



REVIEW

Sex difference in anti-sperm antibodies

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Abstract

Background: Some diseases have sex differences. There have been no reports on the relationship between anti-sperm antibodies (ASA) and sex differences.

Methods: ASA are detected by sperm-immobilization test using patients' sera in women. In men, the ASA testing is generally performed by direct-immunobead test.

Main findings: Sperm-immobilizing antibodies in women inhibit sperm migration in their genital tract and exert inhibitory effects on fertilization. ASA bound to sperm surface in men also show inhibitory effect on sperm passage through cervical mucus. The fertilization rate of IVF significantly decreased when sperm were coated with higher numbers of ASA. For women with the antibodies, it is important to assess individual patients' SI_{50} titers. In patients with continuously high SI_{50} titers, pregnancy can be obtained only by IVF. For men with abnormal fertilizing ability by ASA, it is necessary to select intracytoplasmic sperm injection. Production of sperm-immobilizing antibodies is likely to occur in women with particular HLA after exposure to sperm. The risk factors for ASA production in men are still controversial.

Conclusion: Attention to sex differences in specimens, test methods and the diagnosis of ASA should be paid. For patients with ASA, treatment strategies have been established by considering sex difference for each.

KEYWORDS

anti-sperm antibody, autoimmune disease, sex difference, sperm-immobilizing antibody

1 | INTRODUCTION

Anti-sperm antibodies (ASA) are one of the causes of immune infertility, and they are produced in some infertile men and women. In the infertility outpatient clinic, the ASA testing for women, such as sperm-immobilization test (SIT),^{1,2} is simple for the clinicians because it requires only the patients' sera. In men, however, the world health organization (WHO) recommend to perform the ASA testing for the detection of sperm-bound antibodies simultaneously during the routine semen examination.³ Due to the complexity, it has been

suggested that the actual examinations of ASA for infertile men are rare. Therefore, the option of testing sperm-immobilizing antibody in the infertile patients' sera is discussed for men and women. However, evaluation of sperm-immobilizing antibody in men's sera did not reveal its clinical significance.⁴

In this way, it seems to be generally hard to say that the sex difference in ASA is well recognized. For women, sperm are immunogenic and they do not exist in themselves, and ASA are strictly homologous antibodies. On the contrary, ASA are produced as autoantibodies in men. So far, there have been no reports on the

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relationship between ASA and sex difference. Here, the relationship between the two, which has not been discussed, will be described.

2 | AUTOIMMUNE DISEASES AND SEX DIFFERENCES

In most of the diseases, standard diagnostic and treatment guidelines have been aimed at adult men. However, since epidemiological studies have shown that some diseases have sex differences, it is not always best to apply diagnostic and treatment methods based on men to women.

2.1 | Sex differences in autoimmune diseases

It is reported that 8.5 million people in the United States suffer from autoimmune diseases, 80% of whom are women. In Japan as well, the proportion of women is 2 to 10 times higher than that of men in both systemic and organ-specific autoimmune diseases.^{5,6} The difference between sex hormones and sex chromosomes in men and women can be considered as the causes of sex differences. As for the relationship between autoimmune diseases and sex hormones, it has been shown that estrogens promote immune responses, while androgens suppress immune responses.⁷ Recently, it was revealed that the X escapee *Kdm6a* regulates multiple immune response genes, providing a mechanism for sex differences in autoimmune disease susceptibility.⁷

Systemic lupus erythematosus (SLE) and antiphospholipid antibody syndrome (APS), which are representatives of systemic autoimmune diseases, are common in the childbearing age, and there are many aggravations during pregnancy and puerperal period. Furthermore, since the risk of onset increases when hormone replacement therapy is received after menopause, the involvement of estrogen in the onset and pathogenesis of autoimmune diseases is suggested.⁸ On the other hand, in rheumatoid arthritis (RA), it is difficult to believe that estrogen is involved in the onset of autoimmune diseases, rather than the peak of the onset being before and after menopause, and the symptom is improved during pregnancy, and it has been suggested that the decrease in androgen due to aging is involved in the onset.^{9,10}

2.2 | Sex difference in the frequency of anti-sperm antibody (ASA) production in patients with systemic autoimmune diseases

In patients with autoimmune diseases, autoantibodies that cause tissue damage can reduce reproductive capability, but the majority of such patients are women. In order to ascertain sex differences, the frequency of ASA production was compared between men and women with systemic autoimmune diseases.

