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A MODIFIED AND IMPROVED IMMUNOBEAD TECHNIQUE FOR DETECTING SPERM ANTIBODIES

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A MODIFIED AND IMPROVED IMMUNOBEAD TECHNIQUE FOR DETECTING SPERM ANTIBODIES

By

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ABSTRACT

A MODIFIED AND IMPROVED IMMUNOBEAD TECHNIQUE FOR DETECTING SPERM ANTIBODIES

By

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In this study, the immunobead test (IBT), a novel method for detecting sperm antibodies, was modified and evaluated as an alternate method to screen serum and fluids. The study consisted of modifying the procedure to reduce volumes of buffer and specimens by half. This made it possible to adapt the technique to a microplate and allowed testing of a larger number of patients in a shorter time, at less cost.

A comparison study was performed with the Kibrick or gelatin agglutination test(GAT) on 102 sera and 51 seminal plasma specimens from patients with infertility problems. More than 95% of positive GAT sera and seminal plasmas tested positive by the IBT assay. Groups of controls were assayed in parallel studies and from this a specificity and sensitivity greater than 95% was obtained for the indirect IBT assay.

The IBT technique is therefore an excellent screening and evaluation method for sperm antibodies.

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INTRODUCTION

Infertility, as a clinical entity, can be defined as a state in which a couple cannot achieve pregnancy within a one-year period in the absence of birth control. It has been estimated that up to 20% of the couples in the United States are infertile by this definition (Shulman, 1986). About 10% of these cases are due to the presence of antibodies against sperm in the serum (Shulman, 1986). Fertilization of eggs by sperm, the means by which sexual reproduction takes place in nearly all multicellular organisms, is fundamental to the maintenance of life.

Sperm is made up of tail, midpiece, and head. The tail is involved in the sperm's forward progression. The head contains the nucleus and acrosome. The acrosome is a membrane bound lysosome-like organelle that occupies the anterior region of the sperm head just above the nucleus and beneath the plasma membrane.

The egg in the mammalian is surrounded by a thick extracellular coat called the zona pellucida. Inside the to the zona pellucida is a space called the perivitelline space. The cytoplasmic side of the egg's plasma membrane is covered by lysosome like organelles called cortical

granules. These granules contain various hydrolytic enzymes such as protinases and peroxidase.

The pathway leading to fertilization is similar in all mammalians. It occurs in a compulsory order of steps. First, sperm associates with an egg at the surface of the egg's thick extracellular coat, the zona pellucida. This is a loose nonspecific association between sperm and egg, and is referred to as attachment. Second, the attached sperm can form a tenacious species-specific association with the egg, a step referred to as binding. Binding is mediated by sperm receptors present in the zona pellucida and complementary egg binding proteins present on the sperm plasma membrane. Next, bound sperm then completes what is known as the acrosome reaction in preparation for penetration of the zona pellucida and fusion with the egg plasma membrane. The acrosome reacted sperm then penetrates the zona pellucida by using an atrypsin like protienase, acrosin, that is associated with the inner acrosomal membrane. Finally, sperm that reaches the space between the zona pellucida and egg plasma membrane can then fuse with the egg. Fertilization induces the release of cortical granule contents into the perivitelline space. The contents of the cortical granules enter the zona pellucida and are responsible for inducing the zona reaction. The zona reaction consists of a general hardening of the zona

pellucida as well as loss of its ability to bind sperm. These changes are thought to contribute to a slow block of polyspermy.

Sperm is considered to be antigenic even within the male reproductive tract. Spermatozoa, in the later stages of meioses and subsequent spermatogenesis, are sequestered within the lumen of the seminferous tubules by a blood-testis barrier. Leakage of sperms and seminal products induced by vasectomy, trauma, testicle damage, or infection may, therefore, elicit an autoimmune response in man by exposing the immune system to sperm.

