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Comparative Studies on the Usefulness of Diagnostic Methods of Estimating the Ovulation Process

Porównawcze badania nad wartością metod diagnostycznych przy ocenie procesu jajeczkowania

Сравнительные исследования по оценке диагностических методов анализа процесса овуляции

The correct determination of the fertility and infertility periods during the human menstrual cycle is essential both for the treatment of infertility and for the successful application of birth-control measures (5, 7, 10, 17, 19, 32). There are various clinical and laboratory diagnostic methods which make it possible to determine the time of ovulation or to state its absence. The range of laboratory and clinical tests used for that purpose is wide; we have at our disposal such tests as cytological examination, endometrial biopsy, records of basal body temperature, the Fern Test and spinnbarkeit test of the cervical mucus, determination of the level hormones secreted with urine, etc. In addition to these indirect methods of detecting ovulation, it is possible to study this process by direct observation; culdoscopy, coelioscopy and laparotomy enter into this category.

Comparative investigations on the diagnostic value of all these tests point repeatedly to the fact that the tests should be performed in certain complexes in order to obtain the most reliable results. Discussion is going on in world literature, concerning the usefulness and accuracy of the separate diagnostic tests (25, 30, 31, 33, 41, 42). Cytological examination of vaginal smears has become the most popular method of detecting irregularities of the menstrual cycle and disturbances in ovulation (2, 4, 20). However, it is not free from certain inconveniences, as it must be repeated several times during the menstrual cycle and requires a special, rather complex technique of fixation and staining. Preparations thus obtained can be interpreted correctly by an experienced cytologist only and make it possible to determine the time of the accomplished ovulation with the accuracy of 2 or 3 days. A simpler, easier, but equally reliable method would be therefore highly welcome in clinical practice.

Comparative studies on several diagnostic methods based on personal clinical material enabled the present writer to compare the results and conclusion obtained by other writers with her own observations.

METHODS AND MATERIALS

299 menstrual cycles were studied in 224 women who sought medical advice because of infertility. In some of them two or three successive menstrual cycles were studied in order to obtain more accurate results. In 141 women the following three kinds of tests were carried out: 1. measurements of morning body temperature, 2. cytohormonal tests, 3. tests of mucus crystallization. In 158 women only the first two types of tests were performed. In a few cases Mack's iodine test, endometrial biopsy and Tes-Tape were applied additionally. Apart from that, in all the women blood examination was carried out (blood sedimentation rate and differential blood count), and, if necessary, other examinations, such as determination of hormone level in urine and of basal metabolic rate, and tubal patency tests were added.

The morning body temperature was recorded on a special chart. The studied women had been carefully instructed on the method of taking the body temperature. The temperature was measured under the tongue, immediately after awakening. Intestinal disturbances, infectious diseases, insomnia, etc., were also noted in the charts. The measurements of body temperature were in all cases carried on for a longer time (from 3 months to 2 years). Examinations of vaginal smear and of cervical mucus were repeated 4 or 5 times; the material was taken on the 12th, 14th, 18th, 22nd and 26th day of the menstrual cycle. Vaginal smears were taken by the typical method, and stained according to Schorr. Endometrial biopsy was practised with the use of Novak's sound during the final 2 or 3 days of the menstrual cycle.

The separate menstrual cycles were evaluated with the help of the following data: 1. the characteristic shape of the curve of morning body temperature, 2. description of cytological smears according to Papanicolaou, 3. calculation of acidophilia and caryopycnosis indices according to Langroeder, 4. description and classification of patterns of cervical mucus crystallization according to Wisłocka and Welman, and 5. criteria of the histological appearance of the endometrium, given by Teter and Zaleski.

The studied women were divided into three groups:

Group I — women with regular menses, in whom the results of all performed tests were in agreement and pointed to the ovulatory cycle. The group was divided into subgroups **A** and **B** to differentiate ovulatory cycles in which both phases were normal from those with an inadequate secretion phase.

Group II — women in whom the results of the investigations pointed to a monophasic cycle, without ovulation.

Group III — women in whom the separate tests provided discordant results, so that the exact identification of the type of menstrual cycle was impossible.

The following Table shows the number of investigations in the separate groups of the 224 studied women.

Group	Tests performed		Total
	Basal body temperature Cytohormonal tests Fern Tests	Basal body temperature Cytohormonal tests	
I A	32	26	58
I B	38	28	66
II	68	94	162
III	3	10	13
	141	158	299

RESULTS

Group I. 81 women, aged from 18 to 41 years, were included into this group. They had regular menses, and all performed tests pointed to the presence of the ovulatory cycle. 124 menstrual cycles were studied in this group. The average duration of the cycle was 28 days. In 11 women of this group monophasic, anovulatory cycles were observed occasionally. In 5 cases pregnancy occurred during the first studied cycle; in 13 cases this happened during the next few months.

The first part of the investigations (subgroup A) concerned 45 women, in whom 58 cycles were studied, and in whom all tests confirmed the occurrence of ovulatory cycles. In these cases the temperature curves (Figs. 1, 2, 3, 4, 5, 6, 7, 8 and 9) were typical of the ovulatory cycle

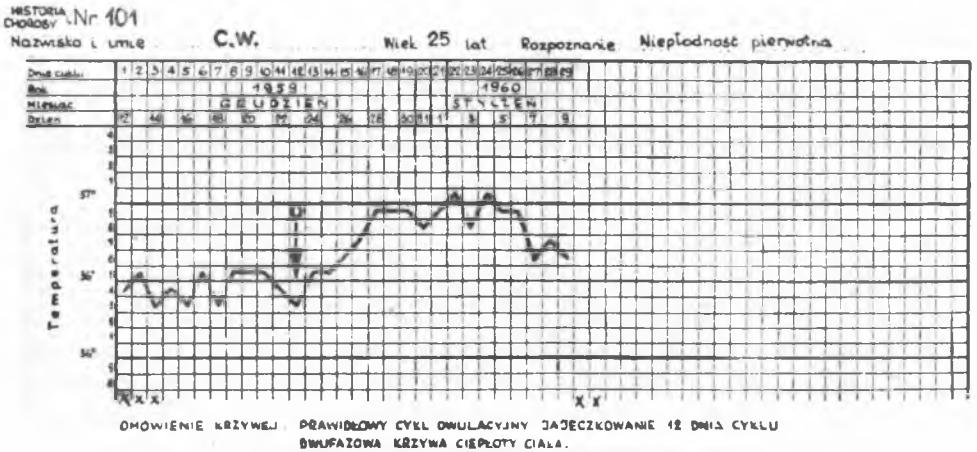

Fig. 1. Case C. W. No 101. Biphasic basal body temperature record. The time of ovulation: 12th day of the cycle

(16, 24, 28, 30, 32, 26, 27, 41). During the menstrual bleeding the body temperature curve shows a characteristic fall, which begins a few days before the appearance of the bleeding and lasts several days. The comparatively low body temperature, ranging between 36.4°C and 36.8°C, which is observed during the proliferative phase, corresponds to the period of growth of the Graafian follicle and of the secretion of oestrogens. During this period the cytological picture reveals flat cells with regular borders, belonging to the superficial layer, and an increasing prevalence of acidophilic cells with small, pycnotic nuclei.

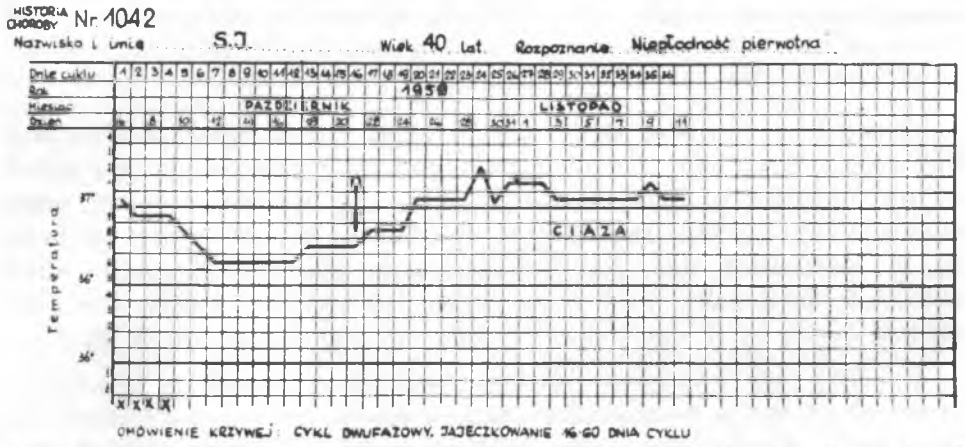

Fig. 2. Case S. J. No 1042. Basal body temperature record as a diagnosis of pregnancy. The time of ovulation: 16th day of the cycle

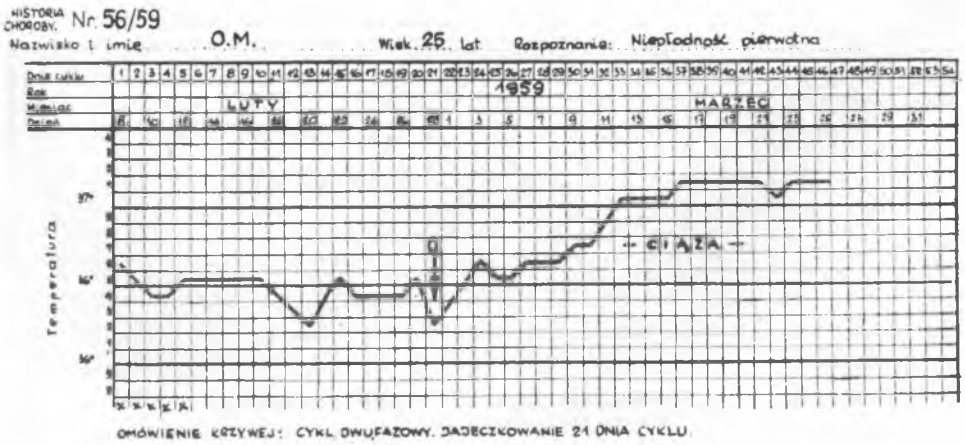

Fig. 3. Case O. M. No 56. Biphasic body temperature record with the prolonged first phase of the cycle. Ovulation and conception on the 21st day of the cycle

