OBSERVATIONS ON SPERM AGGLUTINATING AND IMMOBILIZING ANTIBODY IN THE SERA OF WOMEN OF UNEXPLAINED STERILITY*

by

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Unexplained sterility in married couples still remains the crux of the problem of sterility. In recent years, different immunological tests have been carrred out to assess the immune response against spermetozoa, though antigenicity spermetozoa was discovered as early as 1899 by Landsteiner and Metchnikoff as quoted by Rumke and Hallinga, (1959).

In the present study, sperm agglutinating and immobilrzing antibodies were studied in women of unexplained sterility.

Material and Methods

Fifty cases of primary unexplained sterility were selected from the Gynaecological outpatient Department of the Patna Medical College. Detailed history was elicited from the couples. After clinical examination and investigation, the obvious causes of sterility were excluded.

Sperm agglutinating tests were performed according to the method of Franklin and Dukes (1964 A) and sperm immobilizing tests were performed according to the method of Glass and Vaidya (1971). The tests were carried out as follows:

Sperm Immobilizing Test

Sperm count and motility evaluation were performed on freshly ejaculated specimen. The semen specimens were adjusted by dilution to a concentration of 50 million spermatozoa per ml. The sera of patients and normal women were incubated at 56°C for 30 minutes to destroy complements. Incubated sera, husband's sperm and guineapig complement were taken in three sets of test tubes as detailed in Table I.

The test tubes were incubated at 37°C for 60 minutes and the specimens were then examined microscopically to estimate motility. The percentage of motile sperms in test tube 1 was expressed as T and that in test tube 2, as C, and the ratio C/T (sperm immobilizing index) was estimated. An index value higher than 1.9 was considered positive and indicative of presence of sperm immobilizing antibodies. Test tube 3, which contained no complement, was used to exclude non-specific sperm immobilization. The test was also performed in a group of 35 pregnant ladies as control group.

Sperm Agglutination Test

Semen specimens adjusted to a concentration of 50 million spermetozoa per ml.

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TABLE I

Test tube	Incubated Patient's serum	Incubated Normal Women's serum	Husband's sperm 50 x 10^6/ml.	Guineapig complement/ ml.
	ml.	ml.	ml.	ml.
1.	0.25	—	0.025	0.05
2.	—	0.25	0.025	0.05
3.	0.25	—	0.025	nil

TABLE II

Test tube	Wife's serum ml.	Physiological saline ml.	Husband's sperm 50 x 10^6 ml.
1.	0.5	nil	0.05
2.	0.5 (1:5 dilution)	nil	0.05
3.	nil	0.5	0.05

were mixed with wife's serum and physiological saline for sperm agglutination test as detailed in Table II.

Test tube 3, which contained sperm and physiological saline, was used to exclude non-specific agglutination so that cases showing non-specific agglutination could be discarded.

The three tubes were incubated at 37°C for 4 hours and the specimens were examined microscopically at intervals of 30 minutes during the test period. The aggregation of 4 to 10 motile sperms per high power microscopic field was considered as positive and aggregation of 10 to 20 sperms per high power field, as strongly positive agglutination test. Aggregation of sperm around the cell debris was not taken into consideration.

In cases where sperm agglutination tests were positive, the test was repeated taking 3 additional tubes with serum from the fertile female donor and semen from fertile male donor. Sperm agglutination test was also performed in 35 pregnant women as control.

In the presence of positive immobilizing and agglutinating tests a detailed cervical mucus study was done according to the method of Davajan and Kunitake (1969).

Results

The resuts which were analysed according to age, duration of sterility, blood group incompatibility, antisperm antibody test and sperm penetration of cervical mocus are set out in Table III to IX.

TABLE III

Duration of Sterility

Duration of sterility in years	No. of cases	Percentage
3- 6 years	27	54
7-10 years	13	26
11-14 years	8	16
15-18 years	2	4

The maximum number of cases of unexplained sterility were found in the age of 3 to 6 years.

It appears from Table IV that 10% of the cases had positive sperm agglutination tests.

TABLE IV
Sperm Agglutination Test

Particulars	No. of cases	Positive test	Percentage
Primary sterility	50	5	10
Control	35	1	2.8

It appears from Table V that agglutination was either head to head, tail to tail or mixed.

