

Study of Salivary Matrix to Estimate Ovulation

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Abstract

Introduction - The phenomenon of ovulation is a crucial phenomenon in evolution. Many modalities to detect the same have been developed and time tested. Each of them have their advantages and linked with some shortcomings. This article unravels one more physical property of saliva which is characteristic during the menstrual cycle.

Aim - To study the salivary matrix in women during the course of menstrual cycle.

Materials and method- Early morning salivary smear was obtained from the subjects. They were made to spit and spread the drop of saliva on slide and fix it using the alcohol spray. Each slide was labeled and assessed under the microscope with 10X magnification. The slides labeled were assessed for ferning on first day of the menstruation cycle till the beginning of next menstruation cycle.

Result - The salivary smear showed varied patterns in salivary matrix during the entire course of menstrual cycle.

Conclusion - Ferning assessment is basically a qualitative test of saliva which will be evident around ovulation. This test can be used to plan pregnancy. It cannot be used to prevent pregnancy. An appropriate reagent or kit needs to be developed in order to identify this unique phenomenon.

Keywords - saliva, ferning, ovulation, menstrual cycle, pregnancy

Introduction and Background

Ovulation is a remarkable phenomenon to procreate in human beings. Various methods are available to estimate the ovulatory phase in a woman. For sake

of better interpretation Table 1 is designed to line up various modalities to estimate the ovulation along with the shortcomings of each.

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Table 1: Present day modalities to estimate ovulation alongwith their shortcomings:

Methods	Shortcomings:
Basal Body Temperature	1) Women have to record their temperatures as soon as they wake up, while they are still lying in bed. 2) May not be a good method for women with unpredictable schedules or inconsistent sleep patterns. 3) BBT readings can be affected by factors like illness, infection, stress, anxiety, fatigue, jet lag, insomnia, nightmares, electric blanket use, smoking and exercise. 4) The temperature rise usually occurs after ovulation, making it difficult to identify the fertile days.
Urinary LH surge	1) Patients may dislike collection and handling samples of urine. 2) It should be performed with first urination of the day. 3) An irregular menstrual cycle expands the testing window thus increasing the testing cost. 4) Several health conditions and drugs related to infertility can interfere with the accuracy of test results.
Cervico-vaginal fluid	- Collecting the cervical vaginal mucus sample from women is not always possible as it may be hindered by presence of infection and inflammation in cervix and vagina. In transabdominal route examination of all sides of ovary would be difficult.
Transvaginal Ultrasound	It is invasive, expensive & inconvenient.
Smartphone Applications	It is technology dependent. Validation of slides is required.

Along with all these methods, literature reveals that the salivary matrix beholds the potential to display a phenomenon called “Ferning”. Barbato et al⁽¹⁾ suggested that the appearance of fern formation may be due to the appearance of surge of estrogenic activity which increases concentration of electrolytes in saliva gradually. The fern structures formed in saliva are dried salt or electrolyte crystals present as a result of the electrolyte collected in saliva prior to and during ovulation. Moreover, the ferning is caused by equal proportion of sodium and potassium ions, which cyclically increases under the influence of estrogen⁽²⁾. Thus in the ovulation phase, crystals which formed clearly were observed in saliva, whereas in other phases few crystalline structures were visible. The pattern of ferning in saliva is helpful to detect the ovulation period in women produced by Sodium chloride, which cyclically increase under the influence of ovarian hormone estrogen^(3,4). Hence, assessment of saliva may emphasize that the salivary ferning pattern

can be used for ovulation prediction and such a test can be clinically useful in the identification of ovulation day.

The changes in ions and appearance of fern pattern in ovulatory phase may be due to basic change in estrogen circulation. The direct observation of the quantity of crystals in dried preparations of saliva would favour the knowledge that there are large cyclical variations in the NaCl concentration of the saliva. Many studies⁽⁵⁾ postulate that the level of NaCl would increase in the period of follicular maturing, reach a maximum level at the time of ovulation and intensely decrease in the corpus luteum period. It is possible that the saliva secreted by the glands may differ in composition and physical properties, so that the conditions governing the crystallization process are different at different times in the cycle.

