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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF SALIVARY FERNING VERSUS CERVICAL FERNING TEST AS A PREDICTOR OF OVULATION

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ABSTRACT

Introduction: it is important for the women to know when they are ovulating. Being infertile is the biggest social stigma faced by Indian women. The ability to detect the period of potential monthly fertility is of great importance for women in their reproductive years, both in terms of contraception as well as conception. There is increasing demand of women for cheap self test to predict the fertile period in each menstrual cycle.

Objectives: The purpose of our study is comparative analysis of salivary ferning test by using KNOWHEN ovulation microscope versus cervical ferning test to predict the ovulation and to detect sensitivity and specificity of salivary ferning test and to correlate the salivary ferning with cervical ferning and Trans vaginal sonographic findings (TVS).

Methods: This is a prospective observational type of study conducted on all healthy married women volunteers age between 21 - 40 years attending gynec OPD in sola civil hospital, Ahmedabad, with regular menstrual cycle From April 2016 to September 2016.

Result: Salivary ferning test by KNOWHEN ovulation microscope is accurate method of detecting ovulation. Its accuracy is 86.5% as compare to cervical ferning which has accuracy of 89.4%.

Conclusion: There was strong correlation between salivary ferning and cervical ferning. Hence can be use instead of cervical ferning as both have same accuracy. Salivary ferning test can be used as a self test by using KNOWHEN ovulation microscope so it is more user's friendly than cervical mucus test.

INTRODUCTION

Women probably spent much of their adult life avoiding getting pregnant but when they are actively trying for a baby, it may be taking a little longer time than they hoped. So it is important for the women to know when they are ovulating. Being infertile is the biggest social stigma faced by Indian women. Ovulation is the process that results in the release of one or more eggs from ovary usually once a month. There is only a small window each month during which one can conceive, typically no longer than about six days. This time leading up to ovulation is most fertile period of month and predicting ovulation is central to conception. The ability to detect the period of potential monthly fertility is of great importance for women in their reproductive years, both in terms of contraception as well as conception. There is increasing demand of women for cheap self test to predict the fertile period in each menstrual cycle. New developments in the area of natural family planning have emerged in recent years (Guida, 1997). There are various fertility markers to monitor ovarian function.

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Direct methods

- Trans vaginal ultrasound follicle size, endometrium, corpus luteum, fluid in cul de sac
- Hormonal urinary LH peak, estrogen, progesterone, estrogen conjugates etc.
- Enzymes B glucoronidase, alkaline phosphatase etc.

Clinical markers

• BBT, cervical ferning, saliva electrical resistance, saliva ferning/crystallization.

In recent years, a small hand held microscope (KNOWHEN ovulation microscope) has been developed and marketed for the purpose of self observing ferning patterns in saliva during female fertile period. It is not advised for avoiding pregnancy but helpful in planning pregnancy. The saliva test has been used to ascertain a woman's fertile period with a success rate of > 90% (Galati, 1999). The purpose of our study is comparative analysis of salivary ferning test by using KNOWHEN ovulation microscope versus cervical ferning test to predict the ovulation and to detect sensitivity and specificity of salivary ferning test and to correlate the salivary ferning with cervical

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ferning and Transvaginal sonographic findings (TVS). Transvaginal sonography is most accurate for predicting ovulation by serial monitoring of endometrial thickness and dominant follicle but it is invasive and expensive. If salivary ferning test has high sensitivity and specificity, then it can be used for infertile women for ovulation monitoring as it reduce cost, reduce hospital visits, users friendly and it is self test, woman do it at home, reusable, Non-invasive, suitable for patients who are not willing to undergo repeated per speculum examination or TVS or where USG facility not available.

MATERIALS AND METHODS

This is a prospective observational type of study conducted on all healthy married women volunteers age between 21 - 40 years attending gynec OPD in sola civil hospital, Ahmedabad, with regular menstrual cycle From April 2016 to September 2016.

