

Evaluation of Different Sperm Immunization Methods in Mice

Abolghasem Ajami^{1*}, Amir Esmailnejad Moghaddam², Hasan Motamed¹

¹Department of Immunology, ²Department of Embryology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran.

ABSTRACT

Background: Antifertility effect of naturally occurring antisperm antibody (ASA) in infertile couples and studies on experimental immunization of various animals with sperm antigens represents ASA as immunocontraceptive target. Despite extensive research on the effects of different factors on sperm immunogenicity and ASA production variable result have been reported. **Objective:** To study whole sperm immunization in mice. **Methods:** In an experimental study, whole mice sperm with different adjuvant i.e. complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (ICFA), and cholera toxin subunit- β (CTS- β) were administrated to mice intramuscularly (IM), subcutaneously (SC), intranasally (IN), intra-peritoneally (IP), intrarectally (IR), intravaginally (IVA) and orally. Control groups were inoculated with phosphate buffer saline (PBS) plus corresponding adjuvant. Immunization was carried out on days 0, 7, 14, 28 and ASA titers were detected by indirect immunofluorescence (IFA) technique in sera and vaginal washes of all groups. The IP group was further excluded from the study due to high mortality rate. The results were compared between control and experimental groups by Mann Whitney and Fisher exact tests. **Results:** The number of positive mice for ASA in IM, SC, IN experimental and control groups were significantly different ($P = 0.01$, $P = 0.01$, $P = 0.04$, respectively). However, there were no significant differences between IR, IVA, and oral experimental and control groups. No differences were observed between ASA in vaginal washing of all groups. Due to high mortality in IP group it was excluded from the study. **Conclusion:** It can be concluded that the whole sperm antigen can induce immune response in female mice by IM, SC, IN but not IAV, IR and oral administration routes.

Key words: Antisperm antibody, Immunization, Immunofluorescence, Sperm

*Corresponding author: Dr. Abolghasem Ajami, Department of Immunology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran. e-mail: ajami_ghasem@hotmail.com

INTRODUCTION

It is well documented that germ cell antigens behave more as foreign than as self. Haploid germ cells in the males do not develop until puberty, long after the fetal or neonatal period, when self tolerance is established. This leads to the development of the theory that sperm autoantigens are sequestered behind a strong blood-testis barrier (1). Thus it can generate an immune response in both males and females as allo, iso and even auto antigen (2).

Well documented antifertility effects of naturally occurring ASA in infertile couples and studies on experimental immunization of male or female animals of various species with sperm antigens represent ASA as an immunocontraceptive target (3,4). The effect of different factor i.e. antigens, adjuvant and route of administration on sperm immunogenicity and ASA production have been studied and variable results have been reported. Whole sperm with CFA injected SC to male and female Possums indicated an immuno-contraceptive potential for it (5). High levels of ASA have been detected in sera of mice after SC and IM injection of sperm (6). Intra-gastric administration of sperm in female rat induced a wide range of short to long term infertility (7). This kind of immunization produced secretory IgA in female genital tract and inhibited sperm activity (8). SC and IM immunization of mice with specific sperm antigen produced ASA, the levels of ASA peaked at 48-50 days of the initial injection and subsequently declined to the amount of control group at 255 days (9).

The efficacy of sperm immunization, like other antigens, depends on the nature of target antigen, route of administration, adjuvant and immunization protocol. In order to evaluate the effect of administration route on the whole mice sperm immunization, it was inoculated into female BALB/c mice through different routes and ASA titers were measured in sera and vaginal washes.

MATERIALS AND METHODS

Animals. Male and female albino BALB/c mice, weighting 30-40 grams at age of 10-12 weeks and female rabbits, weighting about 2kg at 5-6 months of age were purchased from Tehran Pasteur Institute (Tehran, Iran). Animals were individually kept in an animal house on a 12 hr light/dark cycle.

Sperm preparation. After spinal dislocation of mice, sperm cells were collected in PBS from tail of epididymis through trans-abdominal epididymal resection. Collected samples were centrifuged at 302 g for 5 minutes and washed with equivalent volume of PBS for 5 times. The samples were subsequently resuspended in PBS for further use.

Adjuvants and immunogens. A solution of $3.8-4.2 \times 10^6$ spermatozoa/ml of PBS was emulsified with an equal volume of CFA (Biogen, Mashhad, Iran) and inoculated IM and SC to the mice as the first immunization dose. The same amount of sperm, emulsified with an equal volume of ICFA (Biogen, Mashhad, Iran), was used as booster immunization. For IN, IR and IVA and oral routes of immunization, 66.6 μ g of CTS- β (Sigma, St. Louis, Mo) was mixed with 1 ml PBS solution

containing $3.8-4.2 \times 10^6$ spermatozoa.

