Evaluation of Salivary Ferning for Predicting Ovulation in Menstruating Women with a Normal Cycle: An Easy, Cost-effective and Noninvasive Approach

Rishika Das\(^1\), Uzma Iqbal Belgaumi\(^2\), Nupura Vibhute\(^3\), Vidya Kadashetti\(^4\), Wasim Kamate\(^5\)

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**ABSTRACT**

**Introduction:** The erudition of the fecundity period is essential for women for both successful conceiving and effective contraception with a need for research in developing a cost-effective, easy-to-use, multiuse, home-based method for prediction of ovulation.

**Aim:** To evaluate the ferning patterns in saliva among various phases of the menstrual cycle (Follicular, Ovulatory, and Luteal) in normally menstruating women using a compound light microscope.

**Setting and design:** A total of 45 healthy women between the ages of 18 and 25 who had a regular menstrual cycle participated in the current study.

**Methods and materials:** Selected individuals were instructed on how to collect samples and when. The presence, absence, and intensity of the ferning pattern were determined by examining the dried saliva smears under a low power objective (10x). Following the Wilcoxon Signed Rank Test, Friedman’s Test was used to statistically analyze the data.

**Results:** The present study’s findings indicate that among all study participants, there is a strong positive link between the occurrence of salivary ferning and the timing of ovulation. The maximum intensity of ferning is observed in the mid-day of the menstrual cycle in a subsection of women who were evaluated on multiple days in the ovulatory phase.

**Conclusion:** The salivary fern test is a non-invasive and cost-effective method can serve as a reliable indicator of ovulation for effective conception or contraception and that the technique can be taught easily and effectively to the participants.

**Clinical significance:** The presence of the salivary ferning pattern throughout the ovulatory phase of the menstrual cycle will serve as an aid in defining the women’s fertile time and, consequently, will further help for successful conception or contraception.

**Keywords:** Estrogen, Menstrual cycle, Ovulation, Salivary ferning.

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**INTRODUCTION**

Ovulation is the process that results in the release of one or more eggs from the ovary usually once a month. It is brought on by an increase in the hormone luteinizing hormone, which in turn raises the body’s estrogen levels. There is only a small window of about 6 days each month during which one can conceive. Hence, it is especially important for a woman to have knowledge about their fertility period for both effective conception and/or contraception.\(^1\),\(^2\)

There are various methods used in assessing the ovulation time. Some of the simple methods are based on basal body temperature and calendar-based method that helps determine the time of ovulation. However, these methods require a daily commitment.\(^3\)

Ultrasonography is a standard reference for ovulation detection since it can be used to observe the maximized follicle (>15 mm on ultrasound). It is used widely as an investigative tool in assisted reproductive techniques. Yet, it requires expensive diagnostic protocols and needs medical assistance. Hormonal tests like luteinating hormone (LH) surge determination in serum or urine, are extremely sentient and provide great accuracy in determining conception capacity.\(^4\) Many over-the-counter home-based methods use this principle. Nevertheless, these ovulation diagnostic kits can be expensive and inaccessible to many.

The ferning pattern, also known as arborization or palm-leaf design, is widely recognized to appear during the ovulatory period as a result of high estrogen levels found in the cervix’s mucus. Saliva samples taken during ovulation show a similar pattern. Cervical smear sampling is frequently accompanied by methodological challenges and contamination risk. Saliva is easier to collect, less contaminated, and equally effective than other biological fluids.\(^5\)

Obtaining saliva is a simple, non-invasive process that has higher sensitivity even with a smaller sample size. The collection of saliva does not require specialized equipment or qualified...
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The processing of saliva is inexpensive and useful for population monitoring. Researchers have become more interested in saliva in recent years for measuring cortisol, HCG enzymes like amylase, other substances like glucose, etc. Saliva can be used to determine the reproductive status because it is simple to collect and also mirrors the cervical fern pattern. To lessen the financial burden, there is a high demand for the promotion of reliable, accurate, and surrogate ovulation detection technologies. The purpose of the current study was to identify ovulation by looking at the fern pattern in smears made from saliva.

METHODS AND MATERIALS

Study Participants

The institutional ethics committee of the host institution examined and approved the whole protocol. (Reference number: KIMSDU/IEC/03/2022, Protocol number: 085/2021-2022, Date: 30/03/2022). Online questionnaires were distributed among 100 females between 18 and 25 years to find out the average duration of their menstruation and menstrual cycle. Women who had PCOS, diabetes, endometriosis, an irregular menstrual cycle, or who were on birth control tablets were not included in the study. Using a computer-generated random sequence, 45 females having a regular menstrual cycle (21–35 Days) and meeting the inclusion criteria were chosen for the study in accordance with the responses and sample size estimation. So, the sample size is 45 participants.

