

Changes of salivary estrogen levels for detecting the fertile period



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ABSTRACT

Objectives: Is the saliva test, *Geratherm* ovu control, as accurate as the established urinary luteinizing hormone (LH) test for detecting ovulation and the following the fertile period?

Study design: The voluntary participants were 74 healthy women with regular menstrual cycles and not using any hormonal contraceptives. The women used *Geratherm* ovu control, a small plastic hand-held microscope, for detecting the fertile period. A drop of saliva from sublingual was put onto the lens of the microscope. Three results were possible: non-fertile (dot pattern), transitional and fertile (ferning pattern).

The participants performed the saliva test from the 5th till the 22nd day of the menstrual cycle and noted the respective result in a table. In addition to *Geratherm* ovu control, the EXACTO test for determining urinary LH concentration and the time of peak fertility was also performed.

Results: Positive LH shows a sharp increase beginning on the 10th cycle day with a maximum on the 17th cycle day. The curve for positive saliva and questionable positive saliva (one curve) is almost parallel with the curve for positive LH, reaching a maximum on the 16th cycle day. There is a high level of conformity for the same test results from the 5th (100%) till the 14th (84%) cycle day and from the 18th (80%) till the 22nd (96%) cycle day which corresponds to the pre- and post-ovulatory period.

Conclusion: The saliva and the LH test both detect the fertile window of a menstrual cycle. Caused by the different hormones (estrogen for the saliva and LH for the LH test) leading to the respective positive test results, saliva turns positive 24 h before LH. Consequently, the saliva test can be used as an ovulation test and help women maximize their chances of conceiving. There is also a high congruence between LH and saliva in the pre- and post-ovulatory period, indicating that the saliva test can also be used for contraception purposes.

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Introduction

For approximately 50 years, urinary hormone assays have been used to help recognize the fertile phase of the menstrual cycle [1,2]. These tests can be applied by women who want to prevent pregnancy by using Fertility Awareness-Based Methods (FABM). In contrast to chemical contraception, this kind of natural family planning works without intervening in the hormonal cycles [3]. The Standard Days Method (SDM), cervical mucus method or basal body temperature are further examples for FABM. The cervical mucus method is one of the most widely used biological markers for self-estimating the beginning and the end of the fertile phase in a menstrual cycle by recognizing cyclical changes in the amount or

consistency of cervical secretions [4–6]. It is usually considered that the start of the fertile interval generally corresponds to a significant rise in estrogen levels which leads to secretion of estrogenic cervical mucus and thus changes in vaginal discharge [7–9]. Similar to self-observing ferning patterns in cervical mucus, saliva changes in a similar manner during the menstrual cycle. The ferning is caused by NaCl which cyclically increases under the influence of estrogen and coincides with the female fertile period [10,11].

In contrast to the cervical mucus method, the urinary testing methods measure the concentration of the hormone to be examined, in order to identify the ovulatory period. Three methods have been discussed and proposed in scientific literature: oestrone-3-glucuronide (E1G), pregnanediol-3a-glucuronide (PDG) and luteinizing hormone (LH) [12,13].

Fig. 1 gives an overview of the hormonal changes during the menstrual cycle which will be important for the analysis of the

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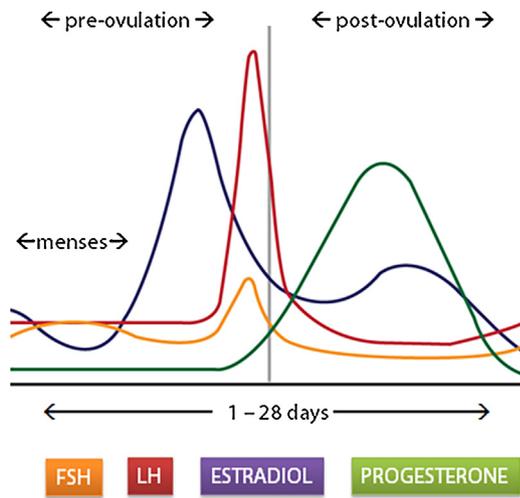


Fig. 1. Hormonal changes during the menstrual cycle.