Immunization procedure. Seven different routes of sperm administration were assayed: IM, SC, IN, IR, IVA, IP and oral. For each routes 15 mice were immunized by whole sperm mixed with adjuvant (cases) and as control group 15 mice were inoculated with adjuvant mixed with only PBS. Administrations were carried out on day 0,7,14 and 28. In all case and control groups, blood samples and vaginal washes were collected 25 days after the last inoculation. In IM and SC groups 100 μ l of previously prepared solution (sperm and Freund's adjuvant) was inoculated. 30 μ l of corresponding immunogen solution (sperm and CTS- β) was used for IN, IR, IVA and oral groups. In IP group 100 μ l of sperm and CTS- β was injected into peritoneal cavity.

Positive and negative control sera. In order to obtain a positive control serum, four female rabbits were immunized with mice sperm. 0.25 ml of PBS solution containing $3.5-4.5 \times 10^6$ mice sperm per ml was emulsified with 0.2 ml of complete Freund's adjuvant (CFA) and injected IM to each rabbit. Boosting was done 14 days after the first immunization with the same amount of sperm but with ICFA. After 72 hours, blood samples were taken by heart puncture and sera were tested by IFA technique. A one to five (1:5) dilution of these sera was used as positive control. Sera prepared from blood sample of healthy rabbits without any injections were used as negative control.

Antisperm antibody detection. 10 μ l of PBS solution containing 2-3 million sperm per ml was placed in each microwell of immunofluorescence slides (Biogen-Mashhad-Iran) and air dried. The slides were then fixed in pure acetone for 2 minutes.

Sera and vaginal washes of case and control mice were diluted 1/5, 1/25, 1/100, and 1/200 in PBS. Each microwell was coated with 10 μ l of each dilution. 1/75 dilution in PBS of polyvalent antimouse antibody conjugated with fluorescein isothiocyanate (FITC) and 1/50 dilution in PBS of polyvalent antirabbit antibody conjugated with FITC (sigma, ST Louis, MO) for positive and negative control microwell were used. These dilutions were confirmed by positive and negative control sera, slides were subsequently observed under fluorescent microscope (Leitze-Germany).

Statistical analysis. Fisher exact and Mann-Whitney tests were used for data analysis.

RESULTS

All but one of the cases in IP group expired during the experimental procedure; therefore the group was excluded from the study. Mortality was also observed in other groups. The number of live mice in each case and control groups at the end of study is shown in table 1.

According to the result of IFA for antisperm antibody in mice sera, significant difference was observed between number of positive cases and controls in IM, SC and nasal groups ($P = 0.01$, $P = 0.01$, $P = 0.04$, respectively). However the difference in IFA result, among cases and controls in IR, IVA and oral experimental groups was not statistically significant (Table 2).

Table 1. The number of live mice in each group at the end of the study. Total number of mice in each group was 15 at the beginning.

Administration routes Group	IM	SC	IR	IVA	Oral	IN	IP
Control	9	9	10	11	15	10	11
Case	7	15	11	11	11	11	1

Table 2. Number of positive and negative mice for ASA in sera of different case and control groups after immunization.

Administration routes	IM		SC		IR		IVA		Oral		IN	
Group	Result		Result		Result		Result		Result		Result	
Control	2	7	2	7	-	10	5	6	-	15	-	10
Case	6	1	10	3	-	11	5	6	3	8	4	7

Table 3: Serum ASA titer of positive case and control mice in different inoculation routes.

Administration routes	IM		SC		IR		IVA		Oral		IN	
Titer	1/5	1/25	1/5	1/25	1/5	1/25	1/5	1/25	1/5	1/25	1/5	1/25
Groups	1	1	2	-	-	-	5	-	-	-	-	-
Control	1	5	5	5	-	-	5	-	3	-	2	2
Case												

No significant differences were observed between ASA in vaginal wash of case and control mice of all experimental groups in different immunization procedures. In most of the positive cases, the ASA titer was 1/5 and sometimes 1/25, the result are presented in table 3.

Comparison between the number of positive ASA cases in SC, IM and IN groups indicated that SC immunization is more effective than IM and IN immunization methods. Percentages of ASA in sera of case and control mice in different groups are shown in figure 1.