The nature of the antigenic determinates on sperm and the degree to which they stimulate antibody production is not clearly understood. Hjort et al. suggested the existence of at least three different antigens, two glycoproteins with rather wide distribution in the membrane and one, as yet uncharacterized, antigen which is apparently restricted to the tail piece. One piece of evidence suggests that in human sperm reactive antibodies may be directed against sperm surface coats derived from the seminal plasma (Bronson, 1984).

Sperm antibodies are detectable in the serum of both male and females, as well as in the cervical mucus of approximately 5% of sexually active women in North America. There are many possible causes of sperm antibodies developing in the female. Unlike the male,

the entire female reproductive tract has immune competent cells, e.g., lymph nodes, macrophages, and T-lymphocytes. The female, therefore, can actively form alloantibodies to a wide variety of sperm antigens. However, the majority of women do not respond to sperm by forming antibodies. There is evidence that the female immune system may regularly respond to antigens present in the ejaculate (Alexander, 1981). In mice there is an increase in the weight of the draining lymph nodes following exposure to sperm (Beer, 1978) It is possible that this lymph node response represents activation of a suppressive immune response. Evidence also has been found that the ejaculate contains factors which blunt the immune response, probably playing a role in allowing coitus without subsequent immunization against sperm (Bronson, 1984). A high molecular weight immunosuppressive factor, identified in seminal plasma, has been shown to impair lymphocyte activation in both mixed lymphocytic cultures and response to mitogens (Lord, 1977).

The extent to which antisperm antibodies are present in the reproductive tract of both males and females would be expected to influence the degree of fertility impairment. In the reproductive tract, the presence of antibodies on the sperm surface may lead to complement mediated cell lysis (Bronson, 1982), or

enhancement of phagocytosis by macrophages (London, 1985). During gamete interaction, antibodies could also interfere with cumulus dispersion (Tazorto, 1979), or sperm binding to the zona pellucida (Bronson, 1982a) and, as a consequence, prevent fertilization.

Circulating antibodies are detected by a battery of laboratory tests that range from simple agglutination and immobilization techniques to the complex Enzymes Linked Immunosorbent Assays (ELISA) and the Immunobead Test (IBT). The two standard methods that are still widely used are the Kibrick agglutination test and the Isojima Immobilization assay.

The Kibrick test was first described in 1952, and has been modified by Shulman et al. (Shulman, 1985). This assay is performed by first diluting a donor semen with an equal volume of 10% gelatin. The serum to be tested is then heat inactivated to destroy complement activity. Equal volumes of diluted serum and sperm suspension are then mixed together. The mixture is transferred to a small tube and incubated at 37C for two hours. The presence of antibodies in serum would result in agglutination of the donor sperm. Flocculation with a clear to slightly turbid background indicates a positive reaction.

The Isojima test was described in 1968 (Isojima, 1968). This assay measures complement dependent

antibodies which have cytotoxic affects and result in the immobilization of sperm. A fresh donor semen with good motility is diluted with buffer. Serum to be tested is heat inactivated, and a mixture of donor semen, complement, and serum is incubated at 37C for one hour. A decrease of 50% or more in the motility of the sperm compared to a control tube that contains everything except complement is considered positive.

The effectiveness of these two tests has been challenged in reference to their specificity, sensitivity, and nonspecific agglutination. High levels of B-lipoprotein in specimen will cause false aggutination with the Kibrick (GAT). As a result, the GAT has a built-in dilution of 1:4 to reduce the levels of B-lipoprotein (Bronson, 1981). Antibodies formed during genital tract infection will cross react with sperm. These antibodies will act like sperm antibodies, and will dissappear with the treatment of infection. These tests are gradually being replaced by better assays including the Immunobead test.

The IBT utilizes polyacrylamide beads which have been coated with rabbit antihuman globulin of IgA, IgG, or IgM types. Some of the beads are also bound to subtypes of immunoglobulins including IgA1 and IgA2. When the beads are mixed with sperm coated with humoral antibodies, agglutination occurs between the sperm and

the beads. This method detects auto-antibodies coating the patient's own sperm in a direct binding assay. It is also possible to identify free sperm antibodies that can be found in serum, seminal plasma, and cervical mucus in an indirect procedure. The specimen is first inactivated at 56C for 30 min. Equal volumes of specimen and adjusted donor semen are mixed and incubated at 37C for 30-60 min. The donor semen is washed three times after the incubation period. Equal volumes of beads and semen are mixed on a slide and the percentage of beads bound is determined.

