

## SODIUM CHLORIDE CONTENT OF CERVICAL MUCUS AND ITS USE IN DETECTION OF OVULATION

by

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The production of cervical mucus as well as certain changes in its physical and chemical properties depends upon the hormone situation. These properties of cervical mucus have been utilized to estimate the hormone status ovulation in women. Pommerenke (1962), Harvey (1960) and Gross (1961) have demonstrated special significance of spinnbarkeit and crystallisation which show marked changes during ovulation.

Herzberg *et al*, (1964) have investigated cyclical variation of sodium chloride of mucus which showed drastic variation in the sodium chloride concentration of dried mucus at different phases of menstrual cycle. They demonstrated that sodium chloride content of fresh mucus of human cervix does not show any cyclical variation and is approximately isotonic ( $\text{NaCl} = 0.93 \pm 0.12$  per cent). On the other hand, percentage of sodium chloride in dry mucus has a cyclical variation exhibiting a very sharp maximum during ovulation time. The salt content of dry mucus at maximum is 40 to 70 per cent as compared with 2 to 20 per cent

during other phases of the cycle. The maximum rise in sodium chloride is not observed in women having anovulatory cycles. The sodium chloride content of cervical mucus in patients having anovulatory cycles never increase over 15 to 20 per cent during midcycle. They suggested that the estimation of sodium chloride in dry cervical mucus is convenient, easy and reliable method for knowing the ovulatory and anovulatory cycles and for exact dating of ovulation. According to them the remarkable increase in sodium chloride content of dry cervical mucus is due to maximal activity of luteinizing hormone.

The present study is an attempt to analyse the place of estimation of sodium chloride content of dry cervical mucus as a test for ovulation.

### *Material and Methods*

Two hundred cases between the age of 20 to 38 years were investigated in this analysis. All these patients came for treatment of primary or secondary infertility. Each patient had usual clinical examinations, and routine investigations like insufflation test and hysterosalpingography. For this special investigation, material was obtained from endocervical canal by aspiration with a sterile pipette. Fern phenomenon of cervical mucus and

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spinnbarkeit was noted. The weight of the specimen with sterile tube was taken overnight for 24 hours at 80°C. On the next day, after cooling the test tube to room temperature in calcium chloride filled desiccator the samples were weighed and dried again for about 2 hours to a constant weight. The dried sample was then diluted with distilled water 50 times its weight, shaken for 5 minutes in a mechanical shake to elute all salts and centrifused for 5 minutes. 0.2 ml. samples were collected in the 0.2 ml. pipette from the supernatant fluid of the above centrifused sample.

**Titration:** Three of the 0.2 ml. samples along with 1.8 ml. of water were titrated with N/200 mercuric nitrate solution using alcoholic solution of diphenyl carbazone as an indicator. The titration was carried out in a white porcelain basin by the method of Shales and Shales as modified by Conway and Foeger.

Four drops (0.06 ml.) of indicator (diphenyl carbazone) was added to the solution to give pale yellow colour. Then N/200 mercuric nitrate solution was added drop by drop from the burette. As more mercuric nitrate solution was added, the solution became colourless. A sharp change of colour to pale violet indicated the end-point of titration. The amount of mercuric nitrate solution required for the titration, was noted down. Again the titration was carried out on 2 ml. of standard chloride solution. The result of the chloride titration was calculated as percentage of sodium chloride.

The calculation is as follows:

The result was converted from mEq.

$$\frac{\text{Milli-equivalent sodium chloride per litre} = \text{ml. of HgNo}_3 \text{ solution required for unknown}}{\text{ml. of HgNo}_3 \text{ solution required for standard}} \times 100$$

sodium chloride per litre to milligram per cent of sodium chloride content of the cervical mucus, which was further calculated from the weight of dry cervical mucus and was expressed as the percentage of sodium chloride in cervical mucus. Sodium chloride cervical mucus arborisation and spinnbarkeit estimation were done serially in the same patient on 8th, 10th, 12th, 14th, 16th and 24th day of menstruation. Endometrial biopsy was taken between 23rd to 26th day of menstruation.

Graph I gives the typical curve of sodium chloride content in dry cervical mucus on various days of menstrual cycle in both ovulatory and anovulatory cycles.

#### Discussion

Herzberg and Joel (1964) have described that increase of sodium chloride content of dry cervical mucus was due to increase in cervical water excretion during normal midcycle which was clearly due to hormonal regulation of female cycle. Li *et al* (1960) suggest that ovulation was governed by sharp increase in the excretion of luteinizing hormone and that luteinizing hormone activity may be responsible for the increase in the level of sodium chloride content in dry cervical mucus.

In the present work the value of estimating sodium chloride in dry cervical mucus as an aid to the diagnosis of ovulation has been analysed and the results thus obtained have been compared with other cervical mucus studies, like spinnbarkeit and crystallisation tests and finally these results have been evaluated

TABLE I  
Showing Correlation Between Crystallisation Test and Endometrial Biopsy in Cases Showing Ovulatory and Anovulatory Cycles

No. of cases	Ovulatory cycle detected by crystallisation test		Ovulatory cycle detected by endometrial biopsy		No. of cases	Anovulatory cycle detected by crystallisation test		Anovulatory cycle detected by endometrial biopsy	
	No. of cases	Percentage	No. of cases	Percentage		No. of cases	Percentage	No. of cases	Percentage
184	180	97.82	184	100	16	12	75	16	100

In 184 cases who had ovulatory cycles as indicated by endometrial biopsy showed ovulatory cycle in 180 cases only by crystallisation test, in 4 cases discrepancies were observed. In 16 cases where anovulatory cycle was present by endometrial biopsy only 12 out of them showed anovulatory cycle by crystallisation test. Therefore, in 25 per cent of the cases where crystallisation test was suggestive of ovulation, were actually anovulatory.