ASA was detected in vaginal secretions of only two individuals in IM and SC

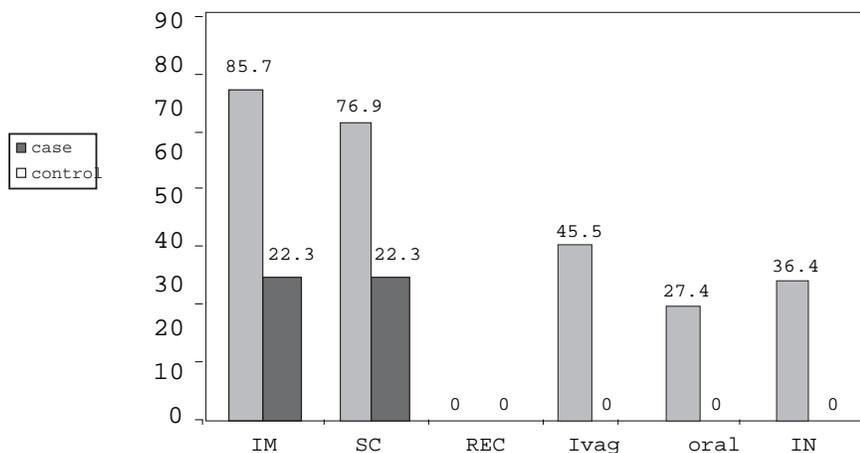


Figure 1. Percentage of ASA positive mice in each case and control groups (case) with dilution of 1/5.

DISCUSSION

Our data indicated that whole sperm administration via IM, SC and IN caused ASA formation in mice serum, however there were no significant differences between case and control groups in IVA, IR and oral administration methods. In SC group the percentage of positive cases of ASA was higher than that of IM and IN groups. Systemic immunization of female possum with whole sperm reduces their fertility by ASA formation in serum and vaginal secretion (5). It is well documented that immunization of a number of different species, including human, with whole sperm results in fertility reduction due to ASA formation (10,11). IM and SC immunization of BALB/c mice with specific sperm antigen caused ASA formation in serum (9). Clinical studies in human suggest that ASA formation in male and female human results in infertility (14). Contrary to these studies, some experiments emphasize that whole sperm cannot be employed for development of a vaccine, because several sperm antigens are present intrinsically and also antigens on the surface of sperms are likely to be shared with various somatic cells (2,13).

In this study, we did not detect any ASA in vaginal wash of our experimental and control mice in all groups. Systemic immunization is used successfully in immune-protection against HSV-2 infection (14,15). SC, IM, and IP immunizations induce humoral antibody responses in female genital tract in some species including human (16,17,18,19,20). However systemic immunization with sperm induces only high levels of serum IgG, which do not correlate with specific antibody in reproductive tract (21).

It has been shown that mucosal immunization (oral, IN, IR) successfully induces specific antibody responses in genital tract of animal models and human (22,23,24,25). However, regarding sperm immunization, conflicting results have been reported, specially in rectal methods (7,13,26). In our study oral, IN, IR immunizations were not effective in producing secretory immunoglobulin in reproductive tract. In oral and IR methods systemic antibody also failed. There are several reasons for the failure of whole sperm mucosal immunization routes in inducing immune response. Whole sperm antigen may not be presented to mucosal associated lymphoid tissues, especially in oral and rectal routes of administration. Besides, surface sperm antigen molecules with CTS- β adjuvant may induce tolerance instead of immunization.

We did not detect any antibody in serum and vaginal wash of mice after intravaginal administration of sperm. The local immune response induced after direct intravaginal immunogen administration was low (14,27). Such poor immune responses following vaginal immunization with non replicating antigens are probably due to the poor permeability of epithelium and natural down regulating mechanism against sperm (21).

In interpretation and analyzing data of our study the following research limitations should be considered.

IFA can be informative only about antibody against sperm surface antigen.

Vaginal washing may dilute the antibody concentration in female reproductive tract. According to the result of this study it can be concluded that mouse whole sperm can induce humoral immune response, in systemic inoculation and IN administration routes. However IR, oral and IVA administration of whole sperm is not immunogenic even with adjuvant.

ACKNOWLEDGMENTS

This study was supported by a grant from Mazandaran Medical Sciences University, IRAN. We would like to appreciate the supervision of J. Tavakoli and A. Khalilian, and assistance of Dr Enayatee and Mrs F. Abedian.

