



## Diagnosis and treatment of immunologically infertile women with sperm-immobilizing antibodies in their sera

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### ARTICLE INFO

#### Article history:

Received 6 February 2009

Received in revised form 15 May 2009

Accepted 6 July 2009

#### Keywords:

Sperm-immobilizing antibody

Sperm protein

Gamete immunology

Female infertility

### ABSTRACT

Detection of sperm-immobilizing antibodies in women may have relevance for diagnosis of immunological infertility. Infertile women in whom sperm-immobilizing antibodies are detected can be refractory to conventional treatments such as timed intercourse or intrauterine insemination (IUI) because the antibodies secreted in the female reproductive tract might impair sperm passage, inhibit fertilization, and prevent normal post-fertilization processes. Hence, manipulation of gametes and embryos from patients with sperm-immobilizing antibodies should be carried out with additional care to avoid fertilization failure resulting from the presence of antibodies during *in vitro* fertilization (IVF). Moreover, the reasons for the why majority of women do not develop sperm-immobilizing antibodies on exposure to sperm is not clear. The production of sperm-immobilizing antibodies is likely to occur in women with particular HLA haplotypes after repeated exposure to sperm. Characterization of sperm-immobilizing antibodies may help in the identification and characterization of sperm specific antigens that can be used as candidate antigens for the development of sperm based contraceptive vaccines.

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### 1. Introduction

Half a century has passed since the relationship between antisperm antibody and sterility was identified in female guinea pigs by Isojima et al. (1959) and Katsh (1959). In humans, a high incidence of sperm agglutinin has been identified in women with unexplained infertility (Franklin and Dukes, 1964). However, the relationship has not been widely accepted as causal because some studies have shown that sperm agglutination occurs equally in the sera from both women with unexplained infertility and con-

trol women (Tyler et al., 1967; Israelstam, 1969; Isojima et al., 1972). It may be argued that spontaneous sperm agglutination is common and it is difficult to determine whether the agglutination is due specifically to antisperm antibody activity, or to a non-specific factor (Isojima et al., 1968).

Since these original studies, several assay methods have been developed to detect antisperm antibodies. Now an important consideration in this field is the selection of the method for detection. Amongst them, the 'sperm immobilization test' which detects sperm-immobilizing antibodies, has been shown to be the most reliable assay for detecting antisperm antibodies linked to female infertility (Isojima et al., 1968, 1972). This observation has been confirmed by other studies (Ansbacher et al., 1973; Jones et al., 1973; Petrunia et al., 1976; Cantuaria, 1977). In this review, we describe clinical aspects of sperm-immobilizing antibodies in female immunological infertility.

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### 1.1. Sperm immobilization test and the incidence of sperm-immobilizing antibodies in infertile women

Serum samples were collected with informed consent from infertile women. The sperm immobilization test was performed as previously described by Isojima et al. (1968), and a test value of 2 or more was considered positive for sperm-immobilizing antibody. In addition, all sera with sperm-immobilizing antibodies were tested to determine the antibody titers ( $SI_{50}$ ) by quantitative sperm immobilization test as described by Isojima and Koyama (1976) and Koyama et al. (1988).

The sperm immobilization test was performed for sera from 3015 infertile women and 77 women had sperm-immobilizing antibodies, giving a positive rate of 2.6%. In total, 46 of 1676 (2.7%) infertile women attending the Hyogo College of Medicine between January 1991 and December 1995, and 31 of 1339 (2.3%) infertile women attending Jichi Medical University between May 1999 and 2008, tested positive for the sperm immobilization test. There was no significant difference in the incidence of sperm immobilization test-positivity between the two groups ( $t$ -test,  $P > 0.05$ ).

A follow-up study has shown that  $SI_{50}$  titers were found to be unstable and to fluctuate over a period of several months within each patient, when infertile women with sperm-immobilizing antibodies were evaluated repeatedly during a 3-year period (Koyama et al., 1988).

