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The Detection of a Salivary Ferning Pattern Using the Knowhen Ovulation Monitoring System as an Indication of Ovulation

Hugh Melnick and Vasilios Theodoros Goudas*

Advanced Fertility Services, New York, NY, USA

Abstract

The ability to detect the period of potential monthly fertility is of great importance to a large segment of the female population in their reproductive years, both in terms of contraception as well as conception. In the current study, the KNOWHEN® ovulation monitoring system was used by a group of women who tested their saliva on a daily basis for the presence of a ferning pattern, a known biologic marker of impending ovulation. Transvaginal ultrasound examination, which is the "gold standard" for the detection of ovulation, was employed to visually determine if the cycle was ovulatory, either by demonstrating the presence of a dominant ovarian follicle or a corpus luteum found at the site of follicular rupture. If neither were observed, the cycle was determined to be anovulatory. The presence or absence of a ferning pattern in saliva was correlated with the actual documentation of ovulation with transvaginal ultrasound examination. Twenty two (22) women were studied for a total of 41 menstrual cycles. Salivary ferning was observed in 29 of 30 ovulatory cycles. False positive results in which ferning was present in an anovulatory cycle, occurred twice in 10 anovulatory menstrual cycles. Our findings indicate a strong correlation between the presence of salivary ferning and ovulation, as detected by the Knowhen ovulation microscope (Log Odds ratio 7.64, P<0.01, CI 4.26 to 11.02), thus validating its use. Age and weight did not appear to affect ferning, alone or together (P: NS).

Keywords: Salivary ferning; Estrogen; Graafian follicle; Endocervical glands; Menstrual cycle

Introduction

Whether conception or contraception is desired, women must have an awareness of their menstrual cycles in order to assess the optimal time to have, or to avoid, coitus. There are three methods of determining periods of fecundity, all based upon observations of biophysical changes brought about by hormones that are involved in the cascade of events that culminate in the monthly production of a fertilizable oocyte. The most commonly used natural methods of ovulation monitoring include the measurement of basal body temperature and the self-examination of the cervical/vaginal secretions for the presence of an increase in amount of clear, elastic discharge which supports the survival and progressive motility of spermatozoa in the female genital tract. The rise in the basal body temperature noted in an ovulatory cycle results from the direct effect of progesterone, produced by the corpus luteum which occurs after the egg has been released from the Graafian follicle, on the thermoregulatory center in the brain. The characteristic cellular patterns and arborization of preovulatory vaginal secretions were described by Papanicalou in 1946 [1]. Rydberg, as well as Zondek and Rozin found the same crystallization patterns in dried cervical secretions in the days close to ovulation [2,3].

Ferning, originally referred to as arborization or palm-leaf pattern, is associated with elevations in circulating estradiol-17b levels originating from the granulosa cells which surround the developing oocyte as it reaches its point of full maturation. Observed microscopically, dried preovulatory cervical mucus exhibits a characteristic fern-like pattern of crystallization which is caused by increased concentrations of sodium and potassium chloride [4]. Zaneveld [5] further studied the hormonal changes associated with the ovulatory cycle that are reflected in salivary ferning.

Because salivary estradiol is found in saliva in peak levels at the time of ovulation [6], salivary ferning is found in dried salivary secretions at time of maximal fecundity in the menstrual cycle. Observation of a ferning pattern in the saliva at the time of ovulation was first described in 1968 by Dr. Biel Cassals [7]. Saliva exhibits biologic behavior similar to that of mucus secretions produced by the uterine endocervical glands, both in its enhanced response to elevated circulating estrogen levels, as well as its inhibition by estrogen antagonists. Ferning in both cervical mucus and saliva can be inhibited by an estrogen antagonist, the selective estrogen receptor modulator (SERM) clomiphene citrate [8]. Furthermore, treatment with an expectorant such as guaifenesin or potassium iodide (Super Saturated KI, [SSKI]) causes an increase in liquidity and volume of mucus secretions in patients with abnormally dry bronchial, cervical and salivary secretions. Clinically, many ovulatory patients with scant, thick cervical mucus are treated with expectorants or estrogen to improve the quantity and liquidity of their cervical secretions, to allow sperm survival and progressive motility in the female genital tract [9]. Such similarities in biologic behavior suggest common stimulatory and inhibitory receptors in the cells of both the endocervical and salivary glands.