results. Responding to low levels of estrogen, the hypothalamus sends a signal to the anterior pituitary gland to release follicle stimulating hormone (FSH). In addition to stimulating ovarian follicular growth, FSH stimulates the granulosa cells of the follicle to produce gradually increasing amounts of estrogen. As the estrogen production accelerates, it begins to inhibit FSH and at the same time stimulates luteinizing hormone (LH). This leads to a major surge in LH with a peak 12 to 24 h before ovulation. This surge in LH is accompanied by a parallel surge in FSH and estrogen [14,15]. After the peak of LH, FSH and estradiol, continuing secretion of LH causes the granulosa cells to produce progesterone which is produced for about 10 days in the absence of pregnancy. Afterwards, progesterone and estrogen concentration rapidly decline which leads to the bleeding. Responding to the low levels of estrogen, the hypothalamus again causes release of FSH and the cycle begins again [16].

One method of examining the validity of fertility monitors using saliva is to compare them with established markers of ovulation, such as LH in urine. The aim of this study was to determine the accuracy of the hand-held microscope, *Geratherm* ovu control, by comparing it with the established urinary LH test EXACTO.

Materials and methods

Subjects

The voluntary participants were 74 healthy women with regular menstrual cycles (25–35 days) and not using any hormonal contraceptives or intrauterine devices (baseline period). Pregnancy and breast-feeding were also exclusion criteria. The mean age was 24 years [20–35]. The sociodemographic data are shown in Table 1. There was no ethic committee vote necessary, because not patients, but voluntary women with informed consent were participating in the study.

Table 1
Sociodemographic data.

	Minimum	Maximum	Average
Age (years)	20	35	24
Menstrual cycle (days)	27	32	28.5
Physical examination (vaginal ultrasound)	Positive: 25	Negative: 50	
1. Ovarian cyst	1		
2. Primordial follicle	24		

At study initiation, each woman completed a standard questionnaire and underwent a physical examination. The data collected were current age, length of menstrual cycle and begin and end of the current menstruation. The physical examination, in particular a vaginal ultrasound, took place in the Department of Gynecology and Obstetrics, University Hospitals Schleswig-Holstein, Campus Kiel or in a private practice. All findings, such as an ovarian cyst, were noted. The menstrual cycle ranged from 27 to 32 days (according on their own statements). The test was explained to the participants and they received instructions for use as well. Afterwards the tests were done at home.

Methods

The women used *Geratherm* ovu control, a small plastic hand-held microscope, for detecting the fertile period. The test was performed during the morning or afternoon either before or at least 3 h after eating, drinking, smoking or teeth brushing. A drop of saliva from sublingual was put onto the lens of the microscope and after 10–15 min of drying, it was ready for evaluation. Fig. 2 shows the three possible results: (a) simple dot patterns and lines indicate no ovulation, (b) small ferning patterns or crystal forms appearing among the spots and lines indicate ovulation during the next 3–4 days and (c) “ferning”, crystal-like patterns show the fertile period. Ovulation is about to occur or already occurring and conception is possible.

The participants performed the saliva-test from the 5th till the 22nd day of the menstrual cycle and noted the respective result (not fertile, transitional or fertile) in a table. In addition to *Geratherm* ovu control, the EXACTO test for determining urinary LH concentration was also performed during the same period mentioned above.

EXACTO monitors luteinizing hormone (LH) in urine to identify the time of peak fertility [17]. A positive result is shown at a concentration of 40 mIU/ml LH in urine with a sensitivity of >99.9%. To make sure that the hormone level is concentrated enough for the test to detect, it was important not to drink at least 2 h before doing the test. On the other hand, morning urine was not permitted because of its high concentration and the risk of false-positive results. The test stick was held directly in the urine stream for 10–15 s and the result read off after 3 min. The test is positive when a line is visible both in the control and in the test window which means that ovulation takes place in average within the next 24–36 h. In case of a positive result, the test was repeated after 6–8 h to prove ovulation.

The program “R” was used for statistical analysis and shows the data as a descriptive statistics in the form of graphs.

Results

For better analysis of the collected data, positive saliva and questionable positive saliva were both considered to be positive. In



Fig. 2. *Geratherm* ovu control, possible results: (a) not fertile: simple dot patterns and lines → no ovulation; (b) transitional: small ferning patterns among the spots and lines → ovulation during the next 3–4 days; (c) fertile: ferning, crystal-like pattern → ovulation is about to occur or already occurring.