## 2. Possible roles of sperm-immobilizing antibodies in female infertility

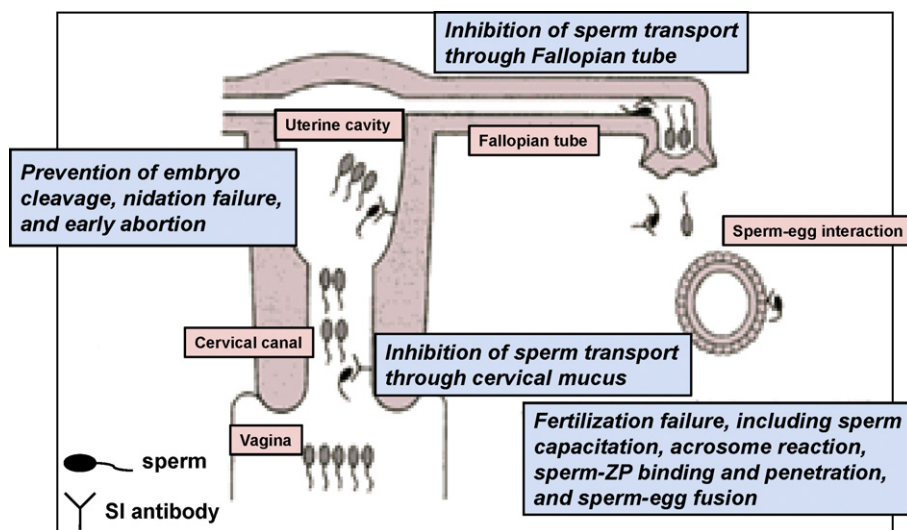
It is well known that infertile women with sperm-immobilizing antibodies are refractory to the conventional fertility treatments of timed intercourse or intrauterine insemination (IUI), presumably because the antibodies secreted in the female reproductive tract might impair

sperm passage, inhibit fertilization, and prevent post fertilization processes (Fig. 1). Several clinical tests are described here which were used to clarify the roles of sperm-immobilizing antibodies in female immunological infertility.

### 2.1. Post-coital test

Koyama et al. (1980) previously reported that the frequency of poor post-coital test results was significantly higher in patients with sperm-immobilizing antibodies in their sera than in those without sperm-immobilizing antibodies. Using the micro method for sperm immobilization testing, they also showed that sperm-immobilizing antibodies exist in the cervical mucus of women with sperm-immobilizing antibodies in their sera (Isojima and Koyama, 1975). They speculated that the sperm-immobilizing antibodies are either transmitted from the blood stream or produced locally in the cervix along with a small amount of complement.

The relationship between the level of serum sperm-immobilizing antibodies and their possible inhibitory effect on sperm migration through cervical mucus in immunologically infertile women has been investigated by our group (Shibahara et al., 2007). In some women with sperm-immobilizing antibodies, the post-coital test was carried out before ovulation and assessed according to the criteria defined by the World Health Organization (1992). Infertile couples with abnormal semen characteristics were excluded from the study. The post-coital test was carried out as close as possible to the time of ovulation as determined by clinical criteria, including usual cycle length, basal body temperature, cervical mucus change and follicular diameter examined by transvaginal ultrasound. Under high ( $400\times$ ) magnification, the average motile sperm count in cervical mucus was examined approximately 6–12 h after coitus. According to the criteria, an abnor-



**Fig. 1.** Possible roles of sperm-immobilizing antibodies in infertility. It is well known that infertile women with sperm-immobilizing antibodies are refractory to conventional treatments including timed intercourse or intrauterine insemination (IUI) because the antibodies secreted in the female reproductive tract might impair sperm transport at the levels of cervical canal or Fallopian tube, inhibit several stages of fertilization, and prevent post fertilization process.

**Table 1**  
Relationship between SI<sub>50</sub> titers and inhibitory effects on sperm transport by sperm-immobilizing antibodies in the female genital tract.

SI <sub>50</sub> titers at the test	No. of normal/no. of tested	
	PCT	PSRT
<10	7/21* (33.3)	2/4** (50)
10≤	0/10* (0)	1/23** (4.4)
Total	7/31 (22.6)	3/27 (11.1)

PCT, post-coital test; PSRT, peritoneal sperm recovery test. Values in parenthesis are percentages.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

mal post-coital test result was defined as that in which less than 10 motile spermatozoa were detected in cervical mucus.