KNOWHEN® is a commercially available Kit that has standardized the process of assessing salivary ferning. It contains a hand-held (patent pending # 13,076,728) pocket mini microscope with an aspherical lens system with 60X which is used by the subject herself and the test is performed daily in the morning. KNOWHEN® is accompanied by a smartphone App (Apple, Windows) that will store a woman's fertility history and issue electronic alerts or text messages when it is an appropriate time to have intercourse to get pregnant or to avoid getting pregnant. App records the test date and provides an ovulation calendar, as well as can tract the sexual activity. The KNOWHEN® Kit also includes an Educational CD with information women need to know about their cycles.

Materials and Methods

We performed an open label prospective study of the KNOWHEN®

***Corresponding author:** Vasilios Theodoros Goudas, Reproductive Endocrinologist, Advanced Fertility Services, 1625 Third Avenue, New York, NY 10128, USA, Tel: 2123698700; Fax: 212-289-8461; E-mail: vasili@goudas.net

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system in order to evaluate its accuracy in predicting ovulation, comparing the detection of a salivary ferning pattern as a predictor of ovulation to transvaginal ultrasonography as the gold standard of ovulation. Healthy female volunteers aged between 21 and 40 years of age who reported menstrual cycles between 26 and 34 days were recruited to participate. Tobacco use and hormonal contraception were exclusion criteria. The participants were trained in the use of the KNOWHEN® pocket microscope. Specifically, they were instructed upon rising each morning to collect their salivary samples from the area at the base of the tongue, and to microscopically examine the air-dried sample for the presence or absence of a ferning pattern. They recorded their results daily. Each woman was instructed to report to the Investigators Center at the midpoint of her menstrual cycle for a transvaginal ultrasound examination to determine if the cycle was ovulatory, as determined by the presence of a dominant ovarian follicle of a minimum mean diameter of 18mm, or by the presence of a corpus luteum. In the absence of a dominant follicle or corpus luteum, the cycle was declared anovulatory. The subjects' daily observations of their morning saliva for the presence or absence of a ferning pattern was compared to concomitant sonographic findings. Descriptive statistics means and standard deviations, as well as 95% confidence intervals were calculated for sensitivity, specificity and positive and negative predictive values. We also used a repeated measures logistic regression model, with generalized estimating equations to account for correlations among observation from the same subject to assess the associations between ovulation and ferning, controlling for age, weight, and the interaction of age and weight.

Results

A total of 22 women were recruited and data were obtained from observation of 41 menstrual cycles. The mean age was 27.8 ± 7.4 (range 22 to 34, median age 28). All volunteers completed their first month study. A total of three volunteers did not participate during the subsequent month, two because they conceived during the first month of the study and one for personal reasons. All volunteers (including the second month drop-out) reported that the KNOWHEN® kit was easy to use and had no difficulty identifying a ferning pattern (Figure 1). Of the 41 cycles studied, 29 were documented as being ovulatory by the sonographic presence of a dominant Graafian follicle or by the detection of a corpus luteum. Twelve (12) cycles were declared anovulatory because of the absence of sonographic criteria of ovulation. Ferning was reported in 28 of 29 ovulatory cycles. A ferning pattern was reported in 2 anovulatory cycles and was not reported in 10 cycles in which ovulation were not documented. These results are shown in Table 1.

