

# IDENTIFICATION OF ACROSOME AS THE MAIN ANTIGEN OF THE SPERM CELLS PROVOKING AUTOANTIBODIES IN VASECTOMIZED IRANIAN MEN

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**Abstract-** Vasectomy is one of the extensively used methods of contraception in family planning programs. Antisperm antibodies (ASA) develop after vasectomy which can result in auto-immune male infertility. The precise sperm antigens involved in the autoimmune response are still poorly defined, therefore we determined the circulating ASA and identified relevant sperm antigens based on localization of binding sites of ASA to sperm cell antigens, using a rapid, inexpensive and clinically relevant assay in vasectomized men. Results showed that 2.5% of men had ASA at the time of vasectomy, whereas 53.5% of the study population subsequently developed ASA. The numbers of men with circulating ASA increased significantly for the first three months after vasectomy. These antibodies were distinguishable into three groups based on their bindings to different sites of sperm cell antigens including against acrosome and tail in 67.56% and 10.8%, respectively; 21.6% of subjects had antibody to the other parts of the sperm cell antigens. The results of this study are discussed in terms of an autoimmune response against sperm antigens and development of ASA.

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**Key words:** Antisperm antibodies, sperm antigens, vasectomy, male infertility

## INTRODUCTION

Vasectomy is a simple and highly effective contraceptive method with a low morbidity rate (1, 2). Approximately 42–60 million men or 5% of married couples of reproductive age rely on vasectomy as a contraceptive method (3, 4). However, the possibility of adverse effects related to an immune response as a result of vasectomy has been raised.

Sperm antigens are normally protected from the

male immune system by a physical barrier. Congenital abnormality or physical damage in the male reproductive tract may result in autoimmune response to sperm antigens and production of antisperm antibodies (ASA) (5). Furthermore, ASA develops frequently after the surgical ablation of the vas deferens during vasectomy (6). Unfortunately, 1-3 per 1000 vasectomized men will request a reversal (7, 8). The success of a reversal, as measured by pregnancy rates, ranges from 30%-60% (9). More recently, evidence from vasectomy reversal patients has confirmed that presence or absence of ASA is the critical factor in determining whether or not pregnancy occurs in the spouse (6).

The correlation of ASA with infertility suggests a role for these antibodies in blocking fertilization, and association with the impairment of sperm function at

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various stages of reproduction (10). The precise antigen involved in ASA formation in the auto-immune infertile men is still poorly defined (11, 19).

The aim of this study was to evaluate the incidence of ASA in vasectomized men and to identify the sperm surface antigens that result in development of ASA, in order to explore methods to control or suppress the immune response against the sperm antigen(s). Furthermore, ASA and identification of relevant antigens from spermatozoa provide the basis for immunologic control of fertility in the form of a birth control vaccine (21-24).

## MATERIALS AND METHODS

Sera were obtained from 80 subjects on the day of vasectomy and from 69 of the same subjects three months after vasectomy. The ages of subjects were between 30-40 years. The study was approved by Ethics Committee of Tehran University of Medical Sciences and we obtained informed consent from all patients.

Detection of IgG antisperm antibodies in serum samples was done based on the indirect mixed erythrocyte-spermatozoa antiglobulin reaction (MAR) test (12, 13) with some modification. Semen samples from normal donor were diluted by one in two volumes of phosphate buffer saline (PBS) containing 0.3% bovine serum albumin, and were washed twice by PBS and centrifuged at 3000 RPM for 5 minutes. The resulting pellet was re-suspended in PBS and it was adjusted to a concentration of 5 million motile sperm per milliliter. Serum samples were complement-inactivated by heating at 56° C for 30 minutes. Then, 100 µl of patient serum was added to the 20 µl of sperm suspension. The mixture was incubated at 37° C for 30 minutes and washed twice by centrifugation at 3000 RPM for 5 minutes. Also, group O Rh-positive red blood cells (RBC) were washed three times in Alsever's solution and resuspended to a hematocrit score of 50%. To one part red cell suspension, two parts of 1 in 5 dilution of serum containing anti-D was added, which was mainly IgG (Behring® diagnostics Germany).

The mixture was incubated at 37° C for 30 minutes. The cells were then washed three times again in Alsever's solution and was adjusted to a hematocrit score of 10% and stored until required for

use in small aliquots at 4° C. The test was done by adding one drop of washed ASA coated sperm cells suspension placed on a microscope slide with one drop of anti-D coated Rh positive RBC suspension and one drop of monospecific anti-human antiserum IgG (Behring® Diagnostics Germany). The three drops were mixed, and the reaction was read within 10 minutes. No interpretation was made unless agglutination of the red cells was observed.

