

Correlation of sperm-bound immunoglobulins with impaired semen analysis in infertile men with varicoceles

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Sperm-bound immunoglobulins were found in 27 (32%) of 84 infertile men with palpable varicoceles. These men were divided into two groups based on the presence (group I; 32%) or absence (group II; 68%) of sperm-bound immunoglobulins, as measured by an enzyme-linked immunosorbent assay. Circulating antisperm antibodies were identified in 52% of patients with sperm-bound immunoglobulins and 14% of group II patients. The presence of sperm-bound immunoglobulins was associated with a small but significant decrease in both sperm concentration and motility. Sperm-bound immunoglobulins are present in a greater percentage of infertile men with varicoceles than infertile men without varicoceles. Their presence may be a marker for damage to the seminiferous epithelia in men with varicoceles and may also contribute to varicocele-associated infertility. *Fertil Steril* 52:469, 1989

A palpable varicocele is present in 30% to 40% of infertile men.^{1,2} This is two to three times the incidence of varicocele in the general male population.^{3,4} MacLeod^{5,6} described an abnormal semen analysis pattern associated with varicocele, consisting of increased tapering forms, immature germinal cells, and severe oligospermia. In addition, 85% of these patients exhibited impaired sperm motility. Varicolectomy results in improved semen parameters in two-thirds of patients and pregnancy rates ranging from 24% to 53%.⁷ Although this data clearly establishes a link between varico-

cele and male infertility, the pathophysiology of varicocele remains incompletely understood.

Recent studies^{8,9} have associated varicocele-related infertility with antisperm antibody formation. Golomb et al.⁸ compared a group of infertile men with varicoceles with a group of infertile men without a palpable varicocele. They found a greater percent of total antisperm antibody (i.e., serum, semen, and sperm-bound) in the infertile men with varicoceles (91%) as compared with infertile men without varicoceles (41%); 38% of their varicocele patients had antibody bound to sperm. Also, sperm-bound antibody was present in 25% of men in the study of Ozen et al.⁹ However, in their study, the use of methanol fixation of sperm is now known to expose antigens other than those bound to the sperm surface. Therefore, the clinical significance of their results is not easily evaluated.

In our prospective study, infertile men with palpable varicoceles were found to have a high incidence of sperm-bound immunoglobulins. In addition, the presence of sperm-bound immunoglobulins was associated with a poorer semen quality.

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MATERIALS AND METHODS

Patients

Eighty-four consecutive infertile men with palpable varicoceles were evaluated. A detailed reproductive history was obtained.¹⁰ All men studied had been attempting to conceive for at least 1 year with partners of either previous proven fertility or having had a negative prior infertility workup. A complete physical examination was performed in a warm room. A test-size orchidometer (REMCAT Trade AB, Vallingby, Sweden) was used to estimate testicular volume. Varicoceles were categorized by a single examiner, with the patient standing, as follows: grade I, small with a distinct impulse felt with Valsalva maneuver; grade II, moderate in size with a palpable dilation detected with the Valsalva maneuver; grade III, large and easily palpable without the Valsalva maneuver; and grade IV, easily visualized through scrotal skin without Valsalva maneuver.

Semen Collection

Semen was collected by masturbation into sterile wide-mouthed containers, after a 3-day abstinence period. After liquefaction, the sample was thoroughly mixed and divided into aliquots for semen analysis and sperm-bound antibody assays.

Semen Analysis

Specimens were examined within 1 hour of collection. Volume, color, pH, and time-to-liquefaction were recorded. Sperm counts were performed with a Makler chamber (Sefi Medical Instruments, Haifa, Israel). Motility was evaluated by videomicrography. The presence of agglutination was recorded. Morphology was assessed after preparation with the Papanicolaou stain. The mean values of samples collected at least 1 month apart was used for data analysis.

