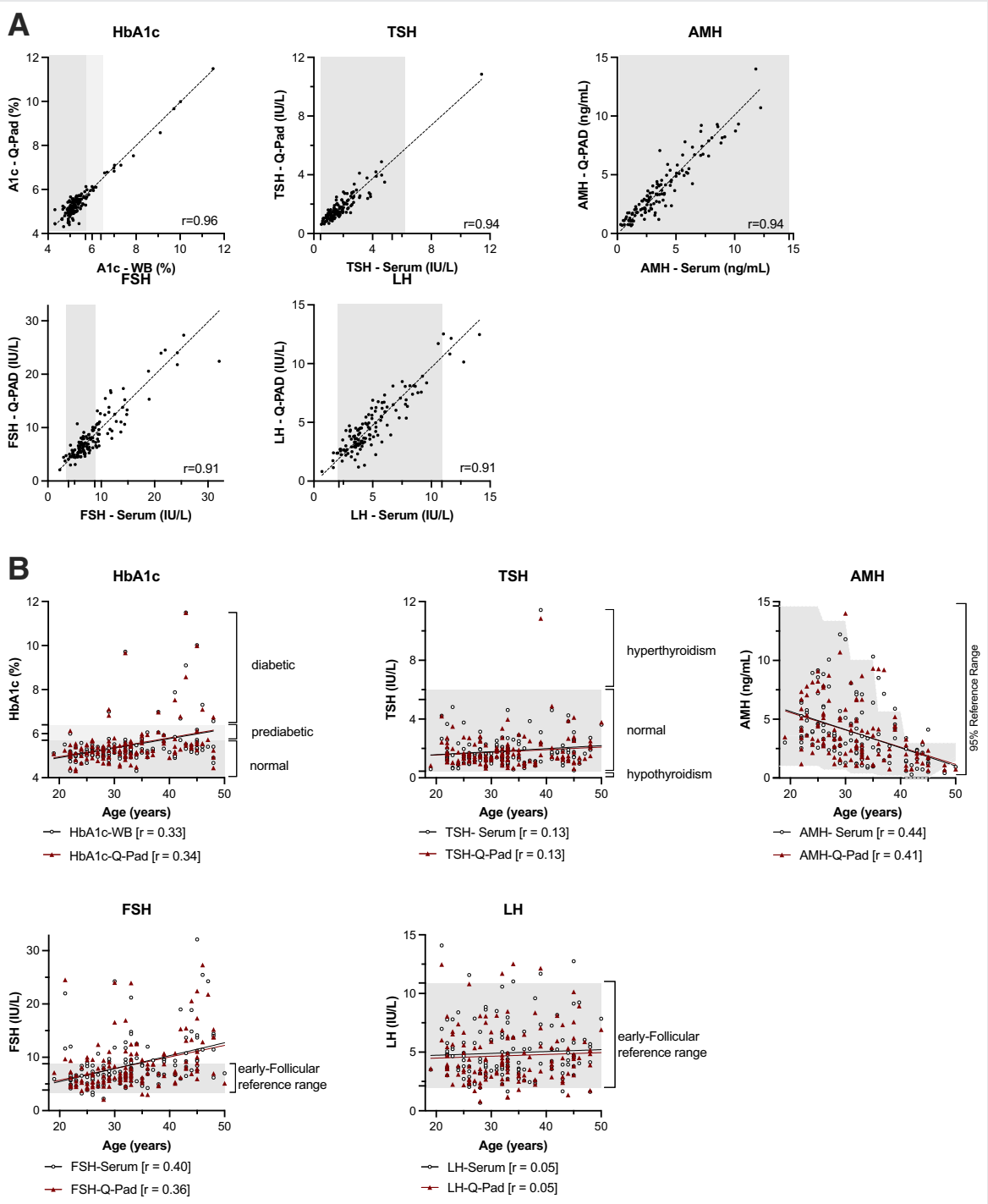
DISCUSSION

This study finds that DBS-based analysis of menstrual blood for HbA1c and reproductive hormone levels is highly correlated to measurements obtained from venipuncture samples. Hormone and HbA1c levels in menstrual samples recapitulate age-related changes in HbA1c, FSH, and AMH levels. Unlike venous blood draws, menstrual blood samples can be acquired noninvasively and stabilized using DBS for testing using standard blood analysis equipment. In a recent study evaluating the suitability of menstrual blood DBS testing for high-risk human papilloma virus infection, 94% of participants reported that they would prefer the modified menstrual pad when it was available as an alternative to clinician-collected cervical sampling (17). Consequently, this technology has the potential to significantly expand the accessibility of hormone and HbA1c testing, especially for rural and other vulnerable populations, where access to medical care is limited. The stability of menstrual DBS samples makes this methodology especially attractive for application in research studies on female reproductive hormone levels, as collected samples can be stored at room temperature for at least 2 weeks without meaningful loss of analyte integrity.

