INCIDENCE OF ANTISEMEN ANTIBODIES IN INFERTILE COUPLES

by

M. C. R. Vyas,* M.D.

K. R. Joshi,** M.Sc. (Med.), M.D., Ph.D.

V. B. Kalra,*** M.D.

(Mrs.) R. Kalra,**** M.S.

and

R. K. Sharma Guleri,† M.B.,B.S.

Introduction

Interest in the immunological aspects of infertility began after the successful attempt of producing antibodies against the spermatozoa in experimental animals by Landsteiner and Metchnikoff (1899). Following the report of Wilson (1954) of 3 cases of male infertility showing the presence of a serum factor capable of causing intense sperm agglutination, the modern concept implicating immunologic basis in the human infertility was formulated. Consequently, the role of antispermatozoal antibodies in human infertility has been extensively studied by various workers. The incidence of high levels of sperm antibodies in infertile male have varied from 3 per cent (Rumke, 1965) to 18 per cent (Nakabayashi *et al* 1961) and from 7 per cent (Israelstam, 1969) to 78.9 per cent (Franklin and Dukes, 1964) in females.

However, studies on the relationship of antispermatozoal antibodies with infertile status of the individual yielded equivocal and often contradictory results (Southerland and Landing, 1961 and Phadke and Padukone, 1964). As such the exact role of antisperm antibodies in causation of sterility still remains an enigma and needs further studies. This paper describes the incidence and titre of antisemen antibodies in healthy fertile couples and infertile couples as detected by various sensitive serologic methods.

*Lecturer in Pathology.
**Reader in Pathology.
***Lecturer in Pathology.
****Lecturer in Gynaecology.
†Senior Demonstrator in Pathology.
Department of Pathology and Gynaecology, S.P. Medical College, Bikaner.

Material and Methods

Forty-five infertile couples were included in this study, requisite clinicopathological investigations including semen examination of male were done in all cases to exclude any obvious organic cause for infertility. These patients were subjected to a battery of serological tests.

Serological studies included, Spermatozoal agglutination test (SPAT) by modified Franklin and Dukes microagglutination technique (1964) and Israelstam (1966) Spermatozoal immobilization test (SPIT), as used by Leslie and Quinlivan (1966); Bentonite flocculation test (BFT), Bloch and Bunim (1959); Tanned red cell agglutination test (passive hemagglutination test) (PHAT) Boyden (1951) technique modified by Doniach and Roitt (1958); Complement fixation

test, Price (1950); Immunodiffusion, procedure followed by Cruickshank with some modifications.

Antigen from whole semen was prepared after liquafation of the semen sample which was then frozen and thawed 6 times and subjected to homogenization by a glass tube manual homogeniser for 30 to 45 minutes. This homogenate was then centrifuged at 2,000 revolution per minute for 15 to 25 minutes to separate the clear supernant from the debris at the bottom of the tube. It was then stored at the bottom of he ube. It was than stored at 20°C in the deep freeze. The similar method was employed for preparation of antigen from semen of both infertile male and healthy control subjects. The anti human semen rabbit antiserum, uscol as control was prepared by injecting into rabbits, a healthy donor semen with complete freuds adjuvent. For control 30 healthy young fertile couples having 2 to 5 issues were included in this study for the presence of antisemen antibodies in both male and female partners by S.P.A.T., S.P.I.T. as basis screening tests and additional P.H.A.T., and B.F.T. using husbands own semen as an antigen.

Observations

The serological studies revealed that antisemen antibodies were detected in 4.4% by SPAT and 0 per cent by S.P.I.T., 11.11 per cent by PHAT 13.33 per cent by BFT and 8.88 per cent by CFI in males; and 20.0 per cent by SPAT, 2.0 per cent by SPIT, 31.11 per cent by PHAT, 33.33 per cent by BFT and 22.22 per cent by CFT in females using husbands own semen as antigen as shown in Table I. When healthy donors semen was used as an antigen the percentage was 0 per cent by SPAT, 0 per cent by SPIT, 2.22 per cent by PHAT, 4.22 per cent by BFI and 2.22 per cent by CFT in males, while in females percentage positively was 8.88 per cent by SPAT, 0.0 per cent by SPIT, 8.88 per cent by PHAT, 11.11 per cent by BFT and 11.11 per cent by CFI (Table II). In both the males in whom spermatozoa agglutinating antibodies were detected, the sperms in their semen appeared autoagglutinated.