In the initial study, our group investigated whether systemic autoimmune diseases could be one of the risk factors for developing

ASA in men.¹¹ Serum samples obtained from 70 men with systemic autoimmune diseases and 80 healthy controls were examined, by using the indirect-immunobead test (I-IBT).¹² Patients in whom the effects of steroids and immunosuppressants could not be ruled out were excluded. The results of I-IBT showed that 5 (7.1%) of 70 men with systemic autoimmune diseases had serum sperm-binding antibodies. However, no positives existed in 80 healthy men ($p < 0.05$, Figure 1), indicating that systemic autoimmune diseases may be one of the risk factors for developing ASA in men. Later, our group additionally examined serum samples obtained from 78 women with systemic autoimmune diseases using the I-IBT.¹³ Among the 78 women, none had serum ASA. A significant sex difference was observed between men and women with systemic autoimmune diseases ($p < 0.05$; Fisher's exact test, Figure 1).

In conclusion, it was found a clear sex difference in the frequency of ASA production in patients with systemic autoimmune diseases.

3 | SEX DIFFERENCES IN ANTI-SPERM ANTIBODIES (ASA)

The frequency of ASA is about 3% in both men^{14,15} and women.¹⁶ ASA are generally detected by the sperm-immobilization test (SIT)^{1,2} using patient's serum in women. On the other hand, ASA are evaluated by the direct-immunobead test (D-IBT)¹² or the mixed antiglobulin reaction (MAR) test¹⁷ using patient's sperm in men. Therefore, in the medical care of ASA, not only the detection method but also the treatment strategy is obviously different for men and women. Therefore, it is very important for the clinicians to recognize that there is sex difference in ASA when they recommend the tests for ASA to the couples and also deciding the strategy when the ASA are found to be positive in either men or women.

3.1 | Measurement and evaluation of ASA

Several assay methods have been developed to detect ASA. However, it is obvious that the most important consideration is the selection of the method. Currently available tests for detecting ASA can be classified into the three groups according to the characteristics of each test (Table 1).¹⁶ Group 1 contains tests for the detection of bioactivity of ASA, including sperm-agglutination test (SAT), SIT, and fertilization-blocking test (FBT) that detects fertilization-blocking antibodies. Group 2 contains tests for the detection of ASA bound for motile sperm, including IBT, MAR test, and Panning test. Group 3 contains tests for the detection of ASA against sperm or sperm extract, including radiolabeled antiglobulin test, indirect immunofluorescent test, fluorescent-activated cell sorter (FACS), enzyme-linked immunosorbent assay (ELISA), and passive hemagglutination for the evaluation of ASA.

As mentioned above, it is necessary to recognize that the selection of the ASA testing is different in men and women.

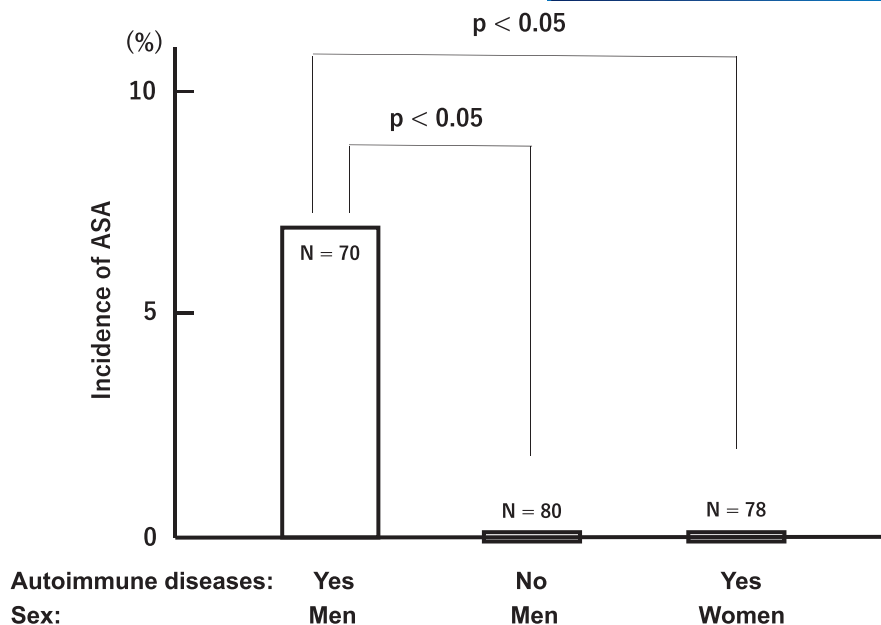


FIGURE 1 Comparison of the incidences of anti-sperm antibodies (ASA) between men with and without systemic autoimmune diseases and those of ASA between men and women with the diseases. In order to ascertain sex differences, the frequency of ASA production was compared between men and women with systemic autoimmune diseases. Serum samples were obtained from 70 men with systemic autoimmune diseases, 80 healthy men and 78 women with systemic autoimmune diseases. These sera were examined by using the indirect-immunobead test (I-IBT). The results of I-IBT showed that 5 (7.1%) of men with systemic autoimmune diseases had serum sperm-binding antibodies. However, no positives existed in 80 healthy men ($p < 0.05$), indicating that systemic autoimmune diseases may be one of the risk factors for developing ASA in men. Among the 78 women, none had serum ASA. A significant sex difference was observed between men and women with systemic autoimmune diseases ($p < 0.05$; Fisher's exact test)

TABLE 1 Available tests for detecting anti-sperm antibody (ASA)¹⁶

Group 1. Tests for detecting bioactivity of ASA.