The correlation between venipuncture and Q-Pad HbA1c levels (r = 0.96) is consistent with previous findings in both healthy and diabetic patient populations (14). Prior data on reproductive hormone levels in menstrual blood is substantially more limited. Zhou et al. (16) reported on measurements of hematocrit, prolactin, LH, FSH, estradiol, and progesterone levels in menstrual and peripheral blood samples collected from 19 regularly cycling subjects. In that study, menstrual levels of FSH were measured significantly lower than blood plasma levels, with a significant positive correlation (r = 0.607). Meanwhile, Zhou et al. (16) reported higher LH levels in menstrual blood, with no correlation to peripheral blood samples. In our data, LH and FSH levels were concordant between the two sample types. Differences in study sample size (19 vs. 120+ participants) and in menstrual blood collection protocols (menstrual cup vs. DBS menstrual pad) may explain the differences between these studies. Follow-up studies should examine potential correlations for analytes not included in our initial panel.

Our study has several limitations. This study represents a limited selection of hormonal biomarkers; additional tests for hormones such as estradiol and testosterone can provide insight for the interpretation of FSH and LH measurements in a healthcare setting, but were outside the scope of this initial study. To ensure accuracy, all samples were analyzed in a CLIA-certified laboratory facility on fully calibrated standard laboratory equipment. However, our approach does not enable the estimation of systemic instrument error in menstrual sample analysis, which represents a unique sample matrix compared with blood serum. Furthermore, our study does not discern whether measured differences between DBS and venous blood samples reflect variation accrued during sample processing and analysis or true biological differences between samples. Participant error accounted for 5%–10% of excluded samples in our study; patient education to ensure subjects understand how to use the Q-Pad will be essential for effective adoption of this technology in research and clinical settings.

FIGURE 2



(**A**) Deming linear regression for analyte measurements in menstrual blood (Q-Pad sample) vs. conventional blood draw. The gray region indicates the reference range of the analyte for healthy populations aged 18–45 years. Follicle-stimulating hormone (FSH) and LH reference ranges provided for early-follicular phase. (**B**) Pearson linear correlation for Q-Pad and venipuncture samples vs. subject age. The gray region indicates the reference ranges for healthy populations. AMH = anti-müllerian hormone; HbA1c = hemoglobin A1c; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid stimulating hormone; WB = whole blood.

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Likewise, effective application of at-home hormonal testing should be paired with appropriate medical oversight and patient education to support appropriate interpretation of test results.

This study examined the correlation between menstrual and peripheral blood samples in a general population of regularly menstruating women. Consequently, our concordance and bias analyses are based on measurements primarily from subjects in the normal reference range for these analytes. Follicular phase LH measurements in eumenorrheic individuals represent a narrow reference range for this hormone, which may exceed 50 IU/L in patients with perimenopausal stage. Further research is needed to establish correlations for samples outside the ranges captured in our study population. For analytes like AMH, FSH, and LH, the Q-Pad shares the same limitations as serum blood tests with difficulty assessing absolute measurements for subjects whose hormone levels are below standard assay measurable ranges. Consequently, analytes like estradiol (whose normal levels during menstruation are $<$50 pg/mL) may require additional development to qualify for evaluation using menstrual DBS. Similarly, further study is needed to confirm the relationship between menstrual pad and venipuncture samples in specific disease populations, as pathologies may differentially impact hormone levels in endometrial tissues vs. systemic circulation. Anti-müllerian hormone and LH levels and LH:FSH ratios are frequently elevated in women diagnosed with polycystic ovary syndrome, who may experience irregular periods, with spotting and vaginal bleeding between cycles (22–24). Our findings that these hormones are concordant between menstrual fluid and blood serum invite research into whether menstrual fluid can be used to detect hormonal imbalances in women with irregular cycles, heavy bleeding, or breakthrough bleeding.

CONCLUSIONS

Our data demonstrate the diagnostic potential of menstrual blood as a monthly window into the hormonal and metabolic milieu reflective of endometrial and reproductive health. In eumenorrheic subjects, DBS-stabilized menstrual blood samples may be analyzed for HbA1c, TSH, FSH, AMH, and LH levels as a functional surrogate for venous blood draw. Further research is needed to establish the diagnostic potential of menstrual blood analysis to interpret longitudinal or pathological hormonal variations in broader populations.

CRediT Authorship Contribution Statement

Sara Naseri: Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maria I. Avrutsky:** Writing – review & editing, Writing – original draft, Formal analysis. **Carlo Capati:** Validation, Data curation. **Khevna Desai:** Project administration, Data curation. **Ruben Alvero:** Writing – review & editing. **Paul D. Blumenthal:** Writing – review & editing.

Declaration of Interests

S.N. is a co-founder and employee of Qurasense Inc., a company that develops laboratory tests for analyzing menstrual fluid. S.N. is listed as an inventor of patents affiliated with Qurasense Inc. M.I.A. is a paid employee of Qurasense Inc., who is the sponsor of this study. C.C. is a paid employee of Qurasense Inc., who is the sponsor of this study. K.D. is serving as a paid employee of Qurasense Inc., who is the sponsor of this study. R.A. reports funding from Celmatix study on molecular markers in infertile women; travel support from ASRM; Board of Directors, American Society for Reproductive Medicine; Hannah Life Technologies, Scientific Advisory Board, Orchid Bioscience, Scientific Advisory Board outside the submitted work. P.D.B. has nothing to disclose.

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