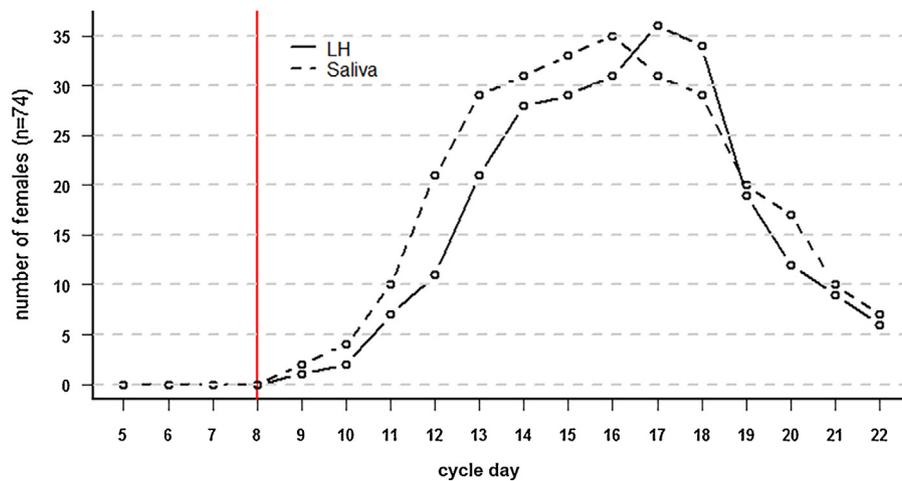


Fig. 3. LH and saliva during the cycle. Nearly parallel curves for positive LH and positive Saliva with a maximum at the 16th (for Saliva) or 17th (for LH) cycle day.

the following, positive saliva describes the sum of positive and questionable positive saliva for the respective cycle day.

Positive LH shows a sharp increase beginning on the 10th cycle day with a maximum on the 17th cycle day, a small descending plateau till the 18.5th cycle day and afterwards a sharp decrease till the 22nd cycle day. The curve for positive saliva and questionable positive saliva (one curve) is almost parallel with the curve for positive LH, reaching a maximum on the 16th cycle day (Fig. 3).

Fig. 4 shows the percent agreement between the LH and the saliva test. There is a high level of conformity for the same test results from the 5th (100%) till the 13.5th (84%) cycle day and from the 18th (80%) till the 22nd (96%) cycle day which corresponds to the pre- and post-ovulatory period. The interval between the 14th and the 18th cycle day shows a less positive match because estrogen, which is responsible for a positive saliva test, increases temporally before the LH peak.

The specificity (true negative rate) describes the proportion of negative results which are correctly identified as such and is complementary to the false positive rate. In contrast, the sensitivity (true positive rate) measures the proportion of actual positives which are correctly identified as such and is complementary to the false negative rate.

The specificity shows a matching rate of 100% from cycle day 5 till cycle day 8. During this period the saliva and the LH test are both negative. Afterwards there is a decrease in specificity to 58%

on the 16th cycle day, followed by a rise to 97% on the 22nd cycle day. Except for cycle day 13.5 to cycle day 17.5 specificity is always higher than 80%. As mentioned above, the lower specificity from the 14th to the 18th cycle day is caused by the different hormone curves increasing one after the other.

Sensitivity shows a similar curve with statistical outliers from the 11th to the 14th cycle day, most likely caused by the small number of cases (Fig. 5).

Comments

The results of this study show cyclical changes in saliva as well as in urinary LH concentration. The maximum for positive saliva was shown on the 16th cycle day, whereas LH had its maximum on the 17th cycle day. This one-day difference concerning the maximum of the two tests is caused by the time sequences of hormonal changes: estrogen (responsible for the saliva test) and LH respectively (responsible for the LH test). These hormonal changes have already been described above (Fig. 1). According to his findings Pauerstein described the relationship between the hormonal curves as follows: The mean interval from the estrogen peak to ovulation was 34 h, the interval from the estrogen peak to the LH peak was 24 h, and that from the LH peak to ovulation was 9 h [18].

