

Chapter 1

Historical Background of Immune Infertility Associated with Anti-sperm Antibody (ASA) in Women



Hiroaki Shibahara

Abstract The sperm was the first target for immunocontraception. The production of sterility in the female by immunizing her to sperm of the same species is a possible solution to excessive fertility. Since Landsteiner and Metchnikoff documented antibody production against sperm in animals in 1899, a number of experiments had been carried out on the relationship between sperm immunity and infertility. Most of the experimental data arose from the immunization of male and female rodents against testicular, epididymal, and seminal antigens. However, the results obtained were inconclusive, with some showing positive relationship, while others arrived at the opposite conclusions.

In human, Rosenfeld was the first to suggest that a woman repeatedly injected with human semen became infertile in 1926. However, it seems to be a harmful and dangerous clinical trial as there might be common antigens against other organs except for reproduction in semen.

In 1964, Franklin and Dukes reported their clinical observations on anti-sperm antibodies (ASAs) in women. Surprisingly, they found 72.1% of women with unexplained infertility had a much higher incidence of sperm-agglutinating activity. There was a supportive appreciation for their clinical findings that they stimulated significant interest in the hypothesis that female immunological reactions to sperm could be involved in the etiology of otherwise unexplained infertility and in the concept of an anti-sperm contraceptive vaccine. However, there was a serious problem for their studies that the phenomenon of sperm-agglutination is not always a specific event by ASA. It has been well known that sperm-agglutination frequently occurs naturally, not related with the existence of ASA.

Because of the nonspecificity of the sperm-agglutination test for detecting ASA, a sperm-immobilization test (SIT), that utilized the function of sperm-immobilization in the presence of complement, was developed by Isojima et al. Other studies also confirmed the reliability of SIT as an assay for detecting ASA closely related to

H. Shibahara (✉)

Department of Obstetrics and Gynecology, School of Medicine, Hyogo Medical University, Nishinomiya, Hyogo, Japan

e-mail: sibahara@hyo-med.ac.jp

female infertility. In the previous review by Koyama, the incidences of sperm-immobilizing antibodies in the sera of unexplained and organic infertile women were found to be 13.2% and 1.4%, respectively, while the incidences of sperm-immobilizing antibodies in the controls, such as children, unmarried women, and pregnant women, were 0%, 0%, and 0.5%, respectively. In conclusion, the SIT proved to be one of the most reliable methods to detect ASA relevant to infertility in women.

1.1 Introduction

Gamete immunology is a major part of reproductive immunology, relating to many aspects of studies on immunological mechanisms in early reproductive phenomena, including gametogenesis and maturation of sperm and oocyte, ovulation, ejaculation, sperm migration, fertilization, and implantation of embryo.

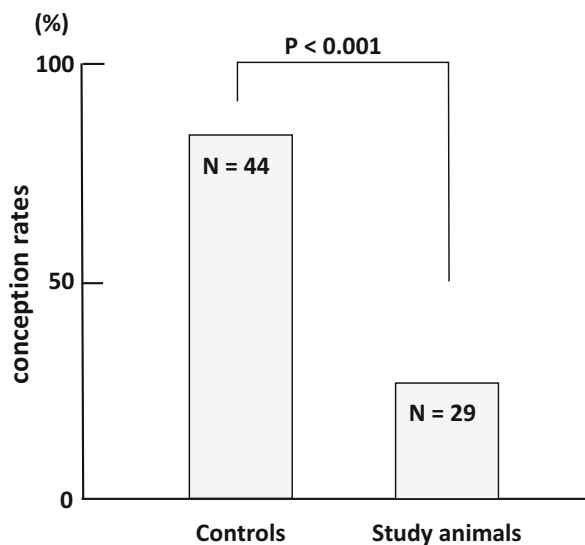
The sperm was the first target for immunocontraception. The induction of sterility in the female by immunizing her to sperm of the same species is a possible solution to excessive fertility. The rationale for a fertility-regulating vaccine directed against sperm has been established for over 120 years, by studies of the induction of infertility in a variety of immunized animals and by the recognition of the role of immunological factors in a proportion of otherwise healthy men and women.

1.2 Immunization of Female Injected with Sperm or Testis in Animals

In 1899, Landsteiner [1], a Nobel Prize winner, documented antibody production against sperm in animal. He repeatedly injected bovine sperm into the peritoneal cavity in guinea pigs. He found that the sperm immediately lost their motility when they were injected into the peritoneal cavity again. In the same year, Metchnikoff [2] independently explored the same phenomena using guinea pigs injected with sperm from various species.