With healthy fertile control, antisemen antibodies were detected in 3.3% by SPAT, 0% by SPIT, 3.3% by PHAT and 3.3% by BFT in males and 10.0% by SPAT, 3.5% by SPIT, 13.3% by PHAT and 13.3% by BFT in females using husbands own semen as an antigen as shown in Table III.

With immunodiffusion using direct reaction in agar gel between wives sera and seminal antigens from husband, only 2 cases showed one faint precepit line after 72 hours incubation. No precepit line was seen with male sera and other groups using donor semen as antigen.

Results showing titres in various techniques in test and control groups is shown in Tables I, II and III. Table IV shows the comparative per cent positivity in test and healthy fertile control group.

Discussion

The present study was under-taken with a view to investigate the role of immunological factors employing various serological techniques. Studies related to the detection of antisemen antibodies (A.S.A.) in the sera of infertile human couples have yielded interesting results. Of a total of 45 infertile human couples, antisemen antibodies were detected in 4.4% of males and 20.0% of females by

TABLE I

Showing Titres by Various Serological Techniques in Male and Female in Test Group (Using Husbands Own Semen as Antigen)

Method	SPAT		PHAT		BFT		CFT		SPIT
Titres	Male	Female	Male	Female	Male	Female	Male	Female	
1 : 1024	—	—	—	+(1)	—	—	—	—	—
1 : 512	—	—	—	+(3)	—	—	—	—	—
1 : 256	—	—	+(1)	+(5)	—	—	—	+(2) 1:60	—
1 : 128	—	—	+(3)	+(1)	—	—	—	+(3) 1:50	2 cases positive in female
1 : 64	—	—	+(1)	+(4)	+(1)	+(6)	+(2)	+(2) 1:40	—
1 : 32	—	+(5)	—	—	+(2)	+(3)	—	—	—
1 : 16	+(1)	+(2)	—	—	+(1)	+(3)	—	—	—
1 : 8	+(1)	+(2)	—	—	+(2)	+(1)	+(2)	+(2) 1:20	—
1 : 4	—	—	—	—	—	+(2)	—	+(1) 1:10	—

SPAT = Spermatozoa Agglutination Test.
PHAT = Passive haemagglutination Test (Tanned red cell).
BFT = Bentonite flocculation Test.
CFT = Complement fixation Test.
SPIT = Spermatozoa immobilization Test.

TABLE II

Showing Titres by Various Serological Techniques in Male and Female in Control Group (Using Donors Semen as Antigen)

Method	SPAT		PHAT		BFT		CFT		SPIT
Titres	Male	Female	Male	Female	Male	Female	Male	Female	
1 : 1024	—	—	—	—	—	—	—	—	—
1 : 512	—	—	—	—	—	—	—	—	—
1 : 256	—	—	—	—	—	—	—	—	—
1 : 128	—	—	—	—	—	—	—	—	—
1 : 64	—	—	+ (1)	+ (3)	+ (2)	+ (2)	—	+ (2)	—
								1:60	—
1 : 32	—	—	—	+ (1)	—	+ (1)	—	—	—
1 : 16	—	+ (1)	—	—	—	+ (2)	—	+ (2)	—
								1:40	—
1 : 8	+ (1)	+ (2)	—	—	—	—	+ (1)	+ (1)	—
								1:30	—
1 : 4	—	+ (2)	—	—	—	—	—	—	—

SPAT = Spermatozoa Agglutination Test.
PHAT = Passive haemagglutination Test (Tanned red cell).
BFT = Bentonite flocculation Test.
CFT = Complement fixation Test.
SPIT = Spermatozoa immobilization Test.

