SELECTIVE FILTRATION OF ABNORMAL SPERMATOZOA BY CERVICAL MUCUS—IN VITRO A SIMPLE NEW TECHNIQUE

by

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Introduction

The uterine cervix and its secretion exert a complex and vital function in the reproductive physiology and it is generally agreed that they are essential for efficient transport of spermatozoa to the higher parts of the female genital tract and may act as a sperm reservoir, storing, and releasing sperm cells at a constant rate into the uterine cavity. Cervical factor may malfunction due to various problems e.g. cervical mucus problem, sperm problem, last but not the least, sperm mucus interaction.

The aim of the present study was to investigate selective filtration of abnormal spermatozoa by cervical mucus-in vitro, with aid of simple, inexpensive, easily available gadget developed by authour (Chaddha) as against expensive, not available (esp. in our country) Kremer sperm penetration meter and thus drastically reducing the cost.

Material and Method

The present study is a preliminary study of the cases of cervical mucus problem in infertility studied at B.Y.L. Nair Hospital and T.N. Medical College, Bombay out of a total of 125 cases of infertility attending infertility clinic.

The Technique of Collection of Cervical Mucus

After exposure of the uterine cervix with a speculum and thorough cleansing by a sterile swab, the mid-cycle cervical mucus was collected with the help of metal cervical aspiration cannula and transfer of mucus thus collected on to a glass slide (fig. 1).

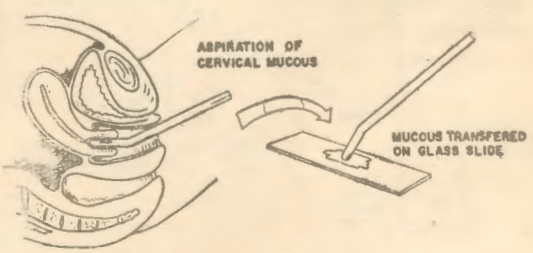

Fig. 1

Fig. 2 shows how the mucus is aspirated in the glass capillary tube with help of an airtight syringe attached to 18 gauze needle. The length of glass capillary is 10 cms with 0.8 mm bore. The needle, after dipping in liquid paraffin is introduced at one end of the capillary tube and mucus

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is thus aspirated to about 4 to 5 cms taking great care to avoid any air bubbles interrupting the length of the mucus column.

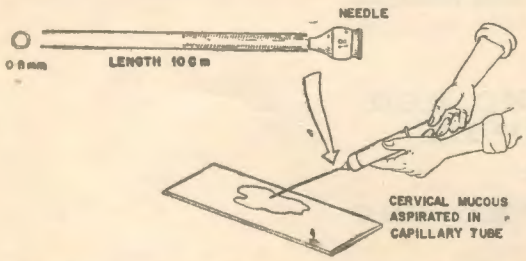

Fig. 2

Fig. 3 shows uninterrupted column of 3 cms length of cervical mucus placed in a bowel containing semen. After 90 minutes incubation at 37°C, the capillary is divided into 3 one cm parts with the help of ordinary, inexpensive, easily available granite glass cutting stone. The segment are marked as segment 1, 2 and 3. Segment 1 is first segment of the column from the bottom, segment 2 is middle segmen and segment 3 is upper segment.

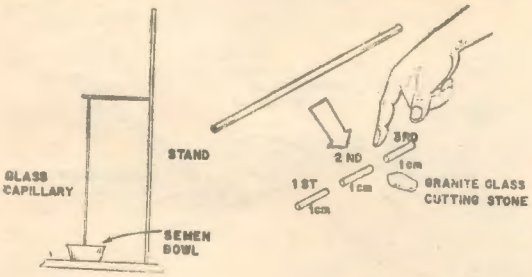

Fig. 3

Fig. 4 shows how the content of each segment is mounted on slides and stained with Papanicouloan stain. The slides are evaluated at 400x magnification, the number of normal and abnormal cells are counted and the abnormal cells are classified into 7 groups.

The physical properties of the mucus

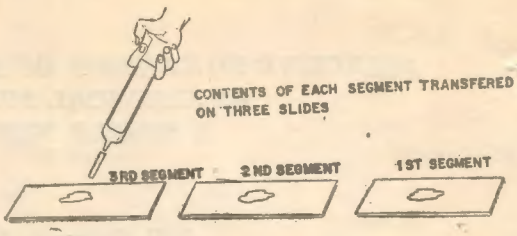

Fig. 4

and by inference its chemical constitution were evaluated by determining the cervical score which is a semiquantitative method using a scoring system of quantity spinnberkeit, ferning, viscosity and cellularity with the maximum score of 15. Mucus sample of score equal to or greater than 8 were only used.

Semen samples were obtained from male infertility clinic and from healthy fertile donors participating in the artificial insemination programme. The criterial in selection of semen samples were quantity 2 to 5 ml with at least 65 million sperm cells per ml 70% spermatozoa exhibiting good, progressive motility at zero time with at least 50% motility at 3 hours and total number of morphologically abnormal forms not exceeding 20%.

Results

Fourteen cases studied out of 125 cases attending infertility clinic showed results as shown in Table I. The abnormal forms studied were absent tail, absent head, large head, bent neck etc. It is also clear from this Table that out of 14 cases studied, 287 pathologically configurated sperm cells were counted at cm 1 and only 34 such cells at cm3 of the mucus column. This difference is statistically significant. Thus, ratio of normal to abnormal cells is 4.57 at cm 1 and 19.20 at cm 3 of the mucus column.

