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Assessment of Salivary Secretion for the Prediction of Ovulation by Comparing It with Cervical Ferning and TVS Ovulation Monitoring

Hemant Deshpande, Madhukar Shinde, Gurkiran Kaur, Pratap Pharande and Chandrakant Madkar*

Department of Obstetrics and Gynecology, Dr. D.Y. Patil Medical College and Research Centre, India

Abstract

Background: Saliva undergoes changes in terms of various physical and biochemical properties in accordance with menstrual cycle.

Aim: To assess salivary secretion for the prediction of ovulation by comparing it with cervical ferning and Transvaginal Sonography (TVS) ovulation monitoring.

Methods: This is an observational study conducted from July 2018 to September 2020 in 100 healthy ovulating females. Informed consent was taken from the patient. During the study visits salivary ferning, cervical mucus and TVS were recorded. Based on comparative findings, sensitivity, specificity, and positive predictive value, negative predictive values were determined for salivary ferning.

Results: Thirty nine percent of the women were in the age group of 26 to 30 years of age. Day 12 to 14 was fertile period indicated by good ferning (+2). Slight ferning indicating beginning or ending of fertile period was observed on day 10 (79%) and day 14 (54%). On day 8, salivary ferning and cervical ferning was absent in 59% women while slight salivary ferning was observed in 41% women without cervical ferning. On day 16, it was seen that 81% women had absence of both salivary and cervical ferning pattern. The sensitivity of diagnosing fertile period by salivary ferning was increased from day 8 to day 16 of cycle while the specificity was decreased from day 8 to day 16 of cycle.

Conclusion: The findings indicated a strong correlation between the presence of salivary ferning and ovulation, thus validating its use.

Keywords: Infertility; Ovulation; Salivary ferning; Cervical ferning; Menstrual cycle; Dysmenorrhea

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Correspondence:
Pratap Pharande, Department of Obstetrics and Gynecology, Dr. D.Y. Patil Medical College and Research Centre, Pune 18, India, Tel: +91-9850053535;
E-mail: pratappharande@gmail.com

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Introduction

Menstruation is a catabolic process influenced by hormones from the pituitary gland and ovaries. Gonadotropin-Releasing Hormones (GnRH) secreted by the hypothalamus have a role in triggering the secretion of gonadotropin by anterior pituitary cells. Gonadotropin-releasing hormones: FSH (Follicle-Stimulating Hormone) and LH (Luteinizing Hormone) stimulate and control the cyclical changes inside the ovaries. During the period of reproduction, endometrium undergoes constant cyclical changes. Every cycle usually has 4 phases related to the activity of ovarian hormones. The phases are proliferative phase, ovulation phase, secretory phase and menstrual phase [1,2].

A woman's reproductive system shows a regular cycle of periodic preparation for fertilization and pregnancy. Infertility is defined by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and WHO as the 'failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [3]. Infertility is widely prevalent. WHO estimates that one in every four couples in developing countries had been found to be affected by infertility [4]. Being infertile is the biggest social stigma faced by Indian women.

Follicular Stimulating Hormone (FSH), which is secreted by the pituitary gland, influences the growth of multiple follicles inside the ovary during the proliferative or follicular phase. Estrogen, a hormone secreted by these growing follicles, subsequently inhibits the FSH through a negative feedback loop involving the pituitary gland, hypothalamus and Inhibin B [5-8].

Assessment of the time of ovulation can be done by a wide range of diagnostic methods such as temperature charts- basal body temperature, cervical mucus examination, measurement of plasma and urinary hormonal assays. These methods, individually or in combination with ultrasound, can help to recognize the fertile period in women and predict ovulation [9].

At-home diagnostic agents have the benefits of being non-expensive, non-invasive, and easy to use and reuse. Ovulation tests help to detect ovulation in females and thus increase their chance of conception, especially females with infertility who are anxious to conceive. Nowadays, saliva is gaining much importance as a medium of testing as its composition is nearly the same as that of plasma. Other advantages of salivary testing are the ease with which the sample can be obtained, cheap, non-invasive.

