Determination of ovulation in women using saliva ferning test

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ABSTRACT

Every human being experiences growth and development, starting from childhood to adulthood. Women who have entered puberty will experience monthly menstrual cycle. One phase of the menstrual cycle is ovulation or the fertile phase in a woman. The fertile period is the period in which there is an egg ready to be fertilized by sperm. At the time of fertility, there is an increase in amount of estrogen and progesterone hormones. Increase in these hormones is also found in saliva. Saliva as a biological fluid in the body can be used as a diagnostic fluid. Woman's fertile period can be assessed from the saliva. Saliva containing high estrogen hormones can form a ferning picture on saliva dried on object glass. The type of research is the study of literature. Literature study was conducted to discuss the determining of the fertile woman with saliva ferning test. The results of previous studies showed the existence of differences in saliva pictures at the time of the fertile period and the infertile period. Salivary ferning was very clearly seen in the woman's fertile period.

Keyword : Fertile period, Ferning, Saliva

ABSTRAK

Setiap manusia akan mengalami pertumbuhan dan perkembangan dimulai dari masa anak-anak hingga dewasa. Wanita yang telah memasuki masa pubertas akan mengalami siklus menstruasi setiap bulannya. Salah satu fase pada siklus menstruasi adalah fase ovulasi atau masa subur pada wanita. Masa subur adalah masa di mana terdapat sel telur yang siap dibuahi oleh sperma. Pada saat masa subur, terjadi peningkatan jumlah hormon estrogen dan progesteron. Peningkatan hormon ini ditemukan juga dalam saliva. Saliva sebagai salah satu cairan biologis di dalam tubuh dapat dijadikan sebagai cairan diagnostik. Masa subur wanita dapat diketahui dari saliva. Saliva yang mengandung hormon estrogen yang tinggi dapat membentuk gambaran ferning pada saliva yang dikeringkan pada gelas objek. Jenis penelitian yang dilakukan adalah studi kepustakaan. Studi kepustakaan ini dilakukan untuk membahas mengenai penetapan masa subur wanita dengan ferning test saliva. Hasil penelitian-penelitian sebelumnya memperlihatkan adanya perbedaan gambaran saliva pada saat masa subur dan masa tidak subur. Ferning saliva terlihat sangat jelas pada masa subur wanita.

Kata kunci: Masa Subur, Ferning, Saliva

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INTRODUCTION

Every human experiences growth and development which start from childhood to adolescence. Entering adolescence, both gender will go through puberty. Puberty is a period when the endocrin system and gametogenic tissue (gonads) mature into their adult state capable of sexual reproduction. Puberty occurs during ages 12-16, although this could be affected by family genetics, race, climate and environment. In women, the first physical manifestation of puberty is *telarche*, the breast development, followed by *pubarche*, the appearance of axillary and pubic hair, and finally marked by *menarche*, the onset of menstruation.[1,2]

Woman reproductive system shows regular cycle known as the periodic preparation of fertilization and pregnancy. This is called menstruation and is noticeable by the monthly blood discharge from the uterus. This bleeding happens when ovum (mature egg cell) is not fertilized. Women can only produce one ovum in a month and only about 400 ova in their lifetime.[1-3] Women reproductive organs which responsible for this cycle are ovarium, fallopian tube, uterus, and vagina.[4]

Menstruation is a catabolic process influenced by hormones from hypophisis and ovarium. Gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus has a role in triggering the secretion of gonadotropin by anterior hypophisis cells. These hormones, FSH (*Follicle-Stimulating Hormone*) and LH (*Luteinizing Hormone*), stimulate and control the cycle changes inside ovarium. During reproduction time, endometrium undergoes constant changes of cycle. Every cycle usually has 4 phases related to the activity of ovarian hormones. The phases are proliferative phase, ovulation phase, secretory phase and menstrual phase.[5,6]

At proliferative phase, follicle maturation takes place inside of the ovarium because of the increase in FSH level. Follicle produces a large amount of estradiol hormone which is as one of the estrogen hormones. Estradiol level will increase until the 13th day and initiates the secretion of LH. The level of LH will finally reach its maximum 10-12 hours before ovulation. This event triggers ovarium to maturate the egg cell and discharge the mature cell on the 14th day. At the same time, basal body temperature is rising by around 0.5^0 C. This is the so-called ovulation when a mature egg cell is released and moves along the fallopian tube.[7,8]