TABLE III

Showing Antisemen Antibodies Titres by Various Serological Techniques in Males and Females in Healthy Fertile Control Group (Using Husbands Own Semen as Antigen)

Method	SPAT		PHAT		BFT		SPIT
Titres	Male	Female	Male	Female	Male	Female	—
1 : 1024	—	—	—	—	—	—	—
1 : 512	—	—	—	—	—	—	—
1 : 256	—	—	—	—	—	—	—
1 : 128	—	—	—	—	—	—	—
1 : 64	—	—	—	—	—	—	1 Positive case in Female
1 : 32	—	—	—	+(1)	—	+(1)	—
1 : 16	—	—	+(1)	—	—	+(2)	—
1 : 8	+(1)	+(1)	—	+(3)	+(1)	—	—
1 : 4	+(2)	—	—	—	—	—	—
1 : 2	—	—	—	—	—	—	—

SPAT = Spermatozoa Agglutination Test.
SPIT = Spermatozoa immobilization Test.
PHAT = Passive haemagglutination Test.
BFT = Bentonite flocculation Test.

TABLE IV

Showing Comparative Per cent Positivity in Both Sterile (Test) Group and Healthy Fertile (Control) Groups as Shown by Various Techniques

Groups investigated	Total No. of cases	Per cent positivity (in terms of antispermatozoan antibody titres males and females)							Antigen used in groups
		SPAT		PHAT		BFT		SPIT	
		Males	Females	Males	Females	Males	Females		
Sterile couples (Test-Group)	45	4.4%	20.0%	11.11%	31.11%	13.33%	33.33%	4.4%	Husbands own semen as antigen in each couple.
Healthy fertile couples (Control Group)	30	3.3%	10.0%	3.3%	13.3%	3.3%	13.3%	3.3%	Husbands own semen used as antigen in each couple testing.
Difference of percentage in fertile control group		1.1%	10.0%	7.8%	17.8%	10.03%	20.03%	1.1%	

TABLE II

Showing Titres by Various Serological Techniques in Male and Female in Control Group (Using Donors Semen as Antigen)

Method	SPAT		PHAT		BFT		CFT		SPIT
Titres	Male	Female	Male	Female	Male	Female	Male	Female	
1 : 1024	—	—	—	—	—	—	—	—	—
1 : 512	—	—	—	—	—	—	—	—	—
1 : 256	—	—	—	—	—	—	—	—	—
1 : 128	—	—	—	—	—	—	—	—	—
1 : 64	—	—	+ (1)	+ (3)	+ (2)	+ (2)	—	+ (2)	—
1 : 32	—	—	—	+ (1)	—	+ (1)	—	1:60 —	—
1 : 16	—	+ (1)	—	—	—	+ (2)	—	+ (2)	—
1 : 8	+ (1)	+ (2)	—	—	—	—	+ (1)	1:40 + (1)	—
1 : 4	—	+ (2)	—	—	—	—	—	1:30 —	—

SPAT = Spermatozoa Agglutination Test.
PHAT = Passive haemagglutination Test (Tanned red cell).
BFT = Bentonite flocculation Test.
CFT = Complement fixation Test.
SPIT = Spermatozoa immobilization Test.

SPAT, using husbands own semen as antigen. A similar incidence has been reported by other investigators with this technique (Kibrick, 1952; Franklin and Dukes, 1964 and 1964 (a), 1968; Bo Fjallbrant, 1965; Israelstam, 1969; Glass and Vaidya, 1970; Ausbacher, 1971 (a) and Mohd. Hanifian, 1972). When PHAT, BFT, CFT techniques were employed using husbands own semen as antigen, the incidence of antisemen antibodies was seen to be higher in both the partners being 11.11 per cent, 13.33 per cent and 8.88 per cent respectively in males and 31.11 per cent 33.33 per cent and 22.22 per cent respectively in females the higher incidence found by these techniques indicated that the PHAT, BFT and CFT are qualitatively much more sensitive than the SPAT. The incidence of occurence of A.S.A. in females in infertile test group was significantly higher (20.0%) and the titres of A.S.A. when compared amongest the positive infertile females and fertile females were significantly higher in the former group (1.32 dilution).

