

# The Role of Sperm-Bound Antibodies in Infertile Men in Iraq

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## Abstract

**Objective:** Antisperm antibodies need to be considered in particular cases of infertility. This study was carried out to determine the prevalence of clinically significant sperm bound antisperm antibodies (ASAs) in infertile men with abnormal seminal fluid parameters, to study the effects of those antibodies on different seminal parameters and to demonstrate the relationship between those antibodies and various male genital tract abnormalities that may coexist or play a role in their development.

**Patients and methods:** 118 infertile patients with abnormal seminal fluid parameters were followed prospectively in the Medical city Hospital between November 2004 and February 2006. Patients were assessed by history, physical examination and investigations that included hormonal assays and Doppler study. Screening for ASAs was by Tray agglutination test (TAT) to determine the degree and type of agglutination. Those with positive TAT were further evaluated by indirect Immunofluorescence technique to observe the type of antibodies and their location on the sperm.

**Results:** Sperm bound ASAs were present in 27 (22.8%) of infertile men with IgA being the predominant one in 26 (96.3%). Sperm agglutination and shaky movement were seen in 25 (92.5%) and 23 (85.1%) respectively of those with immune-infertility respectively. The positive predictive value for sperm agglutination was 86.2% and for sperm shaky movement was 88.4%. Twenty patients (74%) with ASAs had normal sperm count. Sperm motility was significantly reduced in patients with immune infertility. Twenty-five (92.6%) of them had zero to 25% of grade A & B motility. In comparison with infertile patients *without* ASAs 32 out of 91 (35.2 % ) were in the scale of ( 0 – 25 % ) and 55 ( 60.4 % ) in the scale of (26 – 50 % ) and 4 (4.4 % ) in the scale of ( > 50 % ). Leukocytospermia was present in 25 (92.6%) of infertile men with ASAs compared with 10 (11%) of those without ASAs. Leukocytospermia was found in 25 (92.6%) of patients with immune-infertility, it was found in six (22.2%) in patients with physical testicular injuries, six (22.2%) in thermal injuries, three (11.1%) in obstructive lesions, two (7.4%) in cases of unidentified pathology and one (3.7%) in testicular failure. The highest positive predictive value for ASAs detection was seen with physical testicular injuries (85.7%) followed by leukocytospermia (71.4%), genital tract obstruction (50%), thermal injuries (17.1%) and testicular failure (12.5%) successively.

**Conclusions:** immune-infertility is present in a significant percentage of infertile men. IgA is the predominate antibodies. Sperm agglutination and shaky movement are significant predictors for ASAs and have high positive predictive values. Impaired sperm motility is significantly influenced by ASAs. Leukocytospermia is strongly associated with ASAs. The highest positive predictive value for ASAs is seen in physical testicular injuries followed by leukocytospermia and obstructive lesions.

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**Key words:** Antisperm antibodies, infertile men, Iraq

## Introduction

Infertility is defined as the inability of a sexually active non-contracepting couple to achieve pregnancy in one year <sup>(1)</sup>. Most couples achieve conception within one year and approximately 15% of couples are unable to do so <sup>(2)</sup>. Approximately 20% of cases of infertility are caused entirely by male factor, with an additional 30-40% of cases involving both male and female factors; therefore, a male factor is present in half of infertile couples <sup>(2)</sup>. Male infertility has been attributed to many factors including testicular insufficiency, endocrinopathies, and obstructive lesions of the male genital tract, medical and environmental illnesses, varicoceles, sexual problems and ejaculatory disorders <sup>(3)</sup>. Many couples facing infertility issues may have immune system dysfunction. New tests are now available to pinpoint these immune factors in infertility. The seminiferous tubules of the testis are lined with Sertoli cells that rest on the tubular basement membrane and extend into its lumen with a complex cytoplasm. Tight junctions, the strongest intercellular barriers in the body, link Sertoli cells. These junctional complexes divide the seminiferous tubule space into basal (basement membrane) and adluminal (lumen) compartments. This anatomical arrangement forms the basis of blood–testis barrier allowing spermatogenesis to occur in an immunologically privileged site. Spermatozoa are produced at puberty and are considered foreign to the immune system that develops self–recognition during the first year of life <sup>(4)</sup>.

The protection provided by the blood–testis barrier is incomplete. It allows immune system access to the antigens of spermatocytes in the basal compartments of the seminiferous tubules. Intraepithelial lymphocytes are present in the rete testis, epididymis and vas deferens, in addition, macrophages are found in the interstitium of the testis and epididymal lumen.