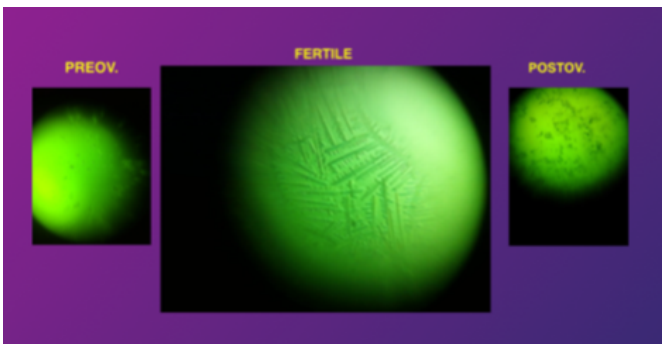

Figure 1: The KNOWHEN microscope enhances visualization of the ferning pattern allowing for easy identification of ovulation.

	Ovulation	No Ovulation	
Ferning	28	2	30
No Ferning	1	10	11
Total:	29	12	41

Table 1: The results of ferning and ovulation for the study. Knowhen was able to identify 28 of the 29 ovulatory months while it was false positive in only 2 cases.

	Repeated measures logistic regression estimates			
Predictor	Coefficient	Standard error	P value	95% CI
Intercept	-25.48	20.23	0.21	-65.12 to 14.16
Ferning	7.64	1.72	<0.01	4.26 to 11.02
Age	1.01	0.77	0.19	-0.51 to 2.52
Weight	0.06	0.14	0.7	-0.22 to 0.33
Age*Weight	0	0.01	0.49	-0.01 to 0.01

Table 2: Summary statistics of predicting ovulation – a repeated measures logistic regression model with generalized estimating equations was used to assess the associations between ovulation and ferning, controlling for age, weight, and the interaction of age and weight.

Results of the regression analyses are shown in Table 2. Ovulation had no significant association with age (range 22-34), weight (range 110-168 lbs), or the interaction of age and weight. Ferning pattern also had no significant association with age, weight, or the interaction of age and weight. However, the presence of a ferning pattern compared to the absence of a ferning pattern is associated with a 7.64 increased log odds of ovulation ($p<0.01$, CI: 4.26 to 11.02), controlling for age, weight, and the interaction of age and weight. This provides evidence to support the use of the KNOWHEN® ovulation monitoring system to detect the period of potential monthly fertility, with the presence of a ferning pattern indicating ovulation. The KNOWHEN® ovulation monitor kit had a positive predictive value of 93.3% (95% Confidence Interval 85.5 to 99.9%) and a sensitivity of 96.5% (95% confidence interval 90.9 to 99.9%). In the 12 anovulatory cycles, the Knowhen kit identified non- ovulation in 10 cases, producing 2 false positive results, for a false positive rate of 16.7% and a negative predictive value of 90.9% (95% CI 82.1 to 99.7%).

Discussion

The detection of a salivary ferning pattern, like that found in preovulatory cervical mucus, is a biologic indicator of potential fecundity and is a recognized, but relatively underutilized method of "at home "ovulation testing" [10]. Since the earliest study by Dr. Cassals [7], who first described the phenomenon of salivary ferning and its relationship to ovulation, there have been several studies published which support the use of salivary testing for a ferning pattern as an indicator of the period of fecundity in the menstrual cycle. In 1991, M. Guida at the Institute of Obstetrics and Gynecology of Naples (Italy) compared the results obtained with the crystallization of saliva with basal temperature, cervical mucus, abdominal pain and ultrasound examination of the ovaries. He reported that in 92% of cases, saliva produced a crystalline pattern in the ovulatory phase of the menstrual cycle. This study was validated in a subsequent paper by Barbato [11].

