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EFFECT OF SPERM IMMUNIZATION OF MALE RABBITS ON SPERM QUALITY, CONCEPTION RATE AND LITTER SIZE

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ABSTRACT

Five Adult male rabbits (7- months old) were used in this study. Among these, three were immunized intramuscularly with washed, sonicated rabbit sperm cells in Freund's adjuvant and two were given intramuscular injection of phosphate buffered saline (PBS) at weeks 0 and 2. Semen and blood samples were collected from each rabbit weekly for 14 weeks, examined for sperm motility and tested for sperm antibodies. Serum sperm antibody titer was inversely correlated with sperm motility in the immunized rabbits ($r = -0.93$, $p<0.001$) and there was no significant difference in response between rabbits ($p>0.05$). The 3 rabbits were reimmunized and the two control rabbits also given PBS as before at week 16. Each of the 5 rabbits was used to breed 4 randomly selected 7-8.month old fertile females. Conception rate (16.67%) and litter size (0.42/female) in females bred by immunized males were significantly lower than in those bred by control males (87.5%, $p<0.02$ and 7.5/female, $p<0.001$). Subsequent breeding of normal females with the previously immunized males at week 22 significantly improved the conception rate (55.56%, $p<0.02$) and litter size (3.05/female, $p<0.001$) compared to the breeding at week 16. It can be concluded that immunization of male rabbits with homologus sperm might reduce fertility and thus conception rate.

Key words: Rabbit, sperm cell, sperm quality, conception rate.

INTRODUCTION

Many antigens have been described for sperm cells. These include sperm coating antigens (Acott and Hoskins, 1981), plasma membrane antigens (Teuscher *et al.*, 1983), cytoplasmic, mitochondrial antigens (Goldberg, 1974), the rabbit sperm tail protein (Wang *et al.*, 1986) and those involved in zona pellucida binding (Naz and Zhu, 1997). It is possible to induce antibody formation by active immunization with these purified antigens, whole sperm or semen. Sperm antibodies interfere with fertility (Castle *et al.*, 1997; Fayemi, 1997) and induction of infertility has been attempted by immunization of female animals with testicular homogenate, sperm or sperm fractions. Immunization of female rabbits with washed ejaculates, epididymal or beta-amylase treated sperm (Kummerfeld and Foote, 1976), testis preparations (Bell, 1969), testis-specific lactate dehydrogenase (LDH-X) (Goldberg, 1974), lithium diodosalicylate (LIS) soluble extracts and NP-40 extract of insoluble fraction of sperm (Menge and Peegel, 1980) reduced the fertility of these animals. Similarly, immunization of male rabbits with testicular cytochrome resulted in conception failure, stillborn and undersized liveborn offsprings when mated with unimmunized females (Kim, 1987). Preinsemination treatment of rabbit semen with antisemen, antifertilin lgG antibodies or antisperm antibodies (Metz and Anika, 1970; Metz, 1972; Hardy *et al*., 1997) or sperm with monoclonal antibodies against rabbit sperm antigens (Naz *et al.*, 1983) also impaired the fertility of inseminated females and antihyaluronidase reduced cumulus cell dispersion *in vitro* (Metz, 1973).

There has been little or no information regarding the effect of immunization of male rabbits with washed sperm cells on conception rate and litter size of females bred to them. The objective of this study was to use the rabbit model to investigate the effect of immunization of males with sperm on conception rate and litter size.

MATERIALS AND METHODS

Preparation of sperm antigen

Semen of good quality was collected with artificial vagina from 10 rabbits that were seronegative for sperm antibodies. The sperm cells from the pooled semen were separated by centrifugation at 1,200g for 5 minutes, washed three times in 0.005M phosphate buffered saline (PBS), resuspended at a concentration of 1×10^6 cells/ml, sonicated 20 strikes with sonicator (Model W380, Heat Systems Ultrasonic Inc.), mixed with an equal volume of Freund's complete adjuvant and used for immunization of male rabbits at week 0. The sperm antigen was mixed with an equal volume of Freund's incomplete adjuvant for the immunizations at weeks 2 and 17.

Immunization of rabbits

Five 7-month old male rabbits yielding good quality semen were purchased. Three were immunized with 2 ml of the sperm antigen preparation and the remaining two were given intramuscular injections of 2 ml PBS at weeks 0 and 2 and served as control. Semen and blood were collected weekly from each rabbit for 14 weeks, starting from the day of immunization. The semen samples were immediately examined for sperm motility and concentration. The sera harvested from the blood samples were stored at –20°C until tested for sperm antibodies.

