

# Objective and subjective data for fertile period diagnosis in women: comparison of methods

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*Summary:* The Authors have evaluated the reliability of various methods, both natural and biochemical, for the identification of the female ovulatory period in women with regular periods. They compared these methods among themselves and with echographical checking of ovulation.

It emerged clearly from the study that the greatest reliability among the natural methods is obtained through the evaluation of the presence of cervical mucus, while among biochemical methods the best results are obtained by a semiquantitative colorimetric test.

Brilliant prospects have also been opened by research on saliva.

**Key words:** Ovulation; Fertile phase; Cervical mucus; Beta Glucoronidase; Home Kits; Natural Family Planning, Saliva.

## INTRODUCTION

The National Research Centre (CNR) in Italy has obtained evidence that 16% of fertile couples rely on periodic abstinence from sexual relations for birth control <sup>(1)</sup>. Only a few couples however, use scientific methods of Natural Family Planning (NFP) <sup>(2,3)</sup>, understood nowadays as the possibility of regulating births without using chemical substances or mechanical methods of any kind but using instead those methods which allow for

the individuation of the fertile period in women, during which to abstain from sexual relation <sup>(4, 5, 6, 7, 8, 9, 10, 11, 12)</sup>.

We wished to experiment on several of these methods, both singly and in combined association <sup>(13, 14, 15, 16, 17, 18, 19, 20, 21, 22)</sup>.

The aim of the present study has been to evaluate the statistics on the validity, efficacy, practicality and all the other characteristics of each one of these methods. Our studies were carried out both prospectively and retrospectively, and we also made parallel evaluations of the various methods in the same women.

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## THE MUCUS AS A MARKER OF THE FEMALE FERTILE PERIOD

Our studies on the cervical mucus as a marker of the female fertile period were principally directed towards:

- the quantitative determination of the mucus withdrawn at the level of the vaginal fornix;

- the fern test;

- the dendritic canalisation;

- the feeling of wetness at vulvar level used as a test of ovulation.

1) Quantitative determination of the mucus withdrawn at the level of the vaginal fornix, ferning and dendritic canalisation.

The cervico-vaginal fluid which accumulates in the upper vagina and in the posterior fornix can be quantitatively correlated to the pre-ovulatory and ovulatory phase of the cycle.

The cervico-vaginal fluid was collected daily by a special aspirator from the posterior vaginal fornix in 5 women of fertile age, and after having read the volume, the mucus was then spread onto two slides.

A first slide not protectively covered served for the analysis of the ferning; a second slide, after the spreading of the mucus, was given protective covering and left for 72 hours at room temperature, in order to count, by optical microscope, the dendritic canals at their highest density. The withdrawals carried out during the fertile period were those of the highest density of mucus, but the intrinsic difficulties involved in the method and the exiguity of the withdrawals did not allow particularly significant evaluation. The same goes for the fern test and for canalisation. It may be remarked, however, that there is an on-going study by the W.H.O. on the subject, from which useful information is expected.

2) Reliability of the subjective feeling of wet at the vulvar level and the echographic diagnosis of the fertile period.

We checked the reliability of the subjective feeling of wet at the vulvar level as a method for diagnosis of the day of ovulation confirmed echographi-

cally, and of the reliability of such a method as a test of ovulation. Two hundred and forty two withdrawals were carried out for a total of 20 ovulatory cycles. The correlation between the feeling of wet (counting from the last day of menstruation) and the echographic diagnosis of ovulation were expressed by 4 conditions as: a) True + (T+); b) False - (F-); c) False + (F+); d) True - (T-) (tabs. 1, 2).