The incidence of normal post-coital test results were compared between infertile patients having high (>10) and low (10≤) SI<sub>50</sub> titers in their sera according to the classification by Kobayashi et al. (1990). Only 7 of 31 women (22.6%) with sperm-immobilizing antibodies and 109 of 137 women (79.6%) without the antibody had normal post-coital test results ( $P < 0.0001$ ). When patients with sperm-immobilizing antibodies were divided into two groups according to the SI<sub>50</sub> titers, normal post-coital test results were obtained in none of 10 patients with high (>10) SI<sub>50</sub> titers. In contrast, 7 out of 21 patients (33.3%) with SI<sub>50</sub> antibody titers <10 had a normal post-coital test, which was significantly ( $P < 0.05$ ) different from data from patients with higher SI<sub>50</sub> antibody titers (Table 1). Thus, SI<sub>50</sub> titer in the serum can predict inhibitory effects on sperm migration through cervical mucus in immunologically infertile women.

## 2.2. Peritoneal sperm recovery test

To test the possible impairment of sperm migration by sperm-immobilizing antibodies in the fallopian tubes, the peritoneal sperm recovery test (Templeton and Mortimer, 1980) was performed to examine the presence of motile sperm in the peritoneal fluid collected by laparoscopy after intrauterine insemination (IUI) (Shibahara et al., 1995). The peritoneal sperm recovery test was carried out in 28 infertile women with sperm-immobilizing antibodies in their sera, and the results were compared with those of 322 infertile women without the antibodies. All the patients had been treated using several cycles of IUI before the peritoneal sperm recovery test.

Among couples with normal semen characteristics (defined using WHO criteria), sperm recovery in the peritoneal fluid was observed in only 3 of 27 (11.1%) patients with sperm-immobilizing antibodies, compared with 72 of 212 (34.0%) patients without the antibodies ( $P < 0.025$ ). When patients with sperm-immobilizing antibodies were divided into two groups according to the SI<sub>50</sub> titers, a normal peritoneal sperm recovery test result was obtained in 1 of 23 (4.4%) patients with high (>10) SI<sub>50</sub> titers, and 2 in 4 (50.0%) patients with low (10≤) SI<sub>50</sub> titers (Table 1). There was a significant difference between the two groups ( $P < 0.01$ ).

In most cases, a similar amount of sperm-immobilizing antibodies was present in the peritoneal fluid and the sera. Though complement activities in peritoneal fluid were generally less than those in sera, the former were still found to be sufficient to immobilize sperm *in vivo*.

These results suggest that complement-dependent sperm-immobilizing antibodies could interfere with sperm migration in the female genital tract at the level of the fallopian tubes. The SI<sub>50</sub> titer in serum can predict inhibitory effects on sperm migration from the uterine cavity to fallopian tubes in immunologically infertile women. Evaluation of the SI<sub>50</sub> titers in patients' sera appears to have value in aiding decision-making in infertile women with sperm-immobilizing antibodies, with respect to assessing their suitability as candidates for assisted conception using IUI.

## 2.3. Hemizona assay

The hemizona assay was used to evaluate the effects of sperm-immobilizing antibodies on sperm-zona pellucida tight binding *in vitro* (Burkman et al., 1988; Shibahara et al., 1991; Shibahara et al., 1993). Serum samples were collected from 24 infertile women with sperm-immobilizing antibodies and 16 women with unexplained infertility without the antibodies. Sera from two postpartum women were used as controls.

Of 24 patients' sera with sperm-immobilizing antibodies, 23 (96%) showed a significant inhibitory effect in the hemizona assay, whereas none of 16 patients' sera with unexplained infertility and without sperm-immobilizing antibodies exhibited any inhibitory effect. The cut-off value of the hemizona index (HZI) of 50 or less was considered a significant inhibition in hemizona assay according to the criteria described by Mahony et al. (1991). A significant difference was found in the average HZI of the two groups ( $17.3 \pm 12.5$  vs  $81.1 \pm 16.1$ ,  $P < 0.0001$ ).

However, we found no correlation between the SI<sub>50</sub> titers and HZI. Therefore, two human (H6-3C4 [Isojima et al., 1987], En46 [Komori et al., 1988]) and two mouse (2H12 [Tsuji et al., 1992], 2C6 [Kameda et al., 1991]) monoclonal sperm-immobilizing antibodies were subjected to hemizona assay. Only one of the four monoclonal antibodies showed a significantly inhibitory effect on the sperm-zona pellucida tight binding. These results were also supported by similar results obtained from experiments using mouse sperm and five monoclonal sperm-immobilizing antibodies in mice for *in vitro* fertilization (IVF) and hemizona assay (Shibahara et al., 1996a).

