

Serum Antisperm Antibodies in Fertile and Infertile Individuals

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Abstract

Background: Presence of antisperm antibodies (ASAs) in infertility and their adverse effects on fertility is a matter of controversy. The aims of this study were to determine the percentage of antibody positive sperms and rate of ASA positive sera in both fertile and infertile men and women, and to find the association between these antibodies and infertility.

Methods: This study consisted of 29 fertile and 60 infertile men and women. The serum immunoglobulin (Ig) M, G, and A antisperm antibodies were evaluated after incubation of the sera with normal and high-quality fresh sperm from healthy donors. The percentage of spermatozoa positive for IgM, IgG, and IgA antibodies and the rate of antisperm antibody positive sera in fertile and infertile groups were measured by flow cytometry.

Results: Mean percentage of antibody positive sperms in fertile and infertile groups showed no significant differences (all $P > 0.05$). The rate of ASA positive sera in fertile and infertile individuals showed no significant differences (all $P > 0.05$). There were no significant differences between the mean percentage of antibody positive sperms and the rate of ASA positive sera in fertile and infertile men and women (all $P > 0.05$).

Conclusions: Presence of ASAs in the serum may not be associated with infertility. Although antisperm antibodies may interfere with fertility, not all types of ASAs can associate with infertility. Current tests cannot differentiate the ASAs that interfere with infertility from those that do not, because the antigenic specificities of these ASAs are not known. The antigens of the infertility-related ASAs must be characterized to allow an accurate detection for individuals with ASAs.

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Keywords • Flow cytometry • spermatozoa • fertility • infertility

Introduction

Immunologic factors are considered as important causes for infertility. One of the immunologic factors proposed for infertility is presence of antisperm antibodies (ASAs) in serum. ASAs can be found in cervical mucus, seminal plasma, and sera of men and women.^{1,2} Etiology of producing ASAs is unknown but it seems to be multifactorial.³

Both men and women have the capability to mount a humoral response to sperm. Either allogenic or autoimmune response could, in turn, adversely affect fertility. ASAs are most commonly

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limited to immunoglobulin (Ig) G, IgM, and IgA isotypes and each subclass have characteristic anatomical localization.

Systematically produced IgG molecules may be found in serum as well as in cervical mucus and semen. Agglutinating antibodies of the IgA class are typically found in cervical mucus and seminal plasma. The larger IgM antibodies have difficulty traversing the genital tract mucosa and therefore are found exclusively in the serum. Antisperm antibodies have also been detected in ovarian follicular fluid.^{1,4} In addition to subclassification by isotype, antisperm antibodies can be free, agglutinating, or bound to motile or immotile sperms. Sperm-bound ASAs can bind to different parts of the outer sperm plasma membrane, including the head, body, or tail. One major challenge in understanding the relationship between ASAs and pathogenesis of infertility, and their impact on fertility.

Prevalence of ASAs in fertile and infertile men and women is different among populations, and the impact and role of these antibodies on infertility remain controversial to date.^{1,5-9} Some reports indicate that ASAs are present in up to 9% to 12.8% of infertile couples.¹⁰ Other studies report that ASAs occur in 1% to 30% of infertile couples.¹¹ ASAs are also found in up to 2.5% of fertile men,⁹ and 4% of fertile women.¹² Some of these reports indicate that presence of ASAs can reduce infertility.^{1,11} The correlation between ASAs and infertility still remains a challenge and the mechanisms by which antisperm antibodies might adversely affect fertility remain a subject of debate and ongoing investigation.¹

The aims of this study were to assess the percentage of antibody positive sperms [i.e., sperms coated by IgG, IgM, and IgA classes], the rate of ASA (IgG, IgM, and IgA classes) positive sera in both fertile and infertile individuals, and the association between these antibodies and infertility.

Patients and Methods

Data regarding gender, age, history of genital infections and surgery of urogenital tract, consuming medicine, being under treatment for infertility were gathered by using a questionnaire. Fertile and infertile men were also inspected for any history of trauma or testicular torsion, vasectomy, varicocele, and cryptorchidism.

Patients and controls selection

This study consisted of 89 men and women: 29 fertile men and women (14 men and 15 women) as the control group, and 60 infertile men and women (29 married couples, and 2 women) as the study group.

