A COMPARISON OF SCREENING TESTS FOR ANTI-SPERM ACTIVITY IN THE SERUM OF INFERTILE WOMEN

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Summary. Evidence of humoral anti-sperm activity was sought in the female partners of 196 couples with unexplained and organic infertility, and in a control group of fifty pregnant women. Two tests were used, sperm micro-agglutination and sperm immobilization. The micro-agglutination test demonstrated little difference in incidence of positive reactions in patients with unexplained and organic infertility and yielded an 18% incidence in the pregnant control patients.

The results of the sperm immobilization test showed a clear disparity in patients with unexplained infertility compared with those in the organic infertility and pregnancy groups. A comparison of these results with those obtained by other workers, using the same and other methods, suggests that the sperm immobilization test provides the most reliable single method for the detection of circulating anti-sperm activity with possible clinical significance.

INTRODUCTION

The immunogenicity of mammalian semen has been studied in a variety of experimental models over the past 80 years (Behrman, 1968; Shulman, 1971). Experimental autoimmune (male) and isoimmune (male and female) infertility can be induced in laboratory animals by immunization with seminal components, and this has established implications for spontaneous and induced infertility in man. This rapidly developing field has been the subject of several recent reviews (Katsh, 1967; Behrman, 1968; Edwards, 1970; Jones, 1971; Shulman, 1971).

Clinical interest in sperm isoimmunization as a possible cause of infertility was aroused by the early report from Franklin & Dukes (1964) of sperm-agglutinating antibodies in the serum of women with otherwise unexplained inability to conceive. Since then, circulating anti-sperm antibodies have been detected by a variety of immunological techniques in both fertile and infertile women and this has led to confusion concerning their clinical significance.

This paper reports a comparison of two tests of humoral anti-sperm activity, sperm micro-agglutination and sperm immobilization, in the female partners of

couples with unexplained and organic infertility, and in a control group of fertile women.

MATERIALS AND METHODS

A total of 196 infertile couples was investigated. Organic factors were demonstrable in forty-three of these, and the remaining 153 were categorized as having unexplained infertility, following routine investigations which included semen analysis, testing for tubal patency, endometrial biopsy and monitoring of the basal body temperature. Patients with organic factors were arbitrarily divided into two groups. The first included those with lesions closely related to the pathogenesis of infertility, such as tubal occlusion and endometriosis. The second comprised those with organic factors of lesser significance, such as oligomenorrhoea corrected by ovulation induction and incidental pelvic surgery. In the unexplained group, there were 111 couples in which the female partner had never become pregnant (primary infertility) and forty-two couples in which conception had occurred previously but in which secondary infertility of at least 2 years' duration had developed.

Patients attended the laboratory where blood was collected from both partners, and a fresh semen specimen was obtained from the male. Sera were incubated at 56° C for 30 min to inactivate complement. Semen specimens were examined immediately: those with sperm densities of less than $40 \times 10^6/\text{ml}$ and with a sperm motility of less than 50% were considered unsuitable for testing.

Sperm micro-agglutination test

The method employed was that of Franklin & Dukes (1964) as modified by Schwimmer, Ustay & Behrman (1967a), using fresh semen from the male partner.

The specimens were prepared in serological tubes by adding 0.05 ml semen to 0.5 ml inactivated serum from the female partner. Semen was used in full strength, and in dilutions of 1:5 and 1:10 in Ringer's solution.

The tubes were incubated at 37° C and after $\frac{1}{2}$, 1, 2 and 4 hr, one drop was removed from each tube by Pasteur pipette and examined under the microscope.

The type of agglutination (head-to-head, tail-to-tail, or mixed) was recorded and the degree of reaction was rated as follows:

- 1+ single agglutination (two or three spermatozoa) per several high power fields (HPF)
- 2+ single agglutination/HPF
- 3+ large aggregate/HPF
- 4+ several large aggregates/HPF
- 5+ massive agglutination.

Agglutination of motile spermatozoa was considered necessary for a positive reaction. The aggregation of dead spermatozoa, or of live spermatozoa and debris, was considered a non-specific reaction.

The test was recorded as positive if it was rated as 1+ in at least two of the

samples or if it was 2+ or greater in one sample. It was found that agglutination was invariably of the head-to-head type.