Since they have reported, a number of works have been performed on the relationship between sperm immunity and infertility. Most of the experimental data arose from the immunization of male and female rodents against testicular, epididymal, and seminal antigens. Between 1920 and 1934, there were at least 12 studies of immunological contraception in women [3]. In some of these reports, infertility and humoral antibody formation were achieved by repeated injections of semen without the use of immunological adjuvants. However, the results obtained were conflicting, with some showing positive relationship, while others showed negative relationship. In them, McCartney's report [4] was the representative of the positive relationship between production of anti-sperm antibodies (ASAs) and infertility in rat, while further work in the field was discouraged by a thoroughly negative report of Henle et al. [5] in 1940, and since then, the subject has been largely neglected. The introduction of Freund's adjuvant for enhancing immunization encouraged researchers to reinvestigate this approach [6].

Fig. 1.1 Comparison of the conception rates between the female guinea pigs treated with homologous testis plus Freund's adjuvant and the controls. Fertility among the controls was 84% (37 of 44), while it was only 24% (7 of 29) in the group injected against homologous testis, which indicates that the active immunization of female guinea pigs with homologous testis or sperm using complete Freund's adjuvant markedly reduced pregnancy rates in immunized animals. There was a significant difference between the two groups ($P < 0.001$)



In 1959, Isojima et al. [7] demonstrated sterility in adult virgin female guinea pigs induced by injection with an emulsion of homologous adult testis and Freund's adjuvant before exposure to males. Fertility among the controls was 84% (37 of 44), while it was only 24% (7 of 29) in the group injected against homologous testis, which indicates that the active immunization of female guinea pigs with homologous testis or sperm using complete Freund's adjuvant markedly reduced pregnancy rates in immunized animals (Fig. 1.1). The testis-injected guinea pigs had also a high titer of antibodies against testis. The authors speculated that there were two possible mechanisms of sterility: one was the cellular immunity, as in Freund's experiment in aspermatogenesis [6], and the second was circulating antibodies which could appear in the vaginal fluid according to their observation or which could cause the uterus to contact with sperm, according to Katsh [8]. Katsh [9] also obtained a similar result by immunizing female guinea pigs with sperm using complete Freund's adjuvant. Later, Otani et al. [10] reevaluated and proved that immunization of female guinea pigs with homologous testis or sperm using complete Freund's adjuvant reduced pregnancy rates in immunized animals.

In mice, McLaren [11] showed the reduction of fertilization in females that received three intraperitoneal injections of live homologous sperm a week for 7 weeks. Females with high titers of anti-sperm agglutinins in their sera showed a marked reduction both in the size of the first litter and in total breeding performance over the 6-month period. They also showed that the reduced fertilization rate in immunized females was due to failure of the sperm to reach the site of fertilization.

Edwards [12] showed that, in his experiments, immunization of mice with sperm and adjuvant has not led to drastic reductions in infertility as found by Isojima et al. [7] in guinea pigs. It is difficult to make comparisons between species when the fertilization rate in the guinea pigs was unknown. The incidence of fertilization in his work was higher and more independent of the amount of circulating antibody than

Table 1.1 History of researches on the production of anti-sperm antibodies (ASA) and infertility in female animals

Year	Author	Ref. no.	Animal	Immunogen	Main results
1899	Landsteiner	[1]	Guinea pig	Sperm (bovine)	Sperm motility was lost
	Metchinikoff	[2]	Guinea pig	Various sperm	Sperm motility was lost
1923	MaCartney	[4]	Rat	Sperm	ASA production and infertility are related
1940	Henle	[5]	Guinea pig	Sperm	ASA production and infertility are not related
1959	Isojima	[7]	Guinea pig	Testis and FA	Reduced pregnancy rates with high ASA titers
	Katsh	[9]	Guinea pig	Sperm and FA	Reduced pregnancy rates with high ASA titers
1963	Otani	[10]	Guinea pig	Testis/sperm and FA	Reduced pregnancy rates with high ASA titers
1964	McLaren	[11]	Mouse	Sperm	Reduced fertilization rate with high ASA titers
	Edwards	[12]	Mouse	Sperm and FA	ASA production did not lead to infertility
1969	Bell	[13]	Mouse	Sperm	Systemic immunization resulted in infertility Intravaginal immunization did not result in infertility

FA complete Freund's adjuvant

was the incidence of infertility found in mice by McLaren [11]. Differences existed between the two investigations in the immunization procedure, and it is possible that a more effective immune response was produced in McLaren's work by the prolonged intraperitoneal immunization. He concluded that the high incidence of fertility remaining in mice after immunization indicates that an immunological method using sperm for the control of fertility has not yet been realized.