These may provide a secondary barrier preventing the escape of non-sequestered antigenic material migrating to the boundary wall of seminiferous tubules at different stages of spermatogenesis where there is a significant amount of degeneration of germ cells <sup>(5)</sup>. Any condition that may disrupt the blood-testis-barrier (BTB) can predispose the patient to development of antisperm antibodies. Multiple risk factors may be involved in the development of ASAs <sup>(1)</sup>. In cases of genital tract obstruction, the epididymis may have a crucial role in ASA development. In certain places, the epididymis ruptures leading to spermatic granuloma formation, which may be an important stimulus of the immune system <sup>(6)</sup>. Infections in the male genital tract may disrupt the BTB and lead to development of ASAs; moreover, antibodies against lipopolysaccharides in the bacterial cell wall may cross react with those on the sperm surface and stimulate ASAs production <sup>(7)</sup>. Hyperthermia as in varicocele, cryptorchidism and associated venous stasis impair testicular metabolism and damage the BTB <sup>(7)</sup>. Physical trauma and disruption of tunica albuginea is an important risk factor in exposing sperm antigens to the immune system and subsequent development of ASAs and immune infertility <sup>(7)</sup>. Other risk factors for the development of ASAs include congenital seminal vesicle agenesis, orchiopexy, torsion, and biopsy.

Antisperm antibodies can be found in three locations <sup>(4)</sup>. Serum, seminal plasma, sperm bound (head, mid piece or tail). Most investigators believe that only antibodies present on the spermatozoal surface are clinically significant. Thus, most recent investigations have been aimed at direct assays determining the presence of sperm bound antibodies instead of the indirect detection of serum antisperm antibodies <sup>(8)</sup>.

Immunoglobulin classes involved in immune infertility are IgG, IgA and IgM. IgG antibody is derived from local production and as a

transudate from the blood stream. IgA is thought to be purely locally derived. IgM antibody is of large molecular weight and rarely, if any, present in the seminal plasma<sup>(4)</sup>.

ASAs can gain access into the male genital tract through the seminiferous tubules, epididymis or prostate<sup>(9)</sup>. Antisperm antibodies can affect sperm function at several different levels including<sup>(10)</sup> impairment of sperm motility, increasing sperm agglutination and inhibition of progression in cervical mucus. IgA ASAs inhibit penetration of cervical mucus and migration through it. It is believed that this is caused by cross-linking of the Fc portion of IgA to the glycoprotein in the high viscosity component of cervical mucus.

The aim of this study is to determine the prevalence of antisperm antibodies (immune infertility) among infertile men with abnormal seminal fluid parameters according to WHO criteria. It is also to study the effects of these antibodies on different seminal fluid parameters and their predictive value. Finally, to demonstrate the relationship between the sperm-bound ASAs and various pathological conditions of the male genital tract. These may coexist or play a role in the development of ASAs.

## Patients and Methods

This prospective study includes one hundred and eighteen patients with a history of primary infertility and with seminal fluid parameters below minimal standards of adequacy as defined by WHO (1999). The study was conducted in the Medical City Hospital (Baghdad, Iraq) in the period from November 2004 to February 2006.

Patients were evaluated by history, including past surgical history (orchiopexy, vasectomy, testicular biopsy, and herniorrhaphy), trauma with or without hematoma and swelling for more than two days. History of urinary tract infections was recorded. Physical examination included

(scrotal examination for testicular size and consistency, epididymis, vas deference, and pampiniform plexus). DRE (digital rectal examination) was performed to assess prostatic and seminal vesicle problems.

Investigations included scrotal ultrasound with color Doppler. Hormonal assays (FSH, LH, serum testosterone prolactin) and seminal fluid analysis (SFA) was done according to WHO (4) criteria.

Two methods for detection of ASAs were employed:

### 1. Tray Agglutination Test (TAT).

Semen samples with several dilutions (using micro titer plate) are mixed with small amount (1 µl) of active sperms from normal healthy donor and examined under inverted microscope (X 400) to observe the degree and type of agglutination (head-to-head, head to tail or tail-to-tail). The highest dilution at which agglutination occurs is called agglutination titer. TAT does not determine the amount of antibodies on sperms surface or the class of immunoglobulin and its location.

Figure 1

### 2. Indirect Immunofluorescence Technique.

Those with positive TAT were further evaluated by indirect Immunofluorescence. This was performed by mixing the seminal plasma examined with donor healthy semen and fluorescent bound antihuman IgA, IgG, and IgM. The mixture was examined in a dark room under Immunofluorescent microscope with (X 650). By this test we observed the site of ASAs, on the head, midpiece or tail of the sperm and the type of these antibodies (IgA, IgG, and IgM). Figure 2 and 3

Data were arranged and tabulated in numbers and percentages. The association between different variables was detected by using Chi-square and Fisher exact test when it is appropriate. P value is considered significant when it is < 0.05.