The study group consisted of consecutive infertile men and women referred to Pars Medical Laboratory (Kermanshah Province, Iran) for infertility evaluation. The control sera were prepared from fertile married men and women with at least one child who referred to the medical laboratory of Taleghani teaching center affiliated to Kermanshah University of Medical Sciences (Kermanshah Province, Iran). The control group members had no history of infertility. Both of the infertile and fertile groups were tested for presence of serum antisperm antibodies (IgM, IgG, and IgA classes).

Inclusion criteria for the infertile group

1) Infertile couples with unexplained infertility, 2) No history of administration of corticosteroids at least 6 months prior to participation in the present study, and 3) No previously diagnosed hormonal or physical causes for infertility.

Inclusion criteria for control group

1) Men and women who had at least one child and voluntarily prevented pregnancy after the last child birth, and 2) No history of administration of corticosteroids at least 6 months before participation in this study.

All individuals with the history of vasectomy or autoimmune diseases were excluded from the study. Informed consent was obtained from all the participants and the obtained information remained confidential and within the institution.

Specimen collection and preparation

Ten ml blood samples without anticoagulant agent were taken from each participant. The samples were allowed to clot in the room temperature and then were centrifuged. The complement of the separated sera was inactivated by incubation in 56 °C for 30 minutes. The sera were aliquoted in 100 µl portions and kept at -20 °C until use.¹³

The semen specimens were collected by routine method from control (fertile) donors and were analyzed immediately after liquefaction according to the World Health Organization standards.¹⁴ Only specimens with counts $>60 \times 10^6/\text{ml}$, mixed antiglobulin reaction (MAR) percentage=0%, and motility rate $>50\%$, were used. The semen samples were also examined for: volume, color, viscosity, pH, count of white blood cells and red blood cells, percentage of abnormal cells, non-existence of antisperm antibodies on the sperms' surfaces, and occurrence of cellular spontaneously agglutination.

Preparation of spermatozoa suspension

Semen samples were incubated in 37°C, for maximum half an hour, for liquefaction. After

performing the above-mentioned evaluations, all samples were washed twice using phosphate-buffered saline (PBS), at pH=7.4. Then, the cell pellets were resuspended in 1 ml of PBS.¹⁵

Fluorescent Flow Cytometric Analysis

Flow cytometry (FCM) was used as an objective method of quantifying sperms coated by ASAs. Prior to running the flow cytometry test, the sera were thawed at room temperature and vortexed to ensure thorough mixing. In each test tube, a 50 µl of each serum sample was added to 50 µl of PBS supplemented with 5% bovine serum albumin (Merck Company, Germany). Ten µl of spermatozoa suspension, prepared by the swim-up method from antibody-negative donor semen containing 125000 motile sperm was added to each sample. Known antisperm antibody-positive and-negative seminal plasma samples were included as controls. A blank sample was included in which sperm were processed in the absence of serum.

The tubes were incubated at 37°C in a 5% CO₂ incubator for 1 hour. Then, the contents of each tube were washed twice with PBS to remove unbound antibody by adding 1 ml of PBS followed by mixing the sample. The tubes were then centrifuged at 500×g for 5 minutes and the supernatants were discarded. The sperm pellet was resuspended with 1 ml of PBS and the wash was repeated. After centrifugation, the pellet was resuspended with 50 µl of a 1:5 solution of fluorescein isothiocyanate conjugate (FITC)-labeled rabbit anti-human immunoglobulin directed against IgG, IgM, or IgA (Dako Company, Denmark) in PBS. For this purpose, for each participant's sample, we considered four test tubes and to each tube a part of this spermatozoa suspension was added. One tube considered as negative control (the sample containing no anti-human immunoglobulins) and FITC-labeled rabbit anti-human immunoglobulin directed against IgG, IgM or IgA was added to each of the other three tubes. All tubes were incubated at 4°C for 1 hour in the dark. Unbound antibody was removed by washing twice with PBS as described earlier and resuspended in 250 µl of PBS and the sperms were analyzed by flow cytometry to assess the number with bound

antibody using a Coulter Epics Flow Cytometer (Coulter Corporation, Miami, Florida, USA).^{13,16}

The sperm population was gated using 90-degree and forward-angle light scatter to exclude debris and aggregates. A total of 5000 sperms were analyzed from each sample, recording single-parameter histograms of the log FITC intensity.^{17,18} Digital subtraction of the blank sample from each test sample using the SYSTEM II™ Software program (Version 1.0., Coulter Corporation, USA) was performed to determine the percentage of sperms in each sample that was positive for antisperm antibody.