The use of the Immunobead procedure for detection of sperm antibodies has been utilized by a group of investigators to study the production of sperm antibodies locally and systemically (Clarke et al., 1985b; Shulman et al., 1985; and Parslow et al., 1985). Clarke et al. used the indirect IBT assay to determine the classes of circulating antibodies in infertile men and women with serum immobilization. Of the 20 infertile men, 100% were found to be positive for sperm bound IgG, 50% positive for IgA, and 0% positive for IgM. Similarly, using the IBT, of 20 infertile females, 95% were positive for IgG, 60% for IgA and 15% for IgM. This study indicated that IgG and IgA are the two major immunoglobulin classes of sperm antibodies in serum (Clarke, 1985b).

Another group of investigators discovered that the type of sperm antibodies produced may vary according to the stimulus for production (Parslow, 1985). Two groups of patients (vasectomy reversal and spontaneously infertile) had similar ranges of antisperm antibody titers in serum and seminal plasma when tested by agglutination methods which have no obvious specificity for particular immunoglobulin classes. The agglutination test failed to distinguish important variations in the classes of the antisperm antibodies present in clinically different groups of patients. When the two groups were evaluated with the indirect IBT technique, the IgG levels showed no difference between the patient groups. However, the IgA levels were significantly higher in the spontaneously infertile group. No explanation for this observation was given by the investigators (Parslow, 1985).

Shulman et al. (1985), in a study similar to the present one, evaluated the indirect IBT test for sperm antibodies in the sera of a number of infertility patients. The results were compared to the corresponding results found for the same serum samples with the Kibrick test. There was excellent agreement between the IBT and the GAT in 90% of the positive serum evaluated. The number of tail binding antibodies was very close to the number that were GAT positive. The most frequent classes

found among the sera were of the IgG type followed by IgA (Shulman, 1985).

The present study consists of two parts. The first part is an evaluation of the indirect IBT in reference to the standard GAT procedure. The indirect IBT that was adopted by the World Health Organization (WHO) was further modified by reducing the volumes of buffer and specimens used in order to enhance the use of this assay for clinical screening. A comparison study was then performed on seminal plasma from infertile patients using the Kibrick (GAT) and indirect IBT.

The second part of the study consisted of adopting the IBT assay to a microplate technique. This technique allowed the testing of a large number of patients in a shorter time and at less cost. A twofold serial dilution was run on specimens found to be positive by the IBT to determine its ability to quantitate sperm antibodies efficiently.

MATERIALS AND METHODS

Materials

Buffers

Dulbecco's Phosphate Buffer containing 0.3% Bovine Serum Albumin (D.PBS 0.3% BSA, PH 7.4) was obtained from Gibco Laboratories for the purpose of washing the donor semen. The D.PBS was dissolved in one liter of distilled water. Then 0.3% BSA was prepared by dissolving 0.6 gram of BSA in 200 ml of D.PBS. The D.PBS 0.3% BSA, PH 7.4, was sterilized by passing it through a 22 um micropore filter using positive pressure. The buffer was then stored in a sterilized bottle at 2-8C.

Immunobead Reagents

The immunobeads were obtained from Biorad Laboratories (Richmond, CA) and consisted of polyacrylamide beads 5-10 um in diameter with covalently bound rabbit antibodies to human immunoglobulin classes IgG, IgA, or IgM. A 5mg/ml suspension of the beads was made with D.PBS and stored at 4C for 4-6 weeks. The IgM beads were not used in this study due to the low titers of IgM antibodies.