More recently, Salmassi et al. [12] found 78% specificity and an 80% sensitivity using a pocket microscope to detect salivary ferning in a group of in vitro fertilization patients. There was an 89.4% agreement between the readings obtained with the pocket microscope and a sophisticated inverted microscope. Further clinical confirmation is found in another study that successfully correlated salivary ferning with estradiol levels in monitoring controlled ovarian hyperstimulation for in vitro fertilization [13]. Other published studies indicate that the detection of salivary ferning may have some limitations as a method of

defining the period of fecundity. In a 1999 study by Guida [14], who had previously reported a 92% correlation between salivary ferning and ovulation [15], reported in a subsequent study that, whereas the urinary Lh correlated 100% with ultrasound evidence of ovulation, the sonographic detection of ovulation with salivary ferning was only 36.8%. In their study, 57.8% of salivary specimens were reported as being "uninterpretable" [14]. The authors suggested that the large percentage of interpretable results could have been due to the fact that many of the patients were not taught to use the microscopes or interpret the slides properly. This point is important because adequate patient instruction is the key to the successful use of this method of ovulatory surveillance. Another possibility that could account for the large number of uninterpretable results in this study is that the actual microscopes used by the patients in this study did not produce an adequate image because of suboptimal illumination or inferior optics. There are currently 13 pocket microscopes designed for detecting ferning in body fluids that have either received patents from, or have filed patent applications with, the U.S. Patent Department. All differ with respect to structure, illumination, magnification and optics which may have an effect on the results which were obtained. Another paper published in The Lancet in 1998 reported a sensitivity of 53% between ovulation, confirmed sonographically, and salivary ferning when a physician used an unspecified pocket microscope to evaluate patients' saliva specimens. This is compared to 86% sensitivity when another physician used a laboratory grade light microscope. This study points out that not all pocket microscopes, and possibly not all observers, are equal in the ability to detect salivary arborisation [16].

Although they confirmed the correlation of serum 17-b-estradiol levels with those found in saliva in a group of 31 patients ($p<0.0001$), the authors found no correlation between 17-b-estradiol levels in saliva and ferning. Another curious finding in this study was the fact ferning was found in the saliva of 8 out 10 post-menopausal women and in 10 of 10 males tested [17]. One would assume that none of the female subjects tested were taking exogenous estrogen replacement therapy, nor were any of the subjects taking expectorants or iodine containing compounds, which cause an increase in salivary secretory output. Males may have low levels of estrogen, the levels are too low to produce a ferning pattern in saliva. Although these findings are in direct contradiction to a large body of confirmed experimental data and clinical experience, we hope to confirm or refute these findings in a future study.

Limitations

We were able to prove good correlation between ferning as identified by the Knowhen kit and ovulation, with a relatively low number of volunteers. Due to the low number of volunteers we were unable to see any correlation between ferning and ovulation and age or weight or age and weight together (Table 2), although these are factors that are known to affect ovulation, a typical type II statistical error due to low N.

Conclusions

Our data, as well as our clinical experience, support the use of the KNOWHEN® monitoring system for women who wish to conceive, as a way of indicating their period of potential fecundity. A ferning or arborization pattern in both cervical mucus and saliva is caused by rising estradiol levels which parallel the development of an oocyte to the point of its release from the ovarian follicle, after which the estradiol level plummets. The closure of the window of fecundity is indicated by the disappearance of the ferning pattern in both the cervical mucus and saliva. The ferning pattern in cervical mucus and saliva are typically seen in normal ovulatory cycles, as well as in menopausal women who are treated with estrogen replacement therapy. A significant number of women who are treated with certain fertility drugs which are estrogen antagonists, such as clomiphene citrate, may ovulate, but may not always produce cervical secretions and saliva which exhibits the ferning phenomenon. Therefore, the use of the KNOWHEN® monitoring system is not advocated for routine use in ovulation monitoring when clomiphene citrate is used for the induction of ovulation. With regard to the use of the KNOWHEN® ovulation monitoring system for contraceptive purposes, there is a small potential risk of conception if coitus occurs on the sixth day prior to ovulation, when ferning may not be detectable in the saliva, but when there may be an adequate amount of cervical mucus to support sperm survival until the point in time that the oocyte is extruded from its follicle. Since there is only a small probability that conception may occur within a time period that may predate ovulation by six days [17], couples who use this system for contraception should maintain careful records of their monthly ferning patterns, so that unprotected coitus can be avoided from at times of potential. An app is provided to insure that a woman using the KNOWHEN® system is able prospectively to anticipate the earliest time of potential conception.

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