Sample Size

The sensitivity of salivary ferning was $95\%^3$ and with 95% confidential interval and 4% allowable error, the desired sample size was as follow.

 $N = z^{2} p (1-p)/d^{2} (4)$ N = Sample Size (menstrual cycles) p = sensitivity (95%) z = Confidential limit which is 95% = 1.96 d = allowable error (4%) $So, N = (1.96)^{2} (0.95) (0.05)/(0.04)^{2}$ = 114

Inclusion Criteria: Healthy married women age between 21 – 40 years with regular menstrual cycle (26-34 days).

Exclusion Criteria

- Pelvic inflammatory disease (PID)
- Patient taking ovulation induction drugs, supplemental estrogen, hormonal contraceptives
- Refuse to give consent

MATERIALS AND METHODS

107 healthy married women volunteers with 114 menstrual cycles were included in our study as per inclusion criteria. All the eligible patients were given patient information sheet and were included in study after obtaining written informed consent. Patient will be asked to come on day 6, 8, 10, 12, 14, 16, 18 of menstrual cycle and post ovulation day 7 to see progestogenic effect on endometrium.

During each visit, following will be examined.

Salivary Ferning

- Test saliva in morning after 2 3 hours of fasting.
- Saliva should be collected from under the tongue as salivary glands located below the tongue. Collect dab of saliva and place it on the surface of lens.
- Try to avoid air bubbles and allow the sample to dry for at least five minutes and viewing the presence or

absence of salivary ferning and degree of ferning by KNOWHEN ovulation microscope and compare with concomitant sonographic findings and cervical ferning.

• Ferning pattern is describe as negative "0" (Non Fertile period), Positive "+1" (Slight ferning indicate beginning or ending of fertile period), Positive "+2" (good ferning indicate fertile period) (Pardo-Carmona, 2010).

Cervical Mucus

- Cervical mucus should be collected by syringe and viewing for its stretchability ("spinnbarkeit") and its ability to fern.
- Ferning pattern will be seen by laboratory microscope.

Transvaginal Sonography (TVS)

During each visit, following parameters will be reviewed.

- Follicular growth
- Endometrial thickness
- Changes in the pattern of endometrium
- Signs of ovulation
- Presence or Absence of Ecogenic endometrium on day 7 post ovulation.

TVS will be done by MINDRAY MODEL; DP-50

TVS examination are considered as a gold standard for detection of ovulation, by demonstrating the presence of dominant follicle (minimum 18mm diameter) or a corpus luteum at the site of follicular rupture or fluid in pouch of duglas. If none are observed, the cycle determines to be anovulatory (Rollason, 2014).

Following parameters will be measured.

• Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of Salivary Ferning and Cervical Ferning test.

A P value <0.05 was considered statistically significant. Yates" correction was applied when expected frequency was less than 5 in more than 20% of cells. Statistical Analysis will be done using Microsoft excel and Epi Info software.

RESULTS

A total of 107 healthy married women volunteers were recruited in our study as per inclusion criteria and data were obtained from observation of 114 menstrual cycles. Out of 114 menstrual cycles studied, 79 (69%) cycles were documented as being ovulatory cycle by ultrasonographic evaluation and 25 (22%) cycles were documented as being anovulatory cycle because of absence of sonographic criteria of ovulation while 10 (9%) women (1 cycle / woman) lost follow up during their study period and not completed the study. So total 104 menstrual cycles completed the study for salivary ferning, cervical ferning and transvaginal sonography examination.

