Salivary ferning as an alternative to sonographic follicle monitoring for determining ovulation: A comparative study*

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ABSTRACT

Objective: To determine if salivary ferning correlates significantly with sonographic indices in identifying the fertile period, and whether it may be used as a cheaper, and more convenient way to aid infertility patients in achieving pregnancy.

Population: Subjects who complain of difficulty achieving pregnancy and for whom follicle monitoring was indicated were recruited from the Outpatient Department in a tertiary hospital in Manila.

Methodology: Patients (n=40) with Primary or Secondary Infertility from April 2013 to August 2015 who require serial follicle monitoring as part of infertility work up were recruited in the study. For every follicle monitoring by ultrasound done by one sonologist, a salivary sample was obtained from the subject and the ferning pattern was determined and recorded by one pathologist blinded as to the day of the subject's menstrual cycle.

Results: There was a total of 40 subjects who underwent 2 serial follicle monitoring during the study. The 1st TVS (preovulatory) was done between Day 9 to 14 of the cycle with an average of Day 11. Correspondingly, salivary ferning done showed that there were 26 (65.0%) with Salivary Ferning 1 pattern and 14 (35.0%) with Salivary Ferning 2 pattern (p=0.35). This showed no significant difference between follicle monitoring and salivary ferning pattern and either may be used in identifying fertile period preovulatory. The 2nd TVS (postovulatory) was done between Day 12 to 21 with an average of Day 16. All the second ultrasound findings showed signs of ovulation. Correspondingly, there were 1 (2.0%) showed Salivary Ferning 1 pattern, 11 (27.5%) showed Salivary Ferning 2 Pattern and 28 (70.0%) showed Salivary Ferning 3 Pattern 9 (p=0.05). This showed no significant difference between follicle monitoring and salivary ferning pattern, hence, TVS follicle monitoring remains more reliable in identifying that ovulation has occurred.

Conclusion: Salivary ferning corresponded well with ultrasonographic findings during the preovulatory phase of the cycle, while no correlation was noted between the salivary ferning pattern and the postovulatory phase of the cycle. Hence, sonographic follicle monitoring remains a better predictor of ovulation, and more effective in identifying the fertile period.

Keywords: Follicle monitoring, infertility, salivary ferning

INTRODUCTION

Infertility is defined as the inability of a couple to conceive within one to two years of unprotected intercourse. The prevalence of this disease, which causes social and psychological stress to many individuals, may reach up to 9% in the general population (PSREI, 2012). Factors that are responsible for this condition may include male and female pathologies which account for up to 22% and 32%, respectively while the rest is due to both. Because of the wide spectrum of causes which may bring about infertility, working up for the said condition may prove to be a tedious and expensive task. Initially, male factor infertility is ruled out through sperm analysis. This is relatively simpler and cheaper than investigating for female factor infertility beforehand. The latter usually involves more sophisticated, and thus expensive, means of diagnosis such as serial hormonal and ultrasonographic evaluation, hysterosalpingography, and even laparoscopy. Once male and female factors of infertility, both of which require medical or even surgical management, have been ruled out, however, a significant number of infertile couples achieve pregnancy simply by properly timing intercourse with ovulation.

The fertile window within the menstrual cycle has been clearly defined. Evidence shows that it is a 6 day interval which occurs before and includes the day of ovulation. It is believed that pregnancy is achieved more likely if intercourse takes place within this period more specifically if coitus occurs 1 to 2 days before ovulation. (Stanford, et al., 2002). The difficulty, however, lies in the identification of the day of ovulation. This may be relatively easy for women with menstrual cycles occurring at regular intervals. Although poorly accurate, documentation with the calendar method and determination of basal body temperature have been traditionally used for this

