ROLE OF CERVICAL MUCUS IN CONCEPTION CONTROL WITH INJECTABLE STEROID CONTRACEPTIVE

by

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Since the advent of steroid contraceptives and its widespread use, various investigations have been done to assess the changes in different biophysical and biochemical properties of cervical mucus under the effect of these drugs.

The introduction of pure synthetic progestogens administered in injectable form is a promising development for the fertility control. An understanding of the biological changes it produces in the structure of genital tract involved in the biology of reproduction is essential to evaluate its mechanism of action and side-effects.

The purpose of this study is to assess the effect of injectable synthetic progesterone on the cervical mucus and its role as a contributing factor in the conception control.

Materials and Method

Twenty healthy ovulating women between the age 20 and 35 years, who were attending the Family Planning Clinic of Patna Medical College Hospital, were selected for this study. Prior to the study, a thorough medical history was recorded and general, physical and gynaecological examinations were performed on each subject. Every subject served as her own control, the first cycle in every case was an untreated cycle. Injectable contraceptive (Nor-ethisterone enanthate 200 mg) was given on the 5th day of the next cycle. Samples of the cervical mucus were collected on 8th, 10th, 12th, 14th, 16th, 18th, 20th, 22nd and 24th day of menstruation.

The cervix was exposed and cervical mucus was collected by a sterilized tuberculin syringe. The nozzle of the syringe was introduced through the external os, and the mucus aspirated. Samples contaminated with blood were discarded. The mucus was transferred to pre-weighed sterile test tube. For protein estimation only 0.2 ml. of mucus was transferred to test tube. Its appearance and consistency were noted. A sterilized swab stick was then introduced into the cervical canal and mucus withdrawn by rotatory movement of the stick. A portion of the mucus so collected was put on a glass slide and covered with a cover slip. Spinnbarkeit was observed lifting the coverslip from the glass slide. The remaining portion of the mucus in stick was gently rolled over another clean glass slide and left to dry in the air. After half an hour it was examined under the microscope, first under low magnification and then under high magnification to look for the crystal-

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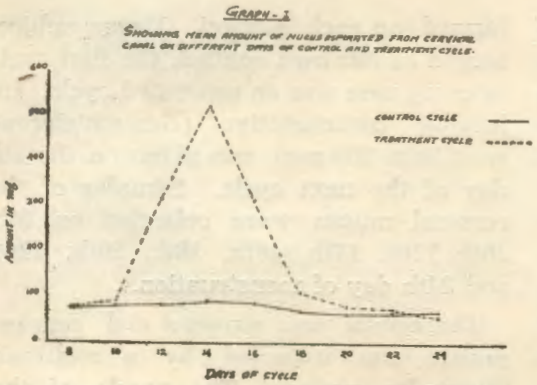
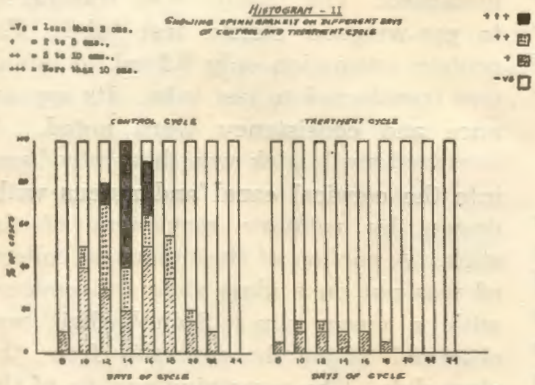
lization and cellularity. Protein, sodium, potassium and chloride of the cervical mucus were estimated on various phases of menstrual cycles.

Graph I gives the amount of cervical mucus in both the control and treatment cycles. There is considerable reduction in the quantity of cervical mucus with treatment by injectable steroid contraceptive.



Graph II gives the Spinnbarkeit of the cervical mucus in the control and treatment cycles.



Graph III gives the dry residue of cervical mucus in the control and treatment cycles.

It is clear from the above table that sodium level was markedly increased during the mid-phase (12th, 14th and

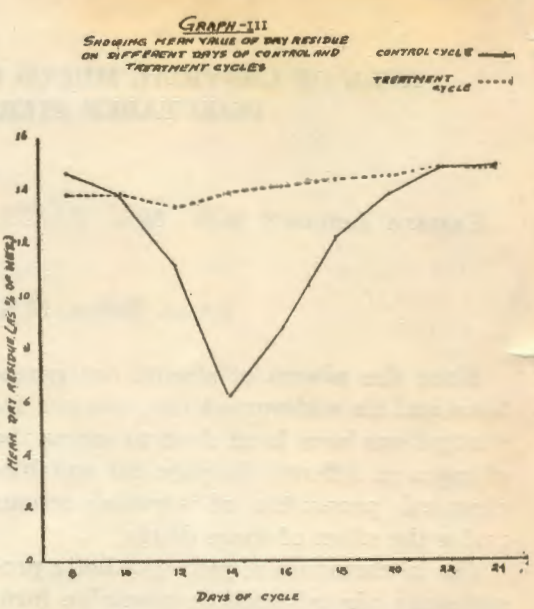


TABLE I

Mean Value of Sodium (micro moles/G of dry mucus) on Different Days of Control and Treatment Cycles

