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Patterns of Salivary Estradiol and Progesterone across the Menstrual Cycle

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Abstract

The aim of this study was to characterize the normality of menstrual cycles on the basis of progesterone and estradiol levels in self-collected saliva samples. Twenty-two women, ages 19–40 years, self-collected whole unstimulated saliva specimens each morning for two consecutive menstrual cycles. On the basis of presence/timing of hormone peaks, two investigators classified 24 cycles as normal, 10 as likely normal, and 10 as clearly not normal with respect to expected profiles. Our results show that whole saliva samples collected at home on a daily basis provide a noninvasive, feasible method of determining menstrual cycle profiles.

Keywords

saliva; estradiol; progesterone; menstrual cycle; hormone; women; pain; temporomandibular dysfunction

INTRODUCTION

Historically, salivary analyses of female sex hormones were used for fertility and pregnancy monitoring.^{1–3} However, recent findings indicate that these assays may be useful beyond the study of reproductive concerns. There is emerging evidence that females are at greater risk for various diseases and experience diseases differently than males. For example, autoimmune diseases, such as Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and autoimmune thyroiditis, are more prevalent in women than in men. Progesterone and estradiol, which are at high levels in women during their reproductive years, may cause increased immunoreactivity responsible for this phenomenon.⁴

Other sex-related differences include differences in pain perception, which have been demonstrated at both experimental and clinical levels. For example, chronic pain conditions, such as migraine headaches and temporomandibular muscle and joint disorders, are more common in women than men, and occur at peak prevalence during the reproductive years.^{5, 6} The effects of female sex hormones are also evident in the study of changes in disease susceptibility, severity, and drug pharmacokinetics in relation to the menstrual cycle.⁷

Frequent serum sampling for hormone analysis is invasive, inconvenient, and requires skilled personnel to draw samples. However, whole saliva provides an excellent specimen for monitoring estradiol and progesterone levels across the menstrual cycle. It can be self-collected

at home on a daily basis and stored in a home freezer for a month or longer before delivery to a laboratory for analysis, and it can be subjected to repeated freezing and thawing without adverse effects on assay results.⁸

There are large differences in the concentration of hormones in each phase of the menstrual cycle and in the timing of menstrual cycle events from woman to woman. In addition, variations exist across multiple cycles for an individual woman, including occurrence of some nonovulatory cycles.⁹ The aim of this study was to characterize the use of salivary progesterone and estradiol in self-collected whole saliva samples to monitor menstrual cycles.

METHODS

Twenty-two healthy women, ages 19–40 years, who were recruited for a study of hormones and orofacial pain, self-collected unstimulated whole saliva specimens each morning for two consecutive menstrual cycles. Subjects were asked to spit the saliva into a 10-mL tube until a 2.5-mL sample was collected. The specimen was dated and stored in the subject's home freezer until the end of the second cycle, when it was picked up by research personnel and stored at -20°C in the laboratory for up to 6 months until analysis.

On the day of analysis, each specimen was thawed and heated at 57°C for 2 h and centrifuged at $9,000\times g$ for 4 min at 10°C . Estradiol and progesterone concentrations were determined by enzyme immunoassay by Saliva Testing and Reference Laboratory (Seattle, WA, USA) with commercial kits (EIA 537 and EIA 574 kits, Pantex, Santa Monica, CA, USA). Laboratory personnel were blinded to the day of the cycle.

Two investigators independently examined plots of the daily salivary hormone levels for each subject over two menstrual cycles ($n = 44$ cycles). On the basis of specified criteria, which included presence/timing of hormone peaks and missing data, cycles were classified as normal, likely normal, or clearly not normal with respect to expected profiles (TABLE 1).

Median hormone levels were calculated for each subject and averaged across subjects. Agreement between the two investigators regarding classification of cycles was assessed using the kappa statistic.

RESULTS AND DISCUSSION

Menstrual cycles ranged from 22–44 days long. The median concentrations for estradiol and progesterone were 2.81 pg/mL and 139.1 pg/mL, respectively. There was a good agreement between the two investigators in categorizing the menstrual cycles ($\kappa = 0.69$). After resolution of disagreements through discussion, 24 cycles were designated as normal, 10 as likely normal, and 10 as clearly abnormal (FIGS. 1–3). If “likely normal” cycles are combined with “normal” cycles, then 22.7% of the samples are abnormal (possibly anovulatory) cycles, which is comparable with serum values.¹⁰

SUMMARY

Our results show that whole salivary samples collected at home by the subject on a daily basis provide a noninvasive, feasible method of determining menstrual cycle profiles. These biomarkers have great potential for studying hormone levels in research on gender differences in health and disease.

Acknowledgements

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FIGURE 1. Representative patterns of salivary estradiol and progesterone levels across the normal menstrual cycle of one subject.

FIGURE 2.

Representative patterns of salivary estradiol and progesterone levels across the likely normal menstrual cycle of one subject. Symbols as in Figure 1.

FIGURE 3. Representative patterns of salivary estradiol and progesterone levels across the clearly abnormal cycle of one subject. Symbols as in Figure 1.

TABLE 1

Criteria for menstrual cycle assessment

Normal cycle:

- 1 Two estradiol peaks are present.
- 2 Primary (earlier) estradiol peak precedes the second estradiol peak by 5 days or more.
- 3 Primary peak is higher than the second peak.
- 4 Progesterone rising over the course of the cycle (a little variation/a few small dips are acceptable).
- 5 Progesterone peaks within 2 days (+ or -) of the second estradiol peak. (e.g., if estradiol peaks on day 27, progesterone peaks on day 25, 26, 27, 28, or 29)

Likely normal cycle:

One or more of the criteria cannot be judged on account of missing data, but the criteria that can be judged are fulfilled.

Clearly abnormal cycle:

Sufficient data are available and one or more of the criteria for normal cycle are not fulfilled.