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purpose. More advanced techniques include serial ovarian ultrasound, urine hormone monitoring, and charting of vaginal discharge, all of which are based on the trend of the woman's hormonal status throughout the cycle. Among these, sonography offers high sensitivity and specificity to determine ovulation. Sonographic indices being used include disappearance or sudden decrease in follicle size, appearance of ultrasonic echoes in the follicle, follicle wall irregularity and the appearance of free fluid in the cul de sac (PSREI, 2012). One disadvantage of ultrasound however, is that positive findings indicate the occurrence of ovulation. It is, however, not a cost effective technique to predict ovulation a few days before it occurs which is the more optimal time for fertilization to take place. There is, thus, an increasing however for newer, more reliable, less expensive and more convenient techniques for predicting ovulation and increasing the likelihood of achieving pregnancy in cases of infertility. In recent years, saliva, which offers a noninvasive, convenient, and cheap alternative to plasma and serum for determining the hormonal status, has been investigated for its use in indicating ovulation, hence, determining the fertile period.

Various studies have shown that there is a direct relationship between salivary estradiol and serum free estradiol. The same pattern applies also to salivary estriol with serum free estriol, and salivary progesterone with serum progesterone levels (Kauffman, et al 2002).

As early as 1986, Bourque et al. conducted a study involving serial saliva collection and blood extraction throughout the menstrual cycle of their subjects. Progesterone levels were determined using direct radioimmunoassay. The results of the study revealed that progesterone levels in saliva correlated well with the levels detected in serum (r=0.68, n=154) during the luteal but not the follicular phase of the cycle. They concluded that salivary progesterone value could probably be used to assess ovulation, although it may be less sensitive than using plasma considering that salivary progesterone increases only up to 4 to 10 fold in contrast with the 100 fold increase of serum progesterone during the luteal phase.

Since then, new devices have been introduced that utilize saliva to predict or determine ovulation. The CUE Ovulation Predictor (Zetek, Inc. Aurora, CO) is a handheld digital monitor with oral and vaginal sensors which determine the electrical resistance of salivary and vaginal secretions which change in response to the cyclical changes in estrogen. Their relationship is due to the indirect effects of estrogen from the dominant follicle on the adrenocorticotrpic hormone and aldosterone levels. Its use was thoroughly investigated and in a descriptive comparative study by Fehring in 1996, it was shown that there was no statistical difference between the number of fertile days in each cycle as determined by the CUE Ovulation predictor and the cervical mucus monitoring method (T= 0.474, p = 0.64).

Using the same mechanism of ionic concentrations in response to cyclic estrogen changes in saliva, a simpler method involving the visualization of a ferning pattern in saliva samples was discovered. Its use was further investigated in a study by Pattanasuttinont and colleagues in 2007. Their objective was to determine ovulation in clomiphene citrate stimulated cycles and their study involved seventy five infertile women with regular menstrual cycles who were given 100 mg clomiphene citrate for 5 days. Saliva samples were then collected daily until 7 days after ovulation and transvaginal ultrasound was also done daily to detect ovulation. The salivary ferning score and ovulation as evidenced by sonography were compared and showed that the two parameters have no correlation (r=0.102, p<.05). Only 7.1% of the cycles had peak ferning patterns consistent with ovulation by ultrasound while in 36.7% of the cases, the peak salivary ferning response occurred within 3 days, before or after ovulation. This was attributed to the possible anti estrogenic effects of clomiphene citrate in salivary glands. A similar study, this time using luteinizing hormone and T4, which are also detectable in saliva through radioimmunoassays, were conducted by Alagendran, et al (2009). The results of their investigation showed that the said hormones are significantly elevated during the ovulatory phase of the menstrual cycle and may therefore be used to predict ovulation using the simple appreciation of the fern pattern of the saliva. Further studies, however, are still recommended to determine whether or not there is a significant correlation between the ferning pattern of saliva and ovulation. If this is indeed proven, it is likely to benefit patients who desire to be pregnant by way of the availability of an inexpensive, convenient way of precisely timing intercourse with that of the fertile period.

OBJECTIVES OF THE STUDY

General Objective

The main objective of this study is to determine whether the use of salivary ferning may provide a cheaper, more convenient yet equally reliable indicator of ovulation compared with that of ovulation indices as proven by ultrasound during follicle monitoring.