Days of cycle	Control cycle	Treatment cycle
8	347.7 ± 70.7	341.0 ± 69.5
10	434.7 ± 166.3	375.0 ± 61.5
12	957.0 ± 793.7	390.5 ± 62.4
14	1873 ± 1137	401 ± 53.3
16	1372.7 ± 723.9	389 ± 53.3
18	618.7 ± 254.7	386.2 ± 58.1
20	473.2 ± 162.9	393 ± 52.2
22	398 ± 92.24	377 ± 56.7
24	371.2 ±	381 ± 55.9

± = S.D.

16th day) of control cycles, but in treatment cycles this increase was not present. There was a large range of variation in the sodium level from case to case specially in mid-phase samples.

The above Table shows that potassium level in the control cycle was at its minimum during mid-phase (12th to 16th day), whereas it was slightly increased

TABLE II

Mean Value of Potassium (micro moles/G or dry mucus) on Different Days of Control and Treatment Cycles

Days of cycle	Control cycle	Treatment cycle
8	278.0 ± 88.8	236.2 ± 84.6
10	231.5 ± 83.0	222.2 ± 82.5
12	178.5 ± 71.2	216.8 ± 85.0
14	114.9 ± 44.8	264.3 ± 92.2
16	124.2 ± 44.1	240.1 ± 95.7
18	162.4 ± 44.8	193.5 ± 67.4
20	200.7 ± 65.4	175.5 ± 68.8
22	237.6 ± 90.5	193.7 ± 77.7
24	267.8 ± 104.6	206.7 ± 77.5

± = S.D.

in the treatment cycles. There was a large day to day and case to case fluctuation in the potassium level during both control and treatment cycles.

TABLE III

Mean Value of Chloride (% NaCl in dry mucus) on Different Days of Control and Treatment Cycles

Days of cycle	Control cycle	Treatment cycle
8	9.96 ± 3.2	11.39 ± 4.8
10	16.61 ± 6.0	15.96 ± 6.1
12	32.86 ± 9.8	19.7 ± 2.9
14	49.34 ± 14.3	20.8 ± 4.4
16	38.21 ± 16.4	20.7 ± 4.0
18	27.75 ± 12.2	20.83 ± 3.0
20	21.91 ± 5.2	14.29 ± 2.8
22	12.93 ± 4.6	18.3 ± 2.5
24	10.6 ± 5.3	16.56 ± 2.9

± = S.D.

On 12th, 14th and 16th day, the mean value of NaCl percentage was maximum (> 32 per cent). Mean value was less than 21 per cent on all days during proliferative and secretory phases. During the treatment cycle the mean value of NaCl was below 21 per cent on all adays.

The above Table shows that there was marked reduction in protein content on 14th, 16th and 18th day of menstruation in control cycles, but in treatment cycles this reduction was not so marked.

Discussion

Maximum secretion of cervical mucus (130 to 800 mg) occurs during ovulatory phase of normal menstruating women and it is scanty in pre-and post-menstrual phases (50 to 90 mg). After giving injectable steroid contraceptives, mucus remains scanty throughout the cycle.

The suppression of mucorrhoea under the effect of steroid contraceptive could be either due to direct effect of the drug on endocervical glands or indirectly by suppressing ovulation. The scanty, thick mucus produced under the effect of these drugs is hostile to spermatozoa and thus produces antifertility effect. Cohen *et al* (1952) are of opinion that mucus inhibiting effect of Nor-ethisterone is due to direct action on endocervical glands.

The mucus is most transluscent with maximum of spinnbarkeit during ovulatory phase of normal cycles. It remains opaque with absent or minimal spinnbar-

TABLE IV

Total Protein Content (microgram/mg. of wet mucus) During Different Phases of Control and Treatment Cycle

Days of cycle	Control cycle	Treatment cycle
8th, 10th and 12th	40.75 ± 11.58	41.61 ± 10.52
14th, 16th and 18th	3.91 ± 1.72	16.34 ± 5.2
20th, 22nd and 24th	55.17 ± 12.34	47.75 ± 7.53

± = S.D.

keit throughout the treatment cycle. Similar were the findings of Cohen (1968), Moghissi *et al* (1973). Sahani (1973) and Zanartu *et al* (1974). The suppression of spinnbarkeit is due to early progestational effect of these drugs.