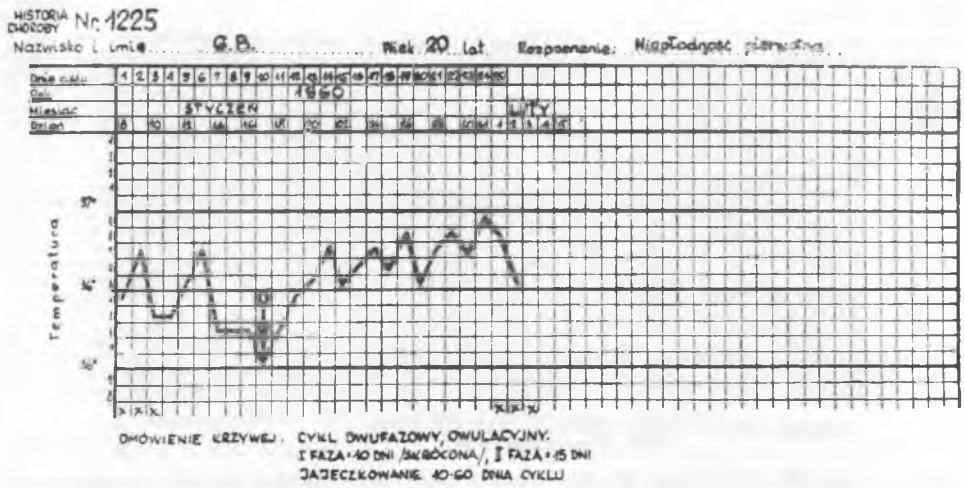

Fig. 4. Case G. B. No 1225. Biphasic basal body temperature record with the shortened first phase of the cycle. The time of ovulation: 10th day of the cycle

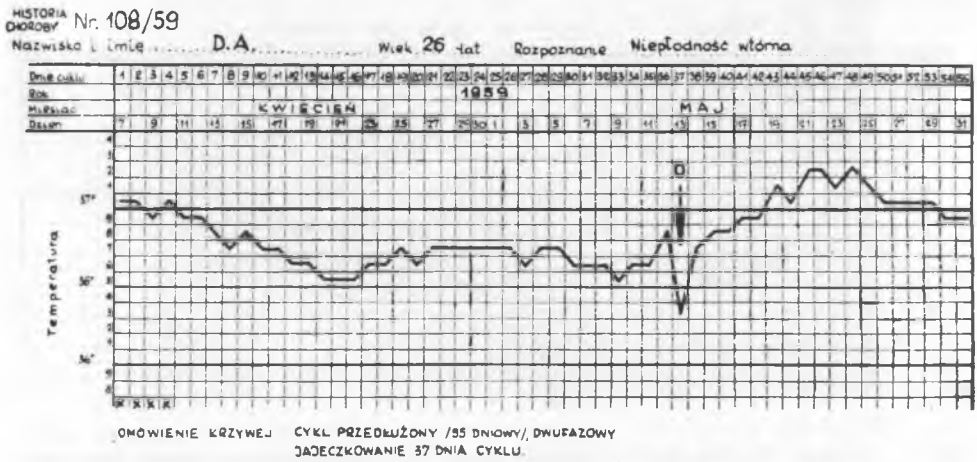

Fig. 5. Case D. A. No 108. Biphasic body temperature record of a prolonged cycle (55 days). The ovulation and conception on the 37th day

There occur also basophilic cells with pycnotic nuclei, belonging to the superficial layer of the epithelium. The number of leucocytes and the amount of mucus gradually decrease (Figs. 10 and 11). As the maturing of the Graafian follicle progresses, the phenomenon of the crystallization of the cervical mucus becomes more distinct (Figs. 12 and 13). In the middle of the menstrual cycle, usually between the 13th and 15th day of its duration, the temperature of the body falls suddenly. The Graafian follicle ruptures and the egg is extruded, which

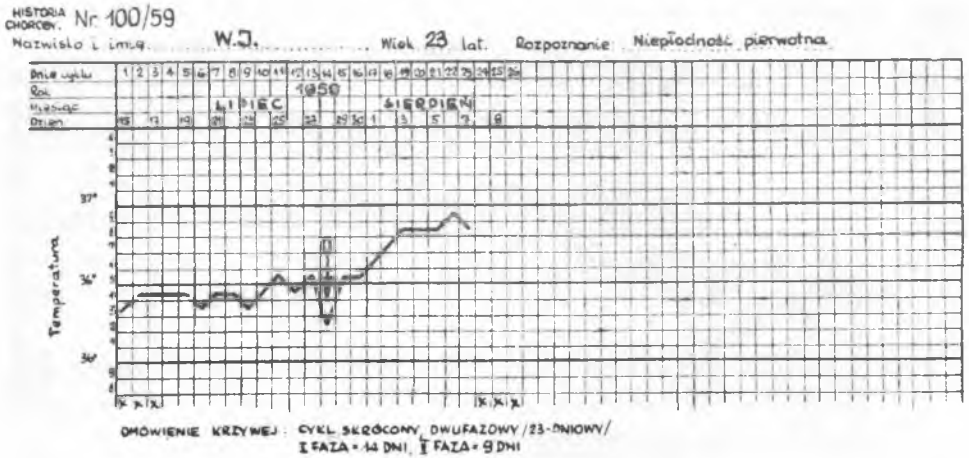

Fig. 6. Case W. I. No 100. Biphasic basal body temperature record of shortened cycle with the ovulation on the 14th day. Shortened second phase of the cycle

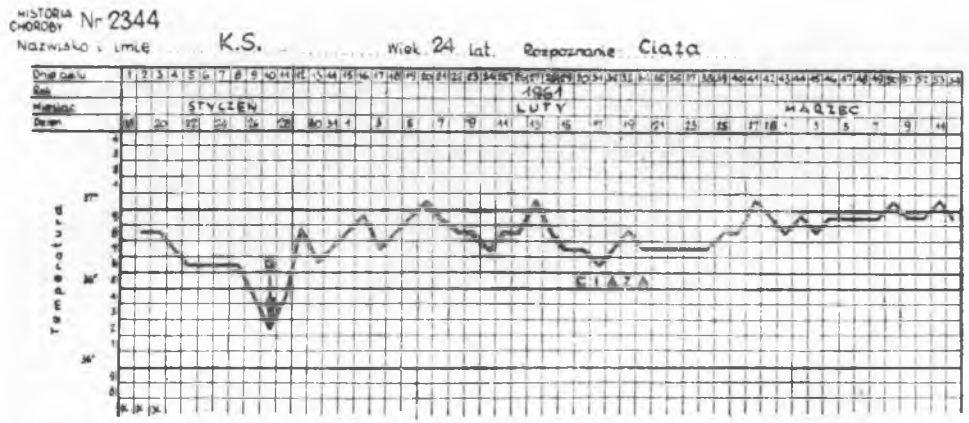

Fig. 8. Case K. S. No 2344. Biphasic basal body temperature record with the ovulation and conception on the 10th day

is followed by a rise in body temperature. During the 2nd phase of the cycle the body temperature remains more or less at a raised level. The microscopical picture becomes clear, devoid of leucocytes and mucus. There appears a great number of superficial, acidophilic cells with pycnotic nuclei. The cells assume a shingle-like arrangement. The degree of crystallization of the cervical mucus (3, 29, 43) reaches then its highest level (Figs. 14 and 15). During the 2nd phase of the menstrual cycle the cytological picture is characterized by the phenomenon of the clustering of cells into groups of various size, and by a ruffling

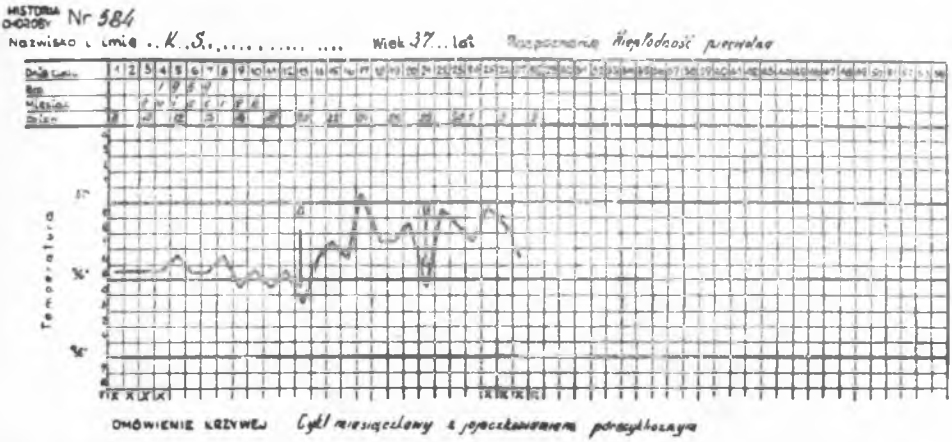

Fig. 9. Case K. S. No 584. Basal body temperature record with the paracyclic ovulation on the 13th and the 21th day of the cycle

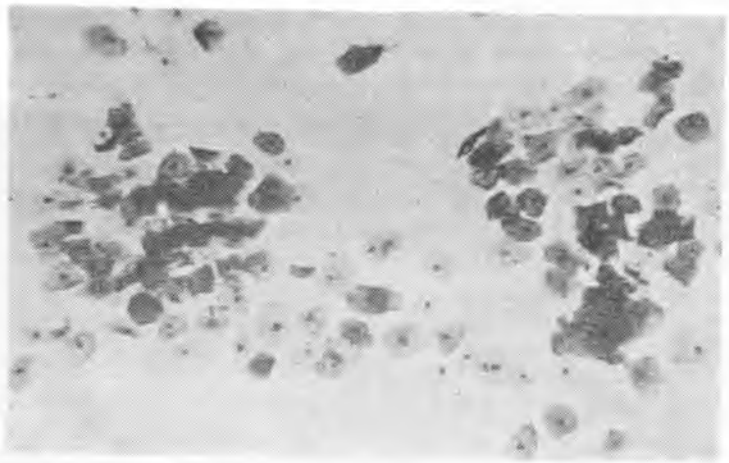

Fig. 10. Case L. H. No 142. Vaginal smear on the 11th day of the biphasic cycle. Magn. 150 x. Clear cytological picture, complete leucopenia and lack of bacteria. Scattered cells distinctly outlined. Picture of the preovulatory phase.
IA 65% IK 80%

of their borders. The cells become smaller, have rolled borders and are mostly of the basophilic type (Figs. 16 and 17). The acidophilia and caryopycnosis indices decrease (Figs. 18 and 19) and no crystallization of the mucus can be observed.