Sperm immobilization test

The method used was that of Isojima, Li & Ashitaka (1968). Inactivated serum (0.25 ml) was added to fresh semen (0.025 ml) and guinea-pig complement (0.05 ml), and sperm motility was expressed as a percentage after incubation for 1 hr at 37° C.

Two tubes were used, the first (A) with the patient's serum and the second (B), a control with normal human serum. The ratio of % sperm motility in Tube B to that in Tube A gave the sperm immobilization value (SIV). An SIV of 2.0 represented a 2:1 ratio of sperm motility in the presence of control serum compared to that in test serum. A value greater than this was considered a positive result.

A control test for non-specific sperm immobilizing activity involved the incubation of test serum with fresh semen in the absence of complement.

Serum from male partners was used in a proportion of these tests but it was found that the interpretation of results was difficult when initial sperm motility was less than 80%. Accordingly, the use of semen from one of two donors of group O and containing at least 80% motile spermatozoa was used in all tests reported here.

Control patients

Fifty women in early pregnancy were utilized as a fertile control group and both tests were performed on their sera, using donor semen in each instance.

RESULTS

The results are summarized in Table 1. The sperm micro-agglutination test was performed for all but three of the 196 couples studied; these were cases of organic infertility in which semen from the male partner was unsuitable for testing. The sperm immobilization test was performed for 181 couples including the three excluded from the micro-agglutination test. Insufficient serum was available from fifteen patients with unexplained infertility to permit testing for sperm immobilization.

There was only a small difference in incidence of positive sperm micro-agglutination in patients with unexplained infertility (both primary and secondary) and those with major organic infertility (Group A). The fertile control group also yielded a significant incidence of positive micro-agglutination tests.

The results of the sperm immobilization test showed a clear disparity in the patients with unexplained infertility compared with those in the major organic infertility and pregnancy groups.

A small number of couples were classified as infertile with minor or indeterminate organic factors (Table 1, Group B). The results in these, though included for completeness, are of uncertain significance.

A comparison of results from 178 couples in which both tests were employed

failed to show a significant correlation between the tests. For 126 couples, both tests were negative; for forty-one, one was positive and the other negative; and for eleven, both were positive. It was noted, however, that when the immobilization test was positive, in only four instances was the micro-agglutination test negative, and for all seven couples with an SIV > 3.0, micro-agglutination was strongly positive.

Table 1. Results of sperm micro-agglutination and sperm immobilization tests

Category	Micro-agglutination		Immobilization	
	No. of patients	No. positive (%)	No. of patients	No. positive (%)
Primary unexplained infertility	111	29 (26.6)	101	12 (11.9)
Secondary unexplained infertility	42	12 (28.6)	37	3 (8.0)
Organic infertility*				
A	28	6 (21.4)	29	0 (0.0)
B	12	3	14	2
Pregnant	50	9 (18.0)	50	0 (0.0)

* A: Patients with lesions closely related to the pathogenesis of infertility (e.g. tubal occlusion; endometriosis); B: Patients with organic factors of indeterminate significance (e.g. oligomenorrhoea corrected by ovulation induction; previous laparotomy and incidental pelvic surgery).

DISCUSSION

The genital tract in the female is well endowed with immunologically competent cells which presumably process seminal antigens and mediate local immune responses. It is well established that cervical mucus contains γ -globulins (Moghissi, Neuhaus & Stevenson, 1960), and there is evidence (Hulka & Omran, 1969) that these include immunoglobulins G and A (including secretory IgA). The human cervix contains cells capable of secreting IgA (Hulka & Omran, 1969) and tissue antibodies have been demonstrated in the vagina, cervix and uterus of rodents (Paine & Behrman, 1968) and humans (Schwimmer, Ustay & Behrman, 1967b). Furthermore, Parish, Carron-Brown & Richards (1967) have reported the presence of complement-fixing antibodies cytotoxic to spermatozoa in the cervical mucus of three out of forty-eight infertile women.

Clearly, immunological effector mechanisms must exist at a cellular and secretory level within the genital tract but their detection and quantification need further refinement. The sperm micro-agglutination test has been widely used as a simple screening test but our data and those of others (Table 2) demonstrate a perplexing variation in the incidence of positive results in both infertile and control subjects. The relevance of the micro-agglutination test to immunological infertility is further questioned by its poor specificity in experiments *in vitro* (Quinlivan, 1966; Israelstram, 1969), and by the demonstration that its activity lies in the β -globulin rather than the γ -globulin fraction of serum (Boettcher & Kay, 1969).