 Table 1. Demographic characteristics (age distributions)

 of study population

Age groups (yrs)	No. of cases	Percentage (%)			
21-25	42	39.25			
26-30	49	45.79			
31-35	14	13.09			
36-40	2 1.87				
Mean \pm SD	27.11 ± 3.43				
median	26				
range	22-36				

 Table 2. Relationship between Salivary Ferning by KNOWHEN Microscope and Ovulation

Salivary Ferning	by	Ovulation		Total
KNOWHEN Microscope		Present	Absent	_
Present		70	5	75
Absent		9	20	29
Total		79	25	104

Table 2 shows Relationship between Salivary Ferning by KNOWHEN Microscope and Ovulation. Ferning was detected by KNOWHEN microscope in 70 out of 79 cases around the time of ovulation while it was detected in 5 cases where ovulation was absent as per the ultrasonographic evaluation. Hence, Detection of salivary ferning by KNOWHEN microscope had a sensitivity of 88.6% and a specificity of 80%, a positive predictive value of 93.3% and a negative predictive value of 69%. Accuracy of KNOWHEN microscope was 86.54%.

 Table 3. Relationship between Cervical Ferning by Laboratory

 Microscope and Ovulation

Cervical Ferning	Ovulation	Ovulation	
	Present	Absent	
Present	73	5	78
Absent	6	20	26
Total	79	25	104

Table 3 shows Relationship between Cervical Ferning by Laboratory Microscope and Ovulation. Cervical Ferning was detected by laboratory microscope in 73 out of 79 cases around the time of ovulation while it was detected in 5 cases where ovulation was absent as per the ultrasonographic evaluation. Hence, Detection of cervical ferning by laboratory microscope had a sensitivity of 92.41% and a specificity of 80%, a positive predictive value of 93.59% and a negative predictive value of 76.92%. Accuracy of cervical ferning was 89.42%.

 Table 4. Comparison of Validity parameters between Salivary

 Ferning by KNOWHEN Microscope and Cervical Ferning by

 Laboratory Microscope

Validity Parameters	Salivary Ferning by KNOWHEN Microscope	Cervical Ferning	P value
Sensitivity (%)	88.6	92.41	0.84
Specificity (%)	80	80	1
Positive Predictive Value (%)	93.3	93.59	1
Negative Predictive Value (%)	69	76.92	0.62
Accuracy (%)	86.54	89.42	0.87

Table 4 shows Comparison of various Validity parameters between Salivary Ferning by KNOWHEN Microscope and Cervical Ferning by Laboratory Microscope. Sensitivity of detecting salivary ferning by KNOWHEN microscope and detecting cervical ferning by laboratory microscope around the time of ovulation were 88.6% and 92.41% respectively and p value was 0.84 which was not statistically significant. Similarly, the Specificity of KNOWHEN microscope for salivary ferning and laboratory microscope for cervical ferning were same (80%) and p value was 1 which was not statistically significant. Positive predictive value of detecting salivary ferning by KNOWHEN microscope and detecting cervical ferning by laboratory microscope around the time of ovulation were 93.3% and 93.59% respectively and p value was 1 which was not statistically significant. Similarly, the Negative predictive value of KNOWHEN microscope for salivary ferning and laboratory microscope for cervical ferning were 69% and 76.92% respectively and p value was 0.62 which was not statistically significant. Accuracy of detecting salivary ferning by KNOWHEN microscope and detecting cervical ferning by laboratory microscope around the time of ovulation were 86.54% and 89.42% respectively and p value was 0.87 which was not statistically significant.

 Table 5. Comparison of Appearance of Ferning in Saliva and Cervical mucus in relation with Ovulation

Day relative to Ovulation	Salivary Ferning by KNOWHEN Microscope (%)	Cervical Ferning (%)	P value
-6	32 (45.71%)	28 (38.89%)	0.53
-4	31 (44.29%)	31 (43.06%)	0.91
-2	4 (5.71%)	13 (18.06%)	0.02
0	0 (0%)	0 (0%)	

The table included only cases where ferning was detected.