Controls and Specimens

Positive and negative controls for both IgG and IgA from previously tested patients were stored in the freezer at -28C. Serum and seminal plasma from infertile patients suspected of immunological infertility were frozen and then stored at -28C. These specimen were evaluated for sperm antibodies using the Kibrick and Isojima assays. Serum and seminal plasma were incubated at 56C for 30 min to inactivate complement activities prior to performing the assay. Seminal plasma was prepared by transferring semen to one or two bullet tubes and centrifuging at 10,000X G for 10 min. The supernatant was then used for testing. Cervical mucus was not evaluated in this study due to its very small recovery from patients. Most, if not all, of it is consumed by testing with the Kibrick and the Isojima assays.

Donor Semen Preparation

Donor semen was collected into a sterile specimen container and delivered to the lab at Sparrow hospital within one hour. A minimum count of 50 million sperm, with a percent motility of 60-80%, normal morphology and a minimum of 2+ forward progression was required. Counts greater than 50 million were diluted with prewarmed D.PBS 0.3% BSA to a final count of 50 million.

Gamma Micro U System Equipment

This system consists of three major parts that include the micro U plates, the Dispense and Wash system, and the Incubation and Time block. The micro U plates are molded from crystalline polystyrene and are permanently numbered to identify the wells in each horizontal and vertical row. The micro U plate contains three rows of seven round bottom micro wells. The Dispense and Wash (D&W) is an enclosed pump designed to deliver a premeasured volume of buffer through a manifold. The Incubation and Time block (I&T) is a dry block incubator which has a clear plastic cover to prevent excessive evaporation of buffer.

Methods

Part One: Performing the IBT Using the Standard Tube Method

Twelve x seventy-five test tubes were used to incubate 100ul of specimen or control with 100ul of donor semen in a 37C water bath for 30-60 min. For washing purposes, 12 ml tubes with a cone shape bottom were used to wash the beads and donor semen after the incubation period. Specimens and controls were transferred to their corresponding tube and were washed three times with 1.5 ml of D.PBS, 0.3% BSA followed by centrifugation for 5 mins. The beads were washed twice with 2.5 ml of the

same buffer. During the washing steps the supernatant was discarded to avoid any neutralization of the beads with free sperm antibodies. After the last wash, the pellets were resuspended with 100ul of buffer.

On a labeled slide, 5ul of the beads were mixed with 5ul of donor semen and a cover slip was applied. The slides were transferred to a moist chamber for 10-15 min at room temperature before evaluation. The slides were read using a phase contrast microscope and a 40X objective. In each field the number of sperms having one or more beads bound as well as the number of unbound sperms were counted to a total of 100 sperm. The number of bound sperm was then divided by the total number of sperm counted (100). This gave a percentage of binding. Any sample with greater than 10% binding was considered positive (Shulman, 1985).

Part 2: Performing the IBT Using the Micro U-Plate Technique

To each labeled well 35ul of specimen or control and 35ul of donor semen were added. The plate was then incubated in the I&T dry block incubator at 37C for 30-60 min. After incubation the plate was transferred to the center of the D & W system. This system allowed a rapid wash of the sperm with 400 ul at buffer.

The plates were then centrifuged and the supernatant removed by placing a micro U clamp on the

rotor, which holds the plate securely in place. While the rotor is inverted, a sharp downward motion over a waste container will remove most of the supernatant. The sperms were washed an additional three times with 400 ul each of buffer. The beads were washed twice just as in part one of this study.

On a labeled slide, 5ul of the beads and 5ul of the washed donor specimen were mixed, and a cover slip was applied. The slides were then transferred to a moist chamber for 15-20 min at room temperature. Samples having a percentage of attachment > 10% were considered positive (Shulman, 1985).

Titration of Positive Serum and Seminal Plasmas

Positive serum and seminal plasmas were titered using twofold serial dilutions. Using the D.PBS, 0.3% BSA buffer, PH 7.4, dilutions varying from 1:2 to 1:512 were carried out using the indirect IBT standard tube method. The end point of the test was the last tube showing less than 10% binding to the beads.

RESULTS

Comparison Between the IBT and GAT in Serum

A total of 102 sera from patients with infertility problems were evaluated. This testing resulted in 22 positive and 80 negative.