According to a study by K.H.Oelker⁽⁵⁾ endogenous 17β-estradiol (E2) and low parenteral doses of exogenous E2 cause vasodilation. Large dose of estrogens, mainly ethinylestradiol (EE) and mestranol, induce the formation of proteins from the liver including coagulation factors, sex hormone binding globulin and angiotensinogen⁽⁶⁾. In the steady state, high plasma levels of angiotensinogen cause very small increase in angiotensin II (AII) and plasma renin activity, because AII inhibits the secretion of renin and reduces plasma renin level. Yet, the increase in AII is enough for a slight reduction in renal blood flow and a slight increase in exchangeable sodium and blood pressure⁽⁵⁾. In menstrual cycle, due to cyclical change in oestrogen level, it produces increase in concentration of aldosterone and angiotensinogen. These hormones cause retention of sodium and other electrolytes which results in formation of ferning in saliva. As estrogens are chemically related to desoxycorticosterone which has strong sodium retaining property, estrogen also causes sodium, chloride and water retention⁽⁷⁾⁽⁸⁾. This property

of estrogen is confirmed by presence of recurrent ankle edema observed in few women before menstruation and in late pregnancy when estrogen level is high⁽⁹⁾⁽¹⁰⁾.

Mechanism of fern formation: When saliva is placed over the lens, there is evaporation of water which further causes increase in concentration of solutes. According to salting out phenomenon used during protein purification procedures, the level of salt continues to rise until a point where the proteins can no longer stay in solution⁽¹¹⁾. The protein is left over at the drop margins as the liquid regresses. The evaporation continues and the concentration of salts continues to rise. When the solubility level of the inorganic salts is reached and spontaneous fernlike crystal formation takes place.

Some research has been attempted to decide whether salivary matrix can aid in ovulation estimation. In order to interpret the outcome of salivary studies, Table 2 enumerates studies on salivary ferning.

Table 2: Salivary Studies attempted and their outcome (latest to old)

Study:	Outcome:
Hugh Melnick et al(12) 2014	Sensitivity of 96.5% and positive predictive value of 93.3%.
A. Salmassi et al(13) 2013	Detect ferning structures in the saliva that occurs during ovulation.
Paul RBrazina(14) 2011	Detection of a salivary ferning pattern, is similar to preovulatory cervical mucus. It can be used as a biological marker for potential fecundity. It was well accepted.
Maurizio Guida(15) 1999	Reported 92% correlation between salivary ferning and ovulation.
Fehring&Gaska(16) 1998	Correlation in occurrence of LH in the urine and appearance of vaginal mucus ferning or salivary ferning.
Didi D M Braat(17) 1998	Found sensitivity of 53% between ovulation detected sonographically and by salivary ferning.
“Determination of Fertile and Infertile Days of the Menstrual cycle of women by using the Saliva Crystallization test”.(18)	Salivary ferning is as effective as cervical mucus analysis and ultra sonographic folliculometer. There was a highly significant relationship between size of follicle and results of ferning tests. A high percentage of positive result was seen when the follicle was larger in size. It proved that the saliva crystallization test has high sensitivity.

Cont... Table 2: Salivary Studies attempted and their outcome (latest to old)

Berardono(11) 1993	Salivary ferning in humans can be seen during the whole menstrual cycle, in post-climateric, pregnant and pre-pubertal women.
Barbato et al,(1) 1993	The investigators concluded, direct association between ferning in saliva and fertile period.
Rotta et al (19) 1992	Done by using mini-microscope PC 2000 IMPCON, examined total of 58 women for 5 months. They established during menstrual cycle fertile and infertile days can be found by examining the crystallization of saliva.
M. Guida et al (20) 1991	Compared the findings obtained with the crystallization of saliva with basal temperature, cervical mucus, abdominal pain and ultrasound examination.
Belkein(21) 1985	Confirmed correlation between salivary ferning and oestradiol level by measuring serum and salivary estradiol level. He was able to compare the results and concluded that determination of E2 in saliva by a convenient, stress-free procedure provides valuable information on ovulation.
Biel Cassals(22) 1968	He was the first to describe the phenomenon of salivary ferning and its relationship to ovulation.