Specific Objectives

1. To determine the salivary ferning score of women on a specified day of their menstrual cycle.
2. To determine the presence of ultrasonographic indices that would indicate ovulation on the same day.
3. To determine if there is a consistent correlation between the salivary ferning score and the ultrasonograpic

indices of ovulation.

4. To determine if the correlation is significant enough such that salivary ferning may be used as a reliable alternative to sonographic follicle monitoring to determine the fertile period.
5. To determine if there is a difference in outcome between groups treated with clomiphene citrate for ovulation induction vs those who were not.

STUDY DESIGN

Study design:

This was descriptive comparative study wherein subjects were recruited from the Outpatient Department of a tertiary hospital in Manila, Philippines. Salivary ferning patterns were observed, scored and correlated with ultrasonographic findings found on the same day.

Inclusion criteria:

Inclusion criteria: (1) married woman, (2) between the age of 20 to 40 years old, (3) diagnosed case of primary or secondary infertility following a thorough history taking and physical examination, (3) whose workup and management required at least 2 serial ultrasound findings of follicle monitoring per menstrual cycle.

Exclusion criteria:

Exclusion criteria: (1) Any use of hormonal therapy for the past three months apart from clomiphene citrate, (2) with a diagnosis of xerostomia, (3) smoking, (4) with an active infection of the oral cavity.

Withdrawal criteria:

Any participant may withdraw from the study at anytime. If during the duration of the study any participant takes hormone replacement therapy apart from clomiphene citrate, or differently from how it was prescribed, or if the subject fails to have an ultrasound done at the time it was requested, or if the request was done in a different institution by a different sonologist, the participant will be withdrawn from the study.

Duration of the study:

This was a two-year study to be conducted from April 2013 to August 2015. Review of literature, and data collection was done followed by statistical analysis.

Sample size calculation

In the local setting of the study site, infertility has a prevalence of 9% among the gynecology outpatient population which reached up to 5,375 last 2012. In calculating the sample size using the Epi Info software program, it was computed that a minimum number of 38 subjects are required for 80% power under 95% confidence interval. The study will include 40 subjects, each of whom will undergo at least two sonographic studies for follicle monitoring, and two salivary ferning tests. One set will be preovulatory, and the other, post ovulation.

MATERIALS AND METHODOLOGY

A detailed medical history was taken and all participants under went general and gynecologic examinations. Once infertility was diagnosed, whether primary or secondary, male factor infertility was ruled out through semen analysis. Once excluded, the need for follicle monitoring through transvaginal ultrasound was established.

The study, its objectives and methodology were thoroughly discussed with the subject and consent was secured. The patient was then scheduled for follicle monitoring conducted by a single sonologist at the same hospital as determined by the patients attending gynecologist. Two serial ultrasound determinations were done for one menstrual cycle. Signs of ovulation such as changes in the follicle size, fluid in the cul de sac, changes in the characteristic of the endometrium, etc, as a response to ovulation were determined and recorded. On the same day of follicle monitoring as determined by the patient's menstrual cycle, saliva sample was collected sublingually. The subject was asked to gargle three times using sterilized water, lasting 5 to 10 seconds each. Using a sterile wooden swab, saliva was collected from under the tongue and placed on the glass slide of an Ovatel™ microscope. A single Pathology resident who was blinded to the subject's day of mensesread the slide and the reading recorded. This was left to dry for 10 minutes, and arborization, characterized by the presence of main stems with leaves with side branches showing tooth like projections, was observed and recorded. The whole slide was examined and the sample scored based on the best pattern seen. The fern formation was graded 1 to 3 (Figure 1) as used by Pattanasuttinont, et al (2007). The miniscope is reusable. After usage, the slide was cleaned using sterile gauze for next use.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

From the number of subjects gathered (minimum number of 40 subjects were required for 80% power under 95% confidence interval).