In this group the results of the separate tests applied for the investigations of the menstrual cycles showed good correlation. However, there were observed differences, ranging from 1 to 4 days, between the results of the separate tests, concerning the determination of the

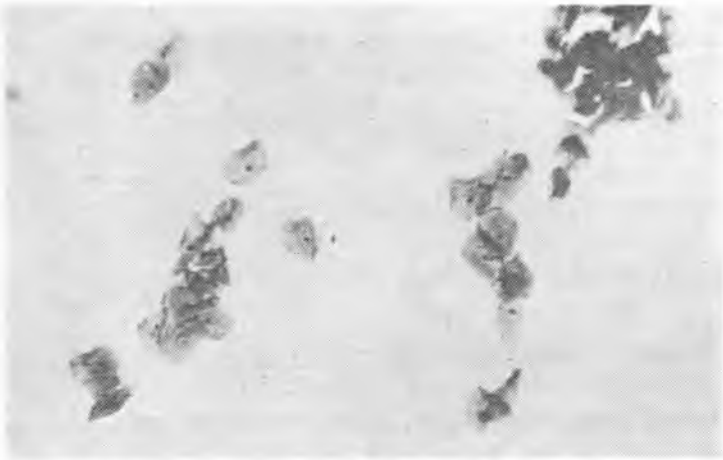

Fig. 11. Case L .H. No 142. Vaginal smear on the 13th day of the biphasic cycle. Magn. 150 x. Clear cytological picture, complete leucopenia and lack of bacteria. Superficial cells with acidophilic multilateral cytoplasm overlapping each other. Picture of the ovulation phase. IA 80% IK 90%

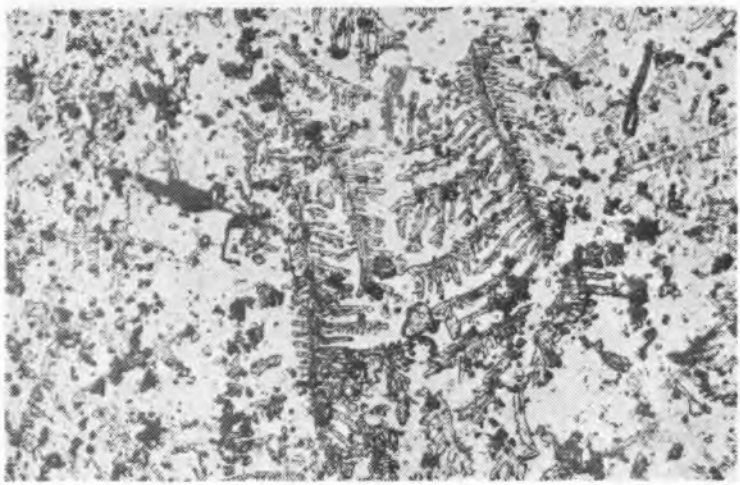

Fig. 12. Case D. K. No 770. Preparation from the 12th day of the biphasic cycle. Fern-test +. Magn. 150 x. Numerous loose crystals, many cellular elements and single formations of fern-like structure

date of ovulation. The determination of this date by recording the morning body temperature becomes difficult in those cases in which there is no sudden, one-day fall of the curve, followed by a constant rise. In such cases the temperature curve showed a step-like rise lasting several days, and the date of ovulation had to be determined with the

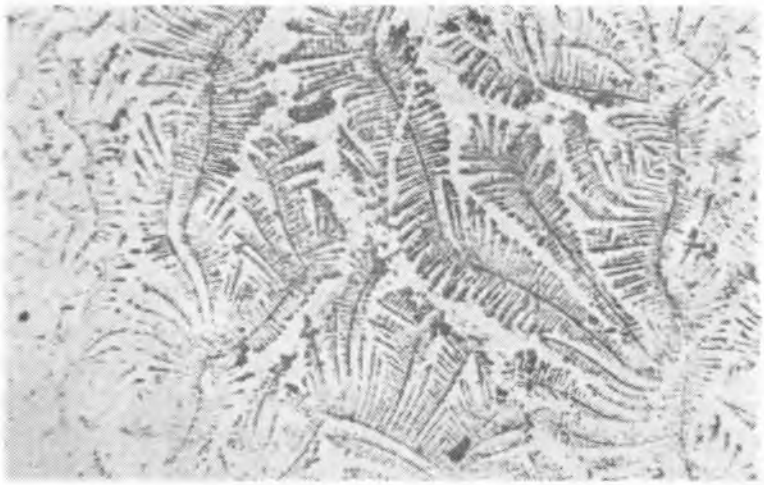

Fig. 13. Case W. Ł. No 22. Preparation from 13th day of the cycle. Fern-test ++. Magn. 150 x. Numerous fern-like phenomena

Fig. 14. Case W. Ł. No 22. Preparation from the 15th day of the biphasic cycle. Fern-test ++. Magn. 150 x. Numerous palm-like phenomena

approximation of several days (Fig. 20). An accurate estimation of the ovulation date by means of cytohormonal investigations was not always possible as the tests were performed at intervals of 2 or 3 days.

The second part of the studies (subgroup B) concerned 54 women, in whom 66 menstrual cycles were investigated. In all of them the biphasic cycle was found, with an inadequate secretion phase. In these cases the morning body temperature curve had a shape characteristic

of ovulatory cycles, with a marked fall of the temperature in the middle of the cycle, followed by a constant rise persisting during the secretion phase. The insufficiency of the secretion phase manifested itself in the temperature curves in a characteristic form, either as frequent and rather considerable oscillations of the temperature, or as an abnormally short rise of the curve during the 2nd phase (Figs. 21 and 22).

There are circumstances which make it difficult to demonstrate progesterone deficiency by means of cytological investigations. It is, therefore, necessary to determine the degree of luteal deficiency above all on the strength of two essential criteria. The acidophilia and caryopycnosis indices should be repeatedly determined during the cycle in order to diagnose the accomplished ovulatory phase, and then the characteristic features of the 2nd phase should be deduced from vaginal smears. Sometimes cytological smears may produce pictures which more or less resemble the luteal activity; this results from pathological luteinization, which occurs in spite of the fact that the ovary contains no yellow body. When an unruptured Graafian follicle undergoes atrophy, there occurs sometimes a pathological luteinization of thecal cells, which produce bodies showing the properties of gestagens. The presence of such changes can be excluded by finding, during the first half of the menstrual cycle, pictures typical of the preovulatory and ovulatory periods, as well as the proper rise in the acidophilia and caryopycnosis indices. Luteal insufficiency may be the result of an

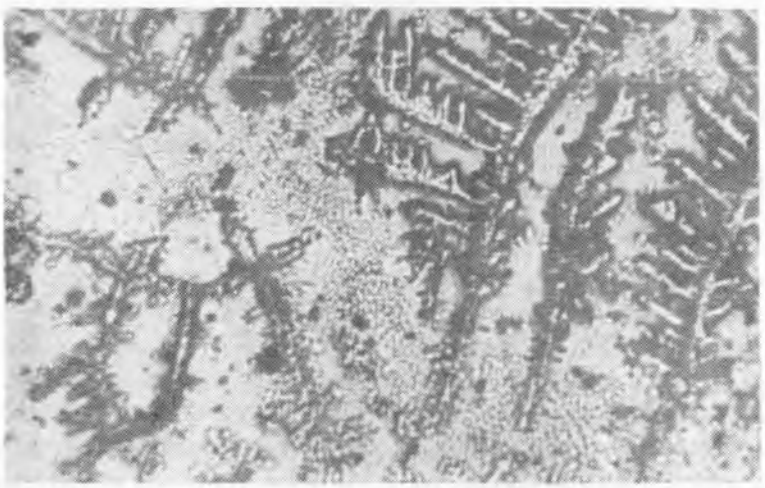

Fig. 15. Case K. J. No 1696. Preparation from the 14-th day of the biphasic cycle. Fern-test +++. Magn. 150 x. Crystalline forms with thick, dark branches. Individual ramifications expand at the angle of 90°

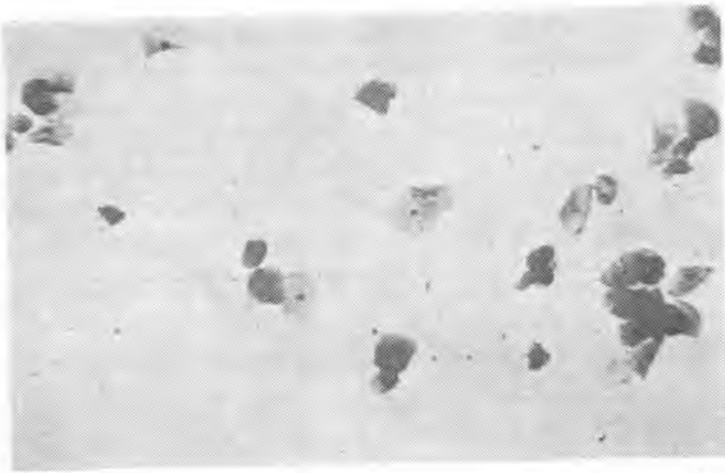

Fig. 16. Case L. H. No 142. Vaginal smear on the 15th day of the biphasic cycle. Magn. 150 x. Acidophilic cells rolled, with pycnotic nuclei. IA 45% IK 70%. Picture of the post-ovulation phase