Table 1. — *Wet sensation test.*

|              | Wet + | Wet - | Total |
|--------------|-------|-------|-------|
| Echography + | 11    | 2     | 13    |
| Echography - | 43    | 186   | 229   |
| Total        | 54    | 188   | 242   |

Table 2. — *Assurance of subjective wet sensation at the vulvar level.*

|                                                                      |     |
|----------------------------------------------------------------------|-----|
| Sensitivity (incidence of T+ in the echo-checked ovulatory period)   | 85% |
| Specificity (incidence of T+ in the echo-checked anovulatory period) | 81% |
| Predictive Value + (incidence of all the positives that are T+)      | 20% |
| Predictive Value - (incidence of all the negatives that are T-)      | 99% |
| Efficacy (Incidence of all the surveys that are true)                | 81% |

3) Temporal relation between the feeling of wet at the vulvar level and the echographic diagnosis of ovulation. This was studied with the aim of determining the fertile period; 47 withdrawals of of the wet for a total of 15 cycles. In 38% of cases the wet sensation was experienced in the three days preceeding ovulation, in 26% on the same day and in 36% during the four days following ovulation (fig. 1).

4) Relation between the physical characteristics, transparent and filamentous, of the mucus withdrawn at the vulvar level and the female fertile period.

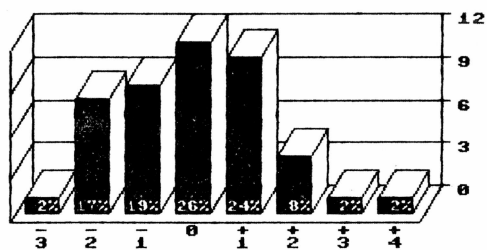


Fig. 1. — Wet sensation.

Another study involved 9 women through a total of 11 cycles and considered the physical characteristics, transparent and filamentous, of the mucus withdrawn at the vulvar level in the aim of determining the female fertile period.

The period of maximum transparency and filamentosity was distributed over the ovulatory and preovulatory periods, echographically monitored according to the percentage reported in tab. 3.

Table 3. — Period of highest filamentosity and transparency.

|                           |     |
|---------------------------|-----|
| – 3 days before ovulation | 5%  |
| – 2 days before ovulation | 11% |
| – 1 day before ovulation  | 6%  |
| 0 Ovulatory day           | 33% |
| + 1 days after ovulation  | 17% |
| + 2 days after ovulation  | 6%  |
| + 3 days after ovulation  | 14% |
| + 4 days after ovulation  | 11% |

In the application of such methods it must be borne in mind that there is a population (20%) which might be defined «at risk»; this refers to women who have few days of mucus production (<sup>4</sup>) at an external examination (<sup>27</sup>).

#### HORMONE ASSAYS

For the use of hormones or their metabolites as markers for the female fertility period (<sup>29, 30, 31</sup>) it is opportune to state the following considerations:

The hematic levels for predicting ovulation cannot be used routinely because they are extremely long and not available on a daily basis, besides being uneconomic and requiring sophisticated equipment and specialised personnel.

The urine level of the steroid hormones in respect to the plasmatic level has the advantage of not being subject to the variations in the pulsatility of the hormone secretion, even if it causes a delay of 16-24 hours in revealing ovarian activity.

1) The utility of the Luminescent Immune Assay (LIA) of Estrone-3-glucuronide in the determination of the female fertile period.

We studied the validity of the determination of the female fertile period by means of the laboratory technique of LIA.

We studied 11 ovulatory cycles in which urinary assays of Estrone-3-glucuronide and Pregandiol-3-glucuronide had been carried out.

Figures 2, 3 and 4 were obtained from the average of the single parameters, and on the basis of the echographic diagnosis of ovulation. In each of the figures we

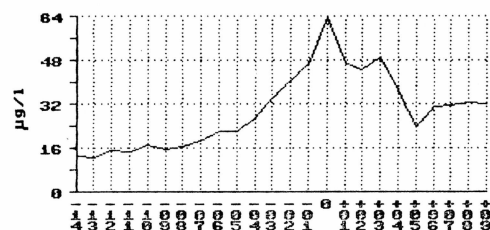


Fig. 2. — Estrone urinary level.

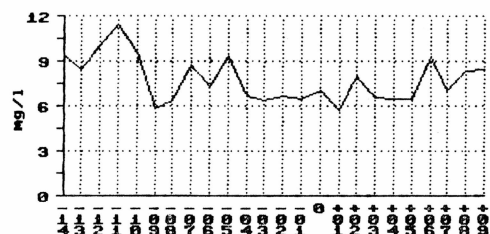


Fig. 3. — Pregandiol urinary level.

