

Salivary ferning and the menstrual cycle in women

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Summary: The Authors report the data relative to the monitoring of menstrual cycle carried out by the observation of salivary ferning. They compare the usual physical and hormonal parameters of four groups of women with the aspect of the crystallisation of the saliva observed both by normal optical microscope and by a special type of pocket microscope which can be used by women themselves. In conclusion the Authors report a careful examination of the biochemical correlation relating the women's hormonal climate and its salivary constituents.

INTRODUCTION

When considering the methods aimed at individuating the female fertile period the tendency today is to direct studies towards techniques which satisfy the demands of simplicity and practicability. With this in view studies have evaluated the biophysical characteristics of the crystallisation of the saliva in relation to the different parameters which undergo cyclical modification during the various phases of the menstrual period. Such an approach proves to be one of the least complicated as it requires less involvement on the part of the woman using it, and is also inexpensive; therefore the object of our work has

This study suggests the examination of salivary crystallisation individuating the woman's fertile period or eventual hormonal disorders, in the case that such emerge from the evidence, and/or of recurrent changes in the time and modality of expression of the characteristics of such crystallisation.

MATERIALS AND METHODS

In this study the data have been analysed relative to 4 groups of women volunteers, classifiable in the following 4 categories:

- A) Unmarried women with regular menstrual cycles.
- B) Married women using Natural Method family planning.
- C) Women submitted to induction of ovulation by clomiphene citrate.
- D) Unmarried women with menstrual disorders.

In the first group the data were studied relative to the 21 spontaneous menstrual cycles of 10 volunteers, university students whose ages ranged between 18 and 30 years. The following parameters were evaluated throughout the entire menstrual cycle: basal temperature, subjective sensation of mucus at vulvar level (dry, damp, been to check the scientific reliability of such a technique.

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wet), characteristics of the cervical mucus (sticky, stringy), and eventual manifestations such as abdominal pains, backache, spotting, breast tension etc.; research for the ovulatory day was also tested by opportune echographic examination.

All these data were correlated with the ferning of the saliva. This biologic fluid was withdrawn daily, fasting, using a special pipette placed sublingually, making sure that the mouth had not been rinsed before sampling, and that it was without foam. The saliva was deposited on a slide and left to dry at room temperature for 3-5 minutes and subsequently studied microscopically.

In the second group 32 cycles were evaluated in 32 women aged between 22 and 40 years who were already using natural methods for birth control.

In the third group data were studied relative to 10 cycles in 10 women aged between 25 and 35 years whose cycles were induced by the administration of clomiphene citrate.

In the fourth group 8 cycles were studied of 4 unmarried women aged between 22 and 30 years who had menstrual problems (premenstrual syndrome - PMS -, dysmenorrhoea).

RESULTS

A) First group

In the first group the dried saliva was analysed for 304 slides relative to 13 ovulatory cycles and from 106 relative to 8 annovulatory cycles, a total of 464 slides. The results of this enquiry are shown in Tables 1 and 2.

Table 1. — *Characteristics of the 304 slides relative to the 13 ovulatory cycles.*

Ramifications:	Absence	Order 1st	Order 2nd	Order 3rd
Periov. period	44%	54%	72%	100%
Extra periov. period	56%	46%	28%	0%

Table 2. — *Characteristics of the 106 slides relative to the 8 annovulatory cycles.*

Ramifications:	Absence	Order 1st	Order 2nd	Order 3rd
	74%	3%	7%	16%

In Table 1 a higher percentage of dendritic arborizations can be seen than in the periovulatory period, a datum that, compared with those which can be evinced from Table 2, does not seem sufficient for the drawing of statistically valid conclusions.

B) Second group

In the second group the slides were examined of 32 women: in 4 cases out of the 32 the aspect of the crystallisation did not appear ascribable to ferning pictures, in the remaining 28 cases the ferning had begun, on average, 8.25 days before the first ovulatory thermic increase (Table 3). Then, comparing the 28 cases in which we observed typical ferning with the peak of cervical mucus, it was ascertained that in 24 cases crystallisation begins on average 6 days before the peak of the mucus, while in the remaining 4 cases it was not possible to note a clearly evident mucus peak (Table 4).