It seems that there are at least two kinds of sperm-immobilizing antibodies, one with both sperm immobilization and blocking of sperm-zona pellucida binding activities and another with the former activity alone. The majority of sperm-immobilizing antibodies reduce binding of sperm to zona pellucida without complement. In most cases treated by IVF, a similar amount of sperm-immobilizing antibodies have been found to be present in follicular fluid and sera (Shibahara et al., 2006). Therefore, it is recommended that the manipulation of gametes and embryos from patients with sperm-immobilizing antibodies should be carried out more carefully than usual to

avoid fertilization failure due to the presence of antibodies in IVF.

#### 2.4. Impairment of embryo development by sperm-immobilizing antibodies following IVF

Koyama et al. (1984) showed that antibodies reacting with rat sperm in the presence of complement can impair *in vitro* development of fertilized rat eggs, indicating that the sperm antigens are still present on the embryo. Later, our group generated clinical data suggesting that sperm-immobilizing antibodies in the sera of infertile women cause poor embryo quality following IVF (Taneichi et al., 2002). The fertilization rate was significantly lower in the medium supplemented with the patient's serum than that with human serum albumin (48.2% vs 86.0%,  $P < 0.0001$ ), indicating that one of the causes of fertilization failure in IVF is sperm-immobilizing antibodies. Embryo quality was assessed by the Veeck's classification (1991); the rate of good quality embryos was significantly lower in the former group than in the latter group (39.0% vs 79.1%,  $P = 0.0003$ ), indicating that sperm-immobilizing antibodies in the sera of infertile women interfere with embryo development *in vitro*.

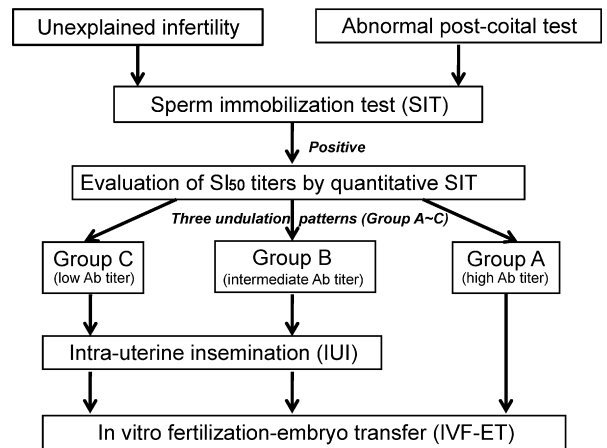
Therefore, the manipulation of gametes and embryos from patients having sperm-immobilizing antibodies should be carefully carried out especially to avoid contaminating the culture medium with patient's serum and follicular fluid, in order to achieve a better IVF result.

### 3. Strategy for the treatment of infertile women with sperm-immobilizing antibodies

It is well known that infertile women having sperm-immobilizing antibodies are refractory to the conventional treatments with timed intercourse or IUI because the antibodies secreted in the female genital tract inhibit sperm migration within cervical mucus and the Fallopian tubes. To overcome this cause of infertility *in vivo*, IVF-embryo transfer (ET) has been applied and satisfactory outcomes resulting from suitable culture conditions for gametes and embryo have been obtained (Sugimoto et al., 1986; Shibahara et al., 1996b).

Kobayashi et al. (1990) 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 and embryo transfer. 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.

A strategy for the treatment of infertile women with sperm-immobilizing antibodies is summarized in Fig. 2. The strategy emphasizes the importance of assessing  $SI_{50}$



**Fig. 2.** Strategy for the treatment of infertile women with sperm-immobilizing antibodies. It is important to assess the  $SI_{50}$  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 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 IUI. If they are not able to be conceived by several cycles of IUI, then the treatment by IVF-ET should be considered.

titers to select treatments for infertile women with sperm-immobilizing antibodies.

### 4. Production of sperm-immobilizing antibodies in infertile women

The factors that affect production of sperm-immobilizing antibodies in some women are not fully understood. Moreover, the reasons why most women do not develop an immune response following exposure to sperm is not clear yet.

#### 4.1. Which women produce antisperm antibodies?

Females do not generally produce antibodies against sperm, however, some infertile women have been found to possess antisperm antibodies which may contribute to their infertility. It has been demonstrated that susceptibility to various immune disorders including autoimmune diseases has a genetic background. Tsuji et al. (2000) analyzed HLA genotypes of 38 infertile women possessing sperm-immobilizing antibodies to examine whether susceptibility to antibody production is influenced by HLA. By examining the frequencies of HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes, they found that infertile women with sperm-immobilizing antibodies had a higher frequency of genes encoding HLA-DRB1\*0901 and HLA-DQB1\*303. Interestingly, another immune disease such as juvenile-onset myasthenia gravis (Matsuki et al., 1990) and systemic lupus erythematosus with antiphospholipid syndrome (Matsushita et al., 1996) are reported to be linked to these genotypes.

