

STUDY OF SPERM AGGLUTININS IN INFERTILE COUPLE

by

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Introduction

The immune response of the spermatozoa is of special significance since it is now viewed as one of the causative factors involved in the problems of unexplained infertility. Landsteiner (1899) and Metchnikoff (1899) separately discovered that spermatozoa are antigenic. The antigenicity of the spermatozoa was used for developing the contraceptive potential of this immune response.

Wilson (1954) demonstrated the presence of auto-antibodies to spermatozoa in the serum of two men and showed that the auto-agglutination had rendered them infertile.

Antibodies against spermatozoa was also demonstrated in the serum of infertile women by Franklin and Dukes (1964a), Schwimmer *et al* (1967), Tyler *et al*

(1967), Hanafiah *et al* (1972) and Mettler *et al* (1974).

The present study has been undertaken to find the incidence of sperm agglutinin in the sera of infertile couples attending the Sterility Clinic of the All India Institute of Medical Sciences.

Material and Methods

A total of 50 infertile couples attending the Sterility Clinic of the AIIMS and 10 fertile couples were examined. They were divided into three groups.

Group A: (25 couples): Couples with no demonstrable organic cause for infertility.

Group B: (25 couples): Couples with demonstrable cause for infertility.

Group C: (10 couples): Couples with known fertility.

Detailed history was taken from the couples and careful clinical examination done and routine investigations performed to find out the cause for infertility.

The sperm-agglutination test (SA test) of Franklin and Dukes (1964a) was used in a slightly modified way. Blood was collected from the antecubital vein of both partners, the serum separated and incubated at 56°C for ½ hour to destroy the complement. The husband provided the semen by masturbation after five days' of abstinence and the specimen was examin-

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ed and the colour, quantity, reaction, liquefaction, motility, count and morphology noted. When sperm count was below 50 million/ml 0.1 ml of semen was used and when the count was above 50 million/ml 0.05 ml of semen was used.

The SA test was carried out in serological tubes as follows:

Tube I: 0.5 ml of wife's serum (undiluted) + 0.05 ml of husband's semen.

Tube II: 0.5 ml of wife's serum (1/10 dilution) + 0.05 ml of husband's semen.

Tube III: 0.5 ml of husband's serum + 0.05 ml of husband's semen.

Tube IV: 0.5 ml of wife's serum + 0.05 ml of infertile donor semen.

Tube V: 0.5 ml of wife's serum + 0.05 ml of fertile donor semen.

Tube VI: 0.05 ml of normal saline + 0.05 ml of husband's semen.

The tubes were incubated in a water bath at 37°C for 4 hours and samples were examined from each tube after 1 hour, 2 hours and 4 hours for evidence of sperm agglutination. Agglutination was graded as 1 + when there was clumping of 2-10 motile sperms, 2 + when there was clumping of 10-20 motile sperms and 3 + when there was clumping of more than 20 motile sperms. Aggregation of spermatozoa around cell debris was not taken into consideration. Normal saline was used to detect non-specific agglutination and fertile couples were taken as control.

Results

Fifty infertile couples and 10 fertile couples were investigated. The period of infertility ranged from 2 years to 18 years and the age varied from 18-45 years. The SA test revealed that 9 out of 50 infertile couples (18%) had a positive SA test as compared to 0% in the fertile group. This is statistically significant ($p < 0.04$).

In Group A, SA test was positive in 20%, in group B 16% and in group C 0%.

The difference is not statistically significant (Table I).

TABLE I

Results of Sperm Agglutination Test in Sera of Infertile Women With Unknown Causes for Infertility, Infertile Women With Known Causes and Fertile Women

Type of couples	No. of couples	Results of Agglutination Test	
		No. +ve	% +ve
A. Infertile unknown causes	25	5	20
B. Infertile known causes	25	4	16
C. Fertile	10	0	0

In the undiluted sera of 9 infertile wives with positive SA test, sperm agglutinating activity was present in 8 cases at the end of one hour, while one serum showed activity at the end of 2 hours. At the end of 4 hours, 4 sera showed enhanced activity while the remaining 5 sera's activity was stationary. In the 9 diluted sera, 6 showed activity after 1 hour, 8 after 2 hours and all the 9 after 4 hours with only one serum showing enhanced activity (Table II).

TABLE II

Sperm Agglutinating Activity in the Wives Sera in Undiluted (N) and Diluted (1/10) Sera at 1 Hour, 2 Hours and 4 Hours

Serial No.	1 hour		2 hours		4 hours	
	N	1/10	N	1/10	N	1/10
1.	++	+	++	+	++	+
2.	-	-	++	+	++	+
3.	++	-	++	+	++	+
4.	+	+	+	+	++	+
5.	+	-	+	-	++	+
6.	+	+	++	+	++	+
7.	+	+	+	+	+	+
8.	+	+	+	+	+	+
9.	++	+	++	+	+++	++

SA test was positive in 3% (1 out of 33) of infertile wives against infertile

donor semen, 5.5% (1 out of 18) of infertile wives against fertile donor and 10% (1 out of 10) of fertile wives against infertile donor semen.

The incidence of autoagglutination in infertile husbands was 2, 4% and in fertile husband it was 1, 10%.

No correlation could be found between SA test and post coital test (Table III), nor against history of abortion in the infertile couples (Table IV).