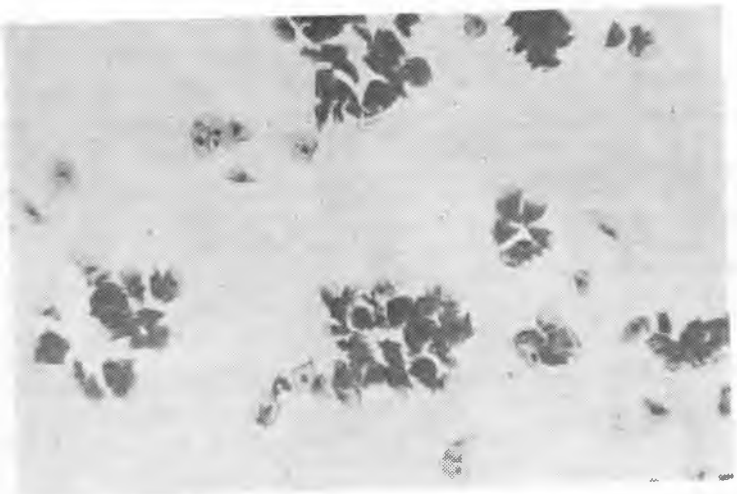

Fig. 17. Case L. H. No 142. Vaginal smear on the 20th day of the biphasic cycle. Magn. 150 x. The cells rolled and clustered in groups. Not numerous intermediate cells. IA 35% IK 45%. Picture of the luteal phase

increased and prolonged production of oestrogens, or it may be caused by a premature regression of the yellow body. Examination of cytological smears revealed an atypical decrease in the A and K indices, and the absence of changes characteristic of the secretion phase. Fern Tests made it sometimes possible to observe the persistence of the mucus crystallization during the second phase of the cycle (6). The estimation

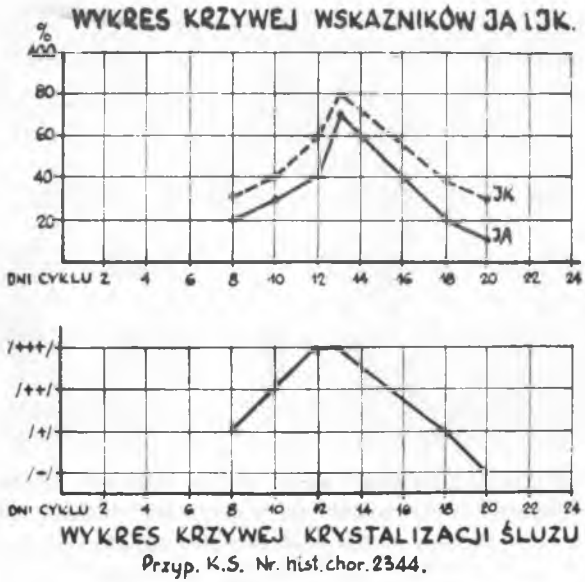

Fig. 18. IA, IK and Fern-Test curves of the biphasic cycle

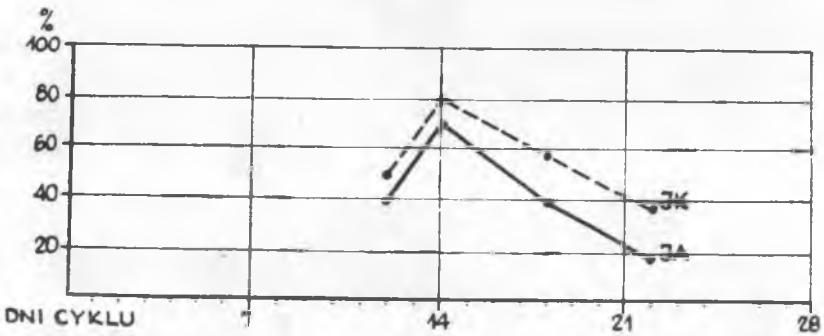

Fig. 19. IA and IK curves of the biphasic cycle. Case S. J. No 1042

of the cytological smears offered the greatest difficulties. In doubtful cases it was necessary to resort to endometrial biopsy (Fig. 23).

Group II. This comprised 133 women between 20 and 42 years of age, with regular menstruation at 23 to 33 days' intervals, seeking advice because of their inability to become pregnant. 162 menstrual cycles were studied in this group. In the majority of cases the morning body temperature curve (Fig. 24) remained at a low level, showing only slight oscillations (13). In 13 cases the temperaure curve had a jumping course, with fairly pronounced fluctuations (Fig. 25). Cytological examination revealed the existence of two different types of

smears. In 63 menstrual cycles, during the 1st half of the cycle, a normal picture of the proliferative phase was found, the indices ranging between: IA 30—60%, IK 60—70%. In the remaining 99 cycles a different character of cytological pictures was found: during the 1st half of the cycle there was obserwed an insufficient development of the features characterizing the proliferative phase, and the absence of the cyclic rise in the index values. During the 2nd phase of the cycle, the absence

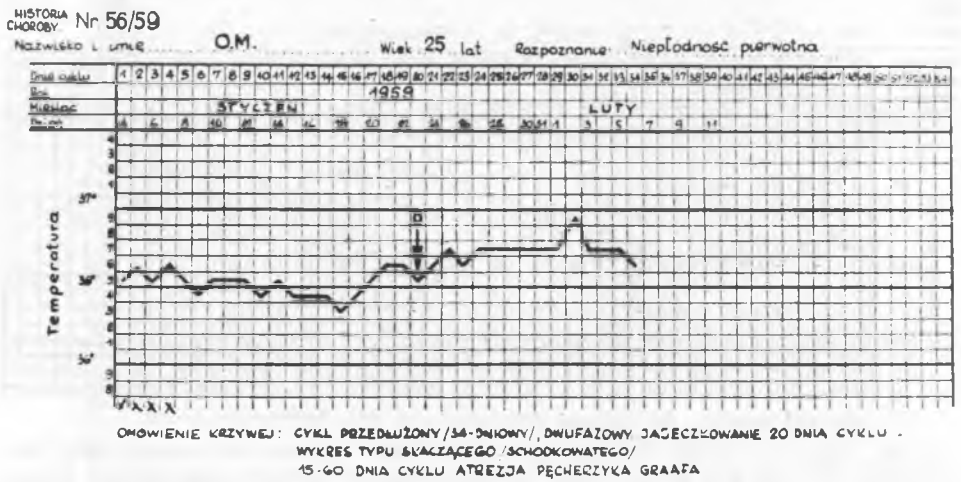

Fig. 20. Case O. M. No 56. Biphasic basal body temperature record of a prolonged cycle (34 days). The time of ovulation 20th day cycle. The droppening cycle. Atresia of the Graafian follicle on the 15th day of the cycle

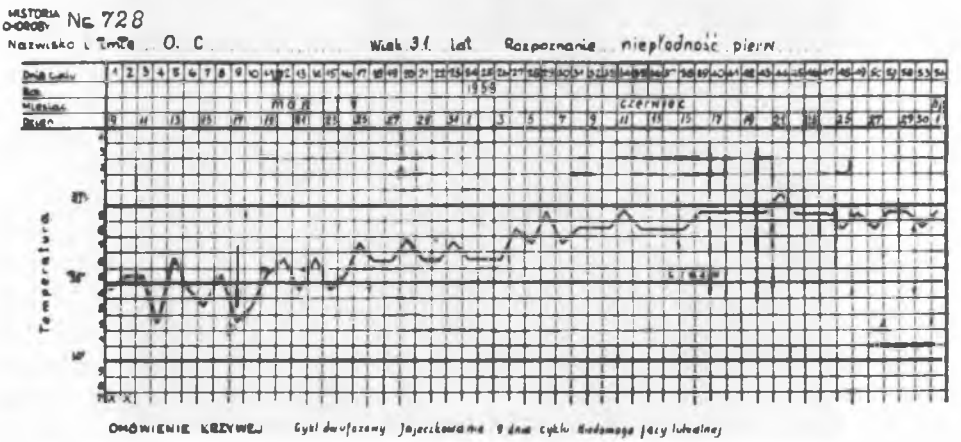

Fig. 21. Case O. C. No 728. Biphasic basal body temperature record with the ovulation on 9th day of the cycle. The curve shows an inadequate luteal phase. In this case there appear symptoms of the threatening abortion

of the luteinization picture was found, which pointed to a biphasic insufficiency of the ovarian secretion. In such cases the false positive diagnosis of the luteal activity of the ovary is caused by the false luteinization of the thecal cells of the atrophic Graafian follicle, which produce bodies related to progesterone. As an instance of the difficulties which offer themselves during the estimation of this type of menstrual cycle will serve the case of M. W., No 930 (Fig. 25). The morning

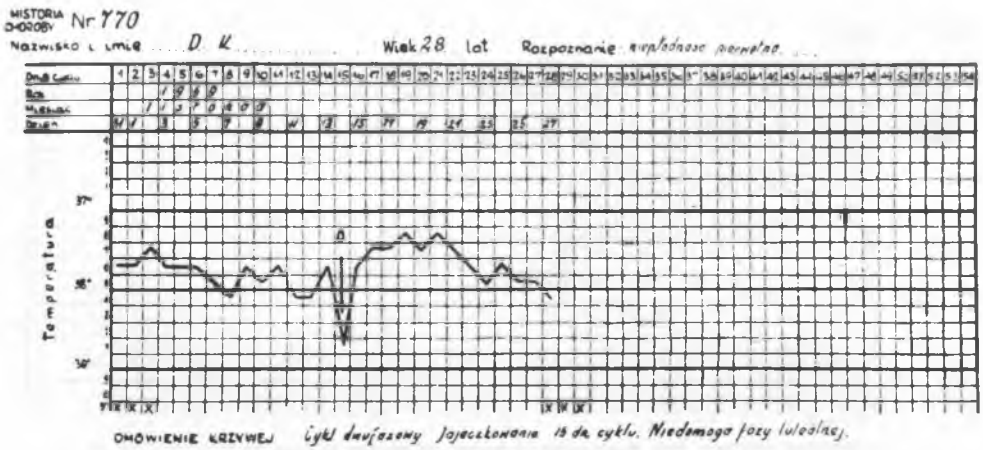

Fig. 22. Case D. K. No 770. Biphasic basal body temperature record with the ovulation on 15th day of the cycle. An early rise in the body temperature record after the menstrual bleeding shows a shortened hormonal activity of the yellow body