- SAT.
- SIT.
- FBT.

Group 2. Tests for detecting ASA bound for motile sperm.

- IBT or IS.
- MAR test.
- Panning test.

Group 3. Tests for detecting ASA against sperm or sperm extract.

- Radiolabeled antiglobulin test.
- Indirect immunofluorescent test.
- FACS.
- ELISA.
- Passive hemagglutination.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FACS, fluorescent-activated cell sorter; FRT, fertilization-blocking test; IBT, immunobead test; IS, ImmunoSpheres; MAR, mixed antiglobulin reaction; SAT, sperm-agglutination test; SIT, sperm-immobilization test.

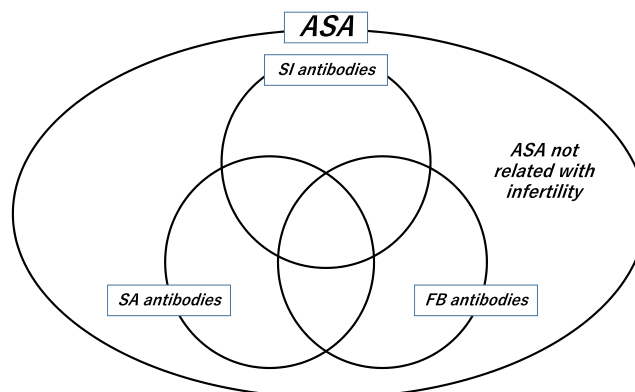


FIGURE 2 Relationship of various kinds of anti-sperm antibodies (ASA) produced in immunologically infertile women.¹⁶ Several assay methods have been developed to detect ASA. The relationship between ASA with bioactivities, including sperm-immobilizing (SI) antibodies, sperm-agglutinating (SA) antibodies, and fertilization-blocking (FB) antibodies, and those without bioactivities is drawn in this scheme. In those methods for the detection of ASA, there are some assays for the detection of ASA that are not related with infertility. Therefore, it has been recommended that clinically specific test for infertile women should be chosen for detecting circulating ASA, that is, the tests for detecting ASA with bioactivities are suitable for choosing as initial testing for infertile women

3.1.1 | Measurement and evaluation of ASA in women

In those methods for the detection of ASA, it has been recommended that clinically specific test for infertile women should be chosen for detecting circulating ASA. The relationship between ASA with bioactivities, including sperm-immobilizing antibodies, sperm-agglutinating antibodies, and fertilization-blocking antibodies, and those without bioactivities is drawn in a scheme of Figure 2.¹⁶ Therefore, the tests for detecting ASA belonged to the group 1 (Table 1) are suitable for choosing as initial testing for infertile women. In them, sperm-immobilizing antibodies are recognized as one of the causes of refractory infertility in women, and the antibodies are detected in patient's serum by the SIT, and their detection is useful for determining the strategy for infertility treatments in women.

Around the year of 1970, Isojima et al.^{1,2} reported that positive reactions in the SIT were given by sera of 17.2% of the patients with infertility of unexplained cause and not by those of normal pregnant and unmarried women. Later, this observation was also confirmed by other studies.¹⁸⁻²² However, it should be noted that the result obtained by the SIT, named as sperm-immobilization value (SIV), is only to be semi-quantitative. As the SIV cannot quantify the antibody exactly, the strategy for the treatment of infertile women with sperm-immobilizing antibodies is not able to be decided.

For the quantitative assay for SIT, Isojima et al.²³ developed the quantitative method for this antibody. In brief, each test serum is serially diluted twofold with control serum. The sperm motility in the test serum (T %) and in control serum (C %) were calculated according to the semi-quantitative assay method. The antibody activity for sperm immobilization is calculated by the formula $(C-T/C \times 100)$, and the value is plotted against the dilutions of the test serum on a semi-logarithmic chart to obtain a sigmoid dose-response curve. The dilution of the test serum at which the sigmoid curve crossed the value of 50 for the antibody activity is determined on the dose-response line and designated as 50% sperm-immobilization unit (SI_{50}). Thus, the amount of sperm-immobilizing antibodies in various sera can be compared. This method is very useful for routine examination of sperm-immobilizing antibody in the sera of infertile women.