Table 5 shows Comparison of Appearance of Salivary Ferning by KNOWHEN Microscope and Cervical Ferning in relation with Ovulation. Salivary ferning by KNOWHEN microscope and cervical ferning by laboratory microscope were first appeared in 45.71% and 38.89% cases respectively on sixth day prior to ovulation and p value was 0.53 which was not statistically significant. Similarly, four day prior to ovulation, salivary ferning by KNOWHEN microscope and cervical ferning by laboratory microscope were first appeared in 44.29% and 43.06% cases respectively and p value was 0.91 which was not statistically significant. Similarly, two day prior to ovulation, salivary ferning by KNOWHEN microscope and cervical ferning by laboratory microscope were first appeared in 5.71% and 18.06% cases respectively and p value was 0.02 which was statistically significant. No Case was detected on the day of ovulation. Hence, ferning in saliva and cervical mucus begins to appear around 4 to 6 day prior to ovulation.

 Table 6. Comparison of Disappearance of Salivary Ferning by

 KNOWHEN Microscope and Cervical Ferning by Laboratory

 Microscope in relation with Ovulation

Day relative to	Disappearan	P value	
Ovulation	Salivary Ferning Cervical Ferning		
	(%)	(%)	
0	0 (0%)	0 (0%)	0.94
+2	44 (62.86%)	39 (53.42%)	0.49
+4	23 (32.86%)	31 (42.47%)	0.34
+6	3 (4.28%)	3 (4.11%)	0.77*

The table included only cases where ferning was detected. *Yates' correction was applied.

Table 6 shows Comparison of Disappearance of salivary ferning by KNOWHEN microscope and cervical ferning by laboratory microscope in relation with ovulation.

Table 7. Comparison of Relationship between Appearance of good (+2) Salivary Ferning by KNOWHEN Microscope and +2 Cervical
Ferning by Laboratory Microscope to Ovulation time interval

Interval (Day relative to	+2 Salivary Ferning by	+2 Cervical	P value
Ovulation)	KNOWHEN Microscope	Ferning	
-4	2 (3.17%)	1 (1.64%)	0.82
-2	39 (61.90%)	39 (63.93%)	0.89
0	22 (34.92%)	21 (34.43%)	0.96

Table 8. Incidence of different signs of ovulation at the time of ovulation

Sign of Ovulation	No. of Cases	Percentage
Dominant Follicle	79	100
Fluid in POD	61	77.22
Corpus Luteum	52	65.82

 Table 9. Comparison of Grade of Salivary Ferning by KNOWHEN Microscope and Cervical Ferning by Laboratory Microscope in relation with follicle size

Follicle size (mm)	0 ferning (%)	0 ferning (%)		+1 ferning (%)		+2 ferning (%)	
	Salivary ferning	Cervical ferning	Salivary ferning	Cervical ferning	Salivary ferning	Cervical ferning	
16	38.10%	46.70%	59.50%	53.30%	2.40%	0%	
18	2.30%	13.00%	90.70%	84.80%	7%	2.20%	
20	0%	10.80%	60%	51.40%	40%	37.80%	
22	0%	0%	56.20%	50%	43.80%	50%	
>=24	0%	0%	33.30%	38.50%	66.70%	61.50%	

 Table 10. Comparison of Relationship of Salivary Ferning by KNOWHEN Microscope and Cervical Ferning by Laboratory Microscope with Endometrial thickness

Endometrial thickness (mm)	Salivary Ferning (%)		Cervical Ferning (%)	
	Present	Absent	Present	Absent
5.1-6.0	4 (5.1%)	75 (94.9%)	2 (2.5%)	77 (97.5%)
6.1-7.0	37 (46.8%)	42 (53.2%)	36 (45.6%)	43 (54.4%)
7.1-8.0	58 (80.6%)	14 (19.4%)	57 (79.2%)	15 (20.8%)
8.1-9.0	63 (94%)	4 (6%)	63 (94%)	4 (6%)

Salivary ferning and cervical ferning were disappeared in 62.86% cases and 53.42% cases respectively two day after ovulation and p value was 0.49 which was not statistically significant while in 32.86% cases and 42.47% cases, salivary ferning and cervical ferning were disappeared respectively four day after ovulation and p value was 0.34 which was not statistically significant. Hence, salivary ferning by KNOWHEN microscope and cervical ferning were disappeared within 2-4 days after ovulation.