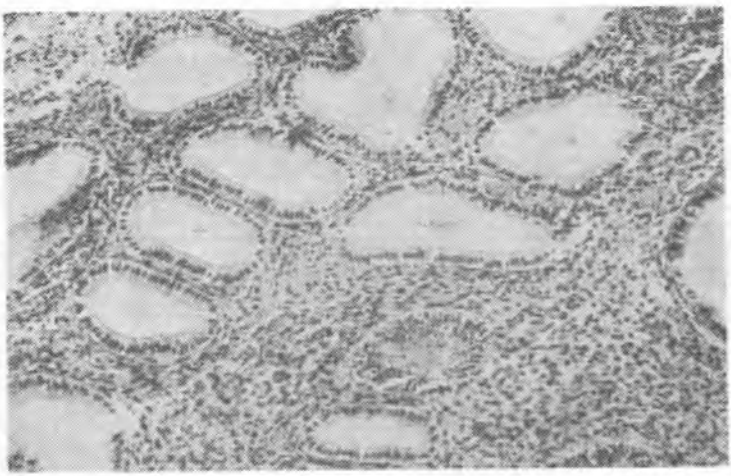

Fig. 23. Case P. S. No 1424. Vaginal cytology and Fern-Test show the biphasic cycle with inadequate luteal phase. Endometrial biopsy performed on the 26th day of the cycle (29 days) shows early secretion phase

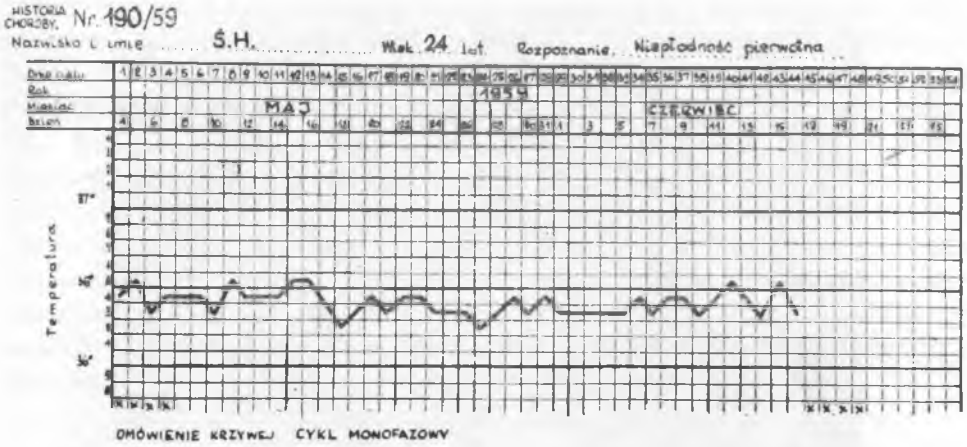

Fig. 24. Case S. H. No 190. Monophasic basal body temperature record

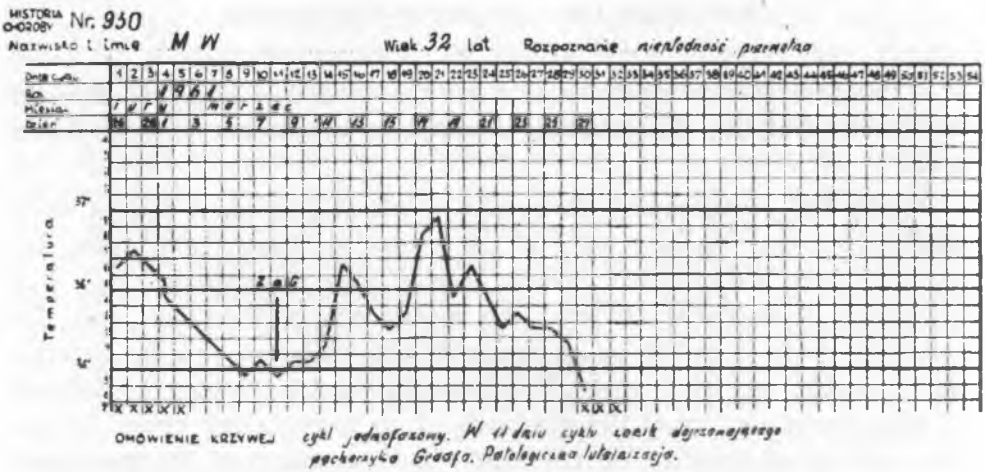

Fig. 25. Case M. W. No 930. Monophasic basal body temperature record. Atresia of the mature Graafian follicle with the pathological luteinization

body temperature curve, though showing a decrease on the 11th day of the cycle, and then a generally elevated level with considerable fluctuations during the 2nd half of the cycle, is not typical for the ovulatory cycle with the normal secretion phase. Cytological examination demonstrated the absence of the behaviour of the acidophilia and caryopycnosis indices characteristic of the ovulatory cycle. In this case the involution of an incompletely mature Graafian follicle and pathological luteinization may be supposed. The examination of the crystallization of the cervical mucus revealed the persistence of the crystallization on the 16th day of the cycle, and the appearance

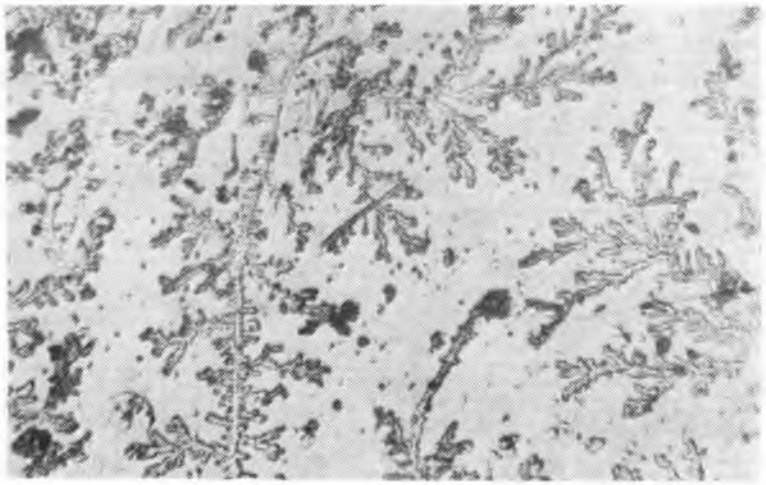

Fig. 26. Case D. Z. No 1658. Preparation from the 22th day of the monophasic cycle. Magn. 150 x. Lycopodium-like structure

of a lycopodium-like crystallization on the 18th day (Fig. 26). Bioptic examination of the endometrium resulted in diagnosing a monophasic cycle.

Group III. It comprised 13 women in whom discordant results were obtained by the separate tests of the menstrual cycle. In 8 cases the morning body temperature records pointed to a monophasic cycle, but cytological examination showed the occurrence of ovulation and the secretion phase (4 cases), or an accomplished ovulation and the insufficiency of the secretion phase (4 cases). Endometrial biopsy performed in these cases confirmed the diagnosis of an anovulatory cycle. In one case cytological examination revealed a normal course of the menstrual cycle, but the body temperature record indicated atrophy of the Graafian follicle and the absence of the secretion phase. In two cases there existed a biphasic body temperature record, but cytological examination failed to confirm the occurrence of ovulation. Endometrial biopsy carried out in these cases gave results agreeing with those of the morning body temperature records. In two cases an ovulatory cycle with luteal insufficiency was diagnosed on the strength of body temperature measurements. Cytological examinations carried out at the same time did not confirm this diagnosis. The assessment of the cytological preparations was false because of changes in the microscopic pictures resulting from the inflammatory condition of the cervix and vagina caused by *Trichomonas vaginalis*.

In the cases under discussion the records of the morning body temperature proved to be a more sensitive and less misleading test in comparison with the cytological test and the Fern Test.

Separate mention should be made of cases in which, apart from the Fern Test, cytohormonal test, and records of the morning body temperature, the „Fertilizing Test" or Tes-Tape were carried out. In all the 11 cases, the last-mentioned test gave results agreeing with those of the tests mentioned before. It should be stressed that the Tes-Tape test has numerous advantages, which may assure it one of the foremost places. Simplicity, accuracy, speedy results make it eminently useful in contraceptive consultation and in the treatment of infertility (11, 18, 36).

DISCUSSION

The most objective test of the accomplished ovulation is the ensuing pregnancy (G r e e n h i l l). Looking for an equally reliable and objective test, research workers dealing with the problem of fertility and infertility in woman discovered a number of tests which, either directly or indirectly, determine the presence or the absence of ovulation.

The direct ovulation tests cannot be applied in all cases and cannot be repeated to confirm the obtained results or to check the efficacy of the adopted treatment. Very often they prove quite useless as they only confirm the occurrence of ovulation *ex post* (14). The remaining methods are based on indirect observation of phenomena produced by the cyclic changes in the biochemical and physical processes going on in the female organism (1, 37). When use is made of these methods, the possibility of a distortion of their results by intrinsic or extrinsic factors should be borne in mind. Some indirect methods of testing the regularity of the menstrual cycle present great technical difficulties, which make it impossible to use them in everyday practice of a hospital or a gynaecologic out-patient clinic (K n a u s' test, testing changes of electric potential). Other methods of detecting ovulation need frequent repetition during the cycle, require a specialized laboratory equipment and are laborious (testing the level of hormones in urine, cytohormonal investigations, determination of the level of reducing bodies in cervical mucus, determination of the acidity of the vagina, endometrial biopsy).

Everyday clinical practice requires a dependable and accurate test, not troublesome for the patient and the physician, and easy to perform even under the most primitive conditions. It is also indispensable that it should determine the present, and not past, ovulation process, and that it might detect additional ovulation. To verify the results of the treatment, the test should allow of frequent repetition during the cycle.

The results of the present investigations point to the morning body temperature test as fulfilling these requirements. It is obvious that only sudden systemic reactions, such as an impetuous rise in basic body temperature, may be the expression of the occurring ovulation. Changes in the cervical epithelium take a longer time to reflect the accomplished ovulation. Usually the morning body temperature records indicate an earlier date of ovulation than cytohormonal examination. This is probably connected with the fact that the sensitivity and quickness of reaction of the epithelial cells to hormonal stimuli are lower than the reaction of the body temperature.

The method of morning body temperature measurements is easy and accurate; it can be used for a longer time and it makes it possible to exclude to a high degree the influence of external factors if the patient is carefully instructed on the conditions under which the temperature should be taken. The interpretation of the basic temperature records is single, and its results are objective. On the other hand, when vaginal epithelial cells are examined morphologically and cytologically, there exist, besides the definite structural properties, subjective criteria resulting from the personal opinion and experience of the examiner. This is proved by the fact that there is always fresh discussion at international congresses concerning the method of evaluation of certain types of cell. It seems that subjectivity in the assessment of cytological preparations results in a comparatively high percentage of false diagnoses. The usefulness of the cytological method for hormonal investigations is also limited by the fact that the assessment of biological phenomena only by morphological methods is insufficient. It is often overlooked that human organism has at its disposition only a limited number of morphological correlates to answer a stimulus. Inflammatory and degenerative cellular inclusions are an additional difficulty in the interpretation of the cytological picture.