Table 1 summarizes data obtained when comparing the standard GAT to the IBT procedure. The IBT was positive in 95% of sera that were positive by the standard GAT. One patient who was positive by the GAT was negative by IBT. The IBT conversely was positive in four sera which were negative by the Kibrick Assay.

Table 1. Summary of Data Obtained When Comparing Standard GAT to the IBT Procedure

GAT	N	IBT(+)	IBT(-)
Positive	19	18(95%)	1(5%)
Negative	83	4(5%)	79(95%)

Note: N = Number of positive or negative serum by GAT.

Comparison Between the IBT and GAT in Seminal Plasma

A total of 51 seminal plasmas were evaluated. This testing resulted in 12 positives and 39 negatives. Table 2 summarizes the data obtained when comparing the standard GAT and IBT assay in the seminal plasmas. The IBT is negative in 87% of seminal plasmas that are also negative by the GAT. The remaining 13% were positive by the IBT technique.

Table 2. Summary of Data Obtained When Comparing the Standard GAT and IBT Assay in Seminal Plasma

GAT	N	IBT(+)	IBT(-)
Positive	6	6(100%)	0
Negative	45	6(13%)	39(87%)

Note: N = Number of positive or negative seminal plasma by GAT.

Comparison of Immunoglobulin Classes in Serum and Seminal Plasma

Table 3 summarizes types of antibodies involved in specimens found to be positive by both assays. Data demonstrates that 61% of sera positive by both assays were of the IgG and IgA types, with 39% of the specimens

Table 3. Summary of the Type of Antibodies Involved in Specimen Found to be Positive by Both Assays

Positive GAT & IBT	N	IgG only	IgA & IgG	IgA only
Serum	18	7(39%)	11(61%)	0
Seminal Plasma	6	0	5(83%)	1(17%)

of the IgG type only. None of the sera was positive for IgA alone.

Of the seminal plasma positive by both assays, 83% were of the IgA & IgG types. Binding to the IgA type only was found in 17% of the seminal plasma. No binding to the IgG type only was found when testing seminal plasma.

Table 4 summarizes the types of antibodies involved in specimens found to be positive by the IBT but negative by the GAT assay. The four sera positive by IBT, and negative by GAT were of the IgA type only. In seminal plasma, on the other hand, the IgA type only was found in 33% of the specimens. Of the remainder of the positive seminal plasma, 67% were combined IgA and IgG classes.

Table 4. Summary of the Types of Antibodies Involved in Specimen Found to be Positive by the IBT but Negative by the GAT Assay

Positive IBT & Negative GAT	N	IgG only	IgA & IgG	IgA only
Serum	4	0	0	4(100%)
Seminal Plasma	6	0	4(67%)	2(33%)

Comparison of Attachment Sites in Both Serum and Seminal Plasma

A primary advantage of the IBT assay is the ability to determine the anatomic attachment sites of antibodies on the sperm's surface. These sites range from binding of the immunobead to the sperm head, tail, and in some cases, to both head and tail (Figures 1, 2 and 3).

Table 5 summarizes different attachment sites on the sperm's surface among positive sera and seminal plasma specimens. In considering the sperm attachment sites in 18 of the positive serum by IBT and GAT assays, IgG was involved in the binding to the tail only in 50% of the specimens. Binding of IgG to the head and tail occurred in 39%. The exclusive binding of IgG to the head only was found in 11% of positive sera. The IgA class in positive sera was involved in the binding to the

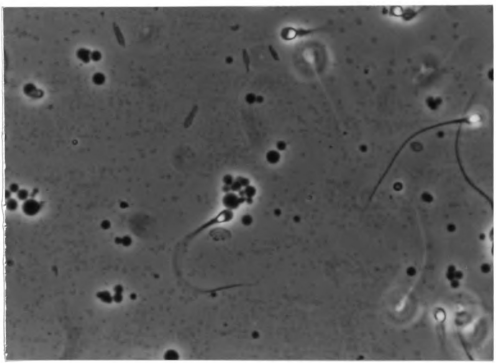

Figure 1. A Photograph of a Positive Immunobead Reaction. The beads are bound to the head. (400x)