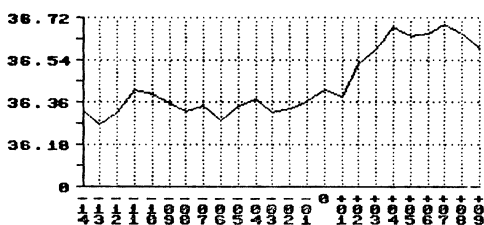


Fig. 4. — Basal temperature course.

considered time «O» of the echographic examination which showed the ovulation accomplished, while the days preceeding and following follicular dehiscence were identified with negative and positive numbers.

In all these cycles fiducial limits were calculated (those in which «real» time average can be found) of the values of Estrone, Pregandiol and Basal Temperature in order to arrive at the conventional limit beyond which values are considered «peak». Only the Estrone assay can be of use for determining the fertile period and its relative peaks, which are reported in tab. 4.

The L.I.A. of Estrone-3-glucuronide can generally be considered reliable, though the data regarding Pregandiol-3-glucuronide are always less so, which still reveal some technical defects in the method, probably due to the selection of the monoclonal antibodies.

2) Biological and individual variability in the interpretation of the urinary values of Estrone and Pregandiol.

Table 4. — Urinary peak of Estrone-3-glucuronide.

|                           |     |
|---------------------------|-----|
| — 6 days before ovulation | 18% |
| — 4 days before ovulation | 9%  |
| — 3 days before ovulation | 9%  |
| — 2 days before ovulation | 18% |
| — 1 day before ovulation  | 28% |
| 0 Ovulatory day           | 9%  |
| + 1 day after ovulation   | 9%  |

The trend of the curves constructed on the basis of Estrone and Pregandiol measured in the urine of different women result co-relatable, but there is a notable heterogeneity both regarding average urinary values (from 12.9 to 49.5 mcg/1 for Estrone and from 3.6 to 16.6 mcg/1 for Pregandiol) and for peaks (from 25.4 to 247.4 mcg/1 for Estrone and from 8 to 50.1 mcg/1 for Pregandiol) in these hormones.

Therefore a parallel study, always carried out on the same samples (11 ovulatory cycles ascertained), which examined the daily values of urinary Estrone and Pregandiol at Basal Temperatures with the aim of studying the biological and individual variability (28) which may represent a limit for the aspecific interpretation of the above-mentioned values.

The average urinary levels of Estrone and Pregandiol of each woman and the average temperature values were submitted to the analyses of variance which permit comparison among more averages, and it was clear that the relation of the variations F (for Estrone and Pregandiol and Basal Temperatures) is the highest of the tabular limits corresponding to their degree of freedom; from this the existence is ascertained up to the level of significance of 5% of difference in the urinary elimination of Estrone and Pregandiol and in the Basal Temperatures among different women, but not in the same women, for whom the contrary has been shown to be probable.

The demonstration that women constantly eliminate in their urine a determined quantity of Estrone and Pregandiol with Basal Temperatures periodically constant requires a knowledge of the values relative to at least one ovulatory cycle specific for each woman; from each of these cycles the limit can be established beyond which values must be considered as peak.

In synthesis:

It is improbable that the same numerical value can be considered a «threshold value» of Estrone, Pregandiol and Basal temperatures common to all women.

On the other hand it is probable that a «threshold value» can be attributed to these parameters for all the cycles in any one woman.

The «interval of variation» (that is, the difference between the minimum and the maximum observed) of the sex hormone rates, particularly marked among different women and within the person of the same woman, did not present any significant oscillations; in fact it may be the expression of a tendency towards the onset of a multiple pregnancy, inasmuch as an excessive production of estrogens invariably involves a hyperstimulation of the ovaries and therefore a contemporary maturation of more egg cells.

In the aim of foreseeing multiple pregnancies in the categories most exposed to such risks (close relatives with twin-pregnancies) fecundation can be avoided during the period in which hormone levels appear particularly high. On these bases improvement should be made in the presentation of colorimetric kits, based on the levels of the above-mentioned metabolites for the determination of the fertile days.