It was found by examination under the phase microscope (D e l n o n) that the products of metabolism of some microorganisms change the structure of the nuclei and cytoplasm of vaginal epithelium cells more often than it has been observed so far. There exists already experimental evidence supporting this opinion. W i e d (quoted by D e l n o n) found that *Streptococcus viridans* added to cell cultures produces a change in the indices of acidophilia and caryopycnosis of the studied cells. It is also a common opinion that *Trichomonas vaginalis* produces degenerative changes in the structure of the epithelial cells (9). In spite of the simplicity of the method of taking vaginal smears, there is always some possibility of error resulting from the failure to observe

all the minute details of the technique of taking, fixing and staining the smears. Delnon demonstrated that changes in the method of taking the smear and spreading it on the glass produce different cytological pictures in the phase microscope. Various endogenous (influence of the nervous system, diseases of the liver) and exogenous (inflammatory conditions of the genital organ, influence of the method of taking vaginal smears, use of contraceptive and cosmetic measures) factors contribute to the situation in which the results of cytohormonal investigations fail to give a true picture of the hormonal interplay going on in the organism of the woman. The proper evaluation of the hormonal activity of the ovary is sometimes hindered by the absence of an adequate reactivity of the receptors. A further obstacle in the proper determination of the phase of the cycle may be seen in the influence of local proliferative processes, either benign (inflammatory changes) or malign, located in the area of the cervix of the uterus. Diagnostic errors, made by using indirect ovulation tests, especially cytohormonal tests, may be the result of the fact that progesterone is produced by mature Graafian follicles even before the ovulation period. It can be also produced owing to pathological luteinization of the granulosa cells and cells of the theca of the Graafian follicle, after its involution. Sometimes an erroneous diagnosis of an accomplished ovulation may be based on the finding of luteal activity resulting from the presence of progesterone or other substance of similar biological action, produced by the adrenals. Under pathological conditions, the ovary can produce certain quantities of androgens, which may resemble progesterone in their action.

In view of all the factors mentioned above, which indirectly or directly influence the sloughing cells of the vagina, some pathologists and cytologists express the opinion that cytology alone is unable to establish a binding diagnosis of disturbances in the menstrual cycle (Stol). Runge thinks that cytology cannot give up cooperation with pathological anatomy and biochemistry. It is, therefore, justified to test the regularity of the menstrual cycle by combining cytological examination with investigations of the hormone level in urine and with endometrial biopsy. For practical reasons, it is not always possible to carry out this complex of tests during the treatment of infertility. It results from the opinions quoted above and from the observations of the present writer that cytological examination alone does not suffice to determine the date of ovulation. Cytohormonal tests are useful as a part of complex investigations aimed at detecting the character of the disturbances in the menstrual cycle and at checking the results of the applied hormonal treatment.

Bioptic examination of the endometrium can disclose an accomplished ovulation. It is, therefore, used in cases when it is desirable to verify the results obtained by other methods or to establish the fact of accomplished ovulation. This method, however, can also give false results, namely when the endometrium fails to react to hormonal stimuli. Moreover, repeated performance of endometrial biopsy during the treatment of sterility may cause much inconvenience.

In their work on the influence of the sex hormones on the biology of the vagina, Schockaert and Ferrin found a close correlation between the amount of glycogen in the cells of the vaginal epithelium, and the level of oestrogenic hormones in the organism. The amount of glycogen varies according to the rise or fall in the oestrogen level. Quantitative changes in the glycogen content of the epithelium occur with a certain delay when compared with the quickly changing level of oestrogenic hormones. Progesterone, produced by the yellow body, inhibits the growth of the vaginal epithelium and the secretion of cervical mucus, and intensifies the production of glycogen in the epithelial cells. The synergetic influence of oestrogenic hormones and progesterone on the increase of the glycogen amount in the cells causes that the highest values of the glycogenic index are found during the secretion phase of the menstrual cycle.

According to Zondek, the bioptic test can be replaced by Mack's glycogenic test and by the Fern Test. Sturgis and Pommerencke (1959) made an enquiry among the members of the American Society for the Study of Sterility concerning the usefulness and reliability of the separate ovulation tests. The obtained answers pointed to the morning body temperature records as the most frequently used and the best method of determining the time of ovulation. 68% of the questioned persons used endometrial biopsy as a criterion of the regularity of the cycle, especially in those cases in which the results obtained by other methods were ambiguous. Almost all of those who used bioptic examination stressed its basic disadvantage, viz. determination of ovulation *ex post*. It resulted in the general conclusion that morning body temperature records (96%) and bioptic examination (68%) were the methods most frequently used to determine the time of ovulation. They were also considered as the best of all known tests (92—80%). Cytohormonal investigations were used only by 29% of the questioned persons, and sufficient adequacy of this test to the determination of the time of ovulation was stated by 31% only.

If the treatment of infertility in woman is to be carried on correctly, it is indispensable to determine the time of ovulation, as well as the character of hormonal disturbances in the anovulatory cycle.

On the strength of morning body temperature measurements, the present writer arrived at the conclusion that many women suffer from clinically undeterminable disturbances in the hormonal activity of the ovary and in the rhythm of ovulation. The moment of ovulation did not arrive regularly in every menstrual cycle; ovulation sometimes occurred a few days earlier or later than it could be expected. Very often this was also connected with the fact that the cycles, which apparently were very regular, varied in their length by one or two days. Topkins (40) found by means of endometrial biopsy that in women with regular menstruation, ovulation occurred always at the same time. This opinion is at variance with the results obtained by other authors (23), including the present writer. It is possible to find in one woman, besides normal diphasic menstrual cycles, those with secretory insufficiency, or even monophasic cycles. This may be especially noticeable in older women, between 35 and 40 years of age.

It results from the present studies that functional insufficiency of the yellow body is a comparatively frequent cause of infertility. Demonstration of progesterone deficiency by means of cytohormonal investigations was connected with difficulties and sometimes gave false positive results. It was much easier to evaluate the regularity of the second phase of the menstrual cycle by plotting the curves of the morning body temperature. It was also possible to establish in this way luteal insufficiency.

The characteristic course of the body temperature curves pointed either to a premature cessation of the activity of the yellow body, or to its insufficient development. In cases when monophasic cycle was diagnosed by means of morning temperature records, it was possible to find out the cause of this condition with the use of cytohormonal investigations or of the Fern Test and Mack's test, which then indicated a diphasic insufficiency of the ovary or absence of the action of the yellow body.

Grant (21) is of the opinion that insufficient decidual transformation of the endometrium may be caused by biochemical deficiences, above all by deficiency in glycogen, basic amino acids, basic phosphates and vitamin C. He studied menstrual cycles in 250 patients by recording morning body temperature and by biopsy carried out on the day preceding the expected menstruation. Cases in which there was a difference between the time of ovulation as determined by morning body temperature records and by biopsy were regarded as instances of

secretory insufficiency of the endometrium. In women with regular menses such difference occurred in 6% only.

Thus it was possible to determine by recording the morning body temperature not only the accurate time of ovulation, but also the regularity or disturbances in the second phase of the menstrual cycle. There occurred differences in the determination of the time of ovulation ranging from 1 to 4 days between the results of cytohormonal investigations and body temperature records. In the majority of cases the time of ovulation determined by plotting the body temperature curves was earlier. This can be explained by the fact that reactions to hormonal stimuli are manifested by generalized systemic changes earlier than by changes in the vaginal epithelium. The evaluation of cytological pictures was often difficult and was impaired by doubts resulting from lack of precision in preparing vaginal smears or from the coexisting inflammatory conditions of the vagina, especially those produced by *Trichomonas vaginalis*. Although cases of *Trichomonas* infection were eliminated from the present study, its presence was sometimes detected in cytological smears, which hindered their correct interpretation. *Trichomonas* infection not only produced desintegration and destruction of the cells of vaginal epithelium, but also influenced the values of the acidophilia and caryopycnosis indices. In some smears there were found abnormally high values of the acidophilia index, due to this infection. It was, therefore, useless to calculate the acidophilia index, as the superficial cells had an increased affinity to acid stains. Detailed examination of such preparations revealed that *Trichomonas* infection produces changes in the appearance of the cell nuclei, which become large and bladder-like.

The conclusion is that none of the indirect methods can reflect faithfully the changes which occur in the hormonal activity of the ovary during the menstrual cycle. Most of these methods (cytohormonal investigations, Fern Test) inform us on the oestrogenic activity of the ovary. Determination of the values of the acidophilia and caryopycnosis indices is the most sensitive test of the actual fluctuations of the oestrogen level. The Fern Test and Mack's test are of little accuracy in evaluating the ovarian activity, but they can be useful as a supplement to the method of morning body temperature records. Endometrial biopsy is of great diagnostic value for the evaluation of the secretory activity of the ovary and for the detection of specific and unspecific inflammatory conditions.

In the present study, the method of the morning body temperature curves proved to be the best and simplest test of the time of ovulation. The possibility of mistakes resulting from a faulty technique of taking temperature could be excluded by careful instruction given to the patient. The reaction of the body temperature to the cyclic processes occurring during the menstrual cycle is sufficiently rapid to assure results reflecting the current condition. The test can be continued for a long time, so that it is possible not only to discover irregularities of the cycle, but also to determine the doses and time and method of administration of hormone preparations. The interpretation of the body temperature curves is easy and makes it possible to diagnose the accomplished ovulation more often than cytohormonal tests.

To find out the often obscure causes of functional sterility, the morning body temperature test, Fern Test and Mack's test should be carried out in the first place. They can be performed in all cases, need no special laboratory equipment and are sufficiently reliable and accurate. The interpretation of their results is easy and can be undertaken by a general practitioner. The tests evaluate the current hormonal activity of the ovary and can be repeated many times to check the results of the treatment.

The recently introduced test known as Tes-Tape is of great value for the determination of the time of ovulation and for the detection of paracyclic ovulation. If practiced every day by properly instructed patients, it can determine the date of the approaching ovulation in each menstrual cycle. This, in turn, will help women who wish to avoid pregnancy to determine the period of sexual abstinence during each menstrual cycle.