Ovulation phase in normal women with 28-day sexual cycle takes place 14 days after menstruation begins.[4] This phase is also known as the fertile period of women. Women fertile period is a time when there is an egg cell available to be fertilized by a sperm cell.[9]

Saliva is generated by salivary glands ending up to the oral cavity and is spread out from blood circulatory system through the ducts located on the surface between tooth and gums. The amount and composition of saliva determine the oral health.[10] Saliva consists of nearly 99% water and a little amount of organic and inorganic substances.[11] which may be used to reflect the whole body health. Inorganic substances contained in the saliva are Ca, Mg, F, HCO_3, K, Na, Cl, and NH_4 while the organic substances are urea, uric acid, free glucose, amino acids, lactate, fatty acids and macromolecules such as protein, amylase, peroxidase, thiocyanate, lysosome, lipid, IgA, IGM and IgG. In addition, saliva also contains CO_2, N_2 and O_2.[12]

Nowadays saliva can be used as a diagnostic fluid which is cheaper and effective in giving the diagnostic results.[13] There are many scientist using sialometry and sialochemistry to diagnose systemic diseases, monitor general health and use it as an indicator of many diseases closely related to oral and systemic health.[14]

Women fertile period

Fertile period of a woman happens when a mature egg cell is released from the ovarian follicle. Fertile period should be considered to plan a pregnancy. The peak of the fertile period is the best time to conceive.[2,15]

LH is necessary for the final growth of follicle and ovulation. Without this hormone, follicle will not proceed to ovulation phase even though FSH is available at a large amount. About 2 days before ovulation, LH secretion rate increaseds dramatically by 6-10 times and at its highest value 16 hours before ovulation. FSH also increases by 2-3 times at the same time ,and then both FSH and LH will work synergically leading up to the

swelling of the follicle which occurs rapidly several days before ovulation. LH has a particular effect toward granulose and theca cell by transforming these 2 cells into progesterone-producing cells. Hence, estrogen secretion rate starts to decrease usually 1 day before ovulation, while progesterone hormone increases in amount of the secretion.[4]

Fertile period detection is an important part of pregnancy planning. A survey from *National Survey of Reproductive and Contraceptive Knowledge* conducted with 1.800 males and females have found that only one-third of the respondents know that fertile period is in between of two menstruation periods.[16] Based on a research, a woman is in her fertility peak several days before ovulation or 14 days before menstruation.[17]

To identify fertile period, a woman having regular menstruations might use a calendar method. Fertile period occurs from the 12th day to the 16th day after the first menstruation.[18] Most women use manual calculation of calendar method to know their fertile period.[19]

Undergoing fertile period, there are many changes in a woman body. These changes can be used to detect the fertile days. These changes are:1) An increase in body basal temperature by 0.2-0.5^0 C[18,20]; 2) Changes in cervical mucus which

Table 1. Comparison of saliva and plasma composition (Hrvatsku et al.)

Analyte	Mixed Saliva	Plasma	Unit
In General			
Water	97 - 99.5	90 – 93	%
pH	(5.6) 6.4 – 7.4 (7.9)	7.4	
Substrates			
Albumin	246 – 344	34 000 – 48 000	mg/l
Cholesterol	3 – 15	150 – 300	mg/l
Creatinine	0.07 – 0.2	< 1.1	mg/l
Glucose	< 2	55 – 115	mg/l
Protein	1.1 – 1.8 (6.4)	66 – 87	g/l
Urea	17 – 41	< 50	mg/l
Uric Acid	0.7 - 0.6	< 7.0	mg/l
Enzymes (37^0 C)			
α- Amylase	11 900 – 305 000	< 220	U/l
AP	< 11	< 270	U/l
SGOT (ASAT)	< 43	< 38	U/l
SGPT (ALAT)	< 11	< 41	U/l
LDH	113 – 609	< 480	U/l
Lysozyme	6 – 12	3 – 9	mg/l
Electrolytes / Minerals			
Calcium	0.88 – 2.05	2.20 – 2.55	mmol/l
Chloride	5 – 40	96 – 108	mmol/l
Magnesium	0.08 – 0.56	0.70 – 1.05	mmol/l
Phosphate	1.4 – 13.2	0.87 – 1.45	mmol/l
Potassium	6.4 – 37	3.3 – 5.1	mmol/l
Sodium	2 – 21	133 – 145	mmol/l
Other			
IgA	42 – 174	850 – 4 000	mg/l
CBG, male	38 ± 18	39 700 ± 6 300	µg/l
CBG, female	72 ± 71	42 200 ± 5 600	µg/l
SHBG, male	19 ± 10	15 – 100	nmol/l
SHBG, female	63 ± 60	15 – 120	nmol/l
Transferrin	< 0.5	250 – 350	mg/dl