Table 3. — *Distribution of the beginning of salivary ferning in relation to the day of rising temperature (0).*

days	-11,	-10,	-9,	-8,	-7,	-6,	-5,	-4,	-3,	-2,	-1,	0
	2	8	5	4	5	1	0	2	1	0	0	0

Table 4. — *Distribution of beginning of salivary ferning in relation to day of cervical mucus peak (0).*

days	-11,	-10,	-9,	-8,	-7,	-6,	-5,	-4,	-3,	-2,	-1,	0
	0	0	1	5	7	4	2	1	2	2	0	0

C) Third group

In the third group the slides were examined of 10 women: in 6 cases the maximum degree of salivary ferning coincided with the day of ovulation echographically determined, while in the remaining 4 cases it was obtained 2 days before or after ovulation echographically observed (Table 5).

Table 5. — *Distribution of maximum ferning in relation to ovulatory day echographically observed.*

— 2 days:	2 cases
— 1 days:	0 cases
0	6 cases
+ 1 day	0 cases
+ 2 days	2 cases

D) Fourth group

In the fourth group a higher percentage of dendritic arborization was observed in the slides relating to the pre-ovulatory period, but this observation does not allow the drawing of statistically significant conclusions. It does, however, seem interesting that an element confirming the role of estrogens in the determination of salivary ferning is given by the observation that, in the cycles referring to 2 volunteers with PMS there was constant evidence of salivary ferning in correspondence with the 21st and 23rd days of the cycle. This might very likely reflect the physiopathological aspect of relative hyperestrogenism or of luteal insufficiency suggested in the pathogenesis of PMS.

Therefore the enquiry into salivary ferning, besides presenting a simple and practical means of checking on fertility to the end of family planning, may also be exploited as a check on women's endocrine situation.

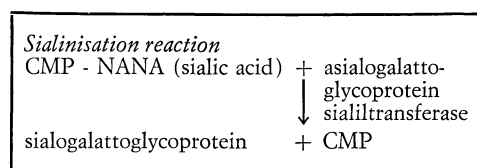
DISCUSSION

It is known that salivary ferning depends principally on the electrolytic concentration and chemico-physical properties of the mucins it contains⁽¹⁾. The biophysical characteristics of the saliva is essentially influenced by the estrogens which impart certain characteristics (ferning in particular) not only to uterine endometrial secretion but also to other secretions such as saliva and nasal mucus⁽¹⁵⁾.

The salivary ferning examination could therefore represent a test for eventual hormonal disorders.

The concentration of salivary electrolytes has been shown to yield a maximum peak about 7 days before ovulation, followed on succeeding days by lower values⁽²⁾.

Other studies have shown that sialic acid with negative macromolecular charge is an importante component of the salivary mucins where it always occupies a terminal place^(1, 3, 4), and that the enzymes active in the binding of the sialic acid structure to the peptidic structure are the sialil-transferases⁽⁵⁾. In sialinisation is catalysed by sialiltransferase, a glycosyltransferase associated with the fraction of the cellular membrane of the Golgi apparatus⁽¹⁶⁾:



Some Authors^(5, 7) have reported that the concentration of sialic acid in the saliva varies during the menstrual cycle, showing a higher concentration during the follicular phase with a peak 4 - 5 days before the preovulatory rise of LH, after which the sialic acid concentration diminishes until ovulation. The peak may be due to a maximum in the activity of the sialic transferases⁽⁵⁾.

On the basis of these premises it has been possible to show that in the ferning process notable importance is assumed by the presence of salts (specially NaCl) that form crystalline nuclei, in which the Na⁺ not effectively neutralised interact with the negative charges of the mucin molecules. Thus a complex structure is determined that could be defined "nematoid" (from the Greek nematos = filament) in

