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A NEW TEST FOR HUMAN FEMALE OVULATION DIAGNOSIS

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Saliva Fertility Tester - Like Maybe Baby

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Abstract

The Authors performed the saliva test on n°328 women using the Saliva Tester. In n°48 cases the test results revealed a fern-like crystallized structures as illustrated in "Figure 3" below. In n°20 cases they performed a Transvaginal Ultrasonography (T.V.U.S.). In n°16 cases (80%) they found an ovarian follicle greater than 2 cm.

Key words: Fertility tester, saliva, fertile period, Transvaginal Ultrasonography.

To know the days of their menstrual cycle when fecundation is possible has always been a demand felt by women and this both in the case when they want to avoid it (1). Many methodologies exist that enable us to verify or not the presence of a mature ovarian follicle or its happened explosion. A very simple methodology was reached with observation and testing of the Saliva Tester; results that may be easily and directly realized by any woman, at any moment of the day, anywhere. The prospective advantages that descend as to the knowledge of the useful days for a fecundation are such that they led us to directly and correctly try this new test.

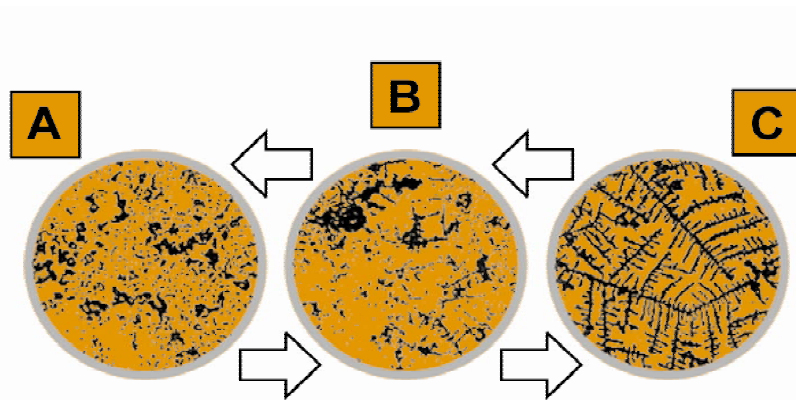
Material and Method

It has been considered the use of a mini-microscope, or "Saliva Microscope", made up of a small cylindrical body or eyepiece where we note an optical part with an opposite small slide where a sample of saliva is placed. As soon as the sample of saliva has dried, the small eyepiece body is put into a cylindrical container, that leads down to a lighting system. By focusing the lens, a fern-like characteristic crystallization is pointed out, in a very near correlation with the ovulation period (Figure 3). Out of the fertile days there are no fern-like

crystallization structures seen, as pictures named "Figure 1" (during the non-fertile period) and "Figure 2" (during the intermediate period) indicate.

We have taken into consideration 328 women between 15 and 50 years old, not using OCP who were placed under our observation at the out-patients' departments of the Institute of Obstetrics and Gynecology of the University "La Sapienza" in Rome. No selective principle has been adopted. We have only invited the patient to leave a sample of saliva on the slide of the mini-microscope, and afterwards age, day of the cycle in which the patient was and length of her cycle were noted. 48 women, whose sample of saliva gave the fern-like characteristically stratification were invited to undergo an ultrasonography with transvaginal sound for the control of the ovary and of the presence or not of the pertinent follicle. 20 women accepted our request, 28 women kindly refused.

Figure A (Infertile) Figure B (Intermediate) Figure C (Fertile)



Results

Results are reported by Tables 1,2 and 3. In Table 1 we see n°48 women of n°328 who, as far as anamnesis is concerned, are in the ovulation period; n°40 of these women gave us the picture "Figure 3" type after test, n°40 gave us the picture "Figure 2" and n°248 the picture "Figure 1" type (Table 2). At the anamnesis, n° 36 women were in the intermediate period; in all 36 cases of this group, we have noted a picture "Figure 2" type. At the anamnesis, n° 244 women were in the non-fertile period and we have noted a picture "Figure 1" type in all the 244 cases. In the Table 3, are pointed out the n°20 cases who, at the saliva test, showed a picture "Figure 3" type and have accepted to undergo sonographic control by means of transvaginal sound..