ETHICAL CONSIDERATIONS

Resource and Funding

No funding aside from that of the investigators

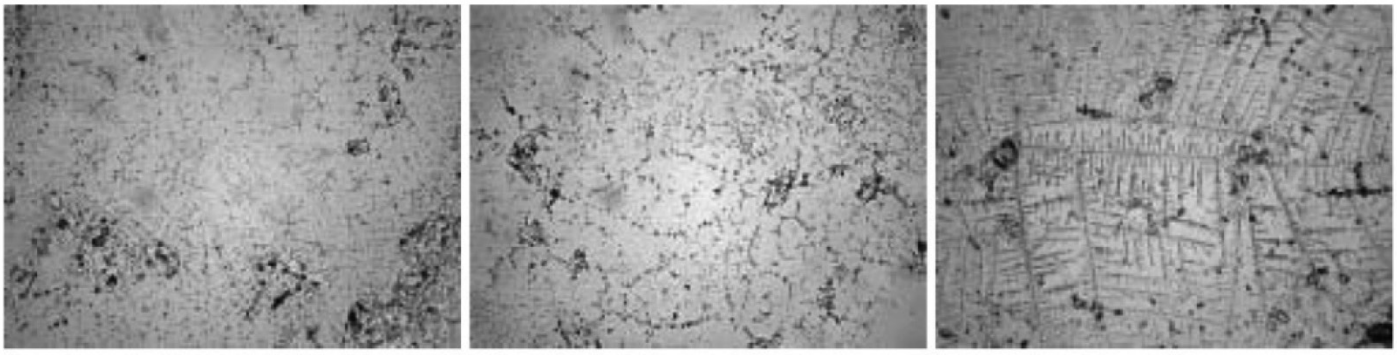
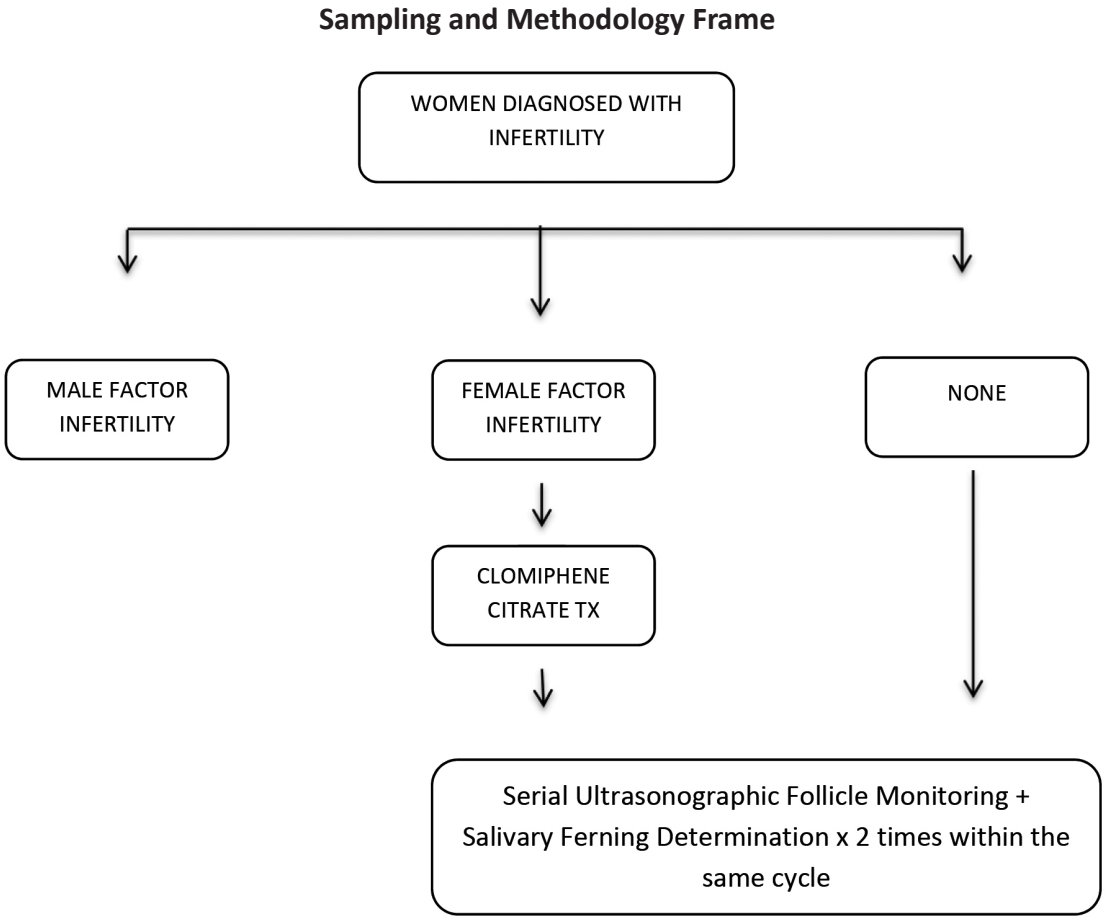