Sample size estimation: \( n = \frac{Z^2pq}{L^2} \)

\( Z = 1.96 \) Type I error at significance level of 5%
\( p = \) accuracy level of ferning pattern = 86.5%
\( q = 100-p = 13.5\% \)
\( L = \) Allowable error = 10%
\( n = \frac{(1.96)^2 \times 86.5 \times 13.5}{100} = 44.8 \)
\( n = 45 \)

Table 1: Comparison of Ferning pattern among three phases (Follicular, Ovulatory and Luteal) by Friedman’s test

<table>
<thead>
<tr>
<th>Phases</th>
<th>Ferning pattern present (%)</th>
<th>Ferning pattern present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular phase</td>
<td>1.50</td>
<td>0</td>
</tr>
<tr>
<td>Ovulatory phase</td>
<td>3.00</td>
<td>45</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>1.50</td>
<td>0</td>
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</tbody>
</table>

Test statistics

\( N = 45 \)
\( \text{Chi-square} = 90.000 \)
\( \text{Df} = 2 \)
\( \text{Asymp. sig.} = <0.001* \)
\( p\text{-value} = <0.001* \)
\( \text{Point probability} = 0.000 \)

Patient education: For computation of time of ovulation, sample collection time and sample preparation.

After obtaining informed consent, participants were explained how to compute the collection date of their samples for the three phases. For example, if a subject had a 28-day cycle, the follicular phase would range from day 1 to day 10, the ovulatory phase would range from day 11 to day 16, and the luteal phase would range from day 17 till the next cycle starts. Participants were also taught the procedure of saliva acquisition and sample preparation with a list of written guidelines. The subjects were asked to collect saliva samples three times (once in the follicular, ovulatory, and luteal phases of the menstrual cycle) based on their average menstrual cycle duration. The subgroup of 15 women was asked to collect samples twice during the ovulatory phase. Sample A was collected randomly during any day other than the mid-day in the ovulatory phase while sample B was collected precisely on the mid-day of their menstrual cycle during the ovulatory phase.

Sample Preparation

The slides received from the participants were coded and given to another evaluator for interpretation to eliminate reporting bias. The saliva smears were viewed under a compound light microscope under 10x magnification. The presence, absence, and intensity of crystallization (ferning) were evaluated.

Statistical Analysis

The data were all entered into Microsoft Excel 2010 for statistical analysis. Using Friedman’s test and Wilcoxon signed rank test for pairwise comparison, the presence or absence of the ferning pattern was assessed in three phases (follicular, ovulatory, and luteal). Wilcoxon signed rank test was performed to compare the degree of ferning on various ovulatory phase days.

Simple graphs were used for graphical representation. The “p” value was regarded as statistically significant for all tests when it was <0.05. Version 19 of SPSS (Statistical Package for Social Sciences) was the program utilized.

RESULTS

According to Friedman’s test for determining the presence and absence of ferning patterns, all 45 cases have shown the presence of ferning patterns in the ovulatory phase (Table 1, Fig. 1). There was a statistically significant difference in the presence or absence of a ferning pattern among the three groups studied with \( p < 0.001* \).
The Ovulatory-follicular phase, Follicular-luteal phase, and Luteal-ovulatory phases were the three compartments identified by the Wilcoxon signed rank test for pairwise comparison of the phases.

There was a significant statistical distinction between the phases corresponding to the existence of ferning in the ovulation phase in the relevant groups with p < 0.001 (Table 2, Fig. 2).

According to the analysis, we conclude that the ferning patterns are seen only in the ovulatory phase among all 45 participants and absent in the follicular and luteal phases (Fig. 3).

In the subgroup studied for the intensity of ferning during the ovulatory phase using Wilcoxon signed ranks test analysis, all 15 cases have shown more intensified ferning patterns in sample B than sample A (Table 3). It was found that a statistically significant difference exists for the intensity pattern for sample A and sample B with p < 0.001*. According to the analysis, irrespective of the duration of the menstrual cycle, the mid-day of the menstrual cycle showed maximum intensity in the ferning pattern (Fig. 4).

**Discussion**

Hormones like luteinizing hormone, follicle-stimulating hormone, and female sex hormones like estrogen and progesterone work in concert to modify the menstrual cycle, which in turn affects the electrolytes and fluid levels in the body. Follicular phases, ovulatory phases, and luteal phases make up its three phases. The significant increase in estrogen levels that occurs during the ovulation phase causes the body’s salt levels to rise. The differences in the chemical composition of saliva cause it to become brinier over time, and this is visible in dried saliva. So, the ferning appearance of saliva is caused by the crystallization of sodium chloride (NaCl). As a result, salivary ferning can be considered one of the finest non-invasive methods to detect ovulation.