become thicker, clearer, white, and slippery. An elastic thread called *spinnbarkeit* is formed when it is drawn out. This mucus serves to facilitate sperms catching and movement to the egg cell. It tastes salty since it contains salts.[18,20,21]; 3) Changes in the character of vaginal ephitelium which could be observed under microscope during menstruation[22]; 4) Changes in the level of estrogen and progesterone level in urine[23] or blood[24], and in the breast milk of breastfeeding mother[25]; 5) Belly pain caused by the stimulation of peritoneal membrane due to the burst of blood vessel or follicle[26,27]; 6) Bloating or water weight because of water retention[20]; 7) Changes in breasts to become more tender and soft.[27,28]

There are several factors affecting fertility. The factors are: 1) Nutrition intake from fruits and vegetables; 2) Healthy lifestyle, such as regular exercise, avoiding cigarettes, drugs or alcoholic beverages, and avoiding overwork; 3) Age, people age over 35 have lower quality of eggs because of the decline in ovarian reserve; 4) Body and reproductive organs health. Obesity could affect fertility because the overproduction of insulin causes an inability in making a good quality ovum. Infection might occur inside the reproductive tracts generating blockage in the ovary tract and hence woman will lose her fertility.[26]

Saliva

Since a very long time, saliva has been known as a unique yet complex body fluid similar with plasma or serum. Unintentionally, saliva has been used as a diagnostic liquid for over than 2000 years. Doctors in the past understanding thoroughly the Chinese traditional medicines have concluded that saliva and blood are "brothers" inside the body because it is originated from the same source. Biomolecules which are distributed in the blood are also found in human saliva (tab 1). It is believed that the changes in saliva indicate the whole human body health. The interest of utilizing saliva as a diagnostic medium has been increasing consistently in the recent decade.[13,9,29]

Oral cavity is anatomically connected with other parts of the body. Oral cavity is the portal to digestive tract. It is connected to posterior nasal, and also linked with the upper digestive tract. Therefore, saliva quality as a whole can reveal the additional components coming from sources other than oral cavity, and those components may point out a wide range of systemic diseases.

Thus, oral cavity environment is a reflection of oral and systemic health and could be utilized to monitor and diagnose specific clinic parameters. Today, many scientist have used sialometry and sialochemistry which are the tools to diagnose systemic diseases, monitor the general health, and to indicate disease risks by analyzing saliva components and its flow rate.[14,32,33]

Saliva contains gingival sulcus exsudate, mucosal transudate, nose and throat expectorant, serum and blood from mouth wound, bacteria, viruses, fungi, ephitelial cells, cell components, and debris. Serum components ,for instance hormones and medicines, are not the normal components of saliva. Biomolecules including drugs and hormones from blood capillaries may come into saliva by intracellular and extracellular mechanisms. Steroid hormones with their small and hidrophylic molecule can be transported from blood capillaries into saliva by means of passive diffusion. Soluble molecules (e.g. immunoglobulin E) might reach into saliva by active transport. Sulfated molecules (e.g. steroids) with their hydrophobic characteristic could be transported to saliva by ultrafiltration. Serum components could be detected in saliva as a consequence of gingival sulcus stream when gingival inflammation happens.[9,13]

Saliva as a diagnostic fluid increasingly takes important roles in dentistry, physiology, internal medicine, endocrinology, pediatry, immunology, clinical pathology, forensic, psycology, pharmacology, and sports medicine. Using saliva, biomarkers (electrolytes, hormones, drugs and antibodies) could be analyzed.[33,34]