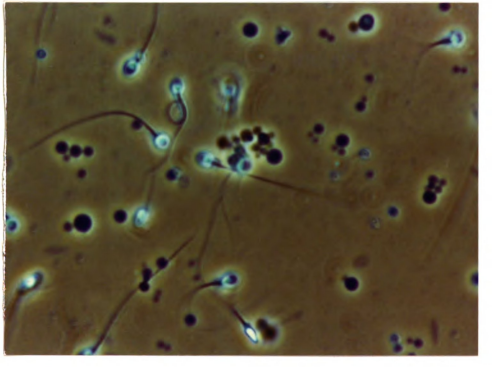

Figure 2. A Photograph of a Positive Immunobead Reaction. The beads are bound to the tail. (400X)

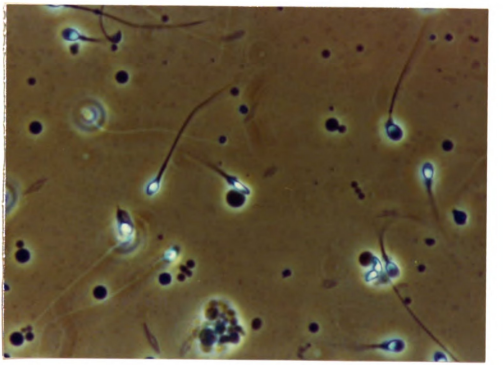

Figure 3. A Photograph of a Positive Immunobead Reaction. The beads are bound to the head and tail. (400X)

Table 5. Comparing Different Attachment Sites Among Positive Serum and Seminal Plasma Specimens

	18 GAT & IBT Positive Sera		6 GAT & IBT Positive Seminal Plasma	
	IgG n=18	IgA n=11	IgG n=5	IgA n=6
Head only	2(11%)	0	0	0
Tail only	9(50%)	7(64%)	3(60%)	4(67%)
Head & Tail	7(39%)	4(36%)	2(40%)	2(33%)

tail in 64%. Binding of IgA to the head and tail was observed in 36% of positive serum by IBT and GAT and no IgA was seen to bind to the head only.

In considering the attachment sites among the 6 positive seminal plasma by both IgG and GAT assays, the IgG class was involved in binding to the tail only in 60% of the specimens. It was found that 40% of the IgG class bound to both the head and the tail. The IgA beads bound to the tail in 67% of positive seminal plasmas by IBT and GAT. The remaining 33% were bound to both head and tail.

Comparison Study Between the Tube and Microplate Methods

In a second study, the microplate method was used to evaluate random positive and negative seminal plasma.

In this study, 51 sera and 25 seminal plasmas from the initial study were retested in a microplate assay. Identical results were obtained between the standard method and the microplate assay.

A group of 52 sera from women who recently delivered and 34 seminal plasmas from apparently normal men were evaluated using both the standard IBT and the IBT modified microplate technique. Table 6 shows that 4% of the negative serum control and 3% of the negative seminal plasma controls were positive by the IBT assays.

Table 6. Control Groups for Both Sera and Seminal Plasma Using Standard Tube and Micro U-Plate Techniques

	Technique	
	Standard Tube	Micro U-Plate
Serum		
Positive-IBT	2(3.8%)	2(3.8%)
Negative-IBT	50(96.0%)	50(96.0%)
Seminal Plasma		
Positive-IBT	1(2.9%)	1(2.9%)
Negative-IBT	33(97.0%)	33(97.0%)

Determining the Sensitivity and Specificity for the IBT Assay

Assuming the GAT assay is the gold standard test for immunoglobulin testing, the sensitivity and specificity of the IBT was calculated. From testing serum and the control groups using the IBT, a sensitivity of 95% and specificity of 96% were established.

Evaluating seminal plasma and their control group, the IBT resulted in a specificity of 97% and a sensitivity of 100%.