The sera were considered antisperm antibody-positive when ≥50% of the sperms were antibody-bound. All flow cytometry assay conditions were chosen after preliminary experiments to determine the optimum preparation of antisperm antibody-negative and -positive sperm populations. The results were reported as percentage of spermatozoa bounded to FITC intensity.^{13,18}

Statistical analysis

SPSS software version 13 was used for statistical analyses. For descriptive statistics Pearson Chi-Square (χ^2), Fisher's exact test, and independent sample *t* test were used. Statistical significance was considered at $P \leq 0.05$.

Results

The mean age in infertile group was 29.5 years (SD= ±8.3) and in fertile group was 42.4 years (SD= ±15.7). The median of duration of infertility among infertile couples was 1 year (minimum 1 and maximum 10 years). In infertile group, six patients had history of undergoing intrauterine insemination (IUI) and one patient mentioned the history of undergoing in vitro fertilization (IVF).

Among the infertile group, in 21 (72.4%) couples, at least one of the partners was ASA positive. Although the rate of positive sera (containing IgA antisperm antibody) in fertile group was high (24.1% v 8.2% in infertile group), it showed no significant differences ($P=0.05$).

Table 1 and figures 1 to 3 show the comparison of the mean percentage of antibody positive sperms [sperms coated by IgG, IgM, and IgA classes of ASAs] in fertile and infertile groups.

Table 1: Comparison of the mean percentage of antibody positive sperms in fertile and infertile groups.

Antibody	Group	No.	Mean percentage of antibody positive sperms	SD	P value
IgG	Infertile	60	30.4%	15.0	0.16
	Fertile	29	35.6%	18.7	
IgM	Infertile	60	56.7%	15.7	0.10
	Fertile	29	62.7%	16.3	
IgA	Infertile	60	35.3%	13.5	0.11
	Fertile	29	40.5%	16.5	

SD= Standard Deviation.

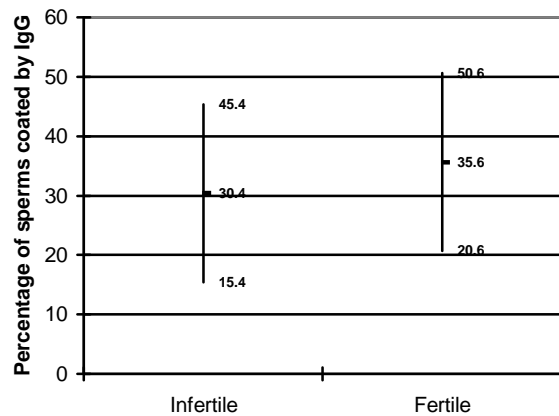


Figure 1: Comparison of percentage of sperms coated by IgG in fertile and infertile groups ($P>0.05$).

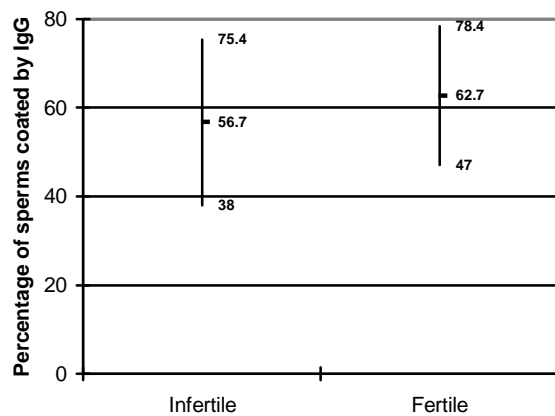


Figure 2: Comparison of percentage of sperms coated by IgM in fertile and infertile groups ($P>0.05$).

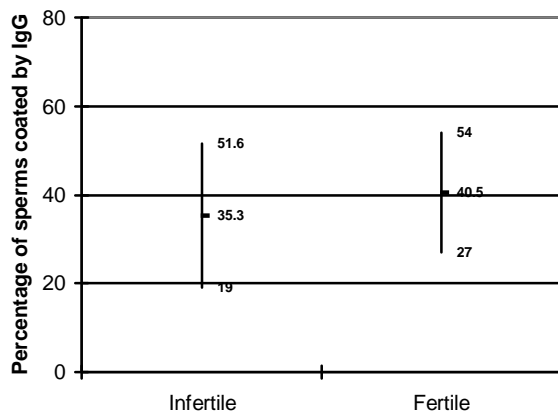


Figure 3: Comparison of percentage of sperms coated by IgA in fertile and infertile groups ($P>0.05$).