Control group comprising of 30 healthy fertile couples when investigated for A.S.A. revealed 3.3% of males and 10.0% of females by S.P.A.T. using husbands own semen as antigen, Another fact regarding the titres of antibodies in this group reveals that the positive cases were of low titre group ranging from 1:2 to 1:16. A similar observation has been reported by other investigator with this technique of SPAT, using husbands own semen as antigen. (Kibrick, 1952; Franklin and Dukes, 1964 and 1968) Bo Fjallbrant, 1965; Israelstam, 1969; Glass and Vaidya, 1970; Ausbacher, 1971 and Mohd. Hanifian, 1972). When PHAT, BFT and CFT techniques have employed using husbands own semen as antigen, the incidence of antisemen antibodies was seen to be higher in both the partners being 11.11% 13.33% and 8.88% respectively in males and 31.11%, 33.33% and 22.22% respectively in females, the higher incidence found by these techniques indicated that the PHAT, BFT and CFT are qualitatively much more sensitive than SPAT. The incidence of occurence of antisemen antibodies in females in infertile test groups by basic screening test of SPAT was significantly higher (20.0%) and the titres of antisemen antibodies when compared amongst the positive infertile females and fertile females were significantly higher in the former group (1:32 ditation).

Control group composing of 30 healthy fertile couples when investigated for ASA revealed 3.3% of males and 10.6% of females by SPAT using husbands own semen as antigen. Another fact regarding titres of antibodies in this reveals that the positive cases were of low titre group ranging from 1:2 to 1:16. A similar observation has been reported by earlier workers. When case to case comparison was made in different groups, it was observed that with the use of PHAT, BFT and CFT, antibodies were not only detected in those cases in which such antibodies were found by SPAT but in addition, additional cases proved to be negative by SPAT techniques were discovered which actually contained antibodies in their sera. These observations force one to draw the invariable conclusion that PHAT, BFT and CFT are better, more reliable and more sensitive techniques in the detection of antisemen antibodies in the infertile human couples. In contrast SPIT and Immunodiffusion techniques were found to be much inferior than the former techniques, in as much as with the former techniques only 4.40 per cent im-

mobilising antibodies were found in females and none in males as compared to 3.3% in females of fertile control group (as shown in Table III).

In 2 cases autoagglutination of spermatozoa was noted in the ejaculates and in both of them High titres of antisemen autoantibodies were also observed Bofjallbrant, 1968, has noticed a significant correlation between sperm agglutination in the ejaculate, the cervical mucous penetrability of spermatozoa and sterility. Higher incidence of occurrence of antisemen antibodies in female partner may be related to their frequent exposure to seminal antigen there by expected to be much more responsive immunologically than the males. Only when antibodies were detected to their husbands semen these were also detected to the donor semen. The high levels of antibodies in females may have significant role in causation of infertility in these patients. On the basis of these findings, it is concluded that women react against the antigen present in the semen to variable extent.

Summary

Antisemen antibody levels were determined in 45 human couples of unexplained infertility by Spermatozoa agglutination test (SPAT), Spermatozoa immobilization test (SPIT), Tanned red cell agglutination test (Passive hemagglutination test) (PHAT) Bentonite flocculation test (BFT), Complement fixation test (CFT) and immunodiffusion procedures using whole semen of healthy donors as well as of infertile males as antigen. Antisemen antibodies were detected in 4.4 per cent by SPAT 0 per cent by SPIT, 11.11 per cent by PHAT, 13.33 per cent by BFT and 8.8 per cent by CFT in male partners and 20.0 per cent by SPAT, 2.0 per cent by SPIT, 31.11 per cent by PHAT, 33.33 per cent by BFT and 22.22 per cent by CFT in female partners using husbands own semen as antigen. When healthy donors semen was used as an antigen the percentage was 0 per cent by SPAT, 0 per cent by SPIT, 2.22 per cent by PHAT, 4.22 per cent by BFT and 2.22 per cent by CFT in males while in females Percentage positivity was 8.88 per cent by SPAT, 0 per cent by SPIT, 8.88 per cent by PHAT, 11.11 per cent by BFT and 11.11 per cent by CFT. For control 30 healthy fertile couples were included for the detection of antisemen antibodies in both male and female partners by SPAT, SPIT, as basic screening tests and additional PHAT and BFT using husbands own semen as an antigen. The antisemen antibodies were detected in 3.3% by SPAT 0.0% by SPIT, 3.3% by PHAT and 3.3% by BFT in males and 10.0% by SPAT, 3.3% by SPIT, 13.3% by PHAT and 13.3% by BFT in females using husbands own semen as an antigen.