## Results

Sperm bound antisperm antibodies were present in 27 patients (22.8 %) with IgA being the predominant antibody seen in 26 out of 27 of them (96.3 %) as in Table 1 and 2. On seminal fluid analysis, 25 patients with sperm-bound ASAs had sperm agglutination (92.5 %) and shaky movement was seen in 23 of them (85.1 %). The positive predictive values for sperm agglutination and shaky movement for ASAs detection were 86.2% and 88.4% respectively. Twenty patients with sperm bound ASAs (74%) had normal sperm count ( $> 20 \times 10^6/\text{ml}$ ); while seven patients (26 %) had sperm count in the range of ( $1- 20 \times 10^6 / \text{ml}$ ), (Table 3).

Sperm motility was in patients with sperm bound ASAs as follows: 25 of them (92.6 %) had sperm motility in the scale of (0 – 25 %) of grade A and B, 2 of them (7.4 %) in the scale of (26 – 50 %), while none of them had above 50 % motility. In comparison with infertile patients without ASAs 32 out of 91 (35.2 %) were in the scale of (0 – 25 %), 55 (60.4 %) in the scale of (26 – 50 %) and 4 (4.4 %) in the scale of ( $> 50 \%$ ) as in Table 4.

Leukocytospermia (peroxidase positive WBCs  $> 1$  million / ml) was found in 25 (92.5 %) of patients with sperm bound ASAs whereas two (7.4 %) of them had WBC count in the range of ( $1 - 2 \times 10^6/\text{ml}$ ). Twelve (44.4 %) of them were in the range of ( $2 - 5 \times 10^6 / \text{ml}$ ). Eleven (40.7 %) had WBC count in the range of ( $>5 \times 10^6 / \text{ml}$ ), (One million WBCs / ml corresponds to 10 – 15 WBCs / HPF)<sup>(2)</sup>. In comparison with infertile patients without ASAs only 10 out of 91 (11%) of them had WBC count in the seminal fluid  $> 1 \times 10^6/\text{ml}$ .

Twenty-five patients (92.6 %) had leukocytospermia. Physical injuries (trauma and biopsy) and thermal injuries (varicoceles) were presented in six patients for each (22.2 %). Three patients (11%) with immune infertility had genital tract obstruction (vasectomy, seminal vesicle agenesis and azoospermia associated with normal testicular biopsy and hormonal assay). No identified pathology could be detected in two (7.4 %) patients. There was primary testicular failure in one patient (3.7%). Table 5 shows the positive predictive value (PPV) for the presence of ASAs with different predisposing factors.

## Discussion

Because it is the sperm, not serum, that reaches the female reproductive tract; direct assays have an advantage of detecting only sperm-bound immunoglobulins. The presence of antisperm antibodies in the serum is not always associated with presence of these antibodies on sperm. In addition, IgM class antibodies that may be present in serum do not usually present in the semen because of it is high molecular weight<sup>(11, 8)</sup>. Antisperm antibodies are present in a high percentage of infertile patients. Their existence was first described by Rumke and Hellinga in 1959. Menge and Beitner<sup>(12)</sup> reported an incidence of 7.8-20.1% among infertile men. Rasanen M<sup>(13)</sup> reported a prevalence of 10%.

The fairly high incidence of sperm bound ASAs in our study (22.8%) may be due to the inclusion of many patients with history of surgical manipulation (mainly testicular biopsy). Those patients have the highest positive predictive value for being affected by ASAs. The type of sperm bound ASA is important in the assessment of patients with immune infertility. Twenty-six out of 27 of our patients (96.3%) were found to have Ig type A.

It is a good screening test but it fails to detect some patients with purely IgG ASAs. Meinertz et al <sup>(14)</sup>, reported on 216 men who had undergone vasectomy reversal. The conception rate was halved if IgA was present. The type of sperm bound ASA is an important prognostic factor in patients with immune infertility. IgM is of high molecular weight and can be present in cases of extensive damage to the BTB. We have shown that sperm agglutination was present in 92.5% of patients with ASAs. Agglutination is not specific for the presence of ASAs. It was present in four patients (4.4%) without ASAs and this can be attributed to genital tract infections and the presence of mucosal threads, which may lead to sperm agglutination. The positive predictive value (PPV) of sperm agglutination for the presence of ASAs was 86.2%. This means that 86.2% of those with sperm agglutination had ASAs. Therefore, the presence of sperm agglutination gives a high index of suspicion for ASAs.