#### IMMUNOENZYMATIC KITS FOR HOME USE

The reliability has been evaluated of 2 immunoenzymatic kits destined for home use, which utilise the urinary peak of LH as a reference point for determining ovulation<sup>(32)</sup>. Kit A represents a quantitative test, Kit B instead, is qualitative and monophasic. Single detections from both kits were compared with echographic diagnoses in order to define their accuracy and characteristics of practicality; rapidity, «end point», possibility of errors in use etc. In a prospective study

Table 5. — T+ condition allotment (Test A).

- a) A colour leap of at least 2 positions to the top of the scale on the same day as echographic diagnosis of ovulation.
- b) Or the above-mentioned positivity colouring was also found on the previous day.
- c) Top scale colour presence on the same day as echographic diagnosis of ovulation.

Table 6. — T+ condition allotment (Test B).

- a) Sign + confirmed by echographic diagnosis of corpus luteum within the two following days.
- b) Sign + for two consecutive days confirmed by echographic diagnosis of corpus luteum on the following day.

we made 150 tests, of which 99 were carried out using Test A in 10 menstruating cycles, and 51 using Test B in 11 menstrual cycles in a group of women volunteers aged between 18 and 35 years.

Each woman in the presumed ovulatory period used Immunoenzymatic Kits A (\*) and/or B daily, marking the results of every withdrawal, on the basis of the colour shown at the tip of the stick in the case of Test A, or on the basis of the appearance of the sign + o — in the trough of each plate used in the case of Test B.

The instructions for carrying out the test with Kit A were slightly modified in relation to the requirements of this clinical study, attributing numerical values on the graduated scale enclosed in the make-up of the Kits; white was assigned to 0, and 1, 2, 3, 4 to the four successive shades of blue.

The data thus obtained were divided into 4 diverse categories:

- a) True + (T+); b) False — (F—); c) False + (F+); d) True — (T—) (tabs. 5, 6, 7, 8, 9).

(\*) Test A = «Clearplan» - Farmades; Test B = «Abbot ovulation test». Abbott - not on sale in Italy.

Table 7. — *F- condition allotment (Test A / Test B).*


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|                                                                                                  |
|--------------------------------------------------------------------------------------------------|
| F- were associated with the absence of the test positivity invalidated by echographic diagnosis. |
|--------------------------------------------------------------------------------------------------|

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Table 8. — *F+ condition allotment (Test A / Test B).*


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|                                                                                              |
|----------------------------------------------------------------------------------------------|
| a) Positivity of the test invalidated by echographic diagnosis.                              |
| b) Positivity of the test on more non-consecutive days or on more than two consecutive days. |

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Table 9. — *T- condition allotment (Test A / Test B).*


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|                                                                                                               |
|---------------------------------------------------------------------------------------------------------------|
| T- is the expression of the correspondence between test negativity and absence of ovulation on a certain day. |
|---------------------------------------------------------------------------------------------------------------|

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Table 10. — *Test A.*


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|              | Test + | Test - | Total |
|--------------|--------|--------|-------|
| Echography + | 3      | 7      | 10    |
| Echography - | 1      | 88     | 89    |
| Total        | 4      | 95     | 99    |

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The data relative to Test A are shown in tabs. 10 and 11.

The data relative to Test B are shown in tabs. 12 and 13.

The prospective study of the female cycle according to the modality reported in this work permits us to affirm the real practical utility (sterility, natural methods etc.) of the 2 Kits immunoenzymatic Test A and Test B.

Test A is a colorimetric test and presents a certain difficulty of interpretation, because the assay following the withdrawal at the initial peak may show a further increase. But even if this difficulty of interpretation can be expressed in poor sensitivity to the ends of diagnosis in real time, in a retrospective study

Test A gives a sensitivity superimposable on that of Test B.

Test B offers a considerable reliability, an end point of easy definition in the period of maximum fertility, a practical indication for reading with the signs + and -, and also a control mechanism (no sign) which allows an indication of a mistaken execution of the test. Finally, it is a rapid and practical method; carrying out the test only takes 5 minutes.