CONCLUSIONS

1. In the present writer's material anovulatory cycles constituted 56.64% (162 cases), cycles with inadequate secretion 23.08% (66 cases), and cycles with regular proliferation and secretion phases 20.28% (58 cases).

2. The time of duration of the 1st and 2nd phase of the menstrual cycle is not constant and can be shorter or longer than 14 days in the 28 days' cycle.

3. Secretory insufficiency may be the result of a premature atrophy of the yellow body or of its insufficient action.

4. In the evaluation of the ovulation process the most accurate results were obtained by recording the morning body temperature.

5. The morning body temperature test makes it possible to determine the duration of the 1st phase, the date of ovulation, the regularity and duration of the 2nd phase of the menstrual cycle, and to discover additional ovulation.

6. Owing to the possibility of multiple repetition, the morning body temperature test is useful not only for the determination of the date of ovulation, but also for checking the results of the hormonal treatment.

7. The morning body temperature test, Fern Test and M a c k's iodine test can be easily performed under all conditions. The combination of these tests makes it possible to determine the date of ovulation and to define the character of hormonal disturbances in the anovulatory cycle; it also furnishes sufficient evidence for the evaluation of the regularity or disturbances of the cycle, without the necessity of resorting to endometrial biopsy.

8. Bioptic examination of the endometrium should be carried out in those cases in which the results of the previous test were discordant. Endometrial biopsy is also indispensable in cases in which inflammatory conditions of the endometrium can be suspected.

9. Cytohormonal investigations alone are not sufficient for an accurate determination of the ovulation date. They should be accompanied by determination of the hormone level in urine and by endometrial biopsy. The determination of the date of ovulation is often difficult and inaccurate. Various exogenous and endogenous factors distort the cytological picture and prejudice the reliability of the results. Investigations used to evaluate the regularity of the 2nd phase of the menstrual cycle present the greatest difficulties.

10. The Fern Test and M a c k's iodine test are insufficient as criteria of the regularity of the menstrual cycle, especially when hormonal disturbances are present. However, these tests can be useful when combined with the morning body temperature records into a complex of three tests.

11. With inflammatory processes existing in the vagina and in the canal of the cervix, the results of the separate morphological tests or of their combinations cannot serve as a basis for diagnostic conclusions on the hormonal activity of the ovary and on the date of ovulation. The existence of an inflammatory condition caused by *Trichomonas vaginalis* produces changes in the staining capability of the superficial cells of the vaginal epithelium, a false increase of the acidophilia index, and structural changes in the nuclei, which become bladder-like. In such cases the morning body temperature test makes it possible to obtain objective results and to determine the date of ovulation.

12. The date of ovulation determined by morning body temperature records is often earlier (by one or two days) than that obtained with the use of cytohormonal investigations.

13. In cycles with an inadequate secretion phase due to atresia of the Graafian follicle, the examination of the cervical mucus fairly often revealed crystallization patterns of a lycopodium-like appearance.

14. When there is no action of progesterone, tests of the crystalization of the cervical mucus performed during the second phase of the cycle reveal to a certain degree the persistence of fern-like pictures.

15. No crystallization of the cervical mucus is found during the secretion phase. When there is a secretory insufficiency or absence of luteinization, slight crystallization is observed and when relative hyperoestrogenism occurs during a monophasic cycle, a high degree of crystallization is often found.

16. Preparations obtained for testing the crystallization of the cervical mucus are not perishable and can be kept for a longer time (2 years).

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STRESZCZENIE

Przedstawiono wyniki badań kilku diagnostycznych testów określających termin jajeczkowania u kobiety. Materiał dotyczył 224 kobiet niepłodnych, u których wykonano w 299 cyklach miesiączkowych pomiary porannej ciepłoty ciała, badania krystalizacji śluzu szyjkowego oraz badania cytohormonalne. Poza tym w przypadkach wątpliwych dokonywano badania bioptycznego błony śluzowej jamy macicy a w 11 przypadkach dokonano także badań przy pomocy testu Tes-Tape. Najbardziej przydatnym testem dla oznaczania terminu jajeczkowania okazał się test porannej ciepłoty ciała i Tes-Tape. Na podstawie przeprowadzonych badań stwierdzono, że wykonanie testu porannej ciepłoty ciała, krystalizacji śluzu szyjkowego oraz próby jodowej Macka jest łatwe i możliwe w każdych warunkach. Wykonanie tych testów kilkakrotnie w czasie cyklu miesiączkowego pozwala na ustalenie terminu jajeczkowania oraz wykrycie charakteru zaburzeń hormonalnych w cyklu jednofazowym, bez konieczności wykonywania badania bioptycznego błony śluzowej jamy macicy. Ze względu na możliwość wielokrotnego wykonywania badania porannej ciepłoty ciała jest ono przydatne nie tylko dla oznaczania aktualnego terminu jajeczkowania, lecz także dla kontrolowania wyników stosowanego leczenia hormonalnego. Przy istnieniu procesów zapalnych w pochwie zarówno wyniki poszczególnych testów morfologicznych, jak i ich zestawienia nie pozwalają na wyciągnięcie wniosków diagnostycznych dla oceny czynności hormonalnej jajnika i oznaczenia terminu jajeczkowania. Pomiary porannej

ciepłoty ciała pozwalają w tych przypadkach na obiektywną ocenę i oznaczenie terminu jajeczkowania.

OBJAŚNIENIA RYCIN

Ryc. 1. Przyp. C. W. nr hist. chor. 101. Wykres prawidłowej, dwufazowej krzywej ciepłoty ciała. Jajeczkowanie 12 dnia cyklu.

Ryc. 2. Przyp. S. J. nr hist. chor. 1042. Wykres ciepłoty ciała w wypadku zajścia w ciążę. Jajeczkowanie 16 dnia cyklu.

Ryc. 3. Przyp. O. M. nr hist. chor. 56. Wykres krzywej ciepłoty ciała w cyklu dwufazowym, z przedłużoną I fazą miesiączkową. Jajeczkowanie i zapłodnienie w 21 dniu cyklu.

Ryc. 4. Przyp. G. B. nr hist. chor. 1225. Wykres krzywej ciepłoty ciała w cyklu dwufazowym ze skróconą I fazą miesiączkową. Jajeczkowanie 10 dnia cyklu.

Ryc. 5. Przyp. D. A. nr hist. chor. 108. Wykres krzywej ciepłoty ciała w cyklu przedłużonym (55-dniowym), dwufazowym z jajeczkowaniem i zapłodnieniem 37 dnia cyklu.

Ryc. 6. Przyp. W. I. nr hist. chor. 100. Wykres krzywej ciepłoty ciała w cyklu skróconym, dwufazowym z jajeczkowaniem w 14 dniu. Skrócenie II fazy cyklu.

Ryc. 7. Przyp. G. M. nr hist. chor. 582. Wykres krzywej ciepłoty ciała w trzech kolejnych cyklach miesiączkowych. Jajeczkowanie w I cyklu w 12 dniu, II — w 9 dniu, w III cyklu jajeczkowanie i zapłodnienie w 9 dniu.

Ryc. 8. Przyp. K. S. nr hist. chor. 2344. Wykres krzywej ciepłoty ciała w cyklu dwufazowym z jajeczkowaniem i zapłodnieniem w 10 dniu cyklu.

Ryc. 9. Przyp. K. S. nr hist. chor. 584. Wykres krzywej ciepłoty ciała w cyklu miesiączkowym z jajeczkowaniem paracyklicznym w 13 i 21 dniu cyklu.

Ryc. 10. Przyp. L. H. nr hist. chor. 142. Preparat z 11 dnia cyklu owulacyjnego. Pow. 150 x. Obraz cytologiczny jasny, brak leukocytów i bakterii. IA 65% IK 80%. Obraz fazy przedowulacyjnej.

Ryc. 11. Przyp. L. H. nr hist. chor. 142. Preparat z 13 dnia cyklu owulacyjnego. Pow. 150 x. Obraz jasny, brak leukocytów i bakterii. Powierzchniowe komórki kwasochłonne o dużej wielobocznej cytoplazmie zachodzą na siebie dachówkowato. Obraz fazy owulacyjnej. IA 80% IK 90%.

Ryc. 12. Przyp. D. K. nr hist. chor. 770. Preparat z 12 dnia cyklu owulacyjnego. Krystalizacja +. Pow. 150 x. W polu widzenia wiele luźno leżących kryształków, mało komórek nabłonkowych oraz pojedyncze gałązki paproci o strukturze drobnej i jasnej.

Ryc. 13. Przyp. W. Ł. nr hist. chor. 22. Preparat z 13 dnia cyklu miesiączkowego owulacyjnego. Krystalizacja ++. Pow. 150 x. W polu widzenia liczne struktury paprociowate.

Ryc. 14. Przyp. W. Ł. nr hist. chor. 22. Preparat z 15 dnia cyklu owulacyjnego. Krystalizacja ++. Pow. 150 x. W polu widzenia liczne struktury krystalizacyjne palmowate.

Ryc. 15. Przyp. K. J. nr hist. chor. 1696. Preparat z 14 dnia cyklu owulacyjnego. Krystalizacja +++. Pow. 150 x. Formy krystaliczne o grubych, ciemnych gałązkach. Poszczególne rozgałęzienia rozchodzą się pod kątem 90°.

Ryc. 16. Przyp. L. H. nr hist. chor. 142. Preparat z 15 dnia cyklu owulacyjnego. Pow. 150 x. Widoczne charakterystyczne zwijanie się brzegów kwasochłonnych komórek kariopyknotycznych. IA 45% IK 70%. Obraz fazy poowulacyjnej.

Ryc. 17. Przyp. L. H. nr hist. chor. 142. Preparat z 20 dnia cyklu owulacyjnego. Pow. 150 x. Komórki o brzegach pozawijanych, zlepiają się w grupy. Pojawiają się nieliczne komórki pośrednie, typu łódkowatego. IA 35% IK 45%. Obraz fazy lutealnej.

Ryc. 18. Wykres krzywej wskaźników A i K i krzywej krystalizacji śluzu w dwufazowym cyklu.

Ryc. 19. Wykres krzywej IK i IA w cyklu owulacyjnym. Przyp. S. J. nr hist. chor. 1042.