REFERENCES

1. Beckerman Karen P: Reproduction and the Immune system. In: Stites Daniel P, Terr Abba I, Tristram G Parslow, eds: Medical immunology, 9th ed. California: Appelton and lang, 1998:613-631.
2. Naz RK, Bhargava KK. Antibodies to sperm surface fertilization antigen (FA-1): their specificities and site of interaction with sperm in male genital tract. *Mol Reprod Dev* 1990; **26(2)**:175-83.
3. Naz RK. Application of sperm antigens in immunocontraception. *Front Biosci* 1996; **1**:e87-95.
4. Bronson RG, cooper G. Rosenfeld D. sperm antibodies: their role in infertility. *Fertile steril* 1984; **42**:171-183.
5. Duckworth JA, Buddle BM, Scobie S. Fertility of brushtail possums (*Trichosurus vulpecula*) immunised against sperm. *J Reprod Immunol* 1998; **37(2)**:125-38.
6. Parr EL, Parr MB, Thapar M. A comparison of specific antibody responses in mouse vaginal fluid after immunization by several routes. *J Reprod Immunol* 1988; **14(2)**:165-76.
7. Allardyce R, Rademaker M. Female genital tract immunity and infertility after oral vaccination with sperm antigens in mice. *Adv Exp Med Biol* 1987; **216B**:1807-13.
8. Cao X, Ben K, Ma L, et al. Secretory monoclonal IgA antibody to human sperm produced by gastrointestinal immunization inhibits human sperm activity and mouse in vitro fertilization. *J Reprod Immunol* 1993; **24(1)**:13-28.
9. Naz RK, Zhu X. Recombinant fertilization antigen-1 causes a contraceptive effect in actively immunized mice. *Biol Reprod* 1998; **59(5)**:1095-100.
10. Frayne J, Hall L. The potential use of sperm antigens as targets for immunocontraception; past, present and future. *J Reprod Immunol* 1999; **43(1)**:1-33.
11. Kozlowski PA, Cu-Uvin S, Neutra MR, et al. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. *Infect Immun* 1997; **65(4)**:1387-94.
12. Baskin MJ. Temporary sterilization by injection of human spermatozoa. *Am J Reprod Immunol Microbiol* 1932; **24**: 892.
13. Bradley MP. Experimental strategies for the development of an immunocontraceptive vaccine for the European red fox, *Vulpes vulpes*. *Reprod Fertil Dev* 1994; **6(3)**:307-17.
14. Bohring C, Krause W. Differences in the antigen pattern recognized by antisperm antibodies in patients with infertility and vasectomy. *J Urol* 2001; **166(3)**:1178-80.
15. Bernstein DI, Ashley RL, Stanberry LR, et al. Detection of asymptomatic initial herpes simplex virus (HSV) infections in animals immunized with subunit HSV glycoprotein vaccines. *J Clin Microbiol* 1990; **28(1)**:11-5.
16. Straus SE, Corey L, Burke RL, et al. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. *Lancet* 1994; **343(8911)**:1460-3.
17. Gallichan WS, Rosenthal KL. Specific secretory immune responses in the female genital tract following intranasal immunization with a recombinant adenovirus expressing glycoprotein B of herpes simplex virus. *Vaccine* 1995; **13(16)**:1589-95.
18. Bouvet JP, Belec L, Pires R, et al. Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination. *Infect Immun* 1994; **62(9)**:3957-61.
19. Miller CJ, Kang DW, Marthas M, et al. Genital secretory immune response to chronic simian immunodeficiency virus (SIV) infection: a comparison between intravenously and genitally inoculated rhesus macaques. *Clin Exp Immunol* 1992; **88(3)**:520-6.
20. Nakao M, Hazama M, Mayumi-Aono A, et al. Immunotherapy of acute and recurrent herpes simplex virus type 2 infection with an adjuvant-free form of recombinant glycoprotein D-interleukin-2 fusion protein. *J Infect Dis* 1994; **169(4)**:787-91.
21. Thapar MA, Parr EL, Parr MB. Secretory immune responses in mouse vaginal fluid after pelvic, parenteral or vaginal immunization. *Immunology* 1990; **70**:121-125.
22. Hordnes K, Tynning T, Brown TA, et al. Nasal immunization with group B streptococci can induce high levels of specific IgA antibodies in cervicovaginal secretions of mice. *Vaccine* 1997; **15(11)**:1244-51.
23. Johansson EL, Rask C, Fredriksson M, et al. Antibodies and antibody-secreting cells in the female genital tract after vaginal or intranasal immunization with cholera toxin B subunit or conjugates. *Infect Immun* 1998; **66(2)**:514-20.
24. Russell MW, Moldoveanu Z, White PL, et al. Salivary, nasal, genital, and systemic antibody responses in monkeys immunized intranasally with a bacterial protein antigen and the Cholera toxin B subunit. *Infect Immun* 1996; **64(4)**:1272-83.
25. Mestecky J, Russell MW. Induction of mucosal immune responses in the human genital tract. *FEMS Immunol Med Microbiol* 2000; **27(4)**:351-5.
26. Crowley-Nowick PA, Bell MC, Brockwell R, et al. Rectal immunization for induction of specific antibody in the genital tract of women. *J Clin Immunol* 1997; **17(5)**:370-9.
27. Alexander NJ, Bialy G. Contraceptive vaccine development. *Reprod Fertil Dev* 1994; **6(3)**:273-80.