To understand the phenomenon of ferning, in Indian women a study was designed after obtaining clearance from the institutional review board. Care was taken that all the subjects in age range of 19-22 were systemically sound and did not consume tobacco or oral contraceptives.

Aim: To study the salivary matrix in women during the course of menstrual cycle.

Objectives:

1. To assess the salivary matrix beginning from the first day of menstrual cycle

2. To assess the salivary matrix during ovulation.

3. To compare the salivary matrix in both the phases.

Study Design: Salivary samples were collected from healthy women in early morning prior to brushing and nil-by-mouth state. Salivary smear was obtained from the subjects. After, the slide was fixed using the alcohol spray. Each slide was labeled and assessed under the microscope with 10 X magnification.

Observations and Result: The pictures obtained were archived and studied closely. As demonstrated in the figure 1, reflected the beginning of menstrual cycle-day 1, wherein there was no ferning.

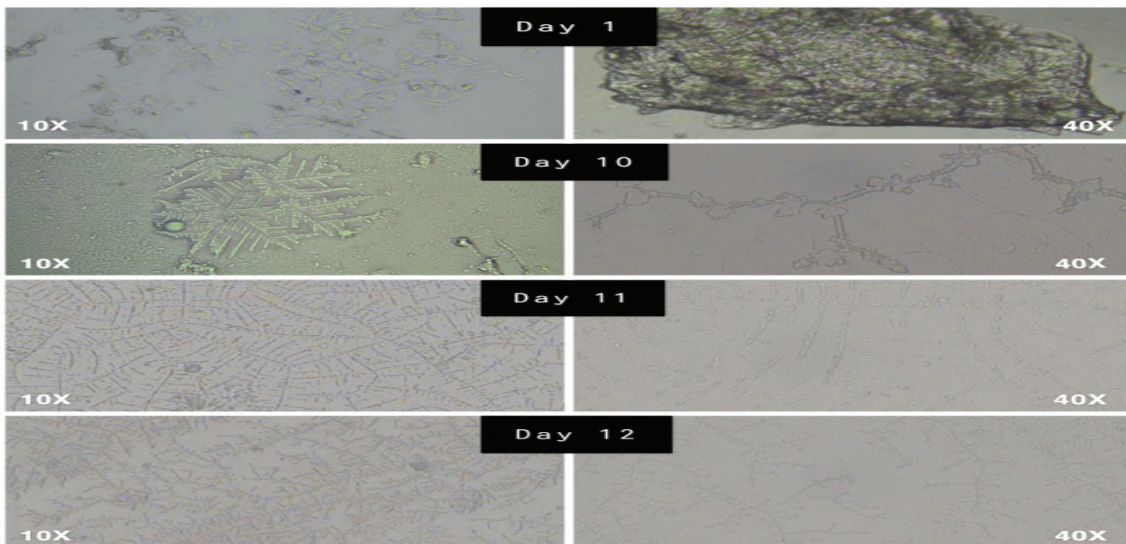


Figure 1: Salivary matrix on Day 1/ 10/11/12.

The same phenomenon continued till day 10, where the salivary matrix revealed orientation of few crystals characteristically. As the days went by, the ferning pattern appeared sharper and well oriented, seen in picture above day 11, day 12.

