



Potential of Sonicated Sperm Proteins as an Immun contraceptive Agent in House Rat, *Rattus Rattus*

KEYWORDS

Antisperm-antibodies, House rat, Immun contraception, Sperm parameters

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ABSTRACT

Immunization of house rats with 500 µg of sonicated sperm proteins (SSP) resulted in maximum production of antisperm-antibodies (ASA) in the form of 93.88% positivity at 21 days post immunization (DPI) as compared to other two prepared antigens i.e 100 and 1000 µg of SSP. Study of various sperm parameters revealed a significant reduction in sperm concentration (million/ml) from 161.84±18.79 (control) to 16.40±0.90, percent sperm motility from 88.33±7.26 (control) to 46.67±1.67 and increase in percent sperm abnormalities from 1.55±0.45 (control) to 67.81±8.31 after immunization with 500 µg of SSP, indicating the potential of this antigenic preparation for its use as an immun contraceptive agent.

INTRODUCTION

House rat, *Rattus rattus* is the most abundant, widely distributed, highly adaptable and cosmopolitan commensal rodent species worldwide, which causes huge losses to the food stuffs and other stored items (Parshad 1999). The regulation of rodent population number constitutes a difficult and largely unsolved contemporary problem because of its prolific reproductive potential and immense adaptive capabilities (Gillespie and Myers 2004). The use of toxic chemicals (rodenticides) especially under commensal situations like in residential areas, animal dwellings and poultry farms etc. poses potential risk of poisoning the non-target mammals including human beings, pets and domestic animals (Fishel 2005). Therefore, reducing fertility by the technique of immun contraception is an effective and safe alternative method to control rodents, preferably the commensal species (Cooper and Larson 2006). The spermatozoa offer the most promising and exciting option to be used as immun contraceptive agent, as raising of antibodies against sperm specific proteins/antigens interfere with sperm functions and sperm-egg binding, thus controlling fertilization (Suri 2005). Therefore, keeping in mind the role of sperms and sperm proteins in fertilization the present study was carried out for testing the potential of sonicated sperm proteins as an immun contraceptive agent.

MATERIAL AND METHODS

Adult house rats were trapped live from poultry farms, grocery shops, godowns, store houses etc. of Ludhiana (Punjab). The mature male rats acclimatized for 10-15 days were dissected to collect cauda epididymal fluid by extirpation of cauda epididymis in normal saline solution (0.9% sodium chloride). Cauda epididymal fluid was centrifuged, the residue having spermatozoa was suspended in known amount of saline solution and the concentration of sperms (number of sperms/ml) was recorded with the help of haemocytometer (Salisbury et al. 1978) and protein content was measured by Lowry et al. (1951). Thereafter, three types of antigenic preparations having 100, 500 and 1000 µg sonicated sperm proteins (SSP) were made. These three SSP antigens were mixed in Complete Freund's Adjuvant, FCA (1:0.67) and were injected intradermally to male rats of groups I, II and III, while group IV (vehicle) rats were injected with adjuvant and group V (control) rats were injected with normal saline (each group was having three male rats). The booster dose with similar types of antigen mixed with Incomplete Freund's Adjuvant, ICFA (1:1) was given on 14 and 21 days post immunization

(DPI). Blood was collected from tail of immunized, vehicle and control rats at 0, 7, 14, 21, 28 and 35 days and serum was collected. Anti-sperm antibodies (ASA) raised against these antigens were detected by ELISA (Crowther 1995). Antibody titre was estimated in terms of % positivity by the formula:

$$\% \text{ Positivity} = \frac{\text{Mean absorbance of treated serum} - \text{Mean absorbance of control serum}}{\text{Mean absorbance of control serum}} \times 100$$

Various sperm parameters viz. sperm concentration (%), sperm motility (%) and sperm viability (%) and were determined by the methods of Salisbury et al. 1978. Sperm morphology/abnormalities were also determined from the rats of immunized, vehicle and control groups. The permission from 'Institutional Animal Ethics Committee' Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was taken before starting the experiments.