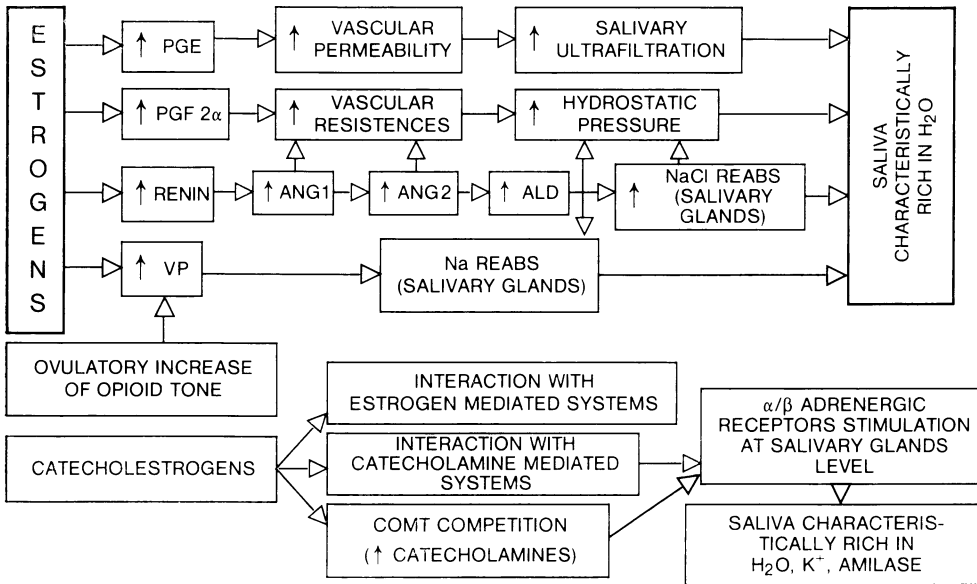


Fig. 1.

view of the ultrastructural aggregation into an ordered filiform organisation.

In this context the role of the estrogens which increase the water content in mid-cycle would seem to be relevant, determining the most favourable condition for the manifestation of a chemico-physical phenomenon defined as epitaxy (from the Greek *epi* = upon and *taxis* = order) (6, 7). This phenomenon consists in the oriented increase and of the fixation of one crystalline substance upon the substrate of another crystalline substance, united so that the crystals of one of them constitute the base for those of the other. The deposit takes place with orientation determined by the reticular characteristics of the two crystalline substances associated (8). This phenomenon requires the presence of regions of low concentration between the above-mentioned structures (6).

We therefore hypothesise that in the determination of a saliva for which the

optimal proportion of water, salts and sialomucins is obtained there is an intervention of factors positively influenced by ovulation: estrogens, catecholestrogens, opioid tone (Scheme 1).

Description of the Scheme

Estrogenic Action:

Estrogens exert an influence on the Prostaglandins (PG), on the renin-angiotensin system and on Vasopressin (VP).

PG: the estrogens stimulate the synthesis $\text{PGF}_2\alpha$ and PGE, probably activating the phospholipase A2 lysosomal and/or the PG synthetase (17, 18); the PG would participate in the production of a saliva characteristically rich in water through an increase of vascular permeability mediated by the PGE (19), with consequent increase in the ultrafiltration, and by an increase of vascular resistance, mediated by the $\text{PGF}_2\alpha$ (19) with consequent increase in hydrostatic pressure.

Renin-angiotensin system: estrogens favour hydrosaline retention through the renin-angiotensin system⁽¹⁵⁾; renin, a proteolytic enzyme secreted by the kidneys, acts on an α -2-globulin of hepatic origin called angiotensin I; this through the releasing activity of a converting enzyme existing prevalently at pulmonary level, is transformed into the octapeptide angiotensin 2, a potent vasoconstrictor with a plasmatic half-life of 1-2 minutes, also capable of direct stimulus on the surrenal secretion of aldosterone⁽²⁰⁾ which has a sodioretentive effect on the salivary glands⁽²¹⁾.

VP: the plasmatic values of VP seem to be correlated to the levels 17- β -estradiol, both in the rat and in man^(22, 23), and a VP release activity may be hypothesised whereby action on the dopaminergic way has an inhibiting effect on the secretion of VP⁽²⁴⁾; a direct action on the cells of the neuro-hypothesis and an indirect action on the secretion of estrogen-dependent neurophysin⁽²⁵⁾ has also been reported; in fact VP is conveyed to an axonal level by a proteic substance called neurophysin whose concentration can be modulated by nicotine and by estrogens⁽²⁶⁾; VP favours the reabsorption of Na⁺ in the ducts of the salivary glands, with reduction of Na⁺ concentration in the saliva⁽²⁷⁾.

Opioid action system: the study of the opioid system and its fluctuations during the menstrual cycles has revealed a reduction in opioid tone in the follicular phase, an increase in correspondence with ovulation and a peak half way through the luteinic phase⁽²⁸⁾; the opioids, LHRH and somatostatin seem to have a stimulatory effect on VP secretion⁽²⁶⁾.