Table 2 shows comparison of the rate of ASA (IgG, IgM and IgA) positive sera in fertile and infertile groups. Comparing the mean percentage of antibody positive sperms and the rate of ASA positive sera in fertile and infertile groups showed no significant differences (all $P>0.05$). There were no significant differences between the mean percentage of antibody positive sperms and the rate of ASA

positive sera in fertile and infertile men and women (all $P>0.05$).

Table 2; Comparison of the rate of antisperm antibody positive sera in fertile and infertile groups.

Antibody	Group	No.	% of antibody positive sera	P value
IgG	Infertile	60	8.2	0.3
	Fertile	29	17.2	
IgM	Infertile	60	60.7	0.4
	Fertile	29	69.0	
IgA	Infertile	60	8.2	0.05
	Fertile	29	24.1	

Discussion

The present study failed to detect association between serum IgM, IgG, and IgA antisperm antibodies (tested by FCM) and infertility. Evaluation of antisperm antibody was not helpful in differentiating between infertile and fertile men and women in our study population.

There are numerous detailed reports on human antisperm antibodies and interference of some of them with reproductive processes. It is supposed that binding of antisperm antibodies to sperm surface inhibits sperm function and fertilization and the presence of circulating antisperm antibodies in serum of women has been implicated as a contributing factor to infertility. In these studies, the incidence of subsequent pregnancy in infertile couples was reduced significantly if one or both partners had antisperm antibodies in serum or in genital tract secretions.^{11,13} According to other reports, the prevalence of ASA positive cases in men and women with unexplained infertility was significantly more than cases with explained infertility.³ This suggests that ASAs may affect fertility. Regarding the aforementioned studies, the concept of antisperm antibodies (ASAs) in fertilization is based on their presence in serum and different secretions of the human reproductive tract.

Although some studies have pointed to the higher prevalence of ASAs in infertile patients,^{1,11,19,20} recent studies suggest that not all ASAs can cause infertility. Current diagnostic evaluations are not able to differentiate between infertility-related ASAs and those that do not interfere with infertility because the antigenic specificities of infertility-related ASAs are unknown.^{5,7,8} Similar to our study, there are other reports revealing that ASAs can be found in a relatively high percentage of both fertile and infertile men and women.^{10,15,21}

The conflicting notions about the significance of antisperm immunity in fertilization may be connected either with the methodological problems,¹⁰ and/or with the problem of adequacy of methods used for detection of ASAs,²² or detecting special antibodies against sperm surface antigens or sperm internal

antigens,^{20,21,23,24} such as CD52.²⁵ ASAs target different antigens. These different antibody binding sites may be positioned on the sperm surface or be a part of internal sperm antigens. Formation of Antigen-antibody complexes, exert diverse impacts on the sperm function. For instance, fertility may affect the formation of CD52-anti CD52 complex on the sperm surface. Such antibodies may lead to a direct cause-and-effect relationship in infertility.^{24,26}

The present findings and the above studies indicate that evaluating the presence of ASAs may not be useful in prognostication of the infertility. These antibodies may contribute to subfertility rather than result in absolute infertility.

Taken together, the present study proposes assessment of ASAs with special effects on sperm (i.e., antibodies which target special antigens and damage the function of gametes, damage fertilization, activate the complement cascade resulting in sperm lysis, etc.) be used in place of total serum ASAs. Additionally, molecular characterization of infertility-related ASAs antigens and the development of appropriate identification assays could prepare an accurate detection and proper treatment for infertile couples with ASAs in the future.

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Conflict of Interest: None declared