3.1.2 | Measurement and evaluation of ASA in men

On the other hand, the clinical significance of sperm-immobilizing antibodies in serum could not be clarified in men.⁴ The reason might be explained by the fact that, even if ASA are present in the sera in men, fertilization of sperm with oocyte never occur in their body.

Therefore, it is considered clinically important in men whether the ASA binds to the sperm when semen are ejaculated in the woman's vagina. It is introduced in the WHO laboratory manual that the D-IBT or the MAR test is useful for the evaluation of ASA in sperm.³ D-IBT has widely been used as a screening test for ASA.^{12,24,25} However, it should be noted that the positive result obtained by the

D-IBT or MAR test means that the patient has only sperm-bound antibodies. If the detected antibodies have inhibitory bioactivities on fertility, they can be the cause of infertility in the patient.

Therefore, to establish the strategy for the treatment of infertile men with ASA, a secondary examination must be carried out. When the result of D-IBT is positive, subsequently the inhibitory effects by ASA on sperm penetration through cervical mucus and fertilizing ability¹⁵ should be evaluated to decide for the strategy of infertility treatments.²⁶

Recently, the IBT was discontinued of its production. However, the ASA test results obtained by the ImmunoSpheres (IS) assay as a substitute was shown in agreement with the results obtained with the IBT test.²⁷

3.2 | Causes of immune infertility in patients with ASA

Some of the ASA have been shown to be highly related to reproductive failures.²⁸ The presence of ASA in women has been shown to inhibit sperm migration in their genital tract. Such antibodies can also exert inhibitory effects on various stages of sperm-egg interaction and subsequent embryo development in vitro.

The presence of ASA in men can reduce fertility as similar as that in women. However, the association between the presence of ASA in men and infertility had previously been disputed before it was clarified that there is a diversity of ASA bound to sperm.^{14,15}

3.2.1 | Causes of immune infertility in women with ASA

Inhibition of sperm migration in the female genital tract by ASA possessed in women

ASA could cause infertility in female at different stages of reproduction including inhibitory effect on sperm transport in the cervical mucus (CM). There have been several reports which found the incidence of poor post-coital test (PCT) results was significantly higher in infertile women with sperm-immobilizing antibodies in their sera than in those without the antibodies.²⁹⁻³² Later, our group revealed that the SI_{50} titer in the serum can predict inhibitory effects on sperm migration through CM in infertile women with sperm-immobilizing antibodies.³³ When patients with sperm-immobilizing antibodies were divided into two groups according to the SI_{50} titers, the abnormal result of PCT was obtained in all 10 patients with high (≥ 10) SI_{50} titers, while that was 14 (66.7%) in 21 patients with low (< 10) SI_{50} titers ($p < 0.05$).³³

To overcome the inhibitory effects of ASA on sperm transport in the CM, infertile women with ASA are generally treated by intra-uterine insemination (IUI). However, patients with ASA, especially sperm-immobilizing antibodies, are less likely to conceive even with IUI.³⁴ One of the reasons is that the sperm-immobilizing antibodies could interfere with sperm migration at

the level of the fallopian tubes. To test the possible impairment of sperm migration in the fallopian tubes, our group performed laparoscopic examinations and investigated the presence of motile sperm in the peritoneal fluid following IUI,³⁵ named peritoneal sperm recovery test (PSRT).³⁶ As a result, sperm recovery in the peritoneal fluid was not observed in 24 (88.9%) of 27 patients with sperm-immobilizing antibodies, while it was not observed in 140 (66.0%) of 212 patients without the antibodies ($p < 0.05$). When the 27 patients with sperm-immobilizing antibodies were divided into two groups according to the SI_{50} titers, the abnormal result of PSRT was obtained in 22 (95.7%) of 23 patients with high (>10) SI_{50} titers, while that was 2 (50.0%) in 4 patients with low (≤ 10) SI_{50} titers ($p < 0.05$).³⁵ These findings suggested that the SI_{50} titer in the serum can predict inhibitory effects on sperm migration from uterine cavity through fallopian tube in infertile women with sperm-immobilizing antibodies.