Bell [13] compared the efficacy of the methods for immunization of female mice between systemic and intravaginal sperm injection. He found that systemic iso-immunization of mice with sperm resulted in a significant reduction ($P < 0001$) of litter size. Re-immunization depressed fertility further to less than half the control level. Serum antibody levels were assayed both by a passive hemagglutination test adapted for detecting antibodies against mouse sperm and by a sperm-agglutination test (SAT). There was a significant regression of numbers of offspring on both sperm-agglutination and hemagglutination titers. Intravaginal iso-immunization with mouse sperm failed to alter the fecundity in short-term breeding periods or during long-term mating or to enhance the sterility induced by systemic injections.

The history of these researches on production of ASA and infertility in female animals is summarized in Table 1.1.

1.3 Immunization of Women Injected with Semen

In 1921, the editorials of the *JAMA* [14] commented that if sperm invade the female tissues and cause the formation of specific antibodies which are capable of preventing fertilization, may not such a process participate in the problem of sterility? They speculated that the traditional sterility of the prostitute depends sometimes on such a process rather than on inflammatory sealing of the tubes. In 1922, Mayer [15] and Vogt [16] stated that sperm overloading leads to sterility and that a natural cure occurs after prolonged abstinence from sexual intercourse.

In human, following some reports from birth control clinics of contraceptive efficacy of up to 20 months' duration by injecting human semen, Rosenfeld [17] was the first to suggest that a woman repeatedly injected with human semen became infertile. He attempted to immunize three women with multiple subcutaneous injections of semen and found serological evidence of immunity to sperm. Mild local reactions were noted but no systemic disturbance occurred. However, it seems to be a harmful and dangerous clinical trial as there might be common antigens against other organs except for reproduction in semen.

Following this report, Baskin [18] also reported infertility in women after immunization with human semen. He reported on 20 women who had received 3 intramuscular injections of 5–10 mL of fresh semen at 7-day intervals. The injections appeared harmless, and in all cases but one, humoral sperm cytotoxicity was induced and persisted for up to 12 months. Repeated testing of immune sera against sperm from different individuals indicated a lack of allospecificity of the reactions. No pregnancies occurred while positive serology persisted, and although the follow-up period was too short to allow a systematic assessment of subsequent fertility, one patient became pregnant 3 months after her serum reverted to negative following 9 months of immunity. Cervical secretions exhibited sperm cytotoxicity in about 50% of cases, but this showed no consistent relationship with serum activity. In 1937, Baskin was issued the US patent for a spermotoxic vaccine, which produced reversible sterilization in fertile women. Subsequent studies confirmed the induction of temporary infertility, without overt side effects in women injected with human sperm [19, 20].

Brunner reported a claim that 30 women had been immunized with the bovine sperm phospholipid and had remained sterile for 6 months in 1941 [21]. The history of these researches on production of ASA and infertility in human is summarized in Table 1.2.

1.4 Clinical Observations

Around World War II, studies for the relation between sperm immunity and infertility lost for a long time. In 1964, Franklin and Dukes [22, 23] reported their clinical observations on ASA in women. They found that 20.1% of 214 women undergoing

Table 1.2 History of researches on the production of anti-sperm antibodies (ASA) and infertility in human

Year	Author	Ref. no.	Immunogen	Main results
1926	Rosenfeld	[17]	Human semen	Repeated injection led to infertility
1932	Baskin	[18]	Human semen	No pregnancies occurred while positive serology persisted
1936	Escuder	[19]	Human sperm	Production of ASA and temporary infertility were induced
	Rodriguez	[20]	Human sperm	Production of ASA and temporary infertility were induced
1941	Brunner	[21]	Sperm phospholipid (bovine)	Temporary infertility was induced

infertility investigations had sperm-agglutinating activity in their serum. Within this group of patients, 31 (72.1%) of 43 women with unexplained infertility had a much higher incidence of sperm-agglutinating activity. The incidence was higher than those with organic causes of their infertility (8.4%) or fertile women (5.7%). They also reported that antibody titers in their sera declined markedly in all 13 women treated by condoms and/or abstained for 2–6 months. Ten women whose antibody titers dropped to undetectable levels were encouraged to resume unrestricted intercourse at the time of expected ovulation with the result that nine became pregnant. Later, they reported that 27 unexplained infertile women with sperm-agglutinating activity were treated by condoms and/or abstained. In 25 of 27 women, the antibody titers dropped to undetectable levels, and finally 20 of 25 women successfully conceived. There was a supportive appreciation for their clinical findings that they stimulated significant interest in the hypothesis that female immunological reactions to sperm could be involved in the etiology of otherwise unexplained infertility and in the concept of an anti-sperm contraceptive vaccine [24].