Water content of mucus is at its maximum (90 to 97.7%) during ovulatory phase of control cycle while the dry residue is at its minimum (2.3 to 10%). This mid-cycle surge in water concentration is absent in treatment cycles. Water is quantitatively the most important component of cervical mucus. The cyclic variation of water in cervical mucus has been subject of great clinical interest. Some of the physical properties of cervical mucus as optical clarity, viscosity, surface tension and spinnbarkeit are directly related to its water content. Blair *et al* (1941) found that the dry matter in bovine cervical mucus was minimal and the water content maximal at the time of ovulation. Pommerenke (1946) observed a similar pattern in human cervical mucus, which also had its highest water content at mid-cycle. The findings of the present study are confirmatory to those of Pommerenke. In the present series of control cycles, the maximum water content i.e. minimum of dry residue of cervical mucus was found during mid-phase in almost all the cases. It ranged from 80 to 97.7% and all the other constituents i.e. dry residue varying from 2.3 to 20 per cent of the weight of fresh mucus. The dry residuce was minimum in the samples collected during mid-cycle of all cases. The increased water content at ovulation facilitates the penetration of cervical mucus by spermatozoa. Bergman (1950 and 1953) has demonstrated that during the water phase of cycle, a decrease of 2 to 3 per cent in the water content changes conditions for sperm survival from optimal to poor, rendering the mucus impenetrable to spermatozoa. Igarashi (1954) observed similar changes in the cyclic water content of the mucus. Kopito *et al* (1973) also observed the dry residue to be minimum at the time of ovulation. Marcus and Marcus (1963) have emphasized that the conditions for sperm migration are maximal when dry residue is less than 5 per cent and if dry residue is more than 7 per cent conditions are poor. Kopito *et al* (1973) are of opinion that the careful determination of water in the mucus may in itself serve as an indicator of ovulation and the physio-chemical measurements which are influenced by the amount of water present in the mucus as spinnbarkeit etc. already served the purpose.

The mid-cycle surge in water concentration of mucus collected during control cycles was absent in cycles treated with injectable steroid contraceptives. The water concentration remained almost equal throughout the cycle in almost all the cases. The dehydrating effect of steroid contraceptives is significant as it causes the loss of small but vital amount of water at the time of ovulation when the water content in mucus is most important. Cohen and Pelaez (1965) using Nor-ethisterone acetate (1 mg, or 2.5 mg. + Ethinyl Oestradiol 0.05 mg) found similar effects. Again in 1968 Cohen confirmed the dehydrating effect of Norethindrone plus Mestranol (Norinyl — 1 mg) upon cervical mucus. Moghissi *et al* (1973) observed similar changes in cervical mucus using microdose Norethindrone. Subjects on sequential therapy do not show the suppression of cervical factor during the period when only oestrogens are given and therefore, are liable to conceive, if ovulation is not

inhibited by oestrogen alone (Cohen and Pelaez, 1965, and Cohen, 1968).

Peak concentration of sodium in dry residue is present during mid-phase of control cycle. This peak is obliterated in treatment cycles. Potassium content of cervical mucus is at its mimimum during ovulatory phase of control cycles, but it is increased in treatment cycles. Chloride level shows maximum level (45-70 per cent) during mid-phase of control cycles. This sharp maximum is not noticeable in treatment cycles. Total protein in fresh mucus is markedly reduced during mid-phase of control cycles (mean average 3.9 micrograms/mg). During treatment cycles the decrease though occurs, but is not so marked.

The concentration of potassium showed cyclic variations in dry residue in most of the control cycles, though it was not that marked as sodium and varied within narrower range. The minimum of every patient was from 45 to 205 micro moles/G of dry mucus and maximum from 165 to 460 micro moles/G of dry mucus. The minimum value was found in mid-cycle samples and coincided with the maximum level of sodium content. There was evidence of reciprocal relationship between sodium and potassium concentration at mid-cycle. Singh and Boss (1973) and Kopito *et al* (1973) studying the control cycles found similar levels of potassium, and reciprocal relationship between sodium and potassium.

During treatment with injectable steroid contraceptives, the potassium level showed slight increase instead of a decrease during mid-cycle. The increased potassium concentration can be explained by increased cellularity of mucus during treatment cycles. In proliferative and secretory phases, the level during control and treatment cycles was almost the same. Present findings resemble those of Kosasky *et al* (1973), and Singh and Boss (1973).

The study of chloride in control cycles of present series showed definite mid-cycle increase, the levels were parallel to those of sodium. Maximum percentage of chloride was found on 14th and 16th day samples, mean average being 49.3 and 38.2% respectively.