TABLE V
Nature of Agglutination

No. of patients	Nature of agglutinat		
	Head to head	Tail to Tail	Mixed type
5	2	1	2

It appears from Table VI that sperm immobilizing antibody was present in 6% of the cases and was absent in the control group.

TABLE VI
Sperm Immobilization Test

Particulars	No. of cases	Positive test	Percentage
Primary sterility	50	3	6
Control	35	0	0

Table VII shows that sperm immobilization value was greater than 1.9 in all the three cases.

TABLE VII
Sperm Immobilization Value in 3 Positive Cases

Motility before incubation	% Motility in test tube 1=C	%Motility in test tube 2=T	Sperm Immobilization value C/T
80	75	25	3
90	80	30	2.6
80	75	15	5

TABLE VIII
Sperm Immobilization Value (C/T) in Relation to Period of Sterilization

Duration of sterility in years	C/T
15	5
10	3
6	2.6

It would appear from the above Table that the patient with longest duration of infertility had the maximum sperm immobilization value.

It is significant to note in Table IX that sperms were absent at levels 2 cm and 2.5 cm in all cases.

Discussion

In the present study sperm immobilizing and sperm agglutinating tests were

TABLE IX
Post Coital Test of Cervical Mucus

Particulars	No. of cases	Presence of sperm at different levels in cervical mucus			
		0.5 cm	1 cm	2 cm	2.5 cm
Positive sperm agglutination only	2	Present	Present	Absent	Absent
Positive sperm agglutination and sperm immobilization	3	Present	Absent	Absent	Absent

performed in sterile women to evaluate comparative value of the two tests, as sperm agglutinating tests have been shown to be of limited clinical usefulness because of the frequency of positive results in the control group (Franklin and Dukes, 1964; Isojima *et al*, 1972; Jones *et al*, 1973).

In the present study, there was no case of positive sperm immobilization in the control group but there was 1 case of positive sperm agglutination out of 35 control cases. Five out of 50 (10%) infertile women showed positive sperm agglutination with the husband's sperm in the concentrated serum as well as in the serum of 1:5 dilution. As the saline control did not show agglutination, the positive results obtained in these 5 cases would be due to serum factor.

There were 3 cases out of 50 (6%) which had positive sperm immobilization test. On analysis it was found that these 3 women had concordant positive sperm agglutination tests as well. Sperm immobilization value had a positive correlation with duration of sterility. The value was highest with the longest duration of sterility.

No correlation could be established between blood group incompatibility and positive antisperm antibody test in the present study.

The work would have been incomplete without the study of post coital cervical mucus. Therefore, attempts have been made to correlate the positive antisperm antibody test with post coital mucus study. Sperms were detected only at 0.5 cm. level of the cervix in the 3 patients with both positive sperm agglutination and sperm immobilization. The sperms were non-motile. No sperm was, however, found at 2 and 2.5 cm level of the cervical canal. Probably the sperms lost their motility at the lower level of cervix due to the antibodies present in cervical mucus. Sinha *et al* (1977) suggest local immunological reaction as a cause of infertility and consequent poor postcoital test. Cervical mucus hostility is further supported by Cantuaria (1977) who found antisperm antibody in 25.6 per cent of the sera and 20.5 per cent of cervicovaginal secretion in cases of infertility. However, according to Hingorani *et al* (1978) no correlation could be established between sperm agglutination test and postcoital test.

In the follow up process, the husbands of infertile women with antisperm antibodies have been advised the use of condom. Periodic check-up of their antibody titre is being done. None of the infertile patients have become pregnant so far.

Summary

1. Fifty cases of unexplained primary sterility were subjected to immunological tests.

2. Out of 50 women of unexplained sterility, five showed positive sperm agglutinating test (10%). The control test was also performed with sera of sterile women and semen of fertile men. The tests with fertile donors did not show positive agglutination test.

3. Three out of 50 (6%) showed a positive test. It is significant to note that the sperm immobilising value was greater than 1.9 in all the 3 cases.

4. There was definite correlation between the antibody in the serum of infertile women and its effect on the sperm motility and its penetration of the cervical mucus.

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