Saliva is able to be used in the diagnoses of systemic and oral diseases. At present, saliva is used in the detection risk of dental caries, periodontitis, mouth cancer, salivary gland disease, and viral infections. Infectional diseases could be detected by the level of antibodies in saliva. Several infectional disease pathogens which could be detected are *human immunodeficiency virus* (HIV); hepatitis A, B, and C viruses; chickenpox virus; Epstein-Barr virus; rubella virus; parvovirus B 19; human herpes 6 virus; *Helicobacter pylory*; and rotavirus. Moreover, the level of hormones (for example, estrogen(estradiol), progesterone, testoteron, dehydroepiandrosterone (DHEA), and

cortisol) could be measured using saliva. Steroid hormone is the indicator of women fertile period, menopause fluctuation, premature birth, low body weight of new-born baby, stress, and other diurnal variations. Several medicines could be detected in saliva. The amount of medicine in saliva could be measured and it might be counted for blood tests replacement.[30,34]

Saliva as a diagnostic fluid has several advantages compared to serum. Saliva detection needs smaller amount of sample; has higher sensitivity; requires non-invasive collecting procedure; makes easier cooperation with patients since sample collection is not stressful; could be collected anywhere; does not required special equipment and trained technicians to collect samples; easily applicable with children and adults; gives more accuracy to detect oral and health diseases compared to blood test; cheaper cost to screen big population; might cut the risk of disease transmission between patients and technicians. Unlike blood which needs centrifugation or filtration to remove blood cells and get the serum or plasma, saliva does not need those processes and therefore it is an ideal diagnostic fluid for diagnoses and tests.[9,13]

However, saliva as a diagnostic fluid has several drawbacks. One of the disadvantages are about the number of the molecules in saliva which are 100-1000 times lower than in blood, so more sophisticated technology is necessary for detection. Identification of saliva biomarker is needed for correlating with onset and development of disease. Biomolecules contained in saliva have circadian rhythms with several implications on the dynamic and kinetic of molecules in saliva and it is important to take it in the right time as a sample for diagnostic use. *Circadian rhythms* are the body biological rhythms for 24-hour period. *Circadian rhythms* might affect sleeping pattern, body temperature, metabolism, heartbeat, blood tension, and hormones level.[29,35,36]

DISCUSSION

Women in their puberty will experience regular cycles change thought to be the preparation for fertilization and pregnancy. Pregnancy takes place when a sperm fertilize a mature egg cell during women fertile period. Fertile period or ovulation phase occurs on the 14th day of 28-day cycle.[5] All Women need to be aware of their fertile period for those whom avoiding pregnancy or even who have a plan to get pregment.

Paying attention to fertile days could be a natural method for family planning. According to WHO, a natural method of family planning is a method to plan or avoid pregnancy through observation of natural signs and symptoms which appear during fertile and unfertile period throughout menstruation cycles without any drugs, contraception, surgery procedure, and sexual intercourse until the fertile period ends.[38] Ovulation method is a natural method of family planning. In the period of ovulation, there are visible changes. These changes are the increase in body basal temperature and cervical mucus.

Fertile period in women can be known by several means and one of newest methods is by using saliva.[38] It has encouraged researchers to find and explore the connection between saliva and women fertile period. Menstruation cycle is affected by hormonal mechanisms.[39] When ovulation happens, FSH and LH increase (graphic 1). This increase also happens with estrogen and progesterone.[4]

Although it is merged, cervix uteri differs from corpus uteri in several aspects. Mucosa of
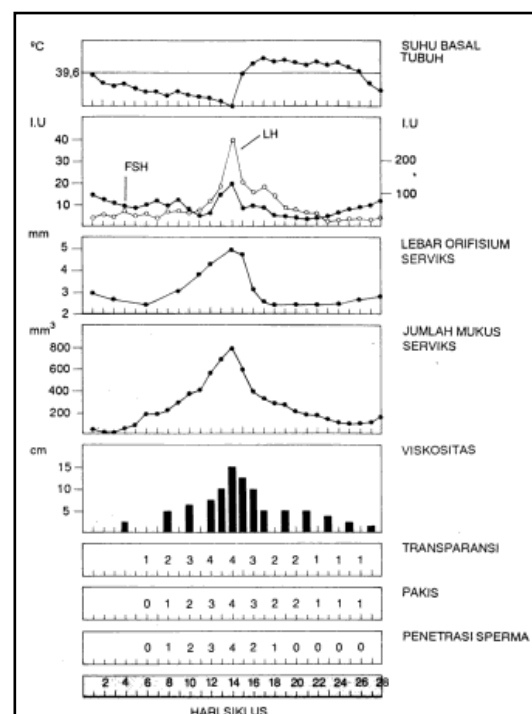
Graphic 1. Physiological changes related to menstruation cycle.[40]