Inhibition of various stages of fertilization by ASA possessed in women

Several diagnostic tests were developed to predict the fertilization potential of sperm, including the hemizona assay (HZA)³⁷ for sperm-zona pellucida tight binding, zona pellucida penetration assay³⁸ for sperm penetration through zona pellucida, and zona-free hamster egg penetration assay³⁹ for sperm-egg fusion. It was reported that the sperm-immobilizing antibodies can exert inhibitory effects on sperm-egg interaction, including acrosome reaction,⁴⁰ zona pellucida recognition, and penetration.⁴¹⁻⁴⁷ Moreover, we found that the ASA block fertilization at specific stages. Some of them may inhibit sperm capacitation and thus prevent all processes of fertilization that follow. Some other antibodies may not affect capacitation and sperm binding to zona pellucida but inhibit the acrosome reaction, followed by the blocking of sperm penetration through zona pellucida and ooplasm.⁴⁸

Infertile women with sperm-immobilizing antibodies are generally treated by in vitro fertilization-embryo transfer (IVF-ET),^{34,49-52} that is an ideal method for the treatment only if a special attention is paid to remove the antibodies adhered to cumulus cells around oocytes before insemination. The contamination of blood in the aspirated follicular fluid should be avoided as much as possible, and the antibodies in the follicular fluid are removed by transferring oocytes several times to the new culture medium. The patient's serum should never be added to the culture medium.

3.2.2 | Causes of immune infertility in men with ASA

Inhibition of sperm migration in the female genital tract by ASA possessed in men

Diverse ASA are bound to sperm surfaces in men.^{14,15} Moreover, immunologically infertile men had a relatively high incidence of asthenozoospermia. We also found that there exists significant inhibitory effect of sperm-immobilizing antibodies in ejaculated human sperm

on sperm motility in immunologically infertile men.⁵³ In this study, all men who were positive on the direct SIT were diagnosed as asthenozoospermia to a varying degree.

ASA bound to sperm surface in infertile men also show significant inhibitory effect on sperm passage through CM.⁵⁴ However, it was also proved by the study that the ability of sperm passage through CM is not inhibited in two-fifths of men with ASA. It indicates there is a diversity of ASA bound to sperm surface in infertile men.

Inhibition of various stages of fertilization by ASA possessed in men

As for the blocking effects on fertilization by ASA bound to sperm surface, there have been conflicting reports. Some investigators have shown that ASA in men have an inhibitory effect on fertilization.⁵⁵⁻⁵⁹ However, others have arrived at the opposite conclusion.⁶⁰ So we analyzed the diverse effects of ASA bound to the surface of ejaculated human sperm on fertilization.¹⁵ Four (57.1%) of seven patients who had IB-bound sperm of $\geq 80\%$, fertilizing ability was inhibited, while none of the eight patients who had $< 80\%$ IB-bound sperm had an inhibitory effect on fertilization ($p = 0.01$).¹⁵

There have been some investigators who demonstrated that the fertilization rate of IVF significantly decreased when the inseminated sperm were coated with higher numbers of ASA.^{56,58} These findings also suggest that in infertile men having positive but lower levels of ASA on the ejaculated sperm, ASA-free sperm might contribute to higher fertilization rates in IVF. In contrast, those with higher levels of ASA have fewer ASA-free sperm, which leads to a significant reduction in fertilization.

3.3 | Treatment strategy for the infertile patients with ASA

As mentioned above, the diagnosis of ASA is different between men and women. Moreover, the causes of infertility in men and women with ASA are not completely same. Therefore, the strategy for the treatment of men and women with ASA should be different each other.

3.3.1 | Strategy for the treatment of infertile women with ASA

For infertile women with ASA, treatments such as condom therapy, abstinence, and steroid suppression therapy have been applied, and they resulted a generally poor consequence.⁶¹ IUI can overcome problems related to sperm passage through the CM, and it has been shown to be useful for the treatment of a part of infertile women with ASA. However, when the SI_{50} titer of sperm-immobilizing antibodies is higher, sperm inserted in uterine cavity by IUI procedure would be impaired by the antibodies secreted from uterine cavity to fallopian tube, and even in follicular fluid.^{34,35} Therefore, the ultrasound-guided intra-tubal insemination (ITI) therapy, that was developed by

Jansen et al.,⁶² for infertile women with sperm-immobilizing antibodies might be effective as a substitute for IUI.^{63,64}

Some success has been achieved with IUI, but not when serum ASA levels are high. To overcome the inhibitory effects of ASA on sperm migration within the CM and the fallopian tubes, IVF-ET has been applied and satisfactory outcomes resulting from suitable culture conditions for gametes and embryo have been obtained. After removing the ASA adhered to cumulus cells around oocytes and avoiding the contamination of blood in the aspirated follicular fluid and moreover removing the antibodies in the follicular fluid by transferring oocytes several times to the new culture medium with replacement serum added to the culture medium, fertilization, and cleavage rates of mature oocytes from the patients with ASA were not different from those in patients without the antibodies.⁵⁰ It is also important that substitution of the patients' serum by replacement serum in the fertilization and embryo growth media may prove to be an effective means of improving IVF treatment for women with ASA.⁵¹