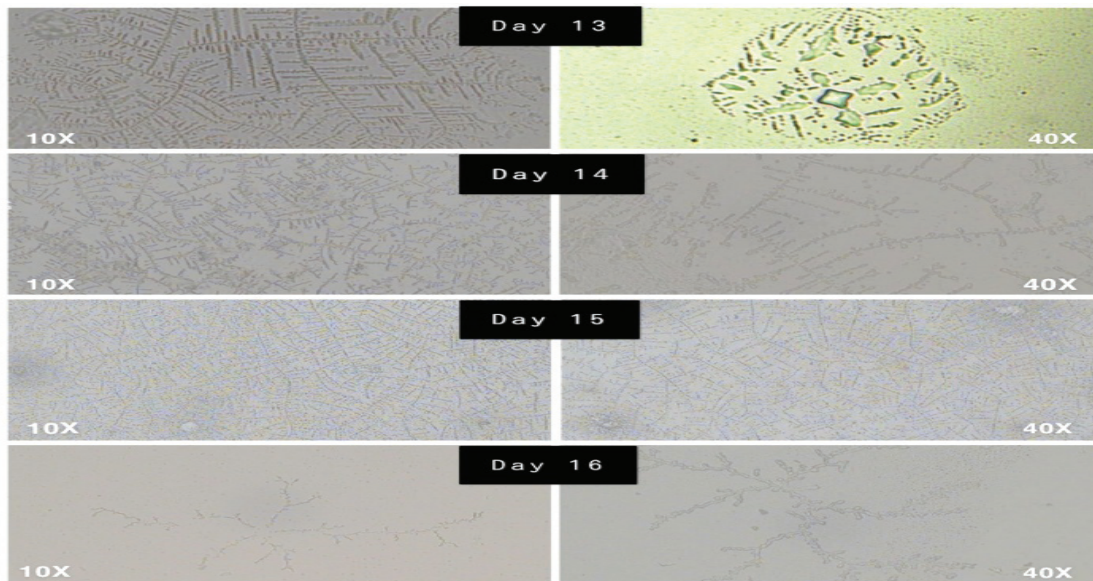


Figure 2: Salivary matrix as seen on Day 13/14/15/16

The phenomenon of Ferning continued to be apparent in the microscope till day 16 as seen in the figure 2. After Day 16, the ferning phenomenon disappeared and salivary matrix failed to show the characteristic orientation of the crystals- the ferning ceases.

The phenomenon of ferning was characteristically seen developing from Day 10 continuing through Day 16 in all women considered in study. The ovulation time of course varied in women with a menstrual cycle of

28 days and those with 30 days. Women with 28 days cycle constituted to 92% in present study ovulated on 14th day, showed ferning pattern around that time of the cycle. Whereas, women with 30 days of menstrual cycle ovulated around 15th day, showed the ferning pattern in salivary matrix.

Discussion

The salivary matrix significantly differed depending

upon the menstrual status of women. This study was an attempt to understand the physical properties mainly the ferning pattern of saliva during the ovulation phase followed by the menstrual cycle. It depicts that salivary fern pattern is very distinct around ovulation period as compared to preovulatory and postovulatory period. The phenomenon is a pathfinder where there is a direct association between ferning in saliva and productive period in women's reproductive life. So salivary ferning may be used as a new method to assist women to know her fertile period along with other methods of ovulation prediction⁽²³⁾.

Obvious advantages of salivary matrix evaluation for ferning facilitates:

- (1) Utilise saliva [non-invasive/ easily obtainable/ patient compliant] matrix for diagnostic purpose,
- (2) Abstain daily withdrawal of blood for serum testing of estradiol or LH,
- (3) Dodge daily visit to sonologist for follicle study,
- (4) Escape invasive procedures like transvaginal ultrasound.

Shortcoming:

We could not compare the ferning pattern of saliva with a cervical mucus. The hormonal levels weren't measured which could have provided valuable information to further increase the strength of study results. When comparing salivary ferning with follicle studies, researchers have found negative test result if the follicle was of small size and high percentage of positive result when the follicle was of larger size. Thus there was a highly significant relationship between size of follicle and results of ferning tests. Also this study proves that the saliva crystallization test is possible in determining fertile and infertile days and is valid with high sensitivity.

Summary:

The results of our study have vital clinical suggestions. Interpretations of salivary ferning revealed that the start of salivary ferning indicates the beginning of fertile period and peak as the time of ovulation and disappearance of ferning as end of fertile period. Thus salivary ferning can be used to identify the fertile period

in the menstrual cycle and timing of ovulation.

Conclusion

Ferning assessment is basically a qualitative test of saliva which will be evident around ovulation. This test can be used to help plan pregnancy. It cannot be used to prevent pregnancy. An appropriate reagent or kit needs to be developed in order to identify this unique phenomenon.

Conflict of Interest: None.

Source of Funding: Nil

Ethical Clearance: Ethical clearance was obtained from institutional ethical committee

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