Saliva being non-invasive, and easily obtainable in nature, has several advantages for the evaluation of ferning patterns as a predictor of ovulation. In comparison to the biochemical analysis of serum estradiol and luteinizing hormone, this technique avoids the procedure of blood collection. It also reduces the number of visits, time, and cost required in imaging tests like ultrasonography. In addition, this method can be used to decrease the discomfort associated with procedures like transvaginal ultrasound, making them more user-friendly and hence increasing patient compliance.

The goal of the current study was to propose a unique method for identifying the ovulatory period in women of reproductive age. Numerous investigations, including those by Maurizio Guida and Salmassi A, have supported the use of saliva for ferning pattern analysis as an indicator of the ovulation period in the menstrual cycle since the initial study by Biel Cassals, who first characterized the feature of salivary ferning and its association to ovulation.

At the Institute of Obstetrics and Gynecology of Naples, Italy, Guida M compared saliva crystallization with basal temperature, cervical mucus, stomach pain, and ultrasound evaluation of the ovaries. He discovered that during the ovulatory phase of the menstrual cycle, saliva created a crystalline pattern in 92% of cases.

A substantial correlation between salivary patterns and follicle size was found by Didi DM Braat and Ratomir Ganovic respectively. When the follicle was larger, they noticed positive results. The remarkable sensitivity of the salivary crystallization test was demonstrated.

![Fig.1: Comparison of ferning pattern among three phases (Follicular, Ovulatory and Luteal)](image)

<table>
<thead>
<tr>
<th>Table 2: Pairwise comparison of Ferning pattern among three phases (Follicular, Ovulatory and Luteal) by Wilcoxon signed ranks test</th>
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<tr>
<td><strong>Wilcoxon signed ranks test</strong></td>
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<td><strong>N</strong></td>
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<tr>
<td>Ovulatory phase–follicular phase</td>
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<td>Follicular phase–luteal phase</td>
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^Statistically significant, a, Ovulatory phase < follicular phase; b, Ovulatory phase > follicular phase; c, Ovulatory phase = follicular phase; d, Follicular phase < luteal phase; e, Follicular phase > luteal phase; f, Follicular phase = luteal phase; g, Luteal phase < ovulatory phase; h, Luteal phase > ovulatory phase; i, Luteal phase = ovulatory phase
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In order to determine ovulation, Fehring, and Gaska looked at the relationship between the LH spike in the urine and the emergence of ferning in vaginal mucous. According to Barbato M, the development of ferns may be a result of a rise in estrogenic activity that gradually raises the concentration of electrolytes in saliva. The current study’s findings support the idea that salivary ferning is a dependable, simple, and non-invasive method for predicting ovulation because they are in line with the studies indicated above. In addition to confirmation of the previous results, the present study utilized an education-based intervention among the participants where the subjects were reinforced on monitoring the duration and changes during their menstrual cycles for a predictable calculation of the time of ovulation and was also trained to collect saliva samples that were optimal for interpretation of salivary ferning. This intervention has helped the women to determine not only the most fertile period in their cycle but also inculcated knowledge about the different phases and durations of menstrual cycles.

Limitations and Future Scope

Though this study was conducted as a pilot study in a small sample, the results are encouraging and validate the usefulness and effectiveness of ferning in saliva as a predictive tool for identifying the ovulatory period. Extrapolating the results in a larger sample size with variable population characteristics will help in the translation of this research hypothesis in the day-to-day practical application of this method for predicting ovulation. Additionally, due to constraints of time and funding, the study population was not subjected to biochemical and imaging studies, and hence the inclusion of these tests will help correlate the biological pathways associated with salivary ferning.

Conclusion

The salivary fern test is a non-invasive and cost-effective method that can serve as a reliable indicator of ovulation for effective conception or contraception. The results of the current study also highlight that such a technique can be taught easily and effectively to the participants which will be the basis of developing an efficient and cost-effective home-based method with minimum financial implications and hence can be easily popularized among low and middle SDI countries.

Clinical Significance

During the ovulatory phase, the level of estrogen is elevated which causes the body’s sodium levels to rise. The increasing brininess in
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the saliva is a result of the variations in the chemical composition and this can be noticed in dried saliva. The differences in the chemical composition of saliva cause it to become brinier over time, and this is visible in dried saliva. So, the ferning appearance of saliva is caused by the crystallization of sodium chloride (NaCl). Thus, the saliva fern pattern is one of the greatest non-invasive methods to identify ovulation which will also aid in successful conception or contraception.

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REFERENCES


Figs 4A and B: Photomicrograph showing salivary smears in the ovulatory phase under 10x magnification. Sample B showed more intensified ferning pattern than sample A.