For infertile women with sperm-immobilizing antibodies, it is important to assess the SI_{50} titers by the quantitative method to select appropriate treatments. Kobayashi et al.³⁴ proposed a strategy for the treatment of infertile women with sperm-immobilizing antibodies according to the undulation patterns of individual patient's SI_{50} titers. They divided patients with sperm-immobilizing antibodies into three groups according to their follow-up SI_{50} titers. Group

A, which consisted of patients with continuously high SI_{50} titers (>10 units), did not conceive by ordinary or repeated IUI, however, a satisfactory pregnancy rate was obtained by IVF-ET. Group B, in which the patients had intermediate SI_{50} titer patterns around 10, showed rare rates of success with IUI. In Group C, the patients with continuously low SI_{50} titers (<10 units), conception by repeated or ordinary IUI was achieved, although the success rates were lower than by IVF-ET. Taken together, a strategy for the treatment of infertile women with sperm-immobilizing antibodies is suggested in Figure 3.⁶⁵

3.3.2 | Strategy for the treatment of infertile men with ASA

Some infertile men with ASA might be treated with the classical treatments such as immunosuppressive therapies using corticosteroids or cyclosporine to reduce production of ASA. However, the randomized controlled trials (RCTs) have not found a therapeutic significance for the corticosteroid therapy for infertile men with ASA.⁶⁶⁻⁶⁹ Moreover, as corticosteroids are well known to have potential side effects, important clinical considerations in the use of corticosteroids to treat infertile patients with ASA are mandatory.⁷⁰⁻⁷² As for the effect of cyclosporine A treatment, no definite conclusions can be drawn so far.⁷³

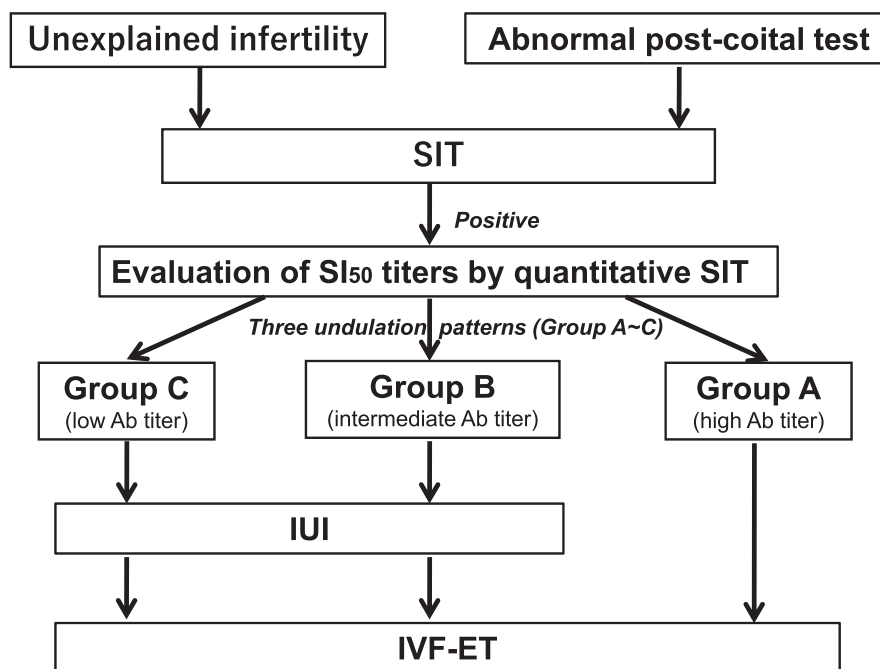


FIGURE 3 Strategy for the treatment of infertile women with sperm-immobilizing antibodies.⁶⁵ It is important to assess the 50% sperm-immobilization unit (SI_{50}) titers to select treatments for infertile women with sperm-immobilizing antibodies. A strategy for the treatments of infertile women with sperm-immobilizing antibodies is shown according to the undulation patterns of individual patient's SI_{50} titers. Patients with sperm-immobilizing antibodies are divided into three groups according to their follow-up SI_{50} titers; Group A: the patients with continuously high SI_{50} titers (>10 units) are recommended to be treated by in vitro fertilization–embryo transfer (IVF-ET). Group B: the patients have intermediate SI_{50} titer patterns around 10, and Group C; the patients with continuously low SI_{50} titers (<10 units) are recommended to be treated by repeated or ordinary intra-uterine insemination (IUI). If they are not able to be conceived by several cycles of IUI, then the treatment by IVF-ET should be considered