In conclusion, colorimetric Kits like that for Test A are offered mainly as a method for the semi-quantitative determination of the level of LH, differing from Kits like the one for Test B, which

Table 11. — *Assurance of Test A.*


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|                                                                      |     |
|----------------------------------------------------------------------|-----|
| Sensitivity (incidence of T+ in the echo-checked ovulatory period)   | 30% |
| Specificity (incidence of T- in the echo-checked anovulatory period) | 99% |
| Predictive Value + (incidence of all the positives that are T+)      | 75% |
| Predictive value - (incidence of all the negatives that are T-)      | 7%  |
| Efficacy (incidence of all the surveys that are true)                | 92% |

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Table 12. — *Test B.*


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|              | Test + | Test - | Total |
|--------------|--------|--------|-------|
| Echography + | 8      | 2      | 10    |
| Echography - | 4      | 37     | 41    |
| Total        | 12     | 39     | 51    |

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Table 13. — *Assurance of Test B.*


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|                                                                      |     |
|----------------------------------------------------------------------|-----|
| Sensitivity (incidence of T+ in the echo-checked ovulatory period)   | 80% |
| Specificity (incidence of T- in the echo-checked anovulatory period) | 90% |
| Predictive Value + (incidence of all the positives that are T+)      | 67% |
| Predictive Value - (incidence of all the negatives that are T-)      | 95% |
| Efficacy (incidence of all the surveys that are true)                | 88% |

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only suggest the presence or otherwise of the level of the LH peak. Therefore the responsibility of the colorimetric kit could probably be considered as the mirror of the endocrine activity of the organism; but this could be better defined in a wider clinical examination.

1) Combined use of the Test and of the subjective feeling of wet at the vulvar level in the determination of ovulation.

Table 14. — *Test B/Wet.*

|              | Test +<br>a/o<br>Wet. + | Test -<br>a/o<br>Wet. - | Total |
|--------------|-------------------------|-------------------------|-------|
| Echography + | 7                       | 0                       | 7     |
| Echography - | 1                       | 34                      | 35    |
| Total        | 8                       | 34                      | 42    |

Table 15. — *Test B and Wet Sensation combined use assurance.*

|                                                                      |      |
|----------------------------------------------------------------------|------|
| Sensitivity (incidence of T+ in the echo-checked ovulatory period)   | 100% |
| Specificity (incidence of T- in the echo-checked anovulatory period) | 97%  |
| Predictive Value + (incidence of all the positives that are T+)      | 87%  |
| Predictive Value - (incidence of all the negatives that are T-)      | 100% |
| Efficacy (incidence of all the surveys that are true)                | 97%  |

Table 16.

| Event occurred | Echo-<br>graphy<br>result | N.<br>times | N.<br>echo. | %<br>condi-<br>tion |
|----------------|---------------------------|-------------|-------------|---------------------|
| Test+ and Wet+ | +                         | 6           | 7           | 85.7                |
| Test+ and Wet- | +                         | 1           | 7           | 14.3                |
| Test- and Wet+ | +                         | 0           | 7           | 0                   |
| Test- and Wet- | +                         | 0           | 7           | 0                   |
| Test- and Wet- | -                         | 23          | 35          | 65.7                |
| Test- and Wet+ | -                         | 9           | 35          | 25.7                |
| Test+ and Wet- | -                         | 2           | 35          | 5.7                 |
| Test+ and Wet+ | -                         | 1           | 35          | 2.9                 |

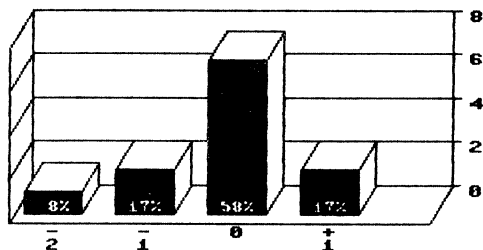


Fig. 5. — Ovulation test.

The efficacy and simplicity of using both Test B and of the subjective evaluation of wet at the vulvar level suggest combined use. Therefore we also evaluated the reliability of the combined use of the two above-mentioned methods in comparison with the echographical examination. The results of this study are reported in tabs. 14, 15, 16.

2) The use of Test B in the determination of the fertile period in women.

In 25% of cases Test B proved positive in the two days preceding ovulation, in 58% on the day of ovulation and in 17% on the day after ovulation diagnosed echographically (fig. 5).