Figure 1. Score 1 = no visible ferning (random and unconnected dots), Score 2 = partial ferning (a combination of dots and ferns), Score 3 = full or peak ferning (complete fernlike patterns).



own money was used in this study. The transvaginal ultrasound for follicle monitoring was shouldered by the participants of the study since these are part of the work ups needed in women with infertility per current clinical practice guidelines. For patients in the clinical division, the procedure cost Php 340, while for private patients, it costs Php 1045. No additional charges were required since no additional views apart from the standard findings will be necessary for the study. Use of the Ovatel™ microscope used to observe for salivary ferning was free as well. The distilled water for cleaning the oral cavity was provided by the investigator.

Informed Consent and Confidentiality

A detailed summary explaining the nature, purpose and methodology of the study was furnished to each participant during the consult in the outpatient department and prior to the follicle monitoring conducted.

The participant was given an opportunity to ask questions regarding the nature, purpose or methodology of the study. All of these were addressed. An informed consent form in Filipino or English was obtained. Written informed consent was secured prior to inclusion of the patient to the study. The identities of the subjects as well as the data obtained from their participation were protected and will remain confidential.

Risks, Benefits and Incentive

The use of transvaginal ultrasound is an invasive procedure which is safe. Insertion of the probe was done after obtaining informed consent from the patient, with the only risk with it causing discomfort. Transvaginal sonography was carried out gently while observing proper aseptic technique. Observation of salivary ferning pattern is non invasive and post no risk to the patient.

Conflict of Interest

The investigators of this study have no conflict of interest with the use of transvaginal ultrasound for follicle monitoring and salivary ferning determination used.

Community Considerations

Infertility is a tedious and expensive disease to work up and treat. This study aimed that should it prove that there is a good correlation between salivary ferning and follicle monitoring through ultrasound, salivary ferning mayprovide a convenient, cheaper yet equally reliable means of determining a woman's fertile period. In doing so, infertile couples may be able to conceive faster and less expensively, their social and psychological concerns addressed more cost effectively.

RESULTS

A total of 40 subjects were included in the study. Table 1 shows the distribution of subjects according to age, gravidity and parity. Their age ranged from 20 to 40 years with a mean age of 30.88 years. There were 20 (50.0%) nulligravida women included. Among the sujects, 15.0% were nulliparous, 70.0% primiparous and 15.0% were multiparous.

Table 2 shows the distribution of subjects according to the day of the 1st and 2nd TVS. The 1st TVS was done as early as the 9th day and as long as at the 14th day with an average 1st TVS done at 11th day. The 2nd TVS was done as early as the 12th day and as long as at the 21st day with an average 2nd TVS done at 16th day. Note that the 2nd ultrasound included in the study were those that showed signs of ovulation.