References

- 1 Berek JS. Berek and Novak's Gynecology. 14th ed. New York: Lippincott Williams & Wilkins; 2007.
- 2 Ulcová-Gallová Z. Mixed antiglobulin reaction and tray agglutination test for detection of sperm antibodies. *Int J Fertil Menopausal Stud* 1994; 39: 185-91.
- 3 Berkowitz RS, Barbieri RL, Ryan KJ, Kistner RW. Kistner's Gynecology: Principles and Practice. 6th ed. St. Louis: Mosby-Year Book; 1995.
- 4 Mandelbaum SL, Diamond MP, DeCherney AH. Relationship of antisperm antibodies to oocyte fertilization in in vitro fertilization-embryo transfer. *Fertil Steril* 1987; 47: 644-51.
- 5 Chiu WW, Chamley LW. Human seminal plasma antibody-binding proteins. *Am J Reprod Immunol* 2003; 50: 196-201.
- 6 Chamley LW, Clarke GN. Antisperm antibodies and conception. *Semin Immunopathol* 2007; 29: 169-84.
- 7 Vujisić S, Lepej SZ, Jerković L, et al. Antisperm antibodies in semen, sera and follicular fluids of infertile patients: relation to reproductive outcome after in vitro fertilization. *Am J Reprod Immunol* 2005; 54: 13-20.
- 8 Chiu WW, Chamley LW. Clinical associations and mechanisms of action of antisperm antibodies. *Fertil Steril* 2004; 82: 529-35.
- 9 Heidenreich A, Bonfig R, Wilbert DM, et al. Risk factors for antisperm antibodies in infertile men. *Am J Reprod Immunol* 1994; 31: 69-76.
- 10 Collins JA, Burrows EA, Yeo J, YoungLai EV. Frequency and predictive value of antisperm antibodies among infertile couples. *Hum Reprod* 1993; 8: 592-8.
- 11 Peters AJ, Coulam CB. Sperm antibodies. *Am J Reprod Immunol* 1992; 27: 156-62.
- 12 Omu AE, Makhseed M, Mohammed AT, Munim RA. Characteristics of men and women with circulating antisperm antibodies in a combined infertility clinic in Kuwait. *Arch Androl* 1997; 39: 55-64.
- 13 Nikolaeva MA, Kulakov VI, Goukasian IA, et al. Flow cytometry study on the effect of serum and peritoneal fluid of women on sperm-binding activity of immunoglobulin G antisperm antibodies. *Fertil Steril* 1997; 67: 680-6.
- 14 Laboratory manual of the WHO for the examination of human semen and sperm-cervical mucus interaction. *Ann Ist Super Sanita* 2001; 37: 1-123.
- 15 Culligan PJ, Crane MM, Boone WR, et al. Validity and cost-effectiveness of antisperm antibody testing before in vitro fertilization. *Fertil Steril* 1998; 69: 894-8.
- 16 Ferrara F, Daverio R, Mazzini G, et al. Automation of human sperm cell analysis by flow cytometry. *Clin Chem* 1997; 43: 801-7.
- 17 Haas GG Jr, Cunningham ME. Identification of antibody-laden sperm by cytofluorometry. *Fertil Steril* 1984; 42: 606-13.
- 18 Nicholson SC, Robinson JN, Sargent IL, Barlow DH. Detection of antisperm antibodies in seminal plasma by flow cytometry: comparison with the indirect immunobead binding test. *Fertil Steril* 1997; 68: 1114-9.
- 19 Bronson R. Detection of antisperm antibodies: an argument against therapeutic nihilism. *Hum Reprod* 1999; 14: 1671-3.
- 20 Haas GG Jr, D'Cruz OJ, DeBault LE. Comparison of the indirect immunobead, radio-labeled, and immunofluorescence assays for immunoglobulin G serum antibodies to human sperm. *Fertil Steril* 1991; 55: 377-88.

- 21 Szczepańska M, Skrzypczak J, Kamieniczna M, Kurpisz M. Antizona and antisperm antibodies in women with endometriosis and/or infertility. *Fertil Steril* 2001; 75: 97-105.
- 22 Helmerhorst FM, Finken MJ, Erwich JJ. Antisperm antibodies: detection assays for antisperm antibodies: what do they test? *Hum Reprod* 1999; 14:1669-71.
- 23 Bhande S, Naz RK. Molecular identities of human sperm proteins reactive with antibodies in sera of immunoinfertile women. *Mol Reprod Dev* 2007; 74: 332-40.
- 24 Shetty J, Naaby-Hansen S, Shibahara H, et al. Human sperm proteome: immunodominant sperm surface antigens identified with sera from infertile men and women. *Biol Reprod* 1999; 61: 61-9.
- 25 Koyama K, Ito K, Hasegawa A. Role of male reproductive tract CD52 (mrt-CD52) in reproduction. *Soc Reprod Fertil Suppl* 2007; 63: 103-10.
- 26 Hasegawa A, Koyama K. Target antigens for sperm-immobilizing antibodies found in infertile women. *Chem Immunol Allergy* 2005; 88: 27-33.

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