The table included only cases where ovulation and +2 ferning were detected: Table 7 shows comparison of Relationship between appearance of +2 salivary ferning by KNOWHEN microscope and +2 cervical ferning by laboratory microscope to ovulation time interval. On the day of ovulation, good (+2) salivary ferning and good (+2) cervical ferning were detected in 34.92% cases and 34.43% cases respectively and p value was 0.96 which was not statistically significant while in 61.90% cases and 63.93% cases, good (+2) salivary ferning and good (+2) cervical ferning were detected respectively, 48 hours prior to ovulation and p value was 0.89 which was not statistically significant. Hence, in majority (97-98%) of cases, good (+2) ferning occurs within 24-48 hours prior to ovulation. Table 8 shows incidence of different signs of ovulation at the time of ovulation. Dominant follicle (minimum 18mm diameter) was seen in 100% of cases while fluid in POD was seen in 77.22% of cases and corpus luteum at the site of follicular rupture was seen in 65.82% of cases.

The table included only cases where ovulation and ferning were detected

Table 9 shows Comparison of Grade of Salivary Ferning by KNOWHEN Microscope and Cervical Ferning by Laboratory Microscope in relation with Follicle size. At 16 mm follicular size by TVS, +1 salivary ferning and +1 cervical ferning were detected in 59.5% and 53.3% cases respectively. At 18 mm follicular size, most cases shows +1 salivary ferning by KNOWHEN microscope and +1 cervical ferning by laboratory microscope and were detected in 90.7% and 84.8% cases respectively. At 20 mm follicular size, +1 ferning were detected in most cases, 60% cases of +1 salivary ferning and 51.4% cases of +1 cervical ferning. Similarly, At 22 mm follicular size, +1 salivary ferning and +1 cervical ferning were detected in 56.2% and 50% cases respectively while +2 salivary ferning and +2 cervical ferning were detected in 43.8% and 50% cases respectively. Similarly, At follicular size of 24 mm or more, most cases shows +2 salivary ferning by KNOWHEN microscope and +2 cervical ferning by laboratory microscope and were detected in 66.7% and 61.5% cases respectively while +1 salivary ferning and +1 cervical ferning were detected in 33.3% and 38.5% cases respectively.

The table included only cases where ovulation was detected.

Table 10 shows Comparison of relationship of salivary ferning by KNOWHEN microscope and cervical ferning by laboratory microscope with endometrial thickness. Salivary ferning and cervical ferning were absent in 95% cases and 98% cases respectively when endometrial thickness was 5.1-6.0 mm while Salivary ferning and cervical ferning were absent in 53% cases and 54% cases respectively when endometrial thickness was 6.1-7.0 mm. At endometrial thickness of 7.1-8.0 mm, salivary ferning and cervical ferning were present in 81% cases and 79% cases respectively. Similarly, both salivary ferning and cervical ferning were present in 94% cases when endometrial thickness was 8.1-9.0 mm.