Herzberg *et al* (1964) determined the sodium chloride level daily in dry mucus and found it to be 2 to 20 per cent during the non-ovulatory period, which rose to 40 to 70 per cent in the 2 to 3 days during which ovulation took place. Achari *et al* (1974) in a study of 200 cases reported similar mid-cycle peak of chloride concentration in normal ovulatory cycles. In their series mid-cycle level varied from 41 to 56 per cent, whereas it was within 2 to 30 per cent during non-ovulatory period. Present findings are comparable to those of Pommerenke (1946), Mesweeny and Sbarra (1964), Herzberg *et al* (1964), MacDonald (1969) and Achari *et al* (1974), all of whom reported definite mid-cycle increase of sodium chloride in dry cervical mucus. Li *et al* (1960) has suggested that the ovulation is governed by sharp increase in secretion of luteinizing hormone, which may be responsible for the increase in the level of sodium chloride content in dry cervical mucus.

The effect of injectable steroid contraceptives on chloride content of cervical mucus was similar to that of sodium. The typical mid-cycle increase observed during control cycles was absent in treatment cycle. In majority of the cases, the level was almost uniform throughout the cycle, only a few cases showing slight increase during mid-cycle.

Herzberg *et al* (1964) reported that the

magnitude of rheological properties of cervical mucus was regulated by its salt content. Sodium chloride being the major salt component has a determining effect on the ionic strength of mucus. The cervical mucus is hydrogel, the gel is immersed in water containing electrolytes and some other dissolved low molecular compounds. Some of the rheological properties of mid-cycle mucus, specially the flow elasticity enables the mucus both to retain the mechanical barrier function between corpus and vagina and simultaneously allow sperm penetration. During treatment with steroid contraceptives the changes produced in electrolyte content of cervical mucus may therefore, responsible for the reduction in sperm motility. The motility of sperm is greatly affected by changes in the electrolyte content of spermatozoal environement Bodnar *et al* (1961).

Total protein in fresh mucus was estimated in only 15 cases in the present series as the amount of mucus obtained was not sufficient in all the cases for all the tests including protein. Total protein was markedly reduced in mid-cycle samples of control series, mean average being 3.9 microgram/mg. with a range of 11 to 7 microgram/mg of wet mucus. The level was much higher in proliferative and secretory phase samples, mean average being 40.75 and 55.1 microgram/mg. respectively. On giving steroid contraceptives the mean average of total protein during mid-cycle was 16.34 microgram/mg (4.3 to 24.2), which is much higher than that of control mid-cycle. During proliferative and secretory phase the levels were 41.6 and 47.7 microgram/mg. respectively which are almost similar to that of control cycle.

After a detailed study of cervical mucus protein Elstein (1970) reported similar mid-cycle fall in normal ovulating women. Highest values were obtained during the secretory and early proliferative phases. Women using chlormadinone acetate (in continuous low doses of 500 micrograms) in his series showed much higher levels in mid-cycle samples than untreated women.

It can be reasonably concluded that various physical and biochemical changes occur during ovulatory phase of normal menstruating women. Injectable steroid contraceptives produce alteration in these properties, so that the mid-phase mucus remains almost similar to early proliferative and secretory phase mucus. These changes in mid-phase mucus may have antifertility effect through inhibition of spermigration and sperm penetration.

The present study supports the view that the search for a better steroid may lead to isolation of a drug which has maximum effect on the servical mucus rendering it impermeable to the spermatozoa with no effect on pituitary ovarian cycle.

Summary

The biochemical property of 20 ovulating subjects were studied after treatment with injectable steroid contraceptives, and it was observed that the maximum secretion of cervical mucus (130 to 800 mg) occurs during ovulatory phase. After giving steroid contraceptive injection, the mucus remains scanty throughout the cycle. The mucus is most translucent with maximum of Spinnbarkeit during ovulatory phase of normal cycles. It remains opaque with absent or minimal spinnbarkeit throughout the treatment cycle. Water content of mucus is at its maximum (90 to 97.7%) during ovulatory phase of control cycle while the dry

residue is at its minimum (2.3 to 10%). This mid-cycle surge in water concentration is absent in treatment cycles. Peak concentration of sodium in dry residue is present during mid-phase of control cycle. This peak is obliterated with treatment cycle. Potassium content of cervical mucus is at its minimum during ovulatory phase of control cycles but it is increased in the treatment cycle. Chloride level shows maximum level (45-70 per cent) during mid-phase of control cycles. This sharp maximum is not noticeable in treatment cycles. Total protein in fresh mucus is markedly reduced during mid-phase of control cycles (mean average 3.9 micrograms/mg). Dur'ng treatment cycles, the decrease though occurs, but is not so marked.

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