Catecolestrogen Action: the catecolestrogens are 2 or 4 hydroxylates, derivatives of the estrogens, and have a double action:

a) they interact with the transport plasmatic proteins and with the cellular membranes of the catecholamine target organs;

b) they interact with cytoplasmatic and nuclear receptors with mechanisms common to the estrogens⁽²⁹⁾.

Since catecolestrogens are double-faced they have the potentiality for interacting both with systems mediated by catecholamines and those mediated by estrogens. They can, besides, compete for COMT (Catechol-O-Methyl-Transferase) with increase of catecholamines⁽³⁰⁾:

Tyrosine \rightarrow Dopamine \rightarrow Norepinephrine -
(COMT) \rightarrow 3 methoxy - norepinephrine

The only hormones which have a sure effect on the salivary glands are the catecholamines⁽³¹⁾. We deduce from this that any increase among them mediated by catecolestrogens can influence the salivary secretion, and at the level of the salivary glands the stimulation of the α receptors determining a secretion rich in K⁺ and H₂O; the stimulation of the β receptors, a secretion rich in amylase⁽³²⁾.

In our opinion no complete evaluation can omit consideration of the factors which may influence the process of dessication of the saliva and its composition. We consider that the salivary dessication process can be influenced in the first place by environmental conditions and principally by air currents which are etched onto the slide. In fact, on this point, it must be underlined that the axis of the ferning formation is parallel to the direction of the air flow, thus confirming that the ferning phenomenon represents an example of the nematoid system whose filiform components reflect individually the direction of the air flow.

Another factor which plays a part in salivary cristallisation is the temperature which, as in the preceding parameter, in-

fluences the time of dessication and therefore, as already mentioned, in the dendritic organisation of the ferning. It is, besides, useful to consider that the production of many of the components of the saliva (protein, aminoacids, mucins, electrolytes, sugars, urea, citric acid and enzymes like amylase and the alkaline phosphates) is regulated by the ovarian hormones; consequently the concentrations vary considerably during the menstrual cycle⁽⁹⁾.

It should also be emphasised that besides the hormonal stimuli correlated to the ovarian function (estrogen and progestogens) in the modifications of the compositions of the saliva, other factors are commonly involved: bacterial flora, inflammatory processes primitive and secondary, clinical or subclinical, and eventual higher susceptibility in premenstrual periods⁽¹⁰⁾; possible alimentary residues; drugs (excretory physiopathology of the salivary gland); smoking^(11, 12); circadian rythms (sleeping-waking⁽¹³⁾); nervous stimuli not correlated to ovarian function (aldosterone)⁽¹³⁾; snoring; etc.

It may besides be interesting to note that fern-patterns can be observed in all those conditions which confirm the event of epitaxis for salivary fluid but are not, however, related to ovulation, as we found in some samples collected from time to time in male subjects. It is worth remembering that for a reliable ferning test the salivary fluid corresponds to the cervical mucus, which has already been studied in depth, with an encouraging documentation of results, giving convincing biological justification⁽¹⁴⁾.

The reliability has also been evaluated of a mini-microscope (PG/53 IT.LAB. BOUTY) of high magnification for the auto-analysis of salivary crystallisation. The readings carried out by volunteers (after opportune training with the PG/53) were compared with those carried out by traditional optical microscopy on the same

slides which were periodically collected, and thus examined by a single observer. The PG/53's yielded an average accuracy of examination in 92% of cases, and, considering the study protocol, such mini-microscopes might usefully be adopted in the research on eventual crystallisation of biologic fluid, in view of the considerations already expressed regarding the physiopathology of salivary ferning. From the results of our study it will be seen that greater correspondence will be found between ferning and ovulation in married women who use the natural methods for family planning.

This greater correspondence is due to many factors; in the first place the particular motive in the application of the method, in the second the stability of the hormonal climate present in this sample, such as to allow the most explicit demonstration of other ovulatory markers too. From this point of view it is important to underline the major preparation and capacity in the interpretation of the various physical signs of ovulation in these women.

In conclusion we can affirm that these first data relative to salivary ferning should encourage further research in the study of a greater number of cases in different clinical conditions. We can, however, maintain that we are confronting a new technique suitable for the individuation of the female fertile period which can usefully be employed alongside other more widely known and experimented methods, above all considering its characteristics of innocuity, practicability and the economy offered.

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