Table 3 shows the distribution of subjects according to salivary ferning. On the 1st salivary ferning done on

Table 1. Demographic Characteristics of Subjects

	Frequency (n=40)	Percentage
Age		
20 – 30	18	45.0
31 – 40	22	55.0
Mean ± SD = 30.88 ± 5.52		
Gravida		
0	20	50.0
1	12	30.0
2	8	20.0
Parity	(n=20)	
0	3	15.0
1	14	70.0
2	3	15.0

Table 2. Distribution of Subjects According to Day of TVS

	Frequency (n=40)	Percentage
1st TVS Day		
9	6	15.0
10	9	22.5
11	7	17.5
12	6	15.0
13	7	17.5
14	5	12.5
Mean ± SD = 11.35 ± 1.65		
2nd TVS Day		
12	1	2.5
13	2	5.0
14	10	25.0
15	8	20.0
16	3	7.5
17	2	5.0
18	2	5.0
19	6	15.0
20	5	12.5
21	1	2.5
Mean ± SD = 16.28 ± 2.52		

Table 3. Distribution of Subjects According to Salivary Ferning

	Frequency (n=40)	Percentage
1st Salivary Ferning		
1	26	65.0
2	14	35.0
2nd Salivary Ferning		
1	1	2.5
2	11	27.5
3	28	70.0

the 1st TVS, there were 26 (65.0%) with 1 salivary ferning and 14 (35.0%) with 2 salivary ferning. On the 2nd salivary ferning done on the 2nd TVS, there were 1 (2.0%) with 1 salivary ferning, 11 (27.5%) with 2 salivary ferning and 28 (70.0%) with 3 salivary ferning.

Table 4 shows the comparison of the TVS day according to salivary ferning (1st and 2nd). In the 1st TVS day and 1st salivary ferning, there was no significant difference noted in the TVS day according to salivary ferning as shown by the p value of 0.31. However, in the 2nd TVS day, there was a significant difference noted as shown by the p value of 0.05. There was an increasing days of TVS with increasing salivary ferning. Salivary ferning of 2 has a median TVS day of 14 while salivary ferning of 3 has a median TVS day of 16.

DISCUSSION

Follicle monitoring by sonogrpahy is highly predictive in identifying the approach to and the occurrence of ovulation. Its limitation includes its cost, availablity of technology as well as trained sonologists for the procedure. (Stanford, et al, 2002)

The pattern of arborization occurs in relation with the level of Na and Cl ions in response to the increase in the level of estradiol in the body. Theoretically, this would allow the use of saliva in determining the fertile period. In earlier studies, LH surge in urine as well as salivary ferning occurred within 3 days of ovulation. (Pattansuttinont, et al, 2007).

The results of this study showed that sonographic findings and salivary ferning pattern during the preovulatory part of the cycle had a significant correlation in that there were indeed no signs of ovulation. Postovulatory results, however, did not show significant correlation.

Saliva in humans is produced by the parotid, sublingual and submaxillary glands. Its arborization is affected by the presence of electrolytes and mucin. During ovulation, as estradiol peaks, a significant increase in the water content is appreciated, and together with the correct proportion

Table 4. Comparison of the TVS Day According to Salivary Ferning

	1st Salivary Ferning			*p-value**
	1 (n=26)	**2** (n=24)		
1st TVS Day				
Mean ± SD	11.15 ± 1.52	11.71 ± 1.90		11.15 ± 1.52
	2nd Salivary Ferning			***p-value****
	1 (n=1)	**2** (n=11)	**3** (n=28)	
2nd TVS Day				
Mean ± SD	20.00 ± 0.00 (20)	15.06 ± 2.29 (14)	16.75 ± 2.52 (16)	0.05 (S)**

- *p-values >0.05- Not significant; p-values ≤0.05-Significant*

** *between 2 and 3 only*

Values are Mean ± SD, values in parenthesis are Median

of salts and sialomucin, ferning pattern in the saliva may be observed. The difference in the expected and the actual results of this study was could have included the temperature at the time of saliva collection and drying, the subject's diet, hydration and supplement intake. In other studies, the timing of saliva collection was controlled and only done premeals (Pattansuttinont, et al, 2007).The small sample size may have also contributed to the poor correlation.

CONCLUSION

Salivary ferning corresponded well with ultrasonographic findings during the preovulatory phase of the cycle, while no correlation was noted between the salivary ferning pattern and the postovulatory phase of the cycle. Hence, sonographic follicle monitoring remains a better predictor of ovulation, and more effective to determine the fertile period. ■

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