Antisperm Antibody Evaluation

An enzyme-linked immunosorbent assay (ELISA) was performed using sperm that were isolated from patients' ejaculate by a swim-up technique.¹¹ Fresh ejaculates were allowed to liquefy at room temperature for 20 minutes. An equal volume of phosphate-buffered saline (PBS), warmed to 37°C, was carefully layered over the semen, and the sample was incubated at 37°C for 60 minutes. Motile sperm migrated into the PBS layer, and a visibly pure popu-

lation of viable sperm was obtained by removing the PBS with a Pasteur pipette. In patients with impaired sperm motility, spermatozoa were collected by low-speed centrifugation. Purified spermatozoa from patients and from fertile donors were diluted to 1×10^6 /mL with PBS, and 0.1 mL of each sample was added to six wells of a microtiter plate. The sperm were pelleted by centrifugation, the supernatants were removed, and the sperm irreversibly bound to the wells by addition of 0.2 mL 0.25% glutaraldehyde in PBS. After 10 minutes at room temperature, the glutaraldehyde solution was removed, and the wells washed three times with 0.2-mL aliquots of PBS plus 0.05% Tween 20 (PBS-Tween). Alkaline phosphatase-conjugated goat antibody to human IgG, IgA, or IgM was diluted 1:200 in PBS-Tween, and 0.1 mL of each was added to two wells and to blank wells. The plates were covered with parafilm and floated in a 37°C water bath. After 60 to 120 minutes, the wells were again washed three times with PBS-Tween, and 0.2 mL of the alkaline phosphatase substrate, *p*-nitrophenyl phosphate (1 mg/mL in diethanolamine buffer, pH 9.8) was added to each well. After 60 minutes (IgG), 120 minutes (IgM), or 180 minutes (IgA), the optical densities at 405 nm of the solutions in the wells were determined with an ELISA plate reader. A positive reading was defined as a value at least twice the absorbance measured in the wells with control sperm. This method has been shown to give data relevant to infertility¹¹ and comparable with the immunobead-binding assay.¹² The presence of any of the three immunoglobulin isotypes placed a patient in group I: sperm-bound immunoglobulins present. Absence of all three isotypes placed the patient in group II: sperm-bound immunoglobulins absent.

Serum antisperm antibody was assayed using the ELISA assay as follows. Aliquots of sperm (5×10^5 /0.05 mL) were pipetted into glass tubes containing an equal volume of sera from the patient and a positive or negative control, diluted 1:8, 1:16, 1:32, and 1:64 in PBS. The samples were incubated in a 37°C water bath for 60 minutes to allow sperm-reactive antibodies to bind to the sperm surface. The sperm were then concentrated by centrifugation, washed three times with PBS, and resuspended in 0.5 mL PBS. Aliquots (0.1 mL) were then added to six wells of a microtiter plate and fixed with glutaraldehyde. Alkaline phosphatase-conjugated antibody to IgG, IgA, or IgM were each added to two of the wells, and bound antibody was quantitated, as described above.

Table 1 A Significantly Poorer Semen Quality is Noted when Sperm-Bound Antibody is Present^a

	Semen volume	Sperm concentration	Motile sperm	Normal morphology	Head defects	Tapering forms	Tail defects
	mL	10 ⁶ /mL	%	%	%	%	%
Sperm-bound antibody present							
Group I (n = 27)	3.0 ± 0.3	35.6 ± 7.0	39.7 ± 2.8	48.4 ± 2.5	28.6 ± 1.8	6.2 ± 1.5	14.7 ± 1.5
Sperm-bound antibody absent							
Group II (n = 57)	3.4 ± 0.2	49.8 ± 6.2	43.9 ± 3.0	52.9 ± 1.8	26.7 ± 1.6	7.7 ± 1.0	12.4 ± 1.0
P ≤	NS ^b	0.05	0.05	0.05	0.05	0.05	0.05

^a Values are expressed as means ± standard error of the mean (SEM).

^b NS, not significant.

Hormone Analysis

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) were measured by a double-antibody radioimmunoassay (RIA) method (Diagnostic Products Corporation, Los Angeles, CA). The lower limit of detection for FSH, LH, and PRL ranged from 1 to 2 IU/mL. Testosterone (T) was measured by a similar RIA method (Radioassay System Laboratories, Inc., Carson, CA). The lower limit of detection using this method was approximately 50 ng/dl. Normal values in our laboratory were as follows: T, 300 to 1,100 ng/dl; FSH, 4 to 19 IU/mL; LH, 8 to 18 IU/mL; and PRL, 7 to 18 IU/mL.