Saliva undergoes changes in terms of various physical and biochemical properties in accordance with menstrual cycle. These changes reflect the changes in hormonal assays associated with the menstrual cycle accurately, thus help to clinically determine the time of ovulation. They act as a potential non-invasive biomarker for the prediction of ovulation. Sodium and chloride were found in high concentrations in the saliva during ovulation phase than in other phases of menstrual cycle [10]. Women with a history of dysmenorrhea and abnormal menstrual bleeding may benefit from more invasive testing.

A key step in assessing women in contraception, conception, child spacing or treatment of infertility is the exact prediction of the timing of ovulation. Many couples choose for themselves when to have children and when not to, to limit the size of the family without putting themselves to the risks posed by hormonal or mechanical contraceptives. Prediction of ovulation is very critical for such couples. Once a reproductive endocrinologist establishes the cause of infertility, the patient will be started on a management plan.

Saibaba et al. [10] studied thirty healthy volunteers for the assessment of physico-chemical changes in saliva. Reproductive cycle was categorized as pre-ovulation phase (5 to 12 days), ovulation phase (13 or 14 days) and post-ovulation phase (15 to 25 days) according to salivary arborization test and hormonal analysis. Estradiol and luteinizing hormone was gradually increased and attained peak at the level of 2.28 ± 0.20 pg/mL and 1.35 ± 0.41 mIU/mL respectively during the ovulation phase. The electrolytes result clearly indicated that the influx of common electrolytes is important for crystallization and help to induce clear ferning pattern in ovulation phase. Sodium (Na) and Chloride (Cl) were found to be high during ovulation phase only. Average salivary pH was 7.5, 7.1 and 7.3 during ovulation, pre- and post-ovulation phases respectively. Buffering capacity of saliva was normal during pre- and post- ovulation phases. In contrast, in ovulation phase the buffer capacity was slightly higher. At the first time, the Scanning Electron Microscopy (SEM) studies revealed the ultra structure difference of saliva during menstrual cycle. During ovulation phase a compact network-shaped mesh appeared; such structure did not appear in pre-and post ovulation phases. Additionally, they observed the saliva is arrayed as a fine mosaic-like structure during ovulation. Based on physico-chemical properties and hormonal levels may lead to develop a detection kit/sensor for detecting the ovulation phase in human.

Fern test is a detection of a characteristic 'fern like' pattern of cervical mucus when a specimen of cervical mucus dries on a glass slide and is viewed under a low-power microscope. This test provides indirect evidence of ovulation and fertility. Sodium Chloride in mucus is responsible for ferning under the effect of estrogen. High levels of estrogen in the body, just before ovulation causes the cervical mucus to form fern-like patterns due to crystallization of sodium chloride on mucus fibers. This pattern is called as 'arborization' or 'ferning'. When progesterone is the dominant hormone in the body, as it is in the second half of a normal menstrual cycle, the fern pattern is no longer identified, and the pattern is completely absent by the 22nd day of a woman's cycle. This disappearance of the fern pattern after the 22nd day of the menstrual cycle is suggestive of ovulation, and its persistence throughout the menstrual cycle suggests an-ovulation or an anovulatory cycle (infertility).

Materials and Methods

This Cross sectional hospital based observational study was carried at Department of Obstetrics and Gynecology, Dr. DY Patil Medical College and Hospital, Pune, Maharashtra from July-2018 to September-2020 in 100 healthy females in ovulating age group. Female patients between 18 to 40 years with regular menstrual cycles without any menstrual abnormalities were included in the study. Patients receiving ovulation induction agents, taking OC pills, progesterone or estrogen that had pelvic inflammatory disease were excluded from study.

In this study, patients attending the gynecology OPD fulfilling the inclusion and exclusion criteria will be asked to sign on the consent form after reading through patient information sheet. General particulars include patient initials; age, address, duration of marriage, date of Last Menstrual Period (LMP), and details of menstrual cycles like average flow, duration of bleeding will be noted.

Patients will visit the hospital on day 8, 10, 12, 14 and 16 of menstrual cycle. A final visit will occur on day 7 post-ovulation. At each visit following examinations will take place.

Salivary ferning testing

Saliva in morning after 2 h to 3 h of fasting will be tested. Then it will be viewed for the presence or absence of salivary ferning and degree of ferning by ovulation microscope and laboratory microscope after drying for at least 5 min. These finding will be compared with concomitant sonographic findings and cervical mucus ferning test. Ferning pattern will be described as below.