cervix uteri does not undergo cyclic desquamation, but regular changes happen in cervical mucus. Estrogen causes mucus to be more basic and thin when it is stretched and this change improves the endurance and motility of sperms. Progesterone causes mucus to be thick when it is stretched, viscous and full with cells. During ovulation, mucus on stretching becomes the thinnest and therefore its elasticity (*spinnbarkeit*) increases until the middle of the menstruation. One drop of mucus could be stretched to be a very thin thread with 8-12 cm length or more. In addition, if a thin specimen is spread on an object glass and allowed to air-dry, the dried mucus will form branching patterns resembling fern leaves which actually consist of salt crystals. After ovulation and before pregnancy, mucus becomes thick and will not form fern-like patterns. Mucus with this characteristic will be easily penetrable by sperm cells.[40,41]

Examination of cervical mucus, one of body fluids, gives an idea to use the other similar fluid which changes during the related cycle, i.e saliva. There are many researches conducted to define cycle fertility by measuring estrogen level (graphic 2) and progesterone level (graphic 3) in saliva, and it is proven that fluctuation patterns of those two hormones in saliva reflect the pattern in blood. Other researches show that saliva has many characteristics in common with cervical mucus, for example they contain salts (especially NaCl), estrogen hormones, and mucus which take part in the formation of ferning. Therefore, both saliva and cervical mucus could be used to monitor cycle fertility.[25,33,42]
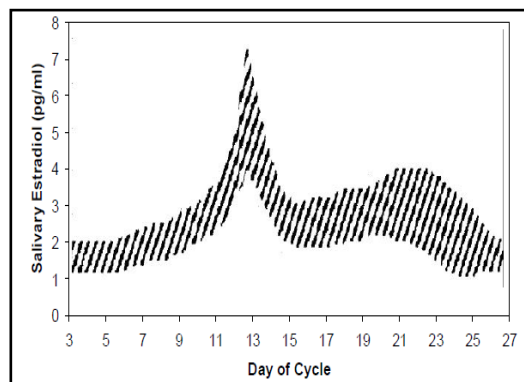
Graphic 2. Monthly Profile of Estradiol in Saliva during Women Menstruation Cycle.[33]
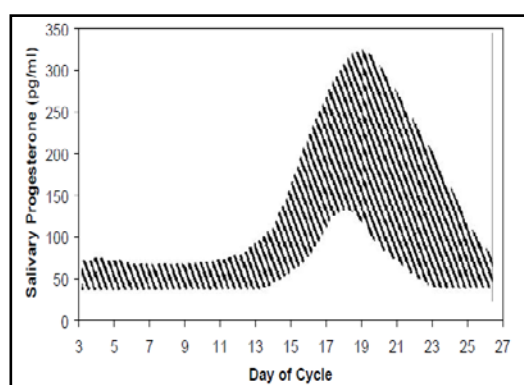
Graphic 3. Monthly Profile of Progesterone in Saliva during Women Menstruation Cycle.[33]

Ferning phenomenon is affected by estrogen and progesterone. At follicular phase of normal menstruation cycle, estrogen level in saliva increases in accordance with the increase of estrogen level in blood. The interaction of estrogen with NaCl and mucus results in ferning
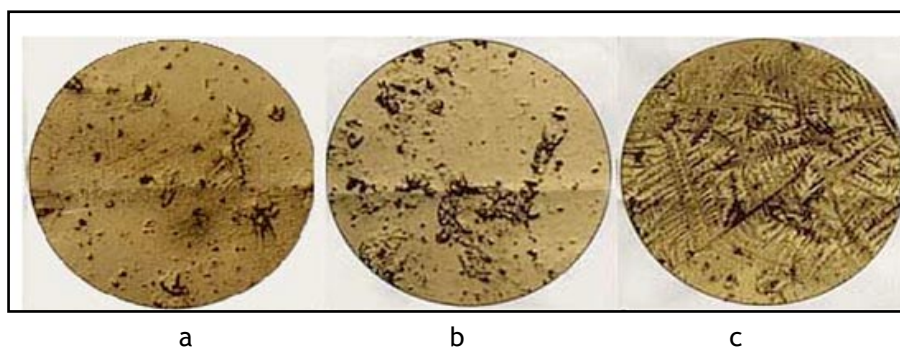

| a | b | c |

Figure 1. Ferning in saliva mccabespharmacy.a. Inpertility estrogen only a couple of dote and dashes, b. Transtition starting to look a few cryrtal structures, c. Fertile time the crstaline form of estrogen is in packem such as fern leaves. (cited 15 Mar 2013)

appearance in saliva (illustration 1), which is a crystal configuration resembling fern leaves.