Table 1 -Number of women undergoing the saliva test, correlated to the supposed fertile or non-fertile period of the cycle.

Day of the cycle	Non-fertile period	Intermediate period	Fertile period	Total
N° of women undergoing test	244	36	48	328

Table 2 -Picture stressed by means of the saliva test.

Stressed picture	Figure 1	Figure 2	Figure 3	Total
N° Cases	248	40	40	328

Table 3 -Echographic control of n° 20 women by means of picture Figure 3 type of the saliva test

Ovarium Echographic Control	Follicle Absence	Follicle < 1 cm	Follicle > 2 cm
N° cases	0	4	16

In 16 cases, equal to 80% of the total, an ovarian follicle has been stressed having dimension over 2 cm, in 4 cases a follicle having diameter less than 1 cm has been stressed.

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Conclusions

A Saliva Microscope can be used to help **determine the fertile period**: the fertile cycle will be identified with a continuous use, cycle after cycle. The Tester can also allow, by repeating every month the tests, to obtain some significant indications about the monthly cycle behavior and the hormonal situation of the woman. The tester can also be used to help **avoid an undesired pregnancy**: the comparison of the figures given in Tables 1 and 2 indicate the following: at the anamnesis **n° 244 women** were in the non-fertile period and we have noted a

picture "Figure 1" type. In all 244 cases at the anamnesis **n° 36 women** were in the intermediate period; in all 36 cases of this group, we have noted a picture "Figure 2" type. In both cases we had a perfect coincidence between supposed fertile or infertile days of the cycle and tests executed with "The Saliva Fertility Tester". At the anamnesis **48 women** were in the fertile period; in 40 of them we have noted a picture "Figure 3" type. Such difference is due to the fact that 4 of them had a meal one hour before testing, thus invalidating the result, and the other 4 may have not correctly performed the test. Therefore, even if we assume that all these **8 women** were in fact fertile (very unlikely), the final result of such test is that **ONLY 8 women** out a panel of 328, i.e. **less than 3%, resulted in false negative** (in fact fertile) at the saliva testing. **WE CAN CONCLUDE STATING THE FOLLOWING:**

- **"TO OUR KNOWLEDGE THE SALIVA MICROSCOPE CAN BE SIGNIFICANTLY HELPFUL IN PROVIDING AN ADDITIONAL AID TO WOMEN WANTING TO IDENTIFY THEIR LIKELY FERTILE PERIOD DURING THE MENSTRUAL CYCLE.**
- **THE OUTCOME IS THAT ITS ACCURACY, IN DETERMINING FERTILE MOMENTS OF THE MONTHLY CYCLE, IS CLOSE TO 98%, HIGHER THAN OTHER CONTRACEPTIVE DEVICES LIKE CONDOM, SPYRAL, DIAPHRAGM, NOT TO MENTION BILLINGS AND OGINO-KNAUS METHODS, PLACED AT THE LOWER END OF THIS SCALE.**
- **TO PREVENT A PREGNANCY ONLY THE CONTRACEPTIVE PILL HAS A HIGHER RATE (99%) ; AT THE SAME TIME EVERYBODY TODAY IS AWARE OF BAD SECONDARY EFFECTS OF THE USE OF THE PILL. THIS TESTER IS NOT INVASIVE AND THERE IS NO NEED OF REAGENTS OR OTHER EVIDENCE SYSTEM.**
- **ANY WOMAN CAN EASILY DO THE TEST. SHE CAN PERFORM IT ANYTIME AND ANYWHERE."**

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In October 1998 Prof. MUDr. M.Kudela, CSc. Conducted tests on a similar product to the Maybe Baby. See below the report.

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Introduction

The assessment of the fertile and the infertile period during the reproductive age of a female has always been a basic question of planned parenthood. There is a spectrum of methods for the determination of the fertile, periovulatory period: basal body temperature measurement, estimation of hormone levels in blood, evaluation of viscosity and crystallisation of cervical mucus, hormonal vaginal cytology, vaginal ultrasonography. Fewer methods are available for the determination of the infertile period. Recently, a revival of the interest in a simple method, e.g. saliva crystallisation, has been observed. Contrary to the cervical mucus crystallisation¹⁰, which is a comparable method, saliva are more suitable to be used in a simple device designed for non-professionals because of the easy, noninvasive collection and the reliable interpretation of results.