Negative - "0" (Non-Fertile period)

Positive - "+1" (Slight ferning indicate beginning or ending of fertile period)

Positive - "+2" (good ferning indicate fertile period)

Cervical mucus

Will be collected by a syringe and will be viewed for its stretchability ("spinnbarkeit") and its ability to fern. Ferning pattern will be seen by laboratory microscope.

Transvaginal sonography (TVS)

Follicular growth, endometrial thickness, changes in the pattern of endometrium, signs of ovulation will be assessed. Presence or absence of echogenic endometrium on day 7 post ovulation.

Observation and Results

A total of 100 healthy married women volunteers were enrolled

Table 1: Day wise comparison of salivary ferning pattern in study group.

Salivary ferning pattern	No of cases					Day 7 post Ovulation
	Day 8	Day 10	Day 12	Day 14	Day 16	
0	59	17	12	12	90	100
+1	41	79	40	54	10	0
+2	0	4	48	34	0	0
Total	100	100	100	100	100	0

Table 2: Day wise comparison of follicle diameter with salivary ferning pattern in study group.

Day of cycle	Salivary ferning pattern		Follicle diameter		F Value/ MW test	P Value
		n	Mean	SD		
8	0	59	16.956	0.58	0.12	0.91
	+1	41	16.941	0.66		
10	0	17	19.024	.6978	1.35	0.27
	+1	79	19.039	.6026		
	+2	4	19.550	.4203		
12	0	12	20.517	.4988	1.87	0.16
	+1	40	20.870	.5730		
	+2	48	20.792	.5515		
14	0	12	23.717	.7720	1.40	0.25
	+1	54	23.267	.9326		
	+2	34	23.424	.7863		
16	0	90	3.663	.8815	0.15	0.88
	+1	10	3.620	.9307		

Table 3: Day wise comparison of endometrial thickness with salivary ferning pattern in study group.

Day of cycle	Salivary ferning pattern		Endometrial thickness		F Value/MW test	P Value
		n	Mean	SD		
8	0	59	5.569	0.28	0.35	0.73
	+1	41	5.590	0.30		
10	0	17	6.500	.3298	0.41	0.66
	+1	79	6.461	.3279		
	+2	4	6.600	.3367		
12	0	12	7.458	.2644	0.23	0.80
	+1	40	7.470	.2672		
	+2	48	7.429	.3066		
14	0	12	8.733	.5158	0.66	0.52
	+1	54	8.778	.4517		
	+2	34	8.876	.4486		
16	0	90	10.024	.3248	0.61	0.54
	+1	10	10.090	.2961		

in the present study and majority of the women were in the age group of 26 to 30 years of age followed by 21 to 25 years of age with mean age of 27.85 ± 5.31 years. Less than five years of marriage was reported by 66% women with mean duration of marriage of 4.60 ± 3.45 years. Mean age of menarche was 12.95 ± 1.36 years.

Day-wise salivary ferning pattern in the study subjects revealed that day 12 to 14 was fertile period indicated by good ferning (+2) with 48% and 34% respectively. Whereas slight ferning indicating beginning or ending of fertile period was observed on day 10 (79%) and day 14 (54%) (Table 1).

Association between salivary ferning and cervical ferning pattern

On day 8 salivary ferning and cervical ferning was absent in 59% women while slight salivary ferning was observed in 41% women without cervical ferning. On day 10 cervical ferning was absent among all the 100 study women while slight salivary ferning was observed among 79% women and good ferning was observed in 4% women. On day 12 salivary ferning and cervical ferning both were absent in 12% women whereas salivary ferning and cervical ferning both were observed in 21% women and the difference observed was statistically significant Chi-square = 9.22, P=0.01. On day 14 it was seen that

Table 4: Day wise comparison of follicle diameter with cervical ferning pattern in study group.

Day of cycle	Cervical ferning pattern		Follicle diameter		t Value	P Value
		n	Mean	SD		
8	Present	0			-	-
	Absent	100	16.950	.6099		
10	Present	0			-	-
	Absent	100	19.057	.6168		
12	Present	40	20.733	.5156	0.84	0.40
	Absent	60	20.828	.5880		
14	Present	87	23.436	.8620	1.85	0.67
	Absent	13	22.962	.8530		
16	Present	10	3.570	.9007	0.34	0.74
	Absent	90	3.669	.8841		

Table 5: Day wise comparison of endometrial thickness with cervical ferning pattern in study group.