TABLE I
In Vitro Penetration of Different Sperm Cells

	CM 1	CM 2	CM 3	Total
Absent Tail	115	26	15	156
Absent Head	55	25	17	97
Large Head	33	3	1	37
Curled Tail	20	—	—	20
Tapering Head	10	2	—	12
Bent Neck	10	5	—	15
Others	44	9	1	54
Subtotal Path. Cells	287	70	34	391
Normal	1313	756	653	2722
Total	1600	831	687	3118

Table II shows penetrability of each group of pathologically abnormal cells.

TABLE II
Percentage of In Vitro Penetration of Different Sperm Cells

	CM 1	CM 2	CM 3
Absent Tail %	7.19	3.12	2.18
Absent Head %	3.44	3.00	2.47
Large Head %	2.06	0.36	0.15
Curled Tail %	1.25	—	—
Tapering Head %	0.63	0.24	—
Bent Neck %	0.63	0.51	—
Others %	2.75	1.08	0.15
Sub-total Path. Cells %	17.94	8.42	4.95
Sub-total Normal %	82.00	90.08	95.05

The percentage share of absent tail fell from 7.19% cm 1 to 2.18% at cm 3; absent head fell from 3.44% at cm 1 to 2.47% at cm 3; large head fell from 2.06% to .15%, while others fell from 2.75% to only 0.15%. These differences are statistically significant.

Fig. 5 summarizes how the cervix acts as filtering mechanism where abnormal sperm cells show a downward trend reducing from 17.94% to only 4.95%. On the other hand, more and more normal spermatozoa are seen in higher segment namely 3rd segment increasing from 82% at 1st segment to 95.05% at 3rd segment.

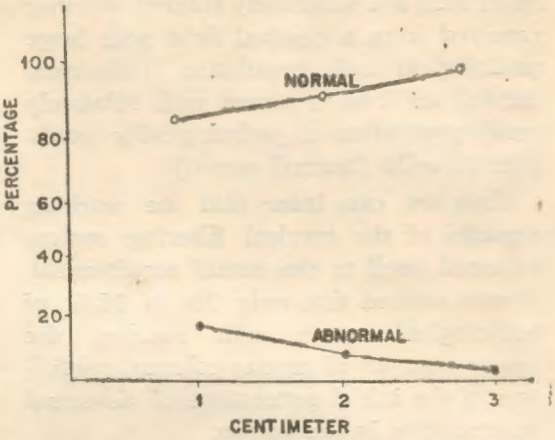

Fig. 5

Discussion

From functional point of view cervix and its secretions fulfill an important task in the reproductive process. Among the various properties of this system, the receptivity to sperm penetration near ovulation and impedence of entry at other times has attracted much attention among intellectuals. Although this hormone dependent function has been intensively studied (Schumacher, 1973; Riestein, 1971) qualitative aspects of sperm penetration through the cervical mucus remained to date at the level of mere hypothesis. Some authors (Botella-Llusia, 1956) have suggested that cervical mucus may have a filtering capacity for abnormally configurated spermatozon while enhancing the passage of normal sperm cells. Others have rejected this hypotheses (Joel, 1963) and linked habitual abortions with the passage of abnormally configurated cells through the cervix and subsequent fertilization of the ovum with these cells.

The present study of in vitro sperm

penetration has revealed the 'Selection' capacity of the cervical mucus. It appears from the study that pathologically configurated cells are selectively filtered whether received from a seminal fluid with large pathological cell population (abnormal semen) or from a semen with relatively small population of pathologically configurated cells (normal semen).

Thus we can infer that the working capacity of the cervical filtering system adjusted itself to the actual requirement. It was noticed that only 10 to 13% of pathological sperm cells reached the upper segment of mucus column, regardless of the initial percentage of abnormal spermatozoa in the semen.

When comparing the working capacity of the cervical filtering system between various segments, it was found that major share of pathological sperm cells were eliminated at lower segment.

Conclusions

1. Cervical mucus of good physical qualities may act as a sperm selector, differentiating between morphologically normal and abnormal sperm cells, while serving concomitantly as an ercellent transit medium for normal spermatozoa.

2. The filtering capacity of the cervical mucus is equally efficient for different types of pathologically configurated spermatozoa.

3. The filtering function of the uterine cervix and its secretion results in similar final ration of abnormal to normal spermatozoa regardless of the initial percentage of abnormally configurated sperm cells in the seminal fluid.

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References

1. Bergman, P.: Int. J. Fertil. 1: 45, 1956.
2. Botella Llusia, J.: Int. J. Fertil. 1: 113, 1956.
3. Elstein, M., Mitchell, R. F., Syratt, J. U.: J. Obstet. Gynec. Brit. 78: 180, 1971.
4. Insler, V., Melmed, H., Eichenbrenner, I., Serr, D. M. and Lunenfeld, B.: Int. J. Gynec. Obstet. 10: 223, 1972.
5. Schumacher, G. F. B.: The Biology of the Cervix. Eds.: R. J. Blandau and K. Moghissi. The University of Chicago Press, Chicago and London 1973, Chapter II, pp. 202-221.