Fertility Indicator is a device (mini microscope) which helps to determine the fertile and the infertile days during menstrual cycle on the basis of a typical microscopic picture of crystallisation, the so called arborisation (ferning)^{2,6} of the dried saliva sample which appears arounds ovulation. Besides the phenomenon of arborisation other examinations in saliva have been described, such as conductivity⁴, concentration of hormones³ and other substances^{7,8}, but these tests are either too difficult to perform or of a questionable validity.

The chemical analysis has shown that the crystals of dried saliva contain predominantly potassium chloride (KCl). The crystallisation of KCl is influenced by many internal and external factors such as pH, viscosity and the content of other salts in saliva. The crystallisation pattern is also influenced by hormonal levels in the peripheral blood. These levels undergo characteristic changes during the physiological menstrual cycle. A marked increase of estrogen levels occurs in the preovulatory period. The crystallisation is positively dependent on the estrogen level unless it is inhibited by the presence of progesterone. The arborisation can also be influenced by the timing of sampling¹, nutritional habits, smoking and the amount of consumed liquid⁵.

The Fertility Indicator can be used either as a „natural" contraception aid for the determination of the fertile period, e.g. the period when couples should avoid sexual intercourse, or for the determination of the optimal fertile period for conception.

The assessment of the fertile period by means of mini microscope is based on the observation of a typical fern-like pattern of the crystals in samples of dried saliva, whilst in the infertile period this phenomenon should not be present. A prospective study of the reliability of the mini microscope as a non-professional aid for the

determination of the fertile and the infertile days of female menstrual cycle has been performed.

Material and methods

A layer of saliva is applied is applied to the lens of the min microscope.

The study has been performed in the Centre of Assisted Reproduction at the Department of Obstetrics and Gynecology of the Palacký University Medical School in Olomouc. The study group of 85 women was composed of motivated student nurses of the Secondary School of Nursing in Olomouc, who wanted to become knowledgeable about the symptomatology of their own ovulation and fertile period and of interested volunteers recruited from health care personnel. The age of included women ranged between 17 and 35 years. In total, 1957 saliva samples from 103 menstrual cycles in 85 healthy women have been tested. Of the 103 cycles 88 (85,4 %) cycles were ovulatory and in 15 cycles (14,6 %) ovulation was not observed.

All women were thoroughly instructed about the use of the min microscope device. Every morning they measured their basal body temperature immediately after waking up, rinsed their mouth by clean water and deposited the saliva sample taken from the sublingual space onto the appropriate field of min microscope. The sample was left to dry for at least 10 min and then evaluated in the minimicroscope. The evaluation was performed according to the preprinted pictures.

The picture was recognised as:

1. Fertile period, characterised by a typical dendritic crystallisation (arborisation, fern-like pattern) (Fig. 1).
2. Infertile period, characterised by a chaotic dot-like pattern without signs of the crystallic dendritic growth (Fig. 2)

As our participants repeatedly reported trouble to distinguish the so called „interim“ period in the small visual field of min microscope, we decided to report the results only as „fertile“ or „infertile“.

The following methods can be used to determine the fertile period of menstrual cycle: the calendar method, detecting „Mittelschmerz“ (in 30 % of women), basal body temperature (BBT) measurement, ultrasound folliculometry and monitoring of LH (luteinizing hormone) levels. In our study we have used the combination of BBT measurement and ultrasound folliculometry.

The ultrasound monitoring was started on the 10th day of menstrual cycle. The ovulation was confirmed on the basis of the dynamics of growth and following absence of the Graafian follicle. The objective assessment of the fertile and the infertile periods was performed by comparison of the BBT curve and ultrasound examination and determined as follows:

1. the objectively fertile period: day of ovulation - 3 days + 1 day, e.g. 5 days. The length of the fertile period was calculated from the fact that spermatozoa survive 72 hours and the oocyte can be fertilized only within 24 hours after ovulation.