TABLE II  
Showing Correlation Between Spinnbarkeit Test and Endometrial Biopsy in Cases Showing Ovulatory and Anovulatory Cycles

No. of cases	Ovulatory cycle detected by spinnbarkeit test		Ovulatory cycle detected by endometrial biopsy		No. of cases	Anovulatory cycle detected by spinnbarkeit test		Anovulatory cycle detected by endometrial biopsy	
	No. of cases	Percentage	No. of cases	Percentage		No. of cases	Percentage	No. of cases	Percentage
184	180	97.82	184	100	16	8	50	16	100

Comparative result of the two tests showed correlation in 97.82 per cent cases and discrepancy in 2.18 per cent, giving a false negative of ovulation in 2.18 per cent of the cases. They appeared to have anovulatory cycle by spinnbarkeit test but actually had ovulatory cycle as shown by endometrial biopsy.

Correlation of result between spinnbarkeit test and endometrial biopsy showed degree of discrepancy (in 50 per cent cases) in anovulatory cycle. Sixteen cases who showed ovulatory cycle by spinnbarkeit test had anovulatory cycle as indicated by endometrial biopsy.

TABLE III  
Showing Correlation Between Sodium Chloride Test and Endometrial Biopsy in Cases Showing Ovulatory and Anovulatory Cycles

No. of cases	Ovulatory cycle detected by sodium chloride estimation test		Ovulatory cycle detected by endometrial biopsy		No. of cases	Anovulatory cycle detected by sodium chloride estimation test		Anovulatory cycle detected by endometrial biopsy	
	No. of cases	Percentage	No. of cases	Percentage		No. of cases	Percentage	No. of cases	Percentage
184	182	98.91	184	100	16	16	100	16	100

Correlation of result revealed discrepancy in only 1 per cent of cases in which sodium chloride estimation test shewed anovulatory cycle but endometrial biopsy suggested ovulatory cycle.

TABLE IV  
Showing Comparative Value of Three Tests, Spinnbarkeit, Crystallisation, and Sodium Chloride Estimation Tests With Endometrial Biopsy in Sterility Cases Showing Ovulatory Cycle

No. of cases	Percentage of ovulatory cycle detected by spinnbarkeit test		Percentage of ovulatory cycle detected by crystallisation test		Percentage of ovulatory cycle detected by sodium chloride estimation test		Percentage of ovulatory cycle detected by endometrial biopsy	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
184	182	97.82	184	97.82	16	98.91	16	100

Among the above three tests the correlation rate of sodium chloride estimation test with endometrial biopsy was highest and it was 98.91 per cent.

TABLE V  
Showing Comparative Value of the Three Tests, Spinnbarkeit, Crystallisation, and Sodium Chloride Estimation Test With Endometrial Biopsy in Sterility Cases Showing Anovulatory Cycle

No. of cases	Percentage of anovulatory cycle detected by spinnbarkeit test		Percentage of anovulatory cycle detected by crystallisation test		Percentage of anovulatory cycle detected by sodium chloride estimation test		Percentage of anovulatory cycle detected by endometrial biopsy	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
16	8	50	12	75	16	100	16	100

Correlation rate of spinnbarkeit test and endometrial biopsy was very poor and was only 50 per cent. Correlation rate of crystallisation test and endometrial biopsy was 75 per cent, whereas the result of sodium chloride estimation test was exactly tallying with the findings of endometrial biopsy (100 per cent correlation). So it is the most accurate method amongst these three tests.

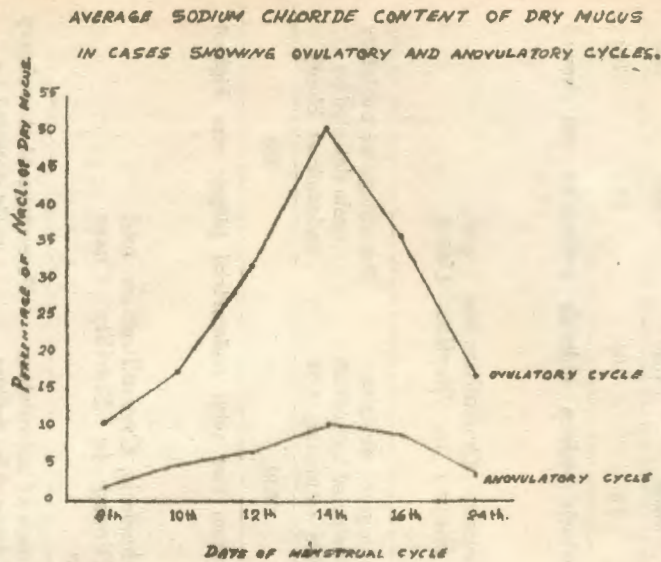


Fig. 3

against the result of endometrial biopsy to assess the accuracy of each test.

The level of sodium chloride content of fresh mucus of the cervix uteri does not show any cyclical variation and is approximately isotonic ( $\text{NaCl} = 0.93 \pm 0.12$  per cent). On the other hand, level of sodium chloride in dry cervical mucus has a cyclical variation exhibiting very sharp maximum during ovulation time. Overall evaluation of the result of spinnbarkeit test, crystallisation test and test of sodium chloride content of dry cervical mucus against the endometrial biopsy indicated that the sodium chloride estimation of dry cervical mucus had given maximum accuracy and correct information regarding the diagnosis of ovulation.

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