The purpose of the technique for removing ASA bound to sperm for infertile men with ASA is the effort to remove sperm-bound antibody from sperm. However, the dissociation of tightly bound antibodies has been found to be difficult. There have been several reports, including simple sperm washing,⁷⁴ split ejaculation into medium containing serum,⁷⁵ immune absorption of ASA,⁷⁶⁻⁷⁸ and enzymatic treatment.⁷⁹⁻⁸² The simple sperm washing did not remove ASA from the sperm surface. The addition of serum to sample collection pots may improve the oocyte fertilization rate. The latter 2 methods have not been clinically applied yet.⁸³

There have been reported other options for the treatments of infertile men with ASA. So far, the usefulness of IUI,^{26,84-86} IVF-ET^{26,57,58,86-89} and intracytoplasmic sperm injection (ICSI)^{26,90,91} for the treatments of infertile men with ASA have been reported. However, for the appropriate strategy to infertile men with ASA, it is necessary to evaluate fertilizing ability of the patient's ejaculated sperm before starting the IUI treatment.²⁶ If there is no inhibitory effect on fertilization by ASA, the treatment using IUI might be useful for some men with ASA who have the negative post-coital test results. For the patient with ASA who is diagnosed to have abnormal fertilizing ability based on the HZA, it is necessary to advise the infertile couple for selecting ICSI-ET as a primary treatment (Figure 4).²⁶

3.4 | Production of ASA

Before the clinical introduction of IVF-ET and ICSI, men and women with ASA were refractory to the classical treatment as mentioned above. If most of men and women, or male and female animals, naturally and easily produce ASA, it might be suggested that such species cease to exist. If not, there is a question what are the causes for the production of ASA. Immuno-contraceptives could be developed in future when the reasons for the production of ASA will be clarified.

3.4.1 | Production of ASA in women

Women do not generally produce antibodies against sperm, however, some infertile women have been found to possess ASA which may contribute to their infertility.⁹² Moreover, the reasons why most women do not develop an immune response following exposure to sperm is not clear yet.

It has been demonstrated that susceptibility to various immune disorders including autoimmune diseases has a genetic background. Therefore, our group examined the frequencies of human leukocyte antigen (HLA)-DQA1, HLA-DQB1, and HLA-DRB1 genes and found that the high frequency of HLA-DRB1*0901 and DQB1*0303 genes in the Japanese population may account for higher frequency of sperm-immobilizing antibody.⁹² Another immune disease such as juvenile-onset myasthenia gravis⁹³ and systemic lupus erythematosus with antiphospholipid syndrome⁹⁴ are reported to be linked to

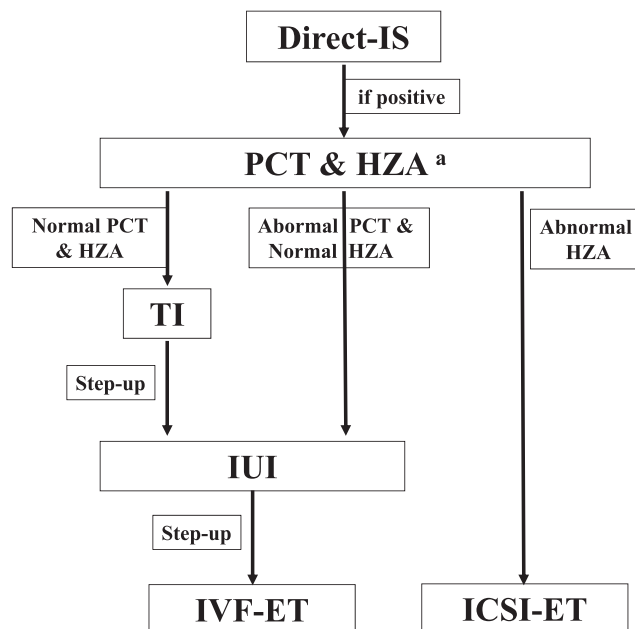


FIGURE 4 Strategy for the diagnosis and the treatment of infertile men with anti-sperm antibodies (ASA).²⁶ To diagnose immunological infertility in men, the direct-ImmunoSpheres (D-IS) test should be carried out as a routine test in the infertility clinic. Then, the post-coital test (PCT) and the hemizona assay (HZA) should be performed as initial screening for infertile men with ASA diagnosed by D-IS, as a basis for decision making. If a patient with ASA has an abnormal hemizona index (HZI), it seems reasonable to advise selecting intracytoplasmic sperm injection-embryo transfer (ICSI-ET) as the primary treatment. For the patients with ASA and normal HZI, selection of timed intercourse (TI) or intra-uterine insemination (IUI) should be advised depending on the PCT result. ^a: If the HZA is not available, infertile men who have IB-bound sperm of $\geq 80\%$ had better be advised for selecting ICSI-ET as a primary treatment

these genotypes. These immune diseases also occur at a higher frequency in the Japanese population.