2. the objectively infertile period consists of the remaining days of the cycle. Statistical evaluation of agreement or disagreement of the fertile and the infertile periods according to either the min microscope readings or the objective assessment was performed only between the 7th and the 25th day of menstrual cycle. In the remaining days on the beginning and the end of menstrual cycle there was always an agreement towards the infertile period. The inclusion of these periods into statistical evaluation would have a negative influence on the sensitivity and the specificity of statistical tests.

After completing the evaluation of the whole cycle by a volunteer, the retrospective evaluation was performed by an instructed and trained staff member. The obtained values were arranged into contingent tables in the Computing Centre, Faculty of Science, Palacký University, Olomouc. We have compared the extent of agreement in detecting the fertile period (test sensitivity) as well as the infertile period (test specificity) in whole and in individual days of menstrual cycle. The agreement between the reading by a volunteer and by a trained staff member was statistically evaluated. The values were compiled into tables and graphs.

Results

The first part of Table I shows the extent of agreement between the objectively fertile period and the period classified by volunteers as fertile during individual days of evaluated cycles. The overall test sensitivity is 31.3 %. The second part of Table I presents the extent of agreement between the objectively infertile period and the period classified by volunteers as infertile. The overall test specificity is 81.8 %.

Table II shows in a similar way the extent of agreement between the objective assessment and the retrospective evaluation by a trained staff member. In this case, the overall sensitivity is 26.7 % and the overall specificity is 88.2 %.

Fig. 1 depicts the course of agreement between the objectively fertile period and the period classified by volunteers as well as by a trained staff member as fertile during evaluated cycles.

Fig. 2 depicts the course of agreement between the objectively infertile period and the period classified by volunteers as well as by a trained staff member as infertile during evaluated cycles.

Fig. 3 express grafically the course of test sensitivity when read by volunteers or by a trained staff member during evaluated cycles.

Fig. 4 express grafically the course of test specificity when read by volunteers or by a trained staff member during evaluated cycles.

Discussion

The validity of the saliva crystallisation test in relationship to fertility was first studied by Biel-Casals². He reported that saliva ferning usually appears around the 10th day of menstrual cycle and disappears around the 18th day. Since this discovery, crystallisation of saliva can be considered as an indirect method of ovulation detection.

In the following years several studies on validity of the detection of the fertile period based on evaluation of crystallisation of dried saliva sample. In the largest study, Rotta et al.⁹ recorded saliva ferning in 66 out of 84 ovulatory cycles, e.g. in 78.6 %. In our opinion this results says little about the reliability of the test. The authors do not specify the length of the periovulatory fertile period and do not discuss the vitality of gametes which is essential for planned conception. Our results in 1957 samples from 85 healthy women proved correlation of the objectively fertile period and adequate ferning pattern in only 31.3 % of cases.

Rotta et al.⁹ report 84 % reliability when used for contraception. Our results, expressed as the test specificity, e.g. 81.8 %, are comparable. The test specificity would achieve even higher values if the first (till the 6th) and last (after the 26th) days of menstrual cycle were included. In this case the test specificity would be 90 % when read by a volunteer and 93 % when read by a trained staff member.

Conclusion

We have evaluated reliability of the Fertility Indicator for the determination of the fertile and infertile periods of female menstrual cycle. Our results show that women are able, after proper instruction, to distinguish between the patterns witnessing of fertile or infertile period. The mini microscope we have tested is a good aid, but it is not 100 % reliable. For a layman use it is always necessary to assume the possibility of a wrong interpretation of findings, especially by beginners or when the recommended procedure is not strictly observed. In our study the device achieved the sensitivity of 31.3 % for the determination of the fertile period and the specificity of 81.8 % for the infertile period (for the days between the 7th and the 25th day of menstrual cycle). **Evaluating the whole cycle (from the first to the 28th day) the test specificity is 90 % when read by a volunteer and 93 % when read by a trained staff member.**

We have observed a high correlation of the evaluation by volunteers with that done by a trained staff member. On the basis of our testing we can recommend the device as a suitable complementary aid for the natural methods of Planned Parenthood.

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