## RESEARCH ON THE SALIVA

The salivary fluid, according to the hormonal condition<sup>(33)</sup>, shows pictures characteristic of the level of glucose<sup>(34)</sup>, sodium<sup>(35)</sup> and some enzymes<sup>(33)</sup>. To the end of identifying the fertile period it is necessary to individuate an enzyme that not only has modification of activity correlated to the menstrual cycle, but also has the property of revealing itself with a reaction easily visible to the naked eye, in such a way as to constitute a Kit of sure and easy application for any woman.

Bearing this in mind we studied beta-glucuronidase, an acid hydrolase, and this for two reasons:

a) in the mouse it has shown itself to be a steroid dependent enzyme<sup>(36, 37)</sup>;

b) in the test-tube reaction its preferential substrate was phenol-phtalene-glucuronide, which under enzyme action changes with a buffer, causing a pink coloration proportional to the enzymatic activity. This might lead to a means of Kit easily usable at home <sup>(39)</sup>.

1) Demonstration of the biochemical activity of a salivary enzyme, beta-glucuronidase.

Twelve women collected samples of 1ml of saliva every day, fasting and without having used dentifrice in order to avoid any interference from fluorides or other salts.

The saliva was analysed prior to homogenisation with 2 ml triethylaniline buffer 0.05 M to pH 7-6 containing dithiotreitol 1mM.

The assay for beta-glucuronidase was carried out according to the method of Lin and Fishman <sup>(38)</sup>.

Fig. 6 represents a typical proceeding of enzymatic activity. In this, three maximums of activity are easily recognisable: the first may be related to the lysis phase of small non-matured follicles, the second practically coincides with ovulation echographically diagnosed, and the third corresponds to the phenomena of the endometrial or corpus luteum lyses.

From the case series examined a certain variability in the width of the second peak can also be noted, probably indicating the length of the fertile phase.

2) Salivary ferning self made test. An important stimulus to the study of the saliva was given by the timely arrival of

special microscopes of pocket size, which women can use in order to recognise the cristallization of their own saliva when it has been deposited on an appropriate slide <sup>(40)</sup> (« PG-53 », Italiana Lab. Bouty).

In the organised crystallization (ferning) of the saliva notable importance is assumed by the presence of salts (especially NaCl) which form crystalline nuclei in which the Na<sup>+</sup> ineffectively neutralised interacts with the negative charge of the mucin molecule that constitutes a nematoid system in which, above all, the slow drying conditions allow for aggregation into an orderly filiform structure. Estrogen action increases the H<sub>2</sub>O content of the saliva at mid-cycle, thus determining the most favourable conditions for «epitaxy» <sup>(41, 42)</sup>. This phenomenon consists of an increase directed towards the fixing of a crystalline substance above the substrate of another crystalline compound, and which, besides, requires regions of low concentration among the above-mentioned structures <sup>(41)</sup>.

Wider studies are still going on, so that it therefore seems practical to await their outcome, in order to establish effective statistical validity <sup>(43, 44, 45, 46, 47)</sup>.

## CONCLUSIONS

The interest aroused in certain sections of public opinion on « natural methods of family planning » has been the principal stimulus which induced us to enquire into its validity. Our experience has allowed us to ascertain this, principally in the case of the immunoenzymatic method on the urine, and in the self-examination of the feeling of wet at the vulvar level, if women are particularly motivated in this enquiry.

The enzymatic salivary method has also aroused interest, while in regard to microscopic self-examination of salivary crystallisation, it may well be that if this is confirmed on a large scale it will be able

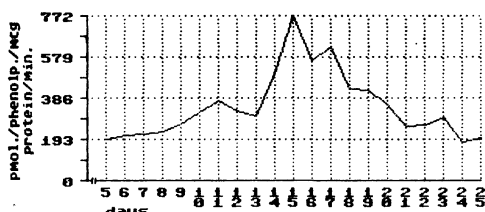


Fig. 6. — Enzymatic activity.



to offer a valid method of self-diagnosis of fertility.

We therefore consider that gynecologists must be accurately informed on the validity of these methods, in order to be able to instruct adequately those women who turn to them, both for the limitation of the fertility and in their desire for conception.

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