There is a high congruence between the LH and the saliva test during the pre- and post-ovulatory period, which means between

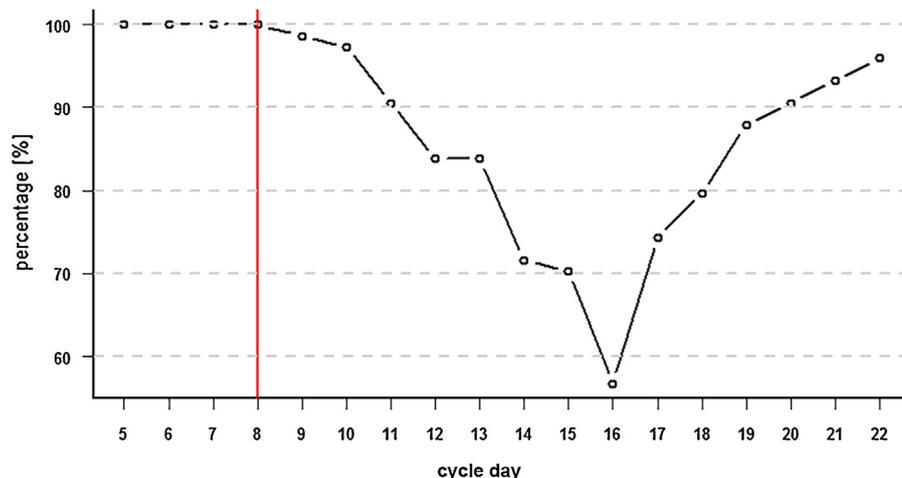


Fig. 4. Positive match of LH and Saliva. Percent agreement between LH and Saliva: high conformity from the 5th (100%) until the 13.5th (84.5%) cycle day, as well as from the 18th (80%) until the 22nd (96%) cycle day.

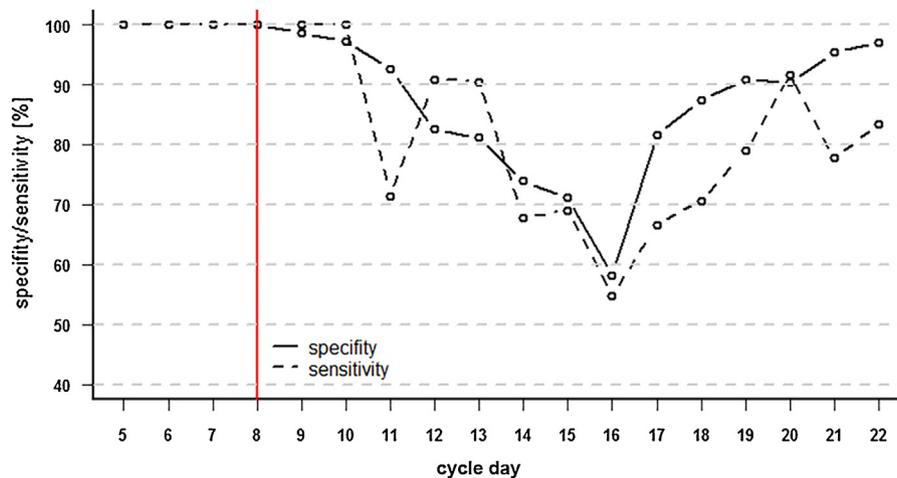


Fig. 5. Specificity and sensitivity (Saliva vs. LH). Specificity: matching rate of 100% (5th–8th cycle day). From cycle day 13.5 until cycle day 17.5 specificity > 80%. Approximately similar curve for sensitivity with statistical outliers caused by small number of cases.

cycle days 5–14 and between cycle days 18–22. During the ovulatory period saliva and LH show a low percent agreement (with a minimum of 57% on the 16th cycle day). The interval between the 14th and the 18th cycle day shows a less positive match because estrogen, which is responsible for a positive saliva test, increases temporally before the LH peak.

These results can be compared to a number of earlier studies correlating positive ferning in cervical mucus and saliva during the fertile period of women [15,19]. A decrease in sialic acid concentration during the pre-ovulatory phase has been observed both in human cervical mucus [20] and human whole saliva [21].

