SPERM AGGLUTINATION PHENOMENA IN CERVICAL MUCUS IN VITRO IN UNEXPLAINED INFERTILITY

by

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Introduction

Infertility or inability to conceive is one of the most distressing problems of the women attending the gynaecological outdoor. Because of her psychological upbearing in our society, the women usually assumed the initial responsibility for failure to produce a child and is usually first to seek the medical care. In contrast, the men may demonstrate their inability to face the possibility of being infertile by avoiding examination. Infertility is a disorder of complex nature and both partners must be evaluated. The problem arises in unexplained cases of infertility where either of the partner does not have any cause which may be responsible for infertility.

It was not until 1899 when the antigenic property of the spermatozoa was discovered by production of heteroantibodies by Landsteiner and Metchnikoff. However the microscopic agglutination of sperms in cervical mucus has never been clearly demonstrated or described, except for the recent paper by Kremer and Lager 1976. The study of spermagglutination because of anti sperm antibodies in cervical mucus and its correlation to infertility is still incomplete. The actual presence of antibodies in the place where the spermatozoa are situated is very significant. It is therefore very important to explore the possible occurrence of sperm antibodies in cervical mucus of women or in some adjacent tissues or mucus. The present study is undertaken to find out sperm agglutination phenomenon in cervical mucus as a responsible cause of unexplained primary infertility.

Material and Methods

One hundred cases of unexplained primary infertility were selected from gynaecological outpatient department of Allahabad Medical College. 34 cases were taken as control.

History was recorded with special reference to name, age, parity, menstrual history, obstetrical history, past history and family history of any prolonged medical disease. Duration of infertility and previous infertility work ups were obtained by interviewing couples.

All the couples had extensive infertility work up including endometrial biopsy, hysterosalpingography, endoscopic exami-

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nation, and all the tests showed normal results.

Seminal analysis and urological examination of male partner were carried out to exclude any cause which might be responsible for infertility. Only those couples who had no detectable cause were taken up for study.

Post coital test was done in as many patients as possible.

For cervical mucus aspiration the women were asked to come 12-15 days after their last menstrual period. They were instructed to maintain sexual abstinence for two days prior to cervical mucus aspiration. Clear endocervical mucus of preovulatory phase was aspirated with a disposable hypodermic syringe attached to a sterile polythylene catheter No. 16 or with the help of a vaginal pipette. Double portion of Sorensen's buffer was added to cervical mucus and agitated frequently for 2 hours at room temperature. Then it was centrifuged at 6000 rpm for 30 minutes. The supernatant were transferred to small tubes and stored at –20°C until the tests were done.

Samples with more than 60 x 10^6 cells ml with over 60% motility were chosen. It was centrifuged at 2000 rpm for 10 minutes and supernatant fluid removed. It was mixed with small amount of Dale's ringer solution and tubes were left standing at 4°C for 1 hour and the supernatant fluid containing spermatozoa removed. The cells were then washed twice and then mixed in Dale's ringer solution to make the volume desired for two test. The final suspension contained 5 x 10^4 to 3 x 10^6 spermatozoa/ml of which 70-80% were motile.

The tests used for the detection of agglutination are as follows:

1. Microscale test of cervical mucus extract.
2. Macroscale test of cervical mucus extract.

Microscale Test of Cervical Mucus Extract

Semen samples of more than 60 x 10^6 cells/ml with over 60% motility were chosen. A volume of 0.01 ml of 50 x 10^6 of adjusted semen was pipetted to the bottom of a serological tube. To this 0.1 ml of cervical mucus was dropped. After gentle shaking for a few seconds the tubes were incubated at 30°C for 2 hours. Then one drop of cervical mucus sperm mixture was pipetted over a slide and microscopic examination was done and agglutinated sperfs counted. Twelve high power fields were examined. The ratio of agglutinated sperms to total sperm was counted. If there was more than 10% agglutination the test was judged as positive for MIS agglutination.

Macroscale Test of Cervical Mucus Extract

Semen adjusted to 40 x 10^6 cells/ml and 10% gelatin were separated for 20 minutes at 37°C. Equal volume of semen and gelatin were mixed. Then 0.1 ml of the mixture was pipetted to the bottom of a serological tube (50 mm x 5 mm) and 0.1 ml of cervical fucus extract was added. The tubes were incubated for 2 hours at 37°C. Within 72 hours there is formation of floccules or a ring at the junction of gelatin and cervical mucus which was judged as positive test for macroscale agglutination or MAS agglutination.

Results

The results were judged according to age, duration of infertility and results of

post coital test. The ages of the patient varied from 18-35 years. Maximum number of cases of primary infertility and control group were between 31-35 years (Table I).

TABLE I
Distribution According to Age

Age (in years)	Unexplained primary infertility		Control group	
	No.	Percentage	No.	Percentage
18-20	8	8.0	5	14.7
21-25	40	40.0	2	5.8
26-30	8	8.0	3	8.2
31-35	44	44.0	25	73.5
Total	100		34	

Duration of Infertility

The duration of infertility ranged from 3 years to as long as 20 years. Maximum number of cases of primary infertility was between 7-10 years (Table II).

TABLE II
Distribution According to Duration of Infertility

Duration of infertility	Unexplained primary infertility	Control group
3- 6	32	8
7-10	52	12
11-14	8	6
15-18	4	4
18-20	4	4
Total	100	34

Microscopic Agglutination Test (MIS Agg. Test)

In unexplained primary infertility, 64% of cases showed positive MIS agg. test while cases of control group it was positive in 41.1% (Table III).