Reimmunization and breeding of females

The three previously immunized males were reimmunized, and the controls were injected with PSB, at week 16. Each male was used to breed 4 randomly selected 7-8 month old fertile females. Each of the 3 previously immunized males was also used to rebreed 6 randomly selected females at week 22. The conception rates (CR) and litter size for each group of females were calculated at parturition.

Sperm antibody detection and quantitation

Sperm antibody titer in each sample was determined by enzyme-linked immunosorbent assay (ELISA) (Harel and Nelken, 1985) with some modifications. Briefly, sperm antigen was prepared from washed, sonicated rabbit sperm cells in PBS, centrifuged twice at 1,500g for 30 minutes (4°C) and the optical density (OD) at 280 nm of the second supernatant was adjusted to 0.2 (equivalent of 0.15 mg/ml protein). Falcon 3912 microplates (Becton Dickinson) were coated with sperm antigen (50 µl/well) overnight at 4°C. The antigen was fixed to the plates with 0.5% gluteraldehyde (50 µl/well), then washed and incubated with 1% bovine serum albumin (BSA) (50 µl/well) overnight at 4°C. Excess BSA was removed by washing and the microplates were incubated with various dilutions (1:50–1:3200) of the test sera (50 µl/well) for 2 hrs at 37°C. The plates were washed and incubated with 50 µl/well of biotin-labeled goat-anti-rabbit 1gG (Kirkegard and Perry Laboratories, Inc. (KPL), 1:4800) for 30 minutes at 37°C, washed and incubated with 50 µl/well of streptavidin peroxidase (KPL, 1:9600) for 30 minutes at 37°C. A mixture of equal volumes of ABTS (2.2 azino-di [3-ethyl-benzthiazoline sulfonate]) and hydrogen peroxide (H_2O_2) (KPL, 50 µl/well) was used as substrate and the plates were kept at room temperature in the dark for 15 minutes before the OD was read at 405 nm with a microELISA reader MR 580 (Dynatech). All washings were done three times with PBS-Tween 20.

Standardization of ELISA

Titration curves of various dilutions of both controls and postimmune samples were made to compare the OD (Fig. 1). The mean OD of various dilutions of the negative samples was calculated and the last dilution of each sample with OD twice of the calculated mean was recorded as the sperm antibody titer in that sample.

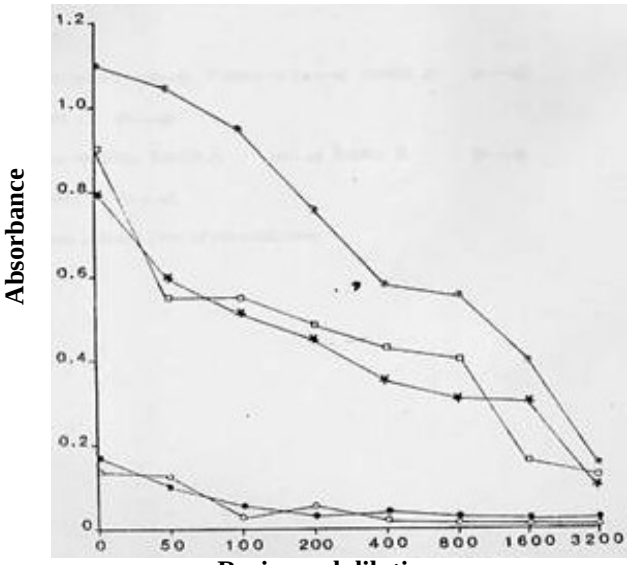

Fig. 1: Titration curve of various dilutions of different rabbit sera in ELISA titer of sample recorded as last dilution with absorbance greater than mean absorbance of negative samples +2SD (Standard Deviation)

Rabbit D, week 0 (o—o); Rabbit E, Week 2 (●—●)
Rabbit A, week 5 (□—□); Rabbit B, Week 7 (✱—✱)
Rabbit C, week 6 (⊙—⊙).

Statistical analysis

The motility and antibody titer were transformed into square roots before subjecting to regression analysis. Comparison of conception rates and litter sizes was done by analysis of variance (Panacea programme, PAN Livestock Services, 1984).

RESULTS

The sperm antibody titer and sperm motility data are presented in Fig. 2. Three weeks after second immunization, the antibody titer reached a peak in 2 out of 3 rabbits (1:1600). This titer was maintained for an additional two weeks before rapidly declining. The antibody titer of the third rabbit peaked on week 7 (1:3200) and then rapidly declined. Sperm motility from the immunized rabbits decreased as the serum sperm antibody titer increased. The sperm motility was less than 10% from weeks 4 to 8 in all the rabbits, but increased to 60-75% at week 14 (Fig. 2). There was a significant negative correlation of sperm motility with sperm antibody titer ($r = -0.93$, $p<0.001$) but there were no significant differences in immunogenic response nor in sperm concentration between the three rabbits.

