DIAGNOSTIC VALUE OF CERVICAL MUCUS ARBORIZATION

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The various methods available at the present time for the determination of ovulation, oestrogen activity and pregnancy involve elaborate procedures. To determine ovulation, for example, one must either do an endometrial curettage, a series of vaginal smears, keep basal body temperature charts or do a combination of these or other procedures. The time involved to obtain the desired results in these tests is prolonged and is costly to the patients, involving trained technicians, and also the extreme co-operation of the patient. A simple test was, therefore, devised, which could provide a good reflection of many endocrine changes that take place in the reproductive organs as does the endometrium. Such a test was the study of the cervical mucus arborization.

Historical Review

The first scientific study of endocervical mucus was made in France in 1837 by Donne. From England originated the first report on the cyclic changes of cervical mucus by Smith in 1855. Later Seguy and Vimmeux (1955) reported the cyclic changes in the character of the cervical mucus, in the normally menstruating woman.

Papanicolaou (1952) demonstrated that when the cervical mucus secretion was spread on a slide and allowed to dry, a certain form of crystallization and arborization took place. Rydberg (1948) had shown this phenomenon to exist and referred to it as a "Fern Leaf" structure.

Campos da Paz (1951), whilst studying the crystallization of cervical mucus and its relationship to cervical receptivity of spermatozoa, states that the cervical mucus undergoes chemical and physical changes during the menstrual cycle.

Ullery et al (1959) state that serum, semen and blood inhibit arborization possibly by a mechanical reaction.

John and Sharda Devi (1960) studying the crystallization of cervical mucus at Patna, mention that the crystallization test can be used for the diagnosis of ovulation and determination of the time of ovulation. They studied 75 cases of sterility, both primary and secondary. They have mentioned that crystallization and arborization of the cervical mucus are an expression of oestrogen activity, the electrolyte substance in the mucus being sodium chloride which is present in a large amount only

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during the period of oestrogen stimulation.

Clift (1945) directs attention to two recently recognised rheologic properties of human cervical mucus — viz., flow elasticity and spinnbarkeit. Rheology is the study of flow and deformation of matter. Spinnbarkeit is the property possessed by the mucus whereby it can be drawn into threads. Clift states that the cervical mucus in pregnancy has a characteristic property of "Plasticity", which is that property which enables a material to be reformed continuously and permanently without rupture. A rheologic law has therefore been laid down as applied to cervical mucus that *"Plasticity predominates in pregnancy, and Elasticity in non-pregnant states especially at the time of Ovulation."*

Shettles (1959), Neumann and Lehfeldt (1955) and Zondek (1954) have all proved that the cervical mucus arborization is negative in pregnancy.

Material and Methods

The present work included 200 cases, which were divided into 5 groups:—

Group 'A' — included 50 women having normal menstrual cycles examined from the 7th to the 14th day of the menstrual cycle.

Group 'B' — included 43 cases examined on the 22nd to the 24th day of the menstrual cycle. These patients were suffering from primary or secondary sterility. The cervical mucus findings were checked with the histopathological study of the endometrium obtained by curettage.

Group 'C' — included 50 cases with a period of amenorrhoea but doubtful of pregnancy.

Group 'D' — This group included 50 cases with definite early pregnancy.

Group 'E' — included 7 post-menopausal women.

The cervical mucus was collected by the method of Zondek and Papanicolaou whereby the mucus was taken up by a glass pipette, 7" long and tapering at its edge with an attached rubber bulb, introduced through the external os into the cervical canal and the cervical secretion aspirated. The cervical secretion was spread on two slides, one on which the spinnbarkeit was measured and the other on which the arborization was noted. The smears were graded, according to the recommendations of Zondek and John and Sharda Devi, into P.L. positive and P.L. negative groups. The P.L. positive group was further divided into 3 Grades:—

(1) *P.L.* +

When the arborization was scarce, and seen only at a very few places on the slide with only the primary branches present.

(2) *P.L.* ++

Those cases in which the arborization was moderate and had both primary and secondary branches.

(3) *P.L.* +++

When the whole slide was densely covered with arborization with presence of primary, secondary and tertiary branches.

Observations

In the course of study of 200 cases

the following observations were made:—

The mucus underwent cyclic changes during the menstrual cycles. These changes applied to or affected all the 3 components viz., viscosity, spinnbarkeit and P.L. reaction of the mucus. The P.L. reaction was found to be one plus(+) in all cases on the 7th day and in 43 cases on the 8th day. The P.L. reaction went on increasing and was found to be maximum between the 12th to the 14th day of the menstrual cycle.

In Group 'B' —the cervical mucus, in 27 of the cases, was found to be negative on the 22nd and 24th days of the menstrual cycle. In the remaining 16 cases it was found to be positive.