Notwithstanding these findings, there was a notable association of strongly

positive micro-agglutination with high SIV values in several instances of unexplained infertility in this study. It is difficult to exclude a possible influence in some of sperm-agglutinating activity in a non-immunological fraction of serum and genital-tract secretions, though this possibility is hard to sustain in the face of the reports of a poor correlation between positive sperm micro-agglutination and an abnormal post-coital test (Schwimmer *et al.*, 1967a; Glass & Vaidya, 1970).

Table 2. Incidence of positive sperm micro-agglutination tests in unexplained infertility

Author	Infertility category	No. of patients studied	Positive tests (incidence %)	Controls (incidence %)		
				Fertile	Unmarried	Organic infertility
Tyler <i>et al.</i> (1967)	Unstated	43	14.0	0.0		
Schwimmer <i>et al.</i> (1967a)	Primary	64	37.5	34.1	20.0	20.0
	Secondary	32	50.0			
Boettcher & Hay (1968)	Unstated	42	19.0			
Malinak <i>et al.</i> (1968)	Primary	127	48.0	13.0		
Israelstram (1969)	Unstated	45	7.0			
Glass & Vaidya (1970)	Primary	77	17.0			4.3
	Secondary	45	24.0			
Isojima (1972)	Unstated	72	37.5*	45.8*	6.3*	22.4
Present series	Primary	111	26.6	18.0		18.0
	Secondary	42	28.6			

* Using donor semen.

Confusion also attends the results of a variety of other methods for the detection of humoral anti-sperm antibodies in infertile women (Table 3). This variation in incidence can, in part, be attributed to the multiple antigen-antibody systems involved (Shulman, 1971), but the lack of clear discrimination in favour of unexplained infertility raises doubts concerning the clinical usefulness of these tests.

Our results with the sperm immobilization test confirm (Table 4) those of Isojima (1972). By contrast with other methods, the absence of positive reactions in the control groups suggests that sperm immobilization provides a reliable means of detecting humoral anti-sperm activity with possible clinical significance. This view is supported by fractionation studies of positive sera from women with unexplained infertility, in which the sperm immobilizing activity is evident in the IgG and IgM fractions (Isojima, 1972; Boettcher, 1972).

There is obviously a need for more complete information relating to the relative importance of seminal antigens, and to their specificity in the induction of immunological infertility. The recent demonstration of the presence of histocompatibility (HL-A) antigens on human spermatozoa (Fellous & Dausset, 1970), foreshadows the application of more precise methodology to the investigation of sperm isoimmunization.

The present study is part of a project designed to establish the natural

Table 3. Incidence of positive tests for anti-sperm activity in infertile patients

Author	Method	No. of patients studied	Positive tests (incidence %)	Controls (incidence %)		
				Fertile	Unmarried	Organic infertility
Rao & Sadri (1959)	Macro-agglutination	10*	60.0	53.8		
Katsh & Katsh (1965)	Immunodiffusion	45*	35.5	16.0		
Behrman (1968)	Haemagglutination	406*	14.0			
Isojima (1969)†	Macro-agglutination	10†	60.0	53.8	11.1	36.4
	Complement fixation	24†	4.2	0.0		0.0
Hjort & Hansen (1971)	Immunofluorescence	29†	65.5	62.0		77.2

* Unselected infertility. † Unexplained infertility. ‡ Using donor semen.

reproductive history of patients with circulating anti-sperm antibodies. It also aims to assess the outcome of a therapeutic approach to such patients, since early reports suggest that, if conversion to negative serological findings can be achieved by an occlusion regimen (male partner using condom), then significant pregnancy rates can be anticipated (Behrman, 1968; Glass & Vaidya, 1970).

Table 4. Incidence of positive sperm immobilization tests in unexplained infertility*

Author	Infertility category	No. of patients studied	Positive tests (incidence %)	Controls (incidence %)		
				Fertile	Unmarried	Organic infertility
Isojima (1972)	Unstated	74	17.6	0.0	0.0	
Present series	Primary	101	11.9	0.0		0.0
	Secondary	37	8.0			

* Using donor semen.

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