The comparison of percentage positivity between infertile test group and fertile control group has been used. However with immunodiffusion precepit lines were seen only in 2 female patients against their own husbands seminal antigen. PHAT, BFT and CFT appear to be more sensitive techniques than SPAT and SPIT.

Antibodies were detected in relatively more patients by using patients semen as antigen as compared with donors semen. Amongst the 2 partners antibodies were detected more frequently in higher levels in females.

References

1. Ausbacher, R.: Fertil Steril. 22: 629, 1971.
2. Ausbacher, R. and Pangan, S. M.: Fertil. Steril. 22: 298, 1971.
3. Bloch, K. J. and Bunim, J. J.: J. Am. Med. Assoc. 169: 307, 1959 (clinical Pathology, Davidson Weils, 893, 1959).

4. Bo Fjallbrant.: Acta. Obstet. & Gynec. Scand. 44: 474, 1965.
5. Bo, Fjallbrant.: Acta. Obstet. & Gynec. Scand. 47: 102, 1968.
6. Boyden, S. V.: J. Exp. Med. 93: 107, 1951.
7. Dukes, C. D. and Franklin, R. R.: Fertil. Steril. 19: 263, 1968.
8. Doniach, D. and Roitt, I. M.: Lancet. 2: 1027, 1958.
9. Franklin, R. R. and Dukes, C. D.: J. Am. Med. Assoc. 190: 682, 1964.
10. Franklin, R. R. and Dukes, C. D.: Amer. J. Obstet. Gynec. 89: 6, 1964.
11. Glass, R. H. and Vaidya, R. A.: Fertil. Steril. 21: 657 1970.
12. Israelstam, D. M.: Fertil, Steril. 20: 275, 1969.
13. Kibrick, S., Belding, D. L. and Merril, B.: Fertil. Steril. 3: 419, 1952.
14. Landsteiner, K.: Zur Kenntinis der spezifish auf Blutkor perchen Wirken den. Sera Zbl. Bakt. 25: 546, 1899.
15. Mohd., J. Hanafiah, Jeannea, Epstein and Sobrero, A. J.: Fertil. Steril. 23: 493, 1972.
16. Metchnikoff, E.: Etudes Surla resorption des cellulens. Ann. X Inst. Pasteur. 13: 739, 1899.
17. Nakabayashi, N. T., Taylor, E. T. and Tyler, A.: Fertil. Steril. 12: 544, 1961.
18. Price I.N.O. (1950): Cited by R. Cruickshank in Medical Microbiology, 1965, E.S. Livingstone Ltd. London.
19. Phadke, A. M. and Padukone, K.: J. Reprod. Fertil. 7: 163, 1964.
20. Robert Cruickshank Medical Microbiology 11 Ed. E.L.B.S. and D.L.S. Livingston LTD (1965) p. 946.
21. Rumke, P.: Ann. N. Y. Acad. Sci. 124: 696, 1965.
22. Southerland, J. M. and Landing, B. H. Lancet, 2: 56: 1961.
23. Wilson, L.: Proc. Soc. Exp. Biol, N. Y. 85: 652, 1954.
24. Leslie, G. and Quinlivan: Fertil. Steril. 17: 722, 1966.