Statistical Methods

The mean and standard error of the mean were calculated. Unpaired *t*-tests and χ^2 analysis were used where applicable.

RESULTS

Sperm-bound immunoglobulins were found in 27 of 84 (32%) infertile men with palpable varicoceles. In these men, IgA was identified in 85% of the samples, while bound IgM and IgG were present in 74% and 67% of the patients, respectively. All three immunoglobulin classes were present 56% (15/27) of the time. Two immunoglobulin types were present 11% (3/27) of the time, and only one class was present 33% (9/27) of the time. When only one immunoglobulin class was present, no specific immunoglobulin type was most prevalent.

Men with sperm-bound immunoglobulins had a significantly decreased sperm concentration, percent motile sperm, percent of sperm with normal morphology, and percent of sperm with tapering forms, when compared with the group not having sperm-bound immunoglobulins (Table 1). The

presence of sperm-bound immunoglobulins was also associated with a greater percent of head and tail defects.

Agglutination was observed in only three of the group I patients and four of the group II patients. Fourteen of 27 men (52%) with sperm-bound immunoglobulins and 13 of 57 men (23%) without sperm-bound immunoglobulins had antisperm antibodies present in their sera. Serum antisperm antibodies were thus present in 27 (32%) and absent in 57 (68%) of these 84 patients. The measured immunoglobulin classes were proportionally the same as those bound to sperm. However, no statistical correlation between circulating antisperm antibody and semen analysis was found in this population.

A progressive decline in semen quality was noted when antisperm antibodies were present in serum, on sperm, or in both serum and on sperm, respectively (Fig. 1).

The group with sperm-bound immunoglobulins had a mean age of 36.2 years, not significantly

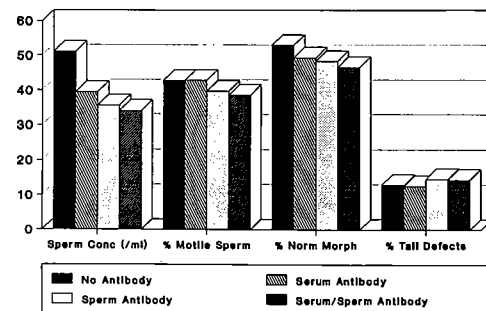


Figure 1 Relation between semen analysis and antisperm antibodies in sera and on sperm. Semen analyses were performed on men with varicoceles who lacked antisperm antibodies (no antibody) or who had antisperm antibodies only in sera (serum antibody), only on sperm (sperm-bound antibody), or both in sera and on sperm (serum/sperm antibody). Sperm concentration is 10⁶ sperm/mL.

different from a mean age of 35.7 years for those without sperm-bound immunoglobulins. Testicular volume was not significantly different between the groups. Right testicular volume averaged 21.5 ± 0.8 (SE) mL, while left testicular volume averaged 21.3 ± 0.7 mL. There was no significant difference in the size of the varicoceles between the two groups.

A significant decrease in FSH ($P < 0.01$), but not serum T, LH, or PRL, was found in men with sperm-bound immunoglobulins present.

DISCUSSION

In the present study, immunoglobulins bound to sperm surface antigens were found in 32% of infertile males with varicoceles. This compares with an incidence of 15% in all infertile men referred to us for sperm antibody testing.¹³ In the infertile male population, as a whole, an immunologic etiology accounts for less than 10% of the total.¹⁴ Rumke and Hekman¹⁵ found an incidence of antisperm antibodies of 3.3% in infertile men and an incidence of 0% in their control group of fertile men. Haas¹⁶ has found the prevalence of antisperm antibody to be 10% in a normal male population. Our data suggest that men with varicoceles may have an increased incidence of antisperm antibodies bound to their ejaculated sperm.