The higher the estrogen level, the more dense the ferning; This is correlated with the increase of estrogen level in blood.[22,41]

Ferning appears because of the increasing estrogen level is such that the electrolytes (specifically Na and Cl) increase in saliva. The presence of estrogen in mucus makes it possible for the salt crystal configuration to resemble fern leaves. Crystals such as those are generated when proteins or other catabolic products interact with certain electrolytes in an adequate amount.[43]

A research has been done on women with ovulatory cycle to figure out the ferning patterns appear in saliva and cervical mucus. Based on the ferning patterns of cervical mucus and saliva throughout the menstruation cycle, the cycle can be divided into 3 phases. The first phase (1st day until 7th day) shows no ferning because the low level of estrogen in blood leads to a very low level of estrogen circulated to salivary glands. In the second phase (8th day until 21st day), estrogen level in blood is increasing and reaching its peak approaching ovulation. It is marked by the more dense appearance of ferning. In the last phase (post ovulation), ferning is beginning to disappear at the same time when progesterone level in blood is increasing so that the patterns are not in the shape of fern leaves anymore.[42]

Salvatore gives additional explanation that the variations of crystal shape in ferning phenomenon depend on the balance of estrogen and progesterone in body fluids. In this case, it is the saliva. The researcher emphasizes that crystallization occurs in the presence of NaCl and mucus although the level of these two components changes as the cycle goes through different phases.[43]

The use of saliva as a diagnostic fluid for determination of fertile period requires paying attention on circadian rhythms which might have an impact on saliva composition including estrogen and progesterone hormones (graphic 4). Researches have already been done to decide the proper time for taking saliva ferning test. The results show that the proper time to do saliva ferning test is in the morning before toothbrushing. There are differences of ferning appearance in saliva in the morning before and after brushing teeth. Before

toothbrushing, ferning pattern is more specific from clinical perspective.
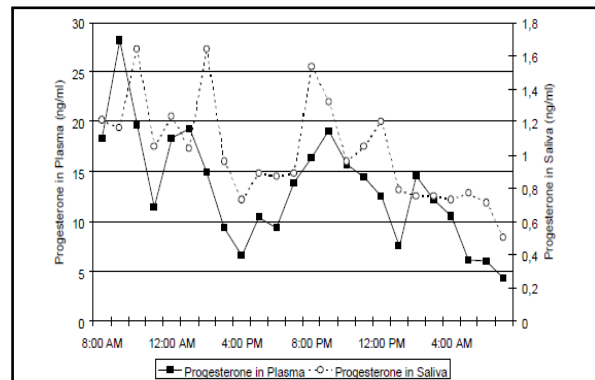
Graphic 4 Fluctuation of Progesterone Diurnal Level in Women.[33]

The appearance of ferning before and after toothbrushing could be observed to monitor cycle fertility. This is important especially for those who want to conceive or prevent pregnancy.[44]

A research on the monitoring of women fertile period has been conducted on women at perimenopause ages. The results give a conclusion that saliva ferning test is able to be used for monitoring fertile period in perimenopause women with irregular ferning appearance.[45]

CONCLUSION

Literature study on the determination of women fertile period using saliva ferning test have shown the pattern of change in saliva during women fertile period. Pattern of change in saliva occurs physiologically along with the change in estrogen and progesterone level during women menstruation cycle. Application of saliva ferning test is proved to be more effective compared to cervical mucus monitoring. Saliva sample collection is much easier than cervical mucus collection. Hence, monitoring the changes of ferning in saliva could be thought as the easiest way to monitor the fertile period in women. Observing pattern of change of ferning in saliva throughout a menstruation cycle could indicate the cycle fertility in a woman.

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