Results of the Two-Fold Dilution

Some of the positive specimens by IBT were titered in a serial twofold dilution to quantitate the level of sperm antibodies (Table 7). There was a fluctuation in the percentage of binding along the twofold dilution. There was no graduated decrease in the level of antibodies as the dilution increased. Some of the specimen had a dilution greater than 512.

Table 7. A Two-Fold Serial Dilution Titer of Positive Serum Using the IBT Standard Tube Method

Positive Serum with IBT		Dilution Factor*								
		2	4	8	16	32	64	128	256	512
P.D.	IgG	12	10	<10						
	IgA	Neg								
F.R.	IgG	96	90	50	60	24	32	14	<10	
	IgA	34	16	22	20	4				
L.M.	IgG	92	86	90	78	74	72	52	<10	
	IgA	Neg								
R.F.	IgG	52	56	68	54	<10				
	IgA	Neg								
K.D.	IgG	80	80	86	88	82	56	56	<10	
	IgA	84	46	18	<10					
J.S.	IgG	98	98	95	94	92	93	86	74	86
	IgA	83	78	77	60	61	29	30	13	<10

*Results indicate % positive.

DISCUSSION AND CONCLUSION

Discussion

The presence of antisperm antibodies has been associated with clinical infertility. These antibodies may be present in male and female reproductive tracts. The molecular biology of the reproductive immunology is only partially understood due to the inability to define a specific antigenic determinante(s) on the membrane of male and female gametes.

In the male and female reproductive tract, the presence of sperm antibodies may lead to complement mediated cell lysis (Bronson, 1982) or enhancement of phagocytosis by macrophages (London, 1985). Additionally, sperm antibodies may directly affect gamete interaction by interfering with cumulus dispersion (Tzortos, 1979), sperm binding to the zona pellucida (Bronson, 1982) and sperm penetration to the zona pellucida (Clarke, 1988).

The IBT has achieved a greater acceptance among researchers and clinical specialist for evaluating sperm antibodies. This is the result of the ability of the IBT to differentiate among the various classes of immunoglobulins. The present study further substantiates

this acceptance. The IBT exhibited an excellent correlation with the standard GAT assay. It was able to detect more than 95% of GAT positive sera and seminal plasma (Tables 1 and 2).

The apparent false positive GAT in (Table 1) may have resulted from the presence of high titers of B-lipoprotein. These proteins are known to interfere with agglutination based assays (Bronson, 1981). The comparison study indicated that some of the negative GAT specimens were positive by the IBT assay (Table 1). Further evaluation indicated that the positive IBT sera were of the IgA class only. This demonstrated that the IBT was capable of detecting classes of immunoglobulin not detected by the gelatin agglutination test. This finding is consistent with the known operational characteristics of the GAT which detects only IgG, IgM, or both (Shulman, 1981).

Seminal plasma testing indicates that 14% of the GAT negative specimens were positive by the IBT (Table 2). The immunoglobulin classes involved, as determined by the IBT, were of the IgA class although a small number were of both the IgA and IgG classes. This testing indicates a limitation of the GAT in detecting sperm antibodies in seminal plasma. As with serum, these results indicate the inability of the GAT to detect certain classes of immunoglobulin. This may have

resulted from the presence of secretory IgG or IgA which are not detected by GAT in seminal plasma.

One of the advantages of the IBT is the ability of this assay to detect a specific anatomic site on the sperm, e.g., tail, head, or a combination of both sites. The detection of binding sites is now being shown to be an important factor in evaluating antisperm antibodies. Carson (1988) has demonstrated that the binding of antibodies to the sperm tail impedes sperm migration through the cervical mucus microenvironment. This binding is thought to have little effect on the ability of sperm to fertilize the egg, yet it is able to markedly decrease the number of sperm that are able to reach the egg. Binding to the head has been demonstrated to play a significant role in interfering with sperm penetration through the zona pellucida of free hamster ova (Bronson, 1982a).