Co-relation of the P.L. Reaction to Endometrial Curettage

Of the 23 cases showing the proliferative phase, four cases had a P.L. reaction negative on all the days of the menstrual cycle. Of the 5 cases having a biphasic endometrial pattern, in 2 cases the P.L. reaction was positive, while in the other three cases it was negative.

Of the 15 cases showing secretory activity in the endometrium, one case had the P.L. reaction positive even on the 24th day of the menstrual cycle.

Of the 43 cases studied, the endometrium on histopathological study was found to have tubercular endometritis in 3 cases.

The findings of the cervical mucus did not correlate with the endometrial curettage in 6 out of the 43 cases.

In Group 'C' — 6 cases gave a positive P.L. reaction, every time the patient was examined; 10 cases gave a variable P.L. reaction. Of these 6 cases the reaction changed to negative on subsequent examination. In 9 cases the P.L. reaction was positive, though on subsequent examination the uterus was found to enlarge and grow in size. Hence in 18% of cases the test gave a false positive result.

Of the 11 cases curetted in this group the cervical mucus findings were in agreement with those of the endometrial curettage.

Group 'D' — Out of the 50 cases, the P.L. reaction was found to be positive, at least once during the course of examination, in 7 cases. Of these 7 cases, 2 had signs of threatened abortion and ultimately were admitted and evacuated for inevitable abortion.

Two cases of habitual abortions were also studied in this group. In both cases the cause of abortion was found to be due to an incompetent internal os.

In Group 'E' — The patients were examined only once and in all these the cervical mucus was scanty, viscid, had a very small spinnbarkeit, and showed a negative P.L. reaction.

Discussion

The theme of the present work was to test the accuracy of the cervical mucus arborization as a test in the determination of ovulation and the diagnosis of pregnancy at a very early stage.

The cervical mucus was found to undergo cyclic changes during the menstrual cycle. These findings are in agreement with those of Smith, Seguy and Vimmeux.

The viscosity was also found to undergo cyclic changes at various periods of the menstrual cycle. It was found that the cervical mucus which was viscous from the seventh to the eighth day of the menstrual cycle slowly became more fluid, glairy and transparent, till the fourteenth day, and then again slowly became viscous by the twenty-second to the twenty-fourth day of the menstrual cycle.

The spinnbarkeit was found to be maximum at 12th-14th day of the menstrual cycle and so may be used as test in cases of sterility requiring artificial insemination.

Mechanism of Arborization. Arborization as such is a non-specific phenomenon. It is produced in the cervical mucus by the action of oestrogen upon the cervical glands which are stimulated to concentrate electrolytes. These electrolytes combined with the mucin to produce arborization. This arborization resembled palm leaves of the South American palms called the Samambaia and Selaginela trees, as has been mentioned by Campos Da Paz; hence the test is called the "P.L. Reaction".

It was found by Landerstrom-Lang that this arborization was due to the presence of crystallization of sodium chloride which was found to crystallize out on drying. Rydberg mentioned that arborization can be also obtained by mixing together egg albumin and sodium chloride (normal saline). The same phenomena was also confirmed in our Departmental Laboratory. However, not being specific elsewhere, this phenomenon is of importance in the cervical mucus as the mucus is under the influence of oestrogens.

When ovulation occurs, the corpus luteum secretes progesterone which nullifies the effect of oestrogen and hence the arborization becomes negative. This test is, therefore, of use in cases of sterility to know whether the patient is ovulating or not. After menopause, as the ovaries undergo atrophy, oestrogen is not produced and so the cervical mucus shows a negative result.

The most important diagnostic value of this test, however, in the present series was in the diagnosis of early pregnancy. It was found to be correct to the extent of 80% positive in the diagnosis of pregnancy. In continuation of this discussion, it may be mentioned that the exclusion of pregnancy is just as important to the clinician as diagnosis of pregnancy. One may state that if in an amenorrhoeic patient we find a soft mass palpable beside a soft and large uterus one can think of either a functioning cyst or an extra-uterine gestation. In one case in our series, a functioning follicular cyst of the ovary was found, and the P.L. reaction was positive although the patient came with a period of amenorrhoea for 5 years.

From the observations of 50 cases one can state that as a rule the cervical mucus during pregnancy does not show arborization, but shows an abundance of vaginal cells and leucocytes mixed with threads of mucus. These findings are in argeement with those of Zondek and Campos da Paz.

As regards the relation of a positive P.L. reaction in pregnancy and an im-

pending abortion, one may state that although progesterone may help in averting an abortion it will have its beneficial effects only if there is a deficiency of this hormone and so it must be used only in selected cases.

Summary and Conclusions

Two hundred cases in five groups have been studied to find out the accuracy and usefulness of the cervical mucus arborization.