Then, the following studies were conducted to clarify the source of immunity for the production of ASA by using severe combined immunodeficient (SCID) mice.⁹⁵ Successful heterotransplantation with human peripheral blood lymphocytes from infertile women with sperm-immobilizing antibodies into SCID mice indicate that the immune response resulting in the production of the antibodies in SCID mice might require the ongoing presence of the eliciting human sperm antigens.⁹⁶

In clinical practice, there was a significant difference in the incidence in sperm-immobilizing antibodies between the women treated by ICSI because of a severe male factor and those whose husbands had a normal sperm count.⁹⁷ Moreover, it was also found that infertile women with past Chlamydia trachomatis infection has significantly higher incidence of sperm-immobilizing antibodies.⁹⁸ Chlamydia trachomatis infection could play a role in the production of sperm-immobilizing antibodies in infertile women.

Therefore, the production of sperm-immobilizing antibodies is likely to occur in women with particular HLA haplotypes after

TABLE 2 Sex difference of anti-sperm antibodies (ASA) in infertile couples

	Women	Men
Classification	Homologous antibody	Autoantibody
Samples	Serum/CM/FF	Motile sperm
Assay	Sperm-immobilization test (Indirect method)	ImmunoSpheres (direct method)
Frequency	Approximately 3%	Approximately 3%
Causes of infertility	<ul style="list-style-type: none"> • Inhibition of sperm transport in CM and uterine cavity to fallopian tubes • Inhibition of sperm-egg interaction 	<ul style="list-style-type: none"> • Asthenozoospermia if ASA show sperm-immobilizing activities • Inhibition of sperm transport in CM
Treatment strategy	IUI or IVF is selected according to the SI ₅₀ titers	<ul style="list-style-type: none"> • ICSI is selected if ASA show inhibitory effect on fertilization • IUI is selected if ASA show only inhibitory effect on sperm penetration through CM • TI is selected if ASA never show biological activities
Production of ASA	<ul style="list-style-type: none"> • Specific HLA genes • Exposure to enough amount of sperm • Genital tract infection such as Ct 	Breakdown of the BTB such as vasectomy followed by vasovasostomy

Abbreviations: CM, cervical mucus; Ct, Chlamydia trachomatis; FF, follicular fluid; ICSI, intracytoplasmic sperm injection; IUI, intra-uterine insemination; IVF, in vitro fertilization; SI₅₀, 50% sperm-immobilization unit; TI, timed intercourse.

repeated exposure to a large enough amount of sperm. Inflammatory disease of the genital tract, such as Chlamydia trachomatis infection, might be a trigger at least for women to produce sperm-immobilizing antibodies.

3.4.2 | Production of ASA in men

In men, the blood-testis barrier (BTB) and immune privilege are critical for fertility, as breakdown can result in the generation of autoimmune T and B lymphocytes, and production of ASA.⁹⁹ This leads to autoimmune-mediated testicular damage, germ cell loss, and impaired sperm function.^{99,100} ASA can affect male fertility by various mechanisms.¹⁰¹ Some of them relate to sperm agglutination and/or complement-dependent sperm immobilization.⁵³ ASA also impair sperm penetration through cervical mucus and interfere with sperm-egg interaction.^{15,54}

The possible risk factors of ASA production in men have been shown such as congenital or acquired chronic obstruction of the male reproductive tract (MRT), infection in the MRT, varicocele, cryptorchidism, testicular injuries, testicular tumors, homosexual men, spinal cord injury, and autoimmune diseases.¹⁰² However, the relationship between these risk factors and the production of ASA is still controversial. Recently, it was suggested that vasectomy followed by vasovasostomy is the only clinical condition that shows almost permanently high titers of ASA in numerous clinical series.¹⁰³

4 | CONCLUSIONS AND FUTURE DIRECTIONS

A summary of the previously unreported sex differences in ASA is shown in Table 2. Since ASA are allogeneic antibodies in women and

autoantibodies in men, it is characterized by having a background different from that of other general diseases. At the time of diagnosis, it is necessary to pay attention to the difference in specimens, the difference in test methods, the mechanism of infertility onset and to treat immune infertility by ASA. Treatment strategies are established for each.

It is strongly expected that the development of immunological contraceptive vaccines using sperm antigens will be materialized in the future as the elucidation of the production mechanism of ASA progresses.

CONFLICT OF INTEREST

Nothing to declare.

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