However, there was a serious problem for their studies that the phenomenon of sperm-agglutination is not always a specific event by ASA. It has been well known that sperm-agglutination frequently occurs naturally, not related with the existence of ASA. Therefore, after their reports, the relationship between sperm-agglutinating antibodies and infertility has been studied inconclusively by many other investigators [25–27]. One of the reasons may lie in the difficulty of discriminating true antibody-specific sperm-agglutination from nonspecific agglutination [28].

Because of the nonspecificity of the SAT for detecting ASA, a sperm-immobilization test (SIT), that utilized the function of sperm-immobilization in the presence of complement, was developed by Isojima et al. [25]. By using this method, it was found that the complement-dependent sperm-immobilizing antibodies were detected exclusively in 3 (12%) of 25 women with unexplained infertility. All of them showed a history of more than 5 years' infertility since marriage.

Later, Isojima et al. [29] compared the results of the SIT and Franklin-Dukes' and Kibrick's SAT by using sera of sterile, pregnant, and unmarried women. Positive reactions in the SIT were given by sera of 17.2% of the patients with sterility of

unexplained cause and not by those of normal pregnant and unmarried women. However, Franklin-Dukes' and Kibrick's SAT gave rather higher percentages of positive sperm-agglutination reactions in normal pregnant women than in cases of sterility of unexplained cause but a very low percentage of positive reactions in unmarried women. They also showed that the sperm-immobilization value did not drop during condom therapy over a long period, while Franklin and Dukes reported a sharp drop in sperm agglutinin in the serum of cases after abstinence or condom therapy for several months [24].

Other studies also confirmed the reliability of SIT as an assay for detecting ASA closely related to female infertility. Ansbacher [30] reported that sperm-immobilizing antibodies appear to have a better correlation with infertility than sperm-agglutinating antibodies.

Jones et al. [31] used 2 tests, including SAT and SIT, to detect ASA in the female partners of 196 infertile couples and in a control group of 50 pregnant women. SAT demonstrated little difference in incidence of positive reactions in patients with unexplained and organic infertility and yielded an 18% incidence in the pregnant control patients. The results of the SIT showed a clear disparity in patients with unexplained infertility compared with those in the organic infertility and pregnancy groups. A comparison of these results with those obtained by other workers, using the same and other methods, suggests that the SIT provides the most reliable single method for the detection of circulating anti-sperm activity with possible clinical significance.

Petrunia [32] studied for the presence of anti-sperm factors in the sera from 102 women with infertility due to a variety of causes and from 40 pregnant women. Three techniques were used: sperm microagglutination, sperm-immobilization, and an indirect immunofluorescent technique for detection of sperm-bound immunoglobulins. There was no correlation between the results obtained using these three different techniques. Of the three, only the results of SIT correlated with primary unexplained infertility. The sperm microagglutination test appeared to measure nonspecific factors. Methanol fixation of sperm used in the indirect immunofluorescent technique apparently resulted in nonspecific binding of immunoglobulins.

Cantuaria [33] collected the sera and cervicovaginal secretions of 39 infertile women without abnormality except apparent cervical hostility and evaluated for the presence of sperm-immobilizing antibodies. The antibodies were found in 25.6% of sera and 20.5% of cervicovaginal secretions. No such antibodies were detected in controls.

In the recent review by Koyama [28], the incidences of sperm-immobilizing antibodies in the sera of infertile women or the control women were shown in Table 1.3. The incidences of the antibodies in unexplained and organic infertile women were found to be 13.2% and 1.4%, respectively, while the incidences of sperm-immobilizing antibodies in the controls, such as children, unmarried women, and pregnant women, were 0%, 0%, and 0.5%, respectively. In conclusion, the SIT proved to be one of the most reliable methods to detect ASA relevant to infertility in women.

Table 1.3 Incidences of sperm-immobilizing antibodies in the sera of infertile women and the controls

Subjects	Incidence
<i>Infertility</i>	
Unexplained	119/901 (13.2%)
Organic	31/2190 (1.4%)
<i>Controls</i>	
Children	0/1013 (0%)
Unmarried women	0/92 (0%)
Pregnant women	1/202 (0.5%)

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