The test was read as negative if no motile mixed agglutination was seen. If motile mixed agglutination was observed, the reaction was graded as follows: positive (++) if 10% to 90% of the motile spermatozoa were attached to the erythrocytes; strongly positive (+++), if more than 90% of the motile spermatozoa incorporated into mixed agglutinates. Statistical analyses were performed using SPSS software version 13.

## RESULTS

This study assessed the incidence of circulating ASA, detectable by indirect mixed erythrocyte-spermatozoa antiglobulin reaction test in 80 subjects before vasectomy and 69 of those subjects three months after vasectomy (Table 1).

Indirect mixed erythrocyte-spermatozoa antiglobulin reaction technique revealed antibodies against distinct sperm antigens. ASA were detected in 2 cases (2.5%) before vasectomy. The incidence of ASA rose to 53.5% (37 cases) three months after vasectomy (Table 2), an increase which was highly significant ( $P < 0.001$ ).

Binding occurred in the acrosomal region, tail and other parts of the sperm cell antigens. These antibodies were distinguishable into three groups based on their binding to different sites of sperm cell antigens. The first group included antibodies to antigens in the acrosome, incidence of which was

**Table 1.** The results of indirect MAR test in men before vasectomy for determination of ASA

Result	No of cases	Percent
Positive	2	2.5
Negative	78	97.5
Total	80	100

Abbreviations: MAR, mixed erythrocyte-spermatozoa antiglobulin reaction; ASA, antisperm antibodies.

**Table 2.** The results of indirect MAR test in men after vasectomy for determination of ASA

Result	No of cases	Percent
Positive	37	53.56
Negative	32	46.44
Total	69	100

Abbreviations: MAR, mixed erythrocyte-spermatozoa antiglobulin reaction; ASA, antisperm antibodies.

67.56% after vasectomy. The second group included antibodies to the sperm tail antigens with incidence of 10.8%. Third group included antibodies to the other parts of the sperm cell antigens which its incidence was 21.6% (Table 3). Most of the ASA in subjects bound to the acrosome and least to the tail region ( $P < 0.05$ ). Only few samples showed binding to other parts of sperm antigen.

## DISCUSSION

After vasectomy, sperm cells are confined to the epididymis and the vas deferens. The epididymis may have a crucial role in ASA development following vasectomy (26). In some conditions, such as obstruction of the epididymis or vas occlusion, the soluble sperm antigens are released and subsequently phagocytized, resulting in ASA formation (17, 25).

Sperm antigens are in abundant supply in vasectomized men because of the continuous resorption of spermatozoa antigens after vasectomy. Some authors have pointed out that ASA have been developed in 50-80% of the vasectomized subjects, decreasing to 30% during long periods (14-16). Others have shown that approximately 20% of vasectomized men develop antibodies to internal nuclear sperm antigens called protamines (11). Similarly, in the present study we have shown that vasectomized subjects have higher incidence of ASA

**Table 3.** Binding of sensitized red cells to different sites of sperm antigens in 37 vasectomized men who had produced ASA

Groups	No	Binding site of Ab	Percent (95% CI)
1	25	Acrosome	67.56 (52/83)
2	4	Tail	10.8 (1/21)
3	8	Other parts	21.6 (8/35)

Abbreviation: ASA, antisperm antibodies; Ab, antibody; CI, confidence interval.

compared to those before vasectomy. Therefore, these finding indicate that it is likely that production of antibody against acrosomal antigen in vasectomized men may have a profound role in autoimmune male infertility. Anti-acrosomal antibodies can block acrosome reaction and interfere with cellular recognition mechanism involved in such process as transport in the female genital tract, binding to the ovum, penetration to the zona pellucida (8, 20).

In conclusion, the available data presented in this article indicate that vasectomy is associated with significant changes in level of circulating ASA, which may affect fertility if reversal is requested. The acrosomal antigen is the main sperm antigen which is involved in ASA formation that may influence acrosome reaction and cellular recognition. Although vasectomy is a safe and the most cost effective form of birth control nevertheless, caution is necessary in advising vasectomy to individuals who may be genetically predisposed to autoimmune disease.

## Conflict of interests

The authors declare that they have no competing interests.

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