TABLE III

Results of Microagglutination Test in Relation to Post-Coital Test

Results of Agglutination Test	Good PCT	Poor PCT	Total
Positive Test	7 (77.8%)	2 (22.2%)	9 (100%)
Negative Test	28 (71.8%)	11 (28.2%)	39 (100%)

TABLE IV

Results of Sperm Agglutination Test and History of Abortion in 50 Infertile Couples

Group	Unknown cause for Infertility		Known cause for Infertility	
	+ve	-ve	+ve	-ve
Total number of couples	5	20	4	21
Number of wives with history of abortion	1	2	1	4
% of wives with history of abortion	20%	10%	25%	19%

In group A there were 19 couples (76%) with primary infertility and 6 couples (24%) with secondary infertility and in group B there were 17 couples (68%) with primary infertility and 8 couples (32%) with secondary infertility. It was found that the incidences of positive SA test in the secondary infertility in both group A and group B (33.3% and 25% respectively) were higher than those

in the primary infertility in the 2 groups, 15.7% and 11.7% respectively (Table V).

TABLE V

Incidence of positive Sperm Agglutination Test in Wives Sera in Primary and Secondary Infertility With Unknown and Known Causes for Infertility

Type of Infertility	No. of couples	Positive agglutination test	
		No.	%
<i>Unknown Causes</i>			
Primary Infertility	19	3	15.7
Secondary Infertility	8	2	33.3
<i>Known Causes</i>			
Primary Infertility	17	2	11.7
Secondary Infertility	8	2	25.0

Discussion

Reports on the relationship between sperm agglutinating antibodies and unexplained infertility show a wide variation. In the present series, 20% of the wives in Group A had positive SA test. This is far below the incidence of 78.9% obtained by Franklin and Dukes (1964a) in 19 cases studied. When the number of cases was extended to 43, Franklin and Dukes (1964b) obtained 72% and after increasing the number to 67 patients Dukes and Franklin (1968) obtained positive reaction in 67.2%. Though the incidence of positive reaction in this series is low, it still suggests a relationship between circulating sperm antibodies and unexplained infertility. Schwimmer *et al* (1967) had 37.5% positive SA test from 64 wives with primary unexplained infertility and 50% from wives with secondary unexplained infertility. Israelstam (1969) obtained 29% positive SA test from 45 infertile couples but he found that 10 patients had also non-specific agglutination as shown in the control tube. He thus obtained only 7% in his series. Tyler *et al*

(1967) reported 14%, Glass and Vaidya (1970), 17% in primary and 24% in secondary unexplained infertility, Hanafiah *et al* reported 9.4% and Mettler *et al* (1974) obtained 17.4%.

Among the 25 couples in Group B, the incidence of positive SA test was 16%, while Franklin and Dukes (1964a) reported 10.37%, Dukes and Franklin (1968) 15.9%, Schwimmer *et al* (1967) 20%, Glass and Vaidya (1970) 4.3% and Hanafiah *et al* (1972) 7.1%. This shows that besides the demonstrable factor for infertility an immunological factor also rendered them infertile.

All the 9 wives' sera which were SA test positive showed individual specific agglutination, i.e. none agglutinated a donor semen. Franklin and Dukes (1964a) reported a serum which agglutinated 28 different semen. However, Glass and Vaidya (1970) found that one quarter of the women who agglutinated their husband's spermatozoa also reacted against donor semen. Moreover, they found that there was never a reaction against donor semen without agglutination of the husband's sperm.

Autoagglutination in the present series was 4% in infertile husbands as compared to 3.2% reported by Rumke and Hellinga (1959) 7.8% of husbands in the primary unexplained infertility and 3% in the secondary unexplained group found by Schwimmer *et al* (1967) and 5.7% of husbands of unknown cause for infertility and 4.5% of husbands of known cause for infertility reported by Hanafiah *et al* (1972). Glass and Vaidya (1970) did not find any case of autoagglutination in their series. No relationship could be established between the presence of autoagglutinins and sperm counts in the 3 husbands (32.0 mil/ml, 96.0 mil/ml and 108.0 mil/ml). Age of the husbands also did not

bear any relationship with autoantibodies. Morphology was within normal limits.

In the fertile group the incidence of autoagglutination was 10% but none of the wives' sera was SA test positive while Franklin and Dukes (1964a) reported 11.8% SA test positive in the fertile women.

There was no concordant positive SA test in both husband and wife in the present series, whereas Hanafiah *et al* (1972) found 3 couples among the positive SA test to have concordant positive test in both husband and wife.

No relationship was found between post-coital test and SA test. There was good post-coital test in 77.8% in SA test positive and 71.8% in SA test negative, while the incidence of poor post-coital test was 22.2% in SA test positive and 28.2% in SA test negative cases. Schwimmer *et al* (1967) and Glass and Vaidya (1970) also did not find any relationship between these two tests.

In the present survey, no relationship could be established between SA test and history of abortion. This is similar to the findings of Hanafiah *et al* (1972). Isojima and Li (1968) postulated from their study in experimental animals that sperm antibodies could not be a cause for foetal death or abortion.

Summary

Sperm agglutination test was carried out in 50 infertile couples and 10 fertile couples. The test was positive in 18% of infertile couples and 0% in the fertile couples. The difference was statistically significant ($P < 0.04$). Further analysis showed that the sperm agglutination test was positive in 20% of wives with unknown causes for infertility and 16% of wives with known causes for infertility. There was no relationship between the results of sperm agglutination test and

post-coital test or history of abortion. Autoagglutination was not related to age of the patient or semen analysis findings.

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