Day of cycle	Cervical ferning pattern		Endometrial thickness		t Value	P Value
		n	Mean	SD		
8	Present	0			-	-
	Absent	100	5.578	0.2911		
10	Present	0			-	-
	Absent	100	6.473	0.3266		
12	Present	40	7.543	0.3054	2.77	0.007
	Absent	60	7.387	0.2534		
14	Present	87	8.813	0.4607	0.37	0.71
	Absent	13	8.762	0.4445		
16	Present	10	9.930	0.2869	1.05	0.30
	Absent	90	10.042	0.3243		

Table 6: Day wise Sensitivity and specificity of salivary ferning test.

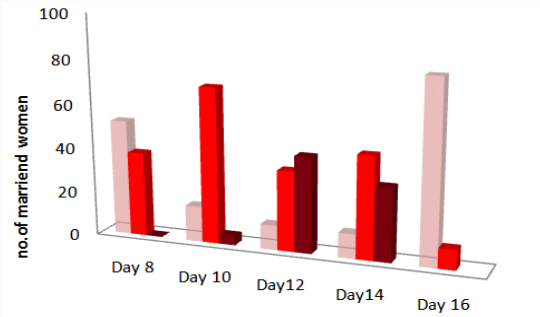
Day of cycle	Sensitivity	Specificity	PPV	NPV
8	42	70	93	12
10	82	10	89	6
12	88	10	90	8
14	88	10	90	8
16	10	90	90	10

87% women had shown cervical ferning pattern, out of them 32% had good salivary ferning and 46% had slight salivary ferning and the difference observed was not statistically significant Chi-square =3.21, P=0.20. On day 16 it was seen that 81% women had absence of both salivary and cervical ferning pattern.

Association between salivary and cervical ferning and TVS ovulation monitoring

The association between mean follicular diameter and salivary ferning pattern (Table 2) and mean endometrial thickness and salivary ferning pattern (Table 3) and mean follicular diameter and cervical ferning pattern (Table 4) and mean endometrial thickness and cervical ferning pattern (Table 5) on various days of cycle were statistically not significant.

The mean follicular size on day 8 was 16.950 ± 0.6099 and was observed to be increasing with maximum size on day 14 (23.374 ± 0.8714) and again 3.659 ± 0.8817 on day 16. The day wise change observed in follicular diameter size was statistically significant.



	Day 8	Day 10	Day12	Day14	Day 16
0	52	16	11	11	81
1	38	70	36	46	9
2	0	4	43	33	

Figure 1: Day wise comparison of ovulation with salivary ferning in study group.

The mean endometrial thickness on day 8 was 5.578 ± 0.2911 and was observed to be increasing with maximum size on day 16 (10.031 ± 0.3212). The day wise change observed in endometrial thickness was statistically significant with P value <0.0001.

It was seen that the sensitivity of diagnosing fertile period by salivary ferning was increased from day 8 (42%) to day 14 (88%) of cycle while the specificity was decreased from day 8 (70%) to day 16 (10%) of cycle (Figure 1) (Table 6 and 7).

Table 7: Day wise comparison of ovulation with salivary ferning pattern.

Day of cycle	Salivary ferning pattern	Ovulation		Chi-square	P Value
		Present (n=90)	Absent (n=10)		
8	0	52	7	FET	0.52
	+1	38	3		
10	0	16	1	0.94	0.63
	+1	70	9		
	+2	4	0		
12	0	11	1	0.05	0.98
	+1	36	4		
	+2	43	5		
14	0	11	1	3.31	0.19
	+1	46	8		
	+2	33	1		
16	0	81	9	FET	1
	+1	9	1		

Table 8: Day wise sensitivity and specificity of cervical ferning test.

Day of cycle	Sensitivity	Specificity	PPV	NPV
8	-	-	-	-
10	-	-	-	-
12	44	100	100	17
14	97	100	100	77
16	0	0	0	0

The sensitivity of diagnosing fertile period by cervical ferning on day 12 was 44% while on day 14 was 97%. However specificity was 100% on both the days (Table 8 and 9).