The data of the present study show a noninvasive way for determining the stages of the menstrual cycle through chemical profiles. Many women are interested in using fertility-awareness methods of contraception, based on identifying the fertile days in the menstrual cycle. Low costs, no side effects and no drug involvement are positive aspects and make this method attractive especially for women in developing countries in which hormonal contraceptives are not widely available.

Nevertheless, saliva undergoes changes caused by fluid intake, body hydration, systemic and oral drugs, salivary glands and systemic diseases. All these factors could modify the characteristics of saliva concerning salivary secretion and solute concentrations and perhaps influence the results of the test with regard to the development and morphology of the dried saliva residual patterns.

Bigelow et al. presented their results in 2004 concerning mucus observations and the probability of conception [4]. They proposed the hypothesis that the consistency of the cervical mucus determines whether sperm will be capable of survival and transport to the ovum and is consequently an essential predictor of the possibility of pregnancy [22]. In Europe, 782 women were recruited from natural family planning centers and daily records of intercourse, basal body temperature (BBT) and changes of cervical mucus were collected. Cervical mucus was divided into four categories ranging from score 1 (no discharge and dry) to score 4 (transparent, stretchy, slippery). The BBT, as a mucus-independent marker, was used to identify the day of ovulation in each menstrual cycle. Bigelow et al. outlined an increasing trend in the day-specific probabilities of pregnancy with increases in the mucus score. The day of the lowest fertility was 5 days before ovulation and the day of the highest fertility was 3 days before ovulation [4]. Within this fertile window, the type of mucus observed on the day of intercourse is more predictive of conception than the timing relative to ovulation. This means

that the probability of conception occurring 2 days before ovulation, when there is no observed discharge, is lower than the conception probability on any day in the 6-day interval when most fertile-type mucus (score 4) is present. Consequently, monitoring of mucus contributes an important clinical marker of days with high conception probabilities.

Another interesting study concerning fertility awareness-based methods was published in 2001 by Dunson et al. [15]. The authors described the *TwoDay Algorithm* as a simple method for identifying the fertile period: conception is possible if cervical secretions are present on that day or were present the day before. A characterization (e.g. consistency, amount) of the mucus is not necessary, the woman has merely to note whether she has any or not. The aim of the study was to evaluate the relationship between secretions and the daily probabilities of pregnancy in order to show the effectiveness of the *TwoDay Algorithm*. The theory was that the presence of secretions predicts not only forthcoming ovulation, but also the day-specific pregnancy probabilities within the fertile interval defined relative to ovulation. Data were collected from a large multinational European fecundability study (European Study of Daily Fecundability, ESDF), where the daily probability of conception on each cycle day relative to ovulation was analyzed [23]. In each menstrual cycle, women kept daily records of basal body temperature (BBT), cervical secretions and coitus [24]. With the help of BBT, the day of ovulation was determined, whereas the interval of potential fertility (−8, 2) was defined as the fertile window beginning 8 days prior to and ending two days after the identified ovulation day. The data collected shows that for most women the *TwoDay Algorithm* is very effective in identifying the fertile days of the cycle. Intercourse on any particular day relative to ovulation had a higher probability of resulting in pregnancy if secretions were present on that day or the day before ($P \leq 0.001$) [25]. Dunson et al. proposed that the probability of pregnancy is reduced by nearly 50% for women who had no secretions on two consecutive days [15].

The results of our study have important clinical implications. Observations of saliva can be used to detect the fertile period in the menstrual cycle [26]. Women can use the saliva test as an ovulation test when planning a pregnancy to help maximize their chances of conceiving. *Geratherm* ovu control is user-friendly, inexpensive and can be used discreetly at home. This one-day difference caused by the time sequences of hormonal changes could be an advantage of the saliva test that it allows to identify the fertile period that much earlier.

Conclusion

The saliva and the LH test both detect the fertile window of a menstrual cycle. Caused by the different hormones (estrogen for the saliva and LH for the LH test) leading to the respective positive test results, saliva turns positive 24 h before LH. Consequently, the saliva test can be used as an ovulation test and help women maximize their chances of conceiving.

Conflict of interest

The authors have no conflicts of interest or financial ties to disclose.

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