Of interest is the distribution of immunoglobulin isotypes bound to sperm. In our study, as well as in Golomb et al.,⁸ IgA and IgM were the most commonly sperm-associated immunoglobulins. IgM is a large immunoglobulin (900,000 d) and, when formed in the vascular space, tends to stay there. It crosses capillary boundaries with difficulty. It is thus tempting to speculate that the measured IgM is produced locally and, together with the locally secreted IgA, is formed in response to a local damage produced by the varicocele. Some support for this comes from the recent work by El-Demiry and James,¹⁷ in which alterations in T-cell subsets lining the genital tract are found with direct injury (e.g., obstruction of the vas deferens).

The occurrence of sperm-bound immunoglobulins was associated with poor semen quality. This relationship was not found when only circulating antisperm antibodies were considered. However, the semen quality was quantifiably similar to that of the group I patients when both sperm-bound and circulating antibodies were present. These findings suggest that men with varicoceles, who also have sperm-bound immunoglobulins, have more exten-

sive damage to the seminiferous epithelia than do men with varicoceles who lack this finding. As noted previously, a progressive decline in semen quality was noted when antisperm antibodies were also present in serum, on sperm, or in both serum and on sperm, respectively (Fig. 1). Thus the presence of circulating and sperm-bound antibodies together might reflect continuing damage to the seminiferous tubular epithelia. A direct comparison between our results and those of Golomb et al.⁸ cannot be made, because their study was designed to compare infertile men with and without varicoceles, and not as in ours with the subgroup of infertile men with varicoceles with or without sperm-bound antibody.

Experimentally produced varicoceles in rats and monkeys have been shown to damage both the Sertoli cells and basal lamina of seminiferous tubules.^{18,19} These lesions would allow immunocompetent cells to gain access to spermatozoal antigens, resulting in activation of a sperm-specific immune response. The slightly higher FSH level found in men negative for sperm-bound immunoglobulins is surprising in view of the better semen analysis in these patients. However, FSH is still well within the normal range for both groups of patients.

Serum antisperm antibodies did not always predict the presence of sperm-bound immunoglobulins. Only 54% of men with sperm-bound immunoglobulins had antisperm antibodies detected in the serum. The predictive value of serum antisperm antibody measurement, i.e., the percent of the time that sperm-bound immunoglobulins will be present when serum antibody is detected, is 30% in the study by Golomb et al.⁸ and 52% in data from our patient population. Only the occurrence of sperm-bound immunoglobulins, and not circulating antisperm antibodies, correlated with a poor semen quality. These data support other recent studies indicating that antibodies bound to sperm may be more relevant to infertility than are circulating antisperm antibodies.^{11,19,20}

We recognize that the sperm-bound immunoglobulin identified in these men may not always be due to the presence of antisperm antibodies. Immunoglobulins,²¹ or antigen-antibody complexes,²² may nonspecifically adhere to sperm, especially sperm with impaired motility or altered surface properties. The relation between sperm-bound immunoglobulins, poorer semen quality, and the high level of sperm-associated IgM in some ejaculates suggests that antigen-nonspecific immunoglobulin

binding to sperm may have occurred in some of the samples. In most of the men, however, the data are consistent with specific antibody binding to the surface of ejaculated sperm. IgA, the isotype detected on sperm at the highest frequency in this study, can be produced locally in the genital tract,²³ is not the immunoglobulin present in the highest quantity in seminal fluid, and is also the most prevalent sperm-associated antibody isotype in normospermic men with immune infertility.¹¹ In addition, the men with sperm-bound immunoglobulins also had a higher incidence of circulating anti-sperm antibodies than did men without immunoglobulins on their sperm. This strongly indicates that, at least in these cases, antibodies to the patients' sperm were being produced.

Our data suggest that varicocele-associated injury to the seminiferous epithelium promotes induction of sperm-bound immunoglobulins and a further impairment of fertility. Although we cannot formally exclude an underlying defect in spermatogenesis that would contribute to autoimmunity, decreased sperm quality, and nonspecific immunoglobulin binding to the sperm surface, the data also suggest that sperm-bound immunoglobulins may play an etiologic role in varicocele-related infertility.

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