Discussion

A total of 100 healthy married women volunteers were enrolled. In the present study mean age of menarche was 12.95 ± 1.36 years. Less than five years of marriage was reported by 66% women with mean duration of marriage of 4.60 ± 3.45 years.

While studying the day-wise salivary ferning pattern in the study subjects, it was observed that day 12 to 14 was the fertile period indicated by good ferning (+2). Whereas, slight ferning indicating beginning or ending of fertile period was observed on day 10 (79%) and day 14 (54%). Thus majority of the women showed salivary ferning on day 10 (i.e. 4 days before ovulation). Dhaval et al. [11] in their study observed that salivary ferning began to appear 5 days before ovulation (i.e. 9th day of cycle). Barbato et al. [12] and Fehring et al. [13,14], in their study found that the salivary ferning began to appear 1 to 2 days before cervical mucus appearance.

Based on above results the salivary ferning seems to appear 2 days before cervical ferning. Similar results were noted by Barbato et al. [12] and Fehring et al. [13,14], where salivary ferning began to appear 1 to 2 days before cervical mucus appearance.

Alagendran et al. [15] conducted a study in 48 women in which they collected salivary samples in three phases namely pre-ovulatory, ovulatory, and postovulatory phases and confirmed that the properties of saliva changed based upon the menstrual period. The observation of fern pattern in pre-ovulatory phase showed few crystalline structures. On inspection of fern pattern in ovulatory phase, obviously formed maximum number (72.91%) of estrogen crystals was found. The postovulatory fern pattern shows ill formed structures of indefinable form.

The association between mean follicular diameter and salivary ferning pattern on various days of cycle was statistically not significant (Table 2). And also the association between mean endometrial thickness and salivary ferning pattern on various days of cycle was

Table 9: Day wise comparison of ovulation with cervical ferning.

Day of cycle	Cervical ferning pattern	Ovulation		Chi-square	P Value
		Present (n=90)	Absent (n=10)		
8	Present			-	-
	Absent	90	10		
10	Present			-	-
	Absent	90	10		
12	Present	40	0	FET	0.005
	Absent	50	10		
14	Present	87	0	FET	<0.0001
	Absent	3	10		
16	Present	0	10	FET	<0.0001
	Absent	90	0		

statistically not significant.

Similarly, the association between mean follicular diameter and cervical ferning pattern on various days of cycle was statistically not significant. The association between mean endometrial thickness and cervical ferning pattern on various days of cycle was statistically not significant.

In the study conducted by Dhaval [11] peak salivary ferning day and day of ovulation detected by TVS showed no correlation.

The mean endometrial thickness on day 8 was 5.578 ± 0.2911 and was observed to be increasing with maximum thickness on day 16 (10.031 ± 0.3212). Ming-zhen et al. [16] conducted retrospective study on 112 patients with infertility. They found that the average endometrial thickness and average diameter of ovulated follicle by TVS were 8.13 mm and 21.6 mm respectively at the time of ovulation.

It is important to note that many factors influence the collection of the cervical mucus sample and it may not be always possible. It may be affected by the presence of infection and inflammation of the cervix and vagina, or vaginal discharge. This makes cervical mucus sample difficult to interpret. Also in this study only transvaginal route of ultrasound was done as in transabdominal route visualization and examination of all sides of ovary would be difficult.

Salivary electrolytes, especially sodium chloride is mainly responsible for salivary ferning and it depends mainly on concentration of sodium chloride. According to a study conducted by Ganesan et al. [17] sodium level in saliva was seen to rise during ovulation phase. Chloride level was seen to start to rise during pre-ovulation phase, with significant and considerable increase during ovulatory phase. Ovulatory and pre-ovulatory phases see a significant increase in concentration of salivary sodium compared to post-ovulatory phases. During ovulation, sodium is lost in excess of water.

Conclusion

Thus, from our study, we can conclude that although cervical ferning has better sensitivity and specificity to detect ovulation, salivary ferning test is also a reliable test to detect the fertile period of menstrual cycle. Hence it can be used for monitoring ovulation in addition to transvaginal ultrasonography.

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