After reimmunization at week 16, when the rabbits were used to breed females, CR was significantly higher in the females bred by the control (87.5%) than in those bred by immunized (16.67%) rabbits ($p<0.001$). The average litter size was also higher for the control (7.5/female) than immunized (0.42/female) males ($p<0.001$). Subsequent rebreeding at week 22 with the previously immunized group resulted in a higher CR (55.56%) than their first breeding ($p<0.02$) and also a higher litter size (3.05/female, $p<.001$) but still had significantly lower CR ($p<0.01$) and litter size ($p<0.01$) than the non-immunized controls (Table 1).

Table 1: Conception rates and average litter size of female rabbits bred by immunized and non-immunized males

Treatment	Males	Conception rate (%)	Litter size
Immunized on weeks 0, 2, 16, and bred on week 17	A	(0/4) 0	0.00
	B	(1/4) 25	0.25
	C	(1/4) 25	1.00
Total		(2/12) 16.67	0.42
Non-immunized, bred on week 17	D	(3/4) 75	6.00
	E	(4/4) 100	8.65
Total		(7/8) 87.5	7.50
Immunized with sperm at week 0, 2 and 16, bred at week 22	A	(3/6) 50	2.83
	B	(3/6) 50	2.50
	C	(4/6) 66.67	3.83
Total		(10/18) 55.56	3.05

DISCUSSION

The results of the first part of the study show that sperm antibodies have negative association with sperm motility. This is similar to the reports of sperm antibody association with sperm motility in humans (Mathur *et al.*, 1986). The mechanism by which the sperm motility is affected is not fully understood but there are immunoreactive proteins in the sperm cell such as the forward motility proteins on the acrosome (Raynaud and Kann, 1986), Zn^{2+}- ion dependent proteins mostly in the flagella (Strzezek and Hopfer, 1987; Strzezek *et al.*, 1987) that enhance sperm motility. These proteins can induce antibody production and thereby reduce sperm motility since antibodies of the IgG type with smaller Fab fragments can transudate into the epididymis and bind to the surface of epididymal spermatozoa (Lee *et al.*, 1987).

The reduction of sperm motility might partly contribute to the lower CR and litter size observed in females bred by immunized males, since conception increases and time of conception decreases as larger numbers of motile sperms pass through the cervical mucus (Hull *et al.*, 1982). The CR and litter size improved at the second breeding when sperm motility must have improved. Rabbit semen mixed with