DISCUSSION

Melnick H et al⁷ had conducted an open label prospective study on 22 women and data were obtained from observations of 41 menstrual cycles. They had found that the mean age was $27.8 \pm$ 7.4 years and the median was 28 years (range 22-34 years) in their study. Pattanasuttinont S et al⁸ had conducted a descriptive study on 90 women and data were obtained from observations of 98 menstrual cycles. They had found that the mean age was 32.9 ± 3.7 years (range 20-40 years) in their study. In Present study, we found that the mean age was 27.11 \pm 3.43 years and the median was 26 years (range 22–36 years). In the study conducted by Melnick H et al⁷, they found that the KNOWHEN microscope had 96.5% sensitivity, 83.3% specificity, 93.3% positive predictive value and 90.9% negative predictive value. In their study, the Accuracy of KNOWHEN microscope was 92.7%. While In present study, the KNOWHEN ovulation microscope had 88.6% sensitivity, 80% specificity, 93.3% positive predictive value and 69% negative predictive value. In present study, the Accuracy of KNOWHEN ovulation microscope was 86.5% and p value was 0.74 which was not statistically significant. Pardo-Carmona B et al⁹ conducted study on 6 adult beagle bitches with normal reproductive activity for saliva crystallization as a means of determining optimal mating time in bitches. They found that salivary ferning first observed in the bitch 4 days before ovulation. In Present study, salivary ferning began to appear 5 days before ovulation. In the study conducted by Barbato M et al¹⁰ and Fehring RJ et al^{11,12,13}, they found that salivary ferning began to appear 1-2 days before cervical mucus appearance. In Present study, salivary ferning began to appear 1 day before cervical mucus appearance. In the study conducted by Pardo-Carmona B et al⁹, salivary ferning disappeared 2-3 days after ovulation has occurred. In Present study, salivary ferning disappeared within 2-4 days after ovulation.

In the study conducted by Barbato M et al¹⁰, the duration of fertile period (duration of ferning) was 6.2 days. Similarly, in the study of Fehring RJ et $al^{11,12,13}$, the duration of fertile period (duration of ferning) was also 6.2 days. In the study conducted by Pardo Carmona B et al⁹, the duration of fertile period (duration of ferning) was 6.6 days. While in Present study, the duration of fertile period was 6.8 days. Study conducted by Pattanasuttinont S et al^8 showed that in 36.7% cases, peak salivary ferning occurred within \pm 3 days of ovulation in clomiphene citrate stimulated cycles. There were two peaks of median salivary ferning, one was two days prior to ovulation and other was five days post ovulation. There was no correlation between the peak salivary ferning day and day of ovulation detected by TVS. In another Study conducted by Fehring RJ et al¹¹ showed that in 76.2% cases, peak salivary ferning occurred within \pm 3 days of LH surge. While in present study, the peak salivary ferning occurred in 96.8% cases within 2 days prior to ovulation. Hence, according to this study, ovulation is most likely to occur within 24-48 hours of peak salivary ferning. DU ming-zhen et al¹⁴ 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. Paramjyot M et al^{15,16} conducted prospective study on 133 spontaneous cycles shows average endometrial thickness and average diameter of ovulated follicle by TVS were 11.2 mm and 20.2 mm respectively at the time of ovulation. While in Present study, the average endometrial thickness and average diameter of ovulated follicle by TVS were 9.17 mm and 21.5 mm respectively at the time of ovulation.

CONCLUSION

There was strong correlation between salivary ferning and cervical ferning. The Sensitivity of detecting salivary ferning by KNOWHEN microscope and cervical ferning were 88.6% and 92.4% respectively and specificity of both microscopes were 80% which was not statistically significant. So it can be use as a substitute of each other. Ferning in saliva and cervical mucus begins to appear around 4 to 6 days prior to ovulation and disappear around 2 to 4 days after ovulation. Ovulation is most likely to occur within 24-48 hours of good (+2) salivary ferning. Average duration of salivary ferning (duration of fertile period) was 6.8 days and cervical ferning (duration of fertile period) was 6.4 days. The commonest sign of ovulation detected by TVS was dominant follicle (>=18mm) followed by fluid in POD and corpus luteum at the site of follicular rupture. So, Salivary ferning test is a reliable test to detect fertile period of menstrual cycle. Hence can be use for monitoring ovulation instead of ultrasonography where facility of ultrasonography is not available. KNOWHEN microscope can be use by woman herself to detect fertile period and it is user's friendly, non invasive and reusable. Salivary ferning for monitoring of ovulation in infertile women with timed ultrasonography visits based on salivary ferning finding will reduce the number of hospital visits and reduce economical burden on women.

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