Macroscopic Agglutination Test (MAS Agg. Test)

60% of unexplained primary sterility and 58.8% cases of control group showed positive MAS agg. test (Table IV).

TABLE III
Results of MIS Age Test

MIS agg test	Unexplained primary infertility		Control group	
	No.	Percentage	No.	Percentage
Total number of cases tested	100	100.00	34	100.0
Cases showing positive results	64	64.0	14	41.1
Case showing negative results	36	36.0	20	59.9

TABLE IV
Results of MAS Agg Test

MAS agg test	Unexplained primary infertility		Control group	
	No.	Percentage	No.	Percentage
Total number of cases tested	100		34	
Cases showing positive results	60	60.0	19	55.2
Cases showing negative results	40	40.0	15	44.8

Post Coital Test and its Relation with Sperm Agglutination

Post coital test was done in cases of primary infertility. The results were considered positive if one or more spermatozoa were found in the endocervical mucus and if no spermatozia were detected (Table V).

TABLE V
Results of PCT in Relation to Positive Sperm Agglutination

Results of agglutination and PCT	Unexplained primary infertility	
+ve agg. +ve PCT	60	75.0
+ve agg. —ve PCT	—	
—ve agg. +ve PCT	18	22.5
—ve agg. —ve PCT	2	2.5
	80	100.0

In 20 cases of unexplained primary infertility PCT could not be done. In 60 cases where PCT was positive with slight cord motility or no motility of sperms of all the sperm agglutination was positive. In 18 cases sperms on PCT showed fair progressive motility and negative agglutination test.

Duration of Infertility

Increasing duration of infertility was associated with higher percentage of positive cases (Table VI).

Type of Agglutination

Agglutination was either head to head type, tail to tail type or mixed type (Table VII).

TABLE VII
Type of Agglutination

Type of agglutination	Primary infertility	Percentage
Head to head type	12	20
Tail to tail type	24	40
Mixed type	24	40
Total	60	100

Discussion

Positive sperm agglutination indicating the presence of sperm agglutinating antibodies in cervical mucus were found in 64% of cases of study group suggesting local production of antisperm antibodies as a responsible cause of infertility. Parish *et al*, 1966 have demonstrated antibodies cytotoxic to spermatozoa in 3 out of 11 patients (27.27%). Parish and Ward

TABLE VI
Sperm Agglutination in Relation to Duration of Infertility

Duration of infertility in years	Total No. of cases tested	Cases showing positive results	% of cases showing positive results
3- 6	32	16	50
7-10	52	32	61.5
11-14	8	6	75.0
15-18	4	2	50
18-20	4	2	50
Total	100	60	

1968 have demonstrated anti sperm antibodies in the cervical mucus in 3 out of 48 cases (6.225%).

Coelingh *et al* (1974) demonstrated positive results in 3 out of 13 patients (23.77%) in cervical mucus by immunoflorescence technique. They supported the concept of local production of sperm antibodies.

Shulman *et al* (1975) have also demonstrated positive sperm agglutination in cervical mucus in their study.

Pacheco, Romero (1973) however showed contradictory results. They demonstrated positive sperm agglutination in only 3 out of 23 infertile women of unexplained infertility. They have suggested that these antibodies do not play an important role in female infertility.

Correlation of antisperm antibodies with post coital test showed that positive sperm agglutination was associated with poor sluggish or no motility of sperms on post coital test. This suggests that probably sperms loose their motility due to antibodies present in the cervical mucus. A poor post coital test is therefore an indication of the study of antisperm antibodies. Parish and Ward in 1968 gave results in favour of above findings. Shulman *et al*, 1975 have observed the same findings and had regarded study of sperm antibodies essential in patients showing poor results on post coital test. However, wall *et al*, 1975 stated that poor results on PCT were because of some factor apart from spermatozoal antibodies. Sinha *et al*, 1977 suggested local immunological reaction as a cause of poor post coital test and infertility. However, Hingorani *et al*, 1978 could not establish any correlation between sperm agglutination and positive post coital test.

Regarding the type of antibody, these were either head to head type, tail to tail type and mixed type depending upon the site of antigen present over the surface of spermatozoa. Glass and Vaidya, 1971 have shown head to head type as the most common type. Shulman in 1973 showed tail to tail type to be most frequent. Mishra and Dass, 1981 have shown all the three types of agglutination in their observation.

So it appears that agglutination of any part of the sperm may be taken as a reliable index for the presence of sperm agglutination antibodies.

With increasing duration of infertility higher prevalence of positive cases have been demonstrated. Mishra and Dass, 1981 showed higher value of antisperm antibodiss with increasing duration of infertility.

Conclusion

Antisperm antibodies present over the surface of spermatozoa resulting in their agglutination are responsible for death and agglutination or poor motility of spermatozoa in cerviral mucus. Sperms have subsequently to swim in abundant endometrial and tubal fluids so that before reaching the site of fertilization they become agglutinated and are incapable of fertilization with increasing duration of infertility chances of positive agglutination go on increasing.

In the follow up process, the husbands of patients showing positive sperm agglutination have been advised to use condom or total abstinence for 2-6 months with the thought that if serum is never allowed to touch tissues of the women for an extended period of time, her antibody level will fall. Periodic check up of these antibodies were recommended.

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