The present study indicates that the most prevalent anatomic site of antibody binding in specimens positive by the GAT and the IBT assays were toward the tail for both the IgG and the IgA class (Table 5). This correlates very well with the accepted concept that GAT positive specimens contain tail directed antibodies (Shulman, 1978).

Subclassification of antibody binding enables the clinician to select an appropriate therapy which would

enhance the probability of achieving fertilization, e.g., the use of Gamete Intrafallopian Transfer (GIFT) or in vitro fertilization (IVF).

A group of controls including 52 sera from recently delivered women and 34 semen from apparently fertile men were evaluated (Table 6). Approximately 4% of the sera and 3% of the seminal plasma specimens were positive by the IBT. The positive IBT in the control group was not thought to be due to a false positive reaction. First, the principle of the IBT test is based on specific antibody-antigen binding. The washing steps in the procedure minimize any false interference with the binding of the beads to the sperm. This was in part due to the removal of free antibodies and high levels of B-lipoprotein that may induce false positive results. Second, these results are consistent with the findings of Bronson et al. (Bronson, 1981) who demonstrated low and intermediate levels of sperm antibodies in an apparently normal individuals. In some individuals, these antibodies have not significantly affected the reproductive potential.

In the second part of this study, the indirect IBT was further refined in terms of volumes of buffer and specimen used. The procedure was adapted to a microplate technique that utilizes the Gamma Micro U- System. This system allows rapid and economic testing of large numbers

of patients. Fifty-one sera and 25 seminal plasmas from previously tested specimens, including the controls, were reevaluated. Identical results were obtained for both the specimens and controls.

No final conclusion about the ability of the twofold dilution technique to quantitate accurately sperm antibodies in positive specimens can be made. The percentage of the beads bound to the sperm did not decrease linearly as the dilution increased as was expected. This may be due to the fact that the number of sperm antibodies required for the binding of one bead is not known. Therefore, the number of beads bound does not necessarily correlate with the amount of antibodies. This was reflected in the fluctuation in the percentage binding of sperm antibodies when positive specimens were titered (Table 7). However, the IBT may be capable of providing a qualitative estimate of the concentration of sperm antibodies. This would be of great help to the physician in following patients undergoing immune suppression therapy for sperm antibodies.

Conclusion

The IBT has been shown to be an excellent screening and evaluation method for sperm antibodies. It provides data on the immunoglobulin classes and regional specificity of the antibodies. However, the present role

such antibodies play in infertility is not totally understood. Immotility and agglutination of sperms are two possible consequences of antisperm antibodies.

Other antibodies, by coating the spermatozoa, may interfere with capacitation or egg penetration. Therefore, the characterization of sperm antibodies is important to an understanding of immunologically mediated infertility.

The present study demonstrated that the IBT is a much better assay than the GAT method for detecting sperm antibodies. The IBT had a sensitivity and specificity greater than 95%, with an agreement of more than 95% with specimens positive by the GAT. The IBT was able to detect specimens with secretory immunoglobulins that tested negative by the GAT method. These classes can play an important role in delaying fertility due to their close association with the sperm.

The reduction in the large volumes of buffer and specimens that have been traditionally used to perform the Immunobead method made it possible for the IBT to become a rapid screening method. It is able to produce specific and precise results with fewer false positives and negatives. Titration of positive specimens by a twofold serial dilution was very helpful in providing a quantitative estimate of sperm antibody concentration.

This will serve as a good tool for the physician in following up patient's therapy.

Performing the serial dilution using the standard tube method proved to be a time-consuming process. Adaption of the IBT procedure to a micro U-plate technique could be helpful in the future in reducing time required to titer positive specimen.

For future evaluation, the IBT assay can serve an an excellent tool in evaluating the relationship of sperm antibodies in serum to cervical mucus. Utilizing beads with antihuman secretory immunoglobulin will also helps determine how many of the sperm's antibodies present in cervical mucus or seminal plasma are due to secretory immunoglobulin classes. Titration of patients, while underoing immunosuppressive therapy using the IBT assay, should provide an idea about the accuracy of using the IBT as a titrating method and to follow up the progress of patients being treated.

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