Ryc. 20. Przyp. O. M. nr hist. chor. 56. Wykres krzywej ciepłoty ciała w cyklu przedłużonym 34-dniowym, dwufazowym z jajeczkowaniem w 20 dniu cyklu. Wykres typu schodkowatego. 15 dnia cyklu atrezja pęcherzyka Graafa.

Ryc. 21. Przyp. O. C. nr hist. chor. 728. Wykres krzywej ciepłoty ciała w cyklu dwufazowym z jajeczkowaniem w 9 dniu. Typ krzywej skaczący świadczy o niedomodze fazy wydzielniczej. U pacjentki w związku z istniejącą niedomogą wydzielniczą wystąpiły objawy grożącego poronienia w 3 miesiącu ciąży.

Ryc. 22. Przyp. D. K. nr hist. chor. 770. Wykres krzywej ciepłoty ciała w cyklu dwufazowym z odbytym jajeczkowaniem w 15 dniu. Zbyt wczesne obniżenie się poziomu krzywej przed miesiączką świadczy o skróconej czynności hormonalnej ciałka żółtego.

Ryc. 23. Przyp. P. S. nr hist. chor. 1424. Badanie cytologiczne i krystalizacji śluzu wykazało cykl dwufazowy z niedomogą lutealną. Badanie histopatologiczne błony śluzowej macicy, pobranej w 26 dniu cyklu (cykl 29-dniowy) przedstawia obraz błony śluzowej w początkowym okresie przemian wydzielniczych.

Ryc. 24. Przyp. S. H. nr hist. chor. 190. Wykres cyklu jednofazowego.

Ryc. 25. Przyp. M. W. nr hist. chor. 930. Cykl jednofazowy. W 11 dniu zanik dojrzewającego pęcherzyka Graafa wraz z jego patologiczną luteinizacją.

Ryc. 26. Przyp. D. Z. nr hist. chor. 1658. Preparat z 22 dnia cyklu jednofazowego. Krystalizacja widłakowata. Pow. 150 x. Kryształy układają się w struktury kształtem swym przypominające pędy widłaka.

РЕЗЮМЕ

Приведены итоги исследования некоторых диагностических тестов, определяющих срок овуляции у женщин. Материал исследований составили 224 бесплодные женщины, у которых произведены в 299 менструальных циклах измерения утренней температуры тела, наблюдения над кристаллизацией цервикальной слизи и цитогормональные исследования влагалищных мазков. В сомнительных случаях применялась биопсия слизистой оболочки тела матки, а в 11 случаях также „Tes-Tape".

Наиболее достоверным и простым тестом для обозначения срока овуляции оказались измерение утренней температуры и тест „Tes-Tape". Установлено, что измерения утр. температуры, кристаллизация цервикальной слизи и йодная проба Жацка даются легко произвести во всяких условиях. Повторные неоднократные их прове-

дение в течение менструального цикла позволяет определить срок овуляции равно как и выявить характер гормональных нарушений закономерностей однофазного цикла, избегая при этом необходимости биопсии слизистой оболочки тела матки. Учитывая возможность многократных утренных измерений температуры тела следует отметить, что они применимы не только для установления данного срока овуляции, но и для контроля успешности применяемого гормонального лечения.

Наличие воспалительного процесса во влагалище и цервикальном канале матки является препятствием для того, чтобы на основании такого или иного морфологического теста либо их сопоставления можно было делать диагностические заключения, касающиеся гормональной функции яичника и срока овуляции. Установление этих фактов является однако в таких случаях вполне возможной при применнении теста утренных температур тела.

Рис. 1. Правильная двухфазная кривая утренней температуры тела. Овуляция на 12 дне цикла.

Рис. 2. Кривая утренней температуры тела при забеременении.

Рис. 3. Двухфазная кривая утренней температуры с удлиненной 1-ой месячной фазой. Овуляция и оплодотворение на 21 дне цикла.

Рис. 4. Двухфазная кривая утренней температуры с укороченной 1-ой месячной фазой. Овуляция на 10 дне цикла.

Рис. 5. Кривая утренней температуры в удлиненном (55 дневном) цикле. Овуляция и оплодотворение на 37 дне цикла.

Рис. 6. Кривая утренней температуры в укороченном 2 фазном цикле с овуляцией на 14 дне цикла. Укорочение второй фазы.

Рис. 7. Кривая утренней температуры в трех очередных месячных циклах. Овуляция на 12-том дне первого, на 9-ом дне второго и на 9-ом дне третьего цикла. В течение последнего наступило оплодотворение.

Рис. 8. Кривая утренней температуры двухфазного цикла с овуляцией и оплодотворением на 10-том дне.

Рис. 9. Кривая утренней температуры в месячном цикле с парациклической овуляцией на 13 и 21-ом дне.

Рис. 10. Влагалищный мазок на 11-ом дне овуляционного цикла. Увелич. 150 х. Четкая цитологическая картина, отсутствие лейкоцитов и бактерий; клетки отчетливо обрисованы, лежат раздельно. А. И. 65%, К. И. 80%. Предовуляционная фаза.

Рис. 11. Влагалищный мазок на 13 дне овуляционного цикла. Чистая цитологическая картина; отсутствие лейкоцитов и бактерий. Большие поверхностные ацидофильные клетки накладывающиеся черепицеобразно. Овуляционная фаза.

Рис. 12. Влагалищный мазок на 12-ом дне овуляционного цикла. Кристализация +. Увеличение 150 ×. В поле зрения много свободно расположенных кристаллов, малое количество эпителиальных клеток и единичные ветки папоротника с мелкой и четкой структурой.

Рис. 13. Влагалищный мазок на 13 дне овуляционного цикла. Кристаллизация ++. Увеличение 150 х. В поле зрения многочисленные папоротниковидные структуры.

Рис. 14. Влагалищный мазок на 15-ом дне овуляционного цикла. Кристаллизация ++. Увеличение 150 х. В поле зрения многочисленные пальмовидные структуры.

Рис. 15. Влагалищный мазок на 14-ом дне овуляционного цикла. Кристаллизация +++. Увеличение 150 ×. Кристаллические формы с толстыми темными ветками разветвляющимися под прямым углом.

Рис. 16. Влагалищный мазок на 15-ом дне овуляционного цикла. Увеличение 150 х. Видно характерное свертывание краев ацидофильных кариопикнотических клеток. А. И. 45%. К. И. 70%. Послеовуляционная фаза.

Рис. 17. Влагалищный мазок на 15-ом дне овуляционного цикла. Увеличение 150 х. Клетки со свернутыми краями слепляются в группы. Появляются немногочисленные промежуточные лодковидные клетки.

Рис. 18. График кривой показателя А. И. и К. И. в овуляционном цикле.

Рис. 19. График А. И. и К. И. в овуляционном цикле.

Рис. 20. График кривой температуры тела в удлиненном 34 дневном двуфазном цикле с овуляцией на 20 дне цикла. График ступенчатовидный. На 15 дне цикла атрезия пузырька Граафа.

Рис. 21. График кривой температуры тела в двухфазном цикле с овуляцией на 9-ом дне. Тип кривой указывает на недомогание секреционной фазы. У больной в связи с недомоганием выделения появились симптомы угрожающего аборта на 3 месяце беременности.

Рис. 22. График кривой температуры тела в двухфазном цикле с овуляцией имевшей место на 15-ом дне. Слишком скорое снижение уровня кривой перед менструацией указывает на укороченную гормональную деятельность желтого тельца.

Рис. 23. Исследования цитологическое и кристаллизации слизи показали на двуфазный цикл недостаточной лютеальной функцией. Гистопатологические исследования слизистой оболочки матки на 26-дне цикла (цикл 29-ти дневной) дают картину слизистой оболочки в начальном периоде изменений в выделении.

Рис. 24. Цикл однофазный.

Рис. 25. Цикл однофазный. На 11-ом дне атрезия созревающего пузырька Граафа и его патологическая лютеинизация.

Рис. 26. Влагалищный мазок на 22-ом дне однофазного цикла. Кристаллизация плауновидная. Увеличение 150 ×. Кристаллы создают структуры, располагающиеся как побеги плауна.

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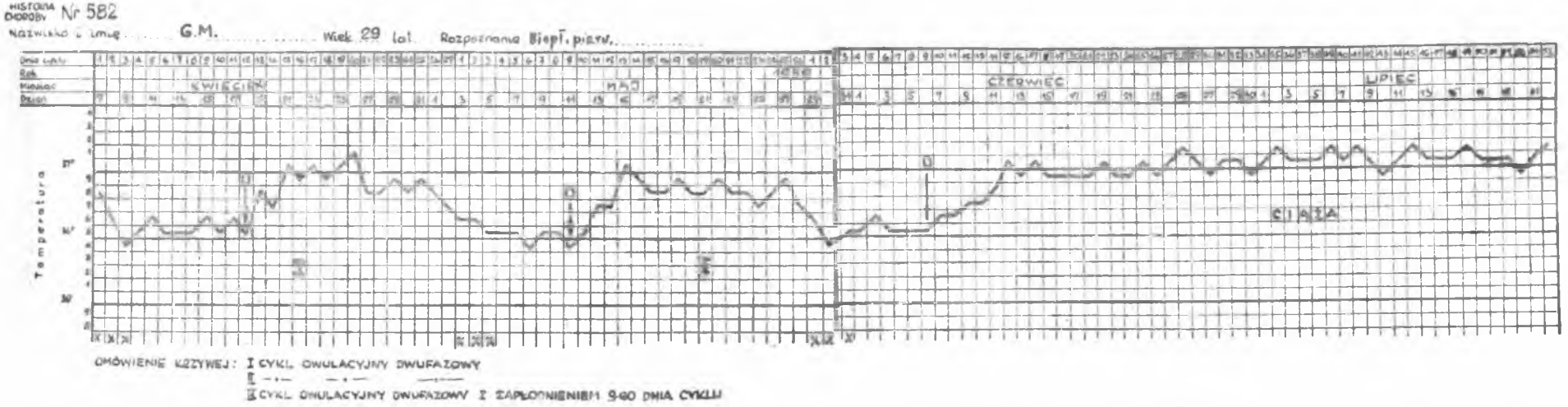

Fig. 7. Case G. M. No 582. Basal body temperature record in the three successive cycles. Ovulation in the first cycle on the 12th day, in the second cycle on the 9th day, and in the third cycle of ovulation and conception on the 9th day