Leukocytospermia (peroxidase positive WBCs in the seminal plasma > 1 million/ml) was significantly associated with the presence of sperm bound ASAs. The reason for the immune cell infiltrate in leukocytospermia is not merely an inflammatory response associated with infection. It can be a result of sensitization of the immune system to sperm antigens in the form of cellular infiltrate in addition to sperm antibodies, moreover, leukocytospermia could be due to a reaction to low grade toxins like cigarette smoke or alcohol<sup>(4)</sup>. Whatever the mechanism, leukocytospermia is significantly associated with sperm bound ASAs specially those with WBC count in the seminal fluid >  $2 \times 10^6$ /ml. The development of immune response against human spermatocytes may be induced secondary to various insults that disrupt the BTB.

In conclusion, this study showed that the most common clinical finding associated with sperm bound ASAs was leukocytospermia followed by physical and thermal testicular injuries. The highest PPV for the presence of ASAs was observed in physical testicular injuries followed by leukocytospermia and obstructive lesions. The majority of patients with ASAs had Ig type A that is associated with a poorer conception rate than other types. Sperm agglutination and shaky movement are present in significant number of patients with ASAs with a high PPV. Sperm count is normal in most patients with sperm bound ASAs. Sperm motility is significantly influenced by ASAs and it is an important predictor for the immune factors specially those with severe asthenospermia. Leukocytospermia, whether a manifestation of genital tract infection or due to sensitization of the immune system to sperm antigens is significantly related to the presence of ASAs and it is the most common clinical finding associated with immune infertility followed by physical and thermal testicular injuries. Physical testicular injuries have the highest predictive value for the presence of ASAs followed by leukocytospermia and obstructive lesions of the male genital tract.

Immune infertility should be remembered as a prevalent medically treatable condition among infertile men and should not be missed during infertility evaluation. It is mandatory to search for and treat immune infertility in many conditions such as when varicocele surgery fails to improve seminal parameters. Corticosteroids suppression is used for high-risk patients. Tray agglutination test is available, practically easy, quantitative, relatively cheap, and can be used as a screening test. When the site of immunoglobulin is an issue, IgA Immunofluorescence is the screening test of choice.

**Table 1: Prevalence of sperm bound ASAs among infertile patients.**

ASAs	<i>No. of patients</i>	<i>Prevalence</i>
Positive	27	22.8 %
Negative	91	77.2 %

**Table 2: Types of sperm bound ASAs among patients with immune infertility.**

No. of patients	Ig type	Prevalence
26	IgA	96.3 %
1	IgG	3.7 %
0	IgM	0 %

**Table 3: Sperm count in patients with ASAs**

Sperm count	<i>Number of patients</i>	<i>% of patients</i>
$> 20 \times 10^6 / \text{ml}$	20	74 %
$< 20 \times 10^6 / \text{ml}$	7	26 %

*Two-sided p value: 0.001134*

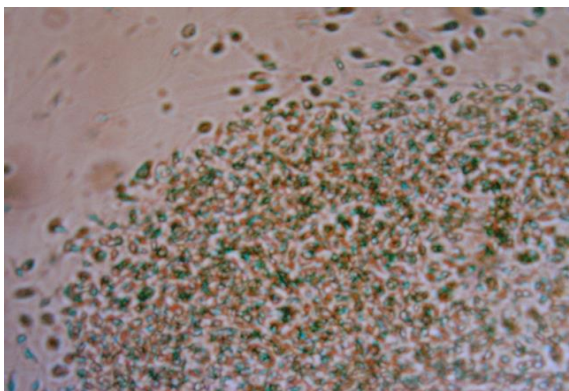
**Table 4: Effects of ASAs on Sperm Motility**

ASAs	<i>Sperm motility ( grade A &amp; B )</i>		
	0 -25 %	26 – 50 %	> 50 %
Positive	25* (92.6 %)	2* (7.4 %)	0* (0 %)
Negative	32* (35.2 %)	55* (60.4 %)	4* (4.4%)

- Number of patients , P value: 0.000001

**Table 5: The positive predictive value (PPV) for the presence of ASAs with different predisposing factors.**

Risk factor	PPV
Physical injury	85.7%
Leukocytospermia	71.4%
Obstructive	50 %
Thermal	17.1%
Testicular failure	12.5 %



**Figure (1) illustrates sperm agglutination  
(Light Microscope X 100 )**



**Figure (2) illustrates head bound ASAs by  
indirect Immunofluorescence Technique.  
( X 625)**



**Figure (3) illustrates tail bound ASAs ASAs by indirect  
Immunofluorescence Technique (X 625)**



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