The cervical mucus is found to undergo cyclic changes, as does the endometrium. It is dependent upon the levels of oestrogen and progesterone. Oestrogens produce arborization whilst progesterone inhibits it.

Its first usefulness is, therefore, in studying the oestrogenic activity in an individual. In all cases of Group A, typical cyclic changes were found to occur in all the patients. Cervical mucus showed maximum spinnbarkeit and P.L. reaction on the 12th to the 14th day of the menstrual cycle in these cases, which coincides with the timing of ovulation. In 5 cases the P.L. reaction remained positive even in the premenstrual phase suggesting that these patients had an anovulatory cycle.

All cases of Group E being postmenopausal showed negative P.L. reaction.

The second use of this test is in the timing of ovulation. In 43 sterile patients it was found that the accuracy of this test was 89.4 per cent which is quite a high figure.

As far as the greatest use of this test is concerned, we may say that it is very useful in the diagnosis of early pregnancy. The accuracy of this test in the present series was found to the extent of 80 per cent.

Cervical mucus, studied in pregnancy, appeared to be thick and viscous, having the property of "Tack", and showed a negative reaction in 86 per cent of cases. Of the remaining 14 per cent showing a positive reaction, in 4 per cent of cases abortion occurred. In the rest the P.L. reaction was found to revert back to a negative reaction spontaneously. In one case of threatened abortion with history of previous abortions, the bleeding was controlled by an injection of depot progesterone. Hence this drug was useful in averting an impending abortion, which was correctly diagnosed by the P.L. reaction.

Hence finally, we may say that the cervical mucus arborization, being a fairly accurate, cheap and quick method for diagnosis of oestrogen activity, timing of ovulation and diagnosis of early pregnancy and in predicting an impending abortion, could be used routinely for these purposes.

References

1. Campos da Paz, A. C.: Transactions of the International and Fourth American Congress on Obst. & Gynec. Supplementary Vol. to Am. Jour. of Obst. & Gynec. Ed. Kosmak G. W. St. Louis, C. V. Mosby Co., **61A:** 790, 1951.
2. Clift, A. E.: Quoted by Greenhill J. P. Year Book of Obst. & Gynaec. Chicago, 1946, The Year Book Publishers. p. 359.
3. Donné A.: Quoted by Leumann G. & Lehfeldt. H. Am. J. Obst. & Gynec. **70:** 650, 1955.

4. John, Mary P. and Sharda Devi.: J. I.M.A. **35:** 206, 1960.
5. Landerstrom, L.: Quoted by Roland M.D. Am. J. Obst. & Gynec. **63:** 81, 1952.
6. Neumann, G. and Lehfeldt, H.: Am. J. Obst. & Gynec. **70:** 650, 1955.
7. Papanicolaou, G. N., Traut, H. F. and Marchetti, A.: Quoted by Roland M.D., Am. J. Obst. & Gynec. **63:** 81, 1952.
8. Pye, A.: Quoted by Neumann G., & Lehfeldt H. Am. J. Obst. & Gynec. **70:** 650, 1955.
9. Rydberg, E.: Quoted by Roland M. Am. J. Obst. & Gynec. **63:** 81, 1952:
10. Seguy, J. and Vimmeux, J.: Quoted by Neumann, G. & Lehfeldt, H. Am. J. Obst. & Gynec. **70:** 650, 1955.
11. Shettles, L.: Obst. & Gynec. **14:** 635, 1959.
12. Smith, W.: Quoted by Roland M. Am. J. Obst. & Gynec. **63:** 81, 1952.
13. Ullery, J., Aboushabanah, E. H., and Livingston, N.: Obst. & Gynec. Survey. **14:** 1, 1959.
14. Zondek, B. and Rozin, S.: Obst. & Gynec. **3:** 463, 1954.

Fig. 1

Shows a one plus (+) P.L. reaction on the seventh day of the menstrual cycle in a case of Group A.

Fig. 2

Shows a two plus (++) P.L. reaction on the tenth day of the menstrual cycle in the same case.

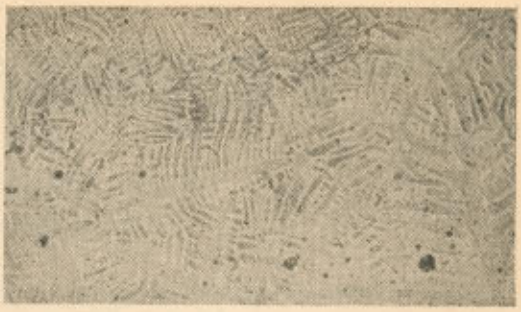

Fig. 3

Shows a three plus P.L. reaction on the twelfth day of the menstrual cycle in the same case.

Fig. 4

Shows a negative P.L. reaction on the twenty-sixth day of the menstrual cycle in the same case.

Note that there is an absence of arborization pattern. The figure shows only leucocytes and mucus.