#### 4.2. Why do some women produce antisperm antibodies?

By using severe combined immunodeficient (SCID) mice that were heterotransplanted with human peripheral blood lymphocytes (Hu-PBLs) from infertile women with sperm-immobilizing antibodies, we previously reported that human sperm could be responsible for the immune response and for the production of sperm-immobilizing antibodies in infertile women (Shibahara et al., 1996c). In brief, we have successfully heterotransplanted Hu-PBLs from infertile women with sperm-immobilizing antibodies following pretreatment of SCID mice with anti-asialo GM1 antibodies and irradiation to deplete NK cells. Reconstruction of SCID mice with Hu-PBLs from women without the antibodies failed to produce antisperm antibodies. These findings might indicate that the immune response resulting in the production of sperm-immobilizing antibodies in SCID mice reconstituted with Hu-PBLs from infertile women with the antibodies might require the ongoing presence of the eliciting human sperm antigens.

It has been shown that some infertile women can conceive after receiving intraperitoneal insemination treatment, while others produced antisperm antibodies after receiving a second insemination procedure without becoming pregnant (Livi et al., 1990). The antibody production observed in this study might be either a transient response to massive antigen stimulation or the first step toward systemic immunity. We also found that the production of sperm-immobilizing antibodies in infertile women is associated with their husbands' sperm count (Shibahara et al., 2003).

These results indicate that production of sperm-immobilizing antibodies in women begins after they have been exposed to a large amount of sperm. Taken together, the production of sperm-immobilizing antibodies is likely to be triggered in women with specific types of HLA after being exposed to a large enough amounts of sperm. These findings might contribute to the development of immunocontraceptives based on sperm-specific antigens, which can induce sperm-immobilizing antibodies at a sufficient level to block sperm penetration through the female reproductive tract and thus inhibit fertilization.

#### 4.3. What are the target antigens of sperm-immobilizing antibodies?

Biochemical and molecular analysis of the target antigens of sperm-immobilizing antibodies would contribute to elucidation of the immunological mechanism for infertility in women, as well as contribute to a better understanding of the physiological functions of sperm surface antigens. To identify human sperm antigens recognized by the sera from infertile women having sperm-immobilizing antibodies, high resolution two-dimensional (2D) gel electrophoresis (established by Naaby-Hansen et al., 1997) was used (Shibahara et al., 2002). Fifty-two human sperm surface proteins reacted with 7 sera from infertile women with sperm-immobilizing antibodies, while 35 of these were reactive with the 6 sperm-immobilizing antibody-negative control sera. The coordinates of four prominent immunoreactive sperm surface proteins which were unique to the

sperm-immobilizing antibodies were identified by on the basis of their high reactivity with the sera from infertile women containing sperm-immobilizing antibodies, but lack of reactivity with any of the sperm-immobilizing antibody-negative infertile sera.

These sperm surface antigens might be considered as the ideal targets in developing immunocontraceptives because the patients with high  $SI_{50}$  titers are refractory to infertility treatments except for IVF-ET. However, it is reported that most of the sperm-immobilizing antibodies in sera of infertile women might be generated to carbohydrate structures of the sperm-coating antigens or sperm membrane proteins (Koyama et al., 1991). Further biochemical studies are necessary before constructing sperm specific immunocontraceptives.

### 5. Concluding comments

In summary, the presence of sperm-immobilizing antibodies is associated with immunological infertility in women, and it is important to assess  $SI_{50}$  titers to select an appropriate treatment modality for infertile women. Characterization of spermatozoa antigens which are targeted by sperm-immobilizing antibodies may help in proposing candidate antigen(s) for development of immunocontraceptive vaccines. Our previous review article detailing the diagnosis and treatment of immunologically infertile males with antisperm antibodies may also be useful in this regard (Shibahara et al., 2005).

### Acknowledgements

We express our sincere thanks to Dr. Shinzo Isojima, M.D., Dr. Nancy J. Alexander, Ph.D., and Dr. John C. Herr, Ph.D. for their useful suggestions and help.

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