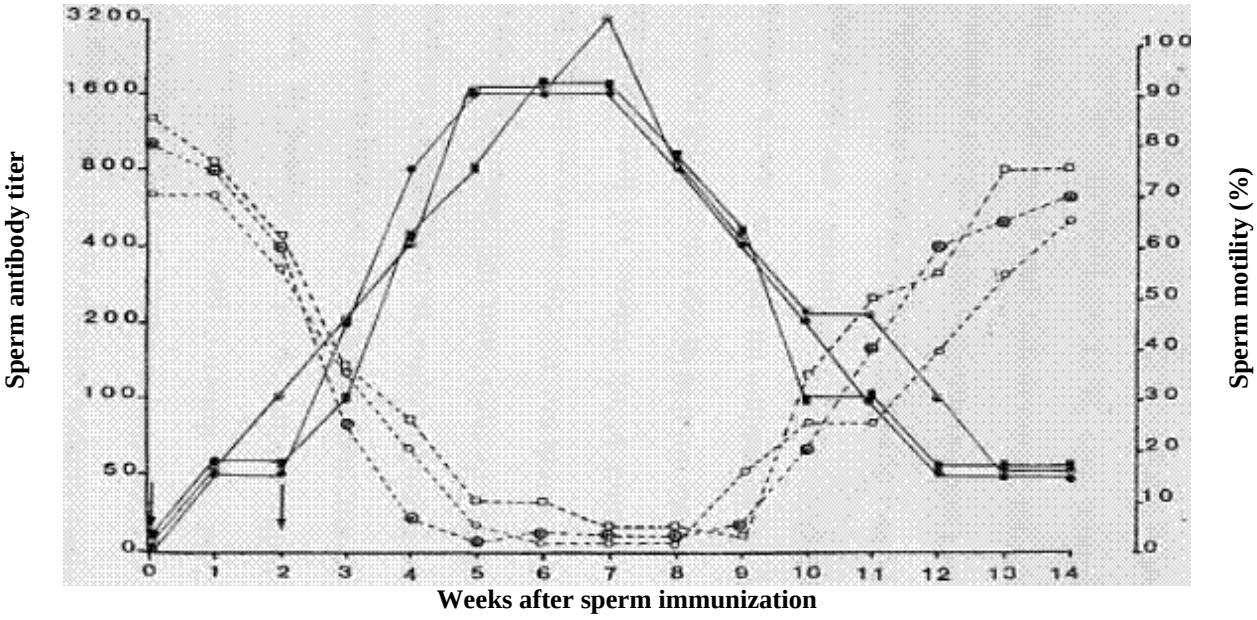

Fig. 2: Serum sperm antibody titer and sperm motility in rabbits A, B and C. Arrow indicates time of immunization.

Serum sperm antibody: Rabbit A **Rabbit B** **Rabbit C**

Sperm motility: Rabbit A **Rabbit B** **Rabbit C**

antiserum to semen had been shown to reduce the number of sperms recovered from oviduct, uterus and vagina and there was a decrease in sperm motility in vaginal flushings, reduced progression of uterine sperms and inhibition of fertilization (Menge, 1971). The reduction in CR and litter size in immunized males is not surprising, because generally a decrease in fertilization rate, as well as an increase in embryonic and foetal mortality had been observed in animals immunized with sperm or testis preparations (Sokamoto *et al.,* 1999) and treatment of semen with antiserum against testis, semen or sperm before insemination had antifertility effect in rabbits (Menge and Protzman, 1967). Possible causes suggested were interference with sperm transport, immobilization of sperm, increased phagocytosis of sperm and blocking of fertilization sites on the sperm membranes. The fertilization failure might be due to failure of sperm in the oviduct to penetrate and disperse the cumulus mass of the ova, with antiserum to RSA-1 which reduced sperm binding and completely inhibited sperm penetration (Amann *et al.,* 1999).

Normally, sperm cells in the proximity of the ova and cumulus mass undergo acrosomal membrane vesiculation as a prerequisite to fertilization. Then there is release of acrosomal enzymes, which disperse cumulus and corona cells and aid sperm penetration of the ova. Antihyaluronidase had been shown to prevent cumulus cell dispersion in vitro (Metz, 1973). Although immunization with acrosin or hyaluronidase did not reduce fertility in rabbits (Syner *et al.*, 1979), the possibility of antibody binding to the sperm membrane, preventing vesiculation and release of these enzymes from the acrosome cannot be totally ruled out.

The spontaneous adherence of rabbit sperm to ova could be prevented by both univalent and bivalent antibodies against sperm (Menge, 1970) and sperm specific antibodies might be blocking sperm adherence to ova, and acrosome reaction, by binding reactive sites on sperm membranes and consequently preventing fertilization. This would reduce CR and litter size.

The antibodies could be acting at various levels of the reproductive tract and the fertility process i.e. sperm transport and migration in the form of reduced progressive motility as observed in the first part of the study, and sperm attachment and penetration of the ova. Sperm agglutination as a cause of the reduced CR was ruled out as it was not observed in the first part of the study.

The improved CR and litter size when the immunized rabbits were used for subsequent breeding suggests that sperm antibody contributed to the reduction in fertility which improved when the antibody titer would have been decreased. The higher CR and litter size in the females bred by unimmunized rabbits was probably due to lack of sperm antibodies. The normal male does not produce sperm antibody because of the blood-testis barrier formed by the tight junctions between sertoli cells that line the seminiferous tubules (Tung, 1980).

The postfertilization effects of the sperm antibodies should also be considered in relation to the reduced litter size in females bred by immunized males. Naz *et al.* (1983) showed that insemination of female rabbits with rabbit sperm treated with monoclonal antibodies resulted in significant reduction in fertility by blocking postfertilization development through inhibition of some steps necessary for viable embryo formation. One of the female rabbits bred by an immunized male died and was found to be pregnant with one fetus but with four other implantation sites without fetuses on post mortem. The litter sizes of ejaculated sperm immunized female rabbits were also shown to be significantly lower than controls (Kummerfeld and Foote, 1976).

In conclusion, this study indicates that immunization of male rabbits with homologous sperm may reduce fertility through reduction of sperm motility, inhibition of sperm binding to ova, and subsequent fertilization leading to reduced conception rate, and possibly by prevention of postfertilization development of viable embryo causing reduction in litter size.

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