RESULT AND DISCUSSION

Results indicated a regular increase in percent positivity/titre of antisperm antibodies (ASA) from 7 to 21 DPI, thereafter it declined till 35 DPI in all the serum samples from house rats injected with different concentrations (100, 500 and 1000 µg) of SSP as antigen. This value was found to be maximum (93.88%) in the serum of rats immunized with 500 µg of SSP (Fig.1). High antibody titre determined with ELISA technique in the serum of rats immunized with this sonicated sperm protein preparation could be an indication towards the reduction in ability of sperm fertility as suggested by Naz and Zhu (1998) and Kocher et al. (2012).

Immunization of house rats with different concentrations of SSP as antigen resulted in significant reduction in sperm concentration (millions/ml) i.e 18.11±5.59, 16.4±0.90 and 20.35±3.57 in the epididymal fluid of house rats immunized with 100, 500 and 1000 µg of SSP respectively as compared to 161.84±18.79 millions/ml in control house rats. However, the values of sperms concentration between vehicle and control was found to be non significant (Table 1). The rats having sperm number less than 20 million/ml are considered to be infertile (Irvine and Aitken 1994). So, significant reduction in sperm concentration (< 20%) after immunization with 500 and 100 µg of SSP can be correlated with the inability of rats producing sufficient sperm number required for effective fertilization.

A significant reduction in percent sperm motility i.e

68.33±8.82, 46.67±1.67 and 65.00±0.00 was observed after immunization with 100, 500 and 1000µg of SSP as antigen as compared to that of vehicle (71.66±4.41) and control rats (88.33±7.26) as shown in table1. Maximum reduction in sperm motility after immunization with 500 µg of SSP can be correlated with the infertility status of the immunized rats, as there are reports indicating that rats having sperm motility less than 50% behave like sterile one (Sigman 1993). Also, maximum antibody production of anti-sperm antibodies was observed after immunization with this particular sonicated protein concentration i.e 500µg (Fig.1), which can also be a cause of decrease in the sperm motility (Yan 1988).

No effect on sperm viability i.e. number of live/dead sperms was observed after immunization with any of the SSP concentrations, as the values of percent sperm viability of all the immunized rats were found to be non-significantly different from that of the control as well as vehicle house rats (Table 1).

In control house rats, average sperm abnormalities (%) were found to be 1.55±0.45 only and this value differed non-significantly with that of vehicle rats, but rats immunized with 100, 500 and 1000 µg of SSP were found to have significantly higher values of sperm abnormalities with its maximum value 67.81±8.31 % obtained in the serum of rats immunized with 500 µg SSP (Table 1). Differential study of various regions of sperms i.e. head, neck, mid piece and tail also revealed that immunization with 500 µg of SSP resulted in maximum head abnormalities i.e 23.39±6.51% as compared to that of 0.58±0.13 % in control rats, 6.56±1.05 % coiling in tail as compared to 0.35±0.04 % in control rats and 26.86±0.93 % breakage as compared to 0.62±0.29% in control rats (Fig. 2). The values of % coiling in neck and mid piece and % multiple abnormalities in the sperms of rats immunized with different concentrations of SSP were found to differ non-significantly from that of control group rats. Also the values of all the parameters of sperm abnormalities varied non-significantly between the vehicle and control group rats (Fig. 2). Significant enhancement in percent head abnormalities after immunization with 500 µg of SSP is a sign towards its effective immunocontraceptive antigenicity, as a high incidence of head abnormalities (> 20 %) usually refer to a pin head and is an

indicative of male infertility (Gopalkrishnan 1995). Significant coiling in the tail region of the sperms from rats immunized with 500 of µg SSP may be involved directly in influencing the motility pattern of the sperms (Alexander 1984).

The present study concluded that out of the three tested antigenic preparations having sonicated sperm proteins @ 100, 500 and 1000 µg, the antigen having 500 µg of SSP was found to have the maximum effect in terms of significant production of antisperm antibodies, reduction in sperm concentration and sperm motility and increase in sperm abnormalities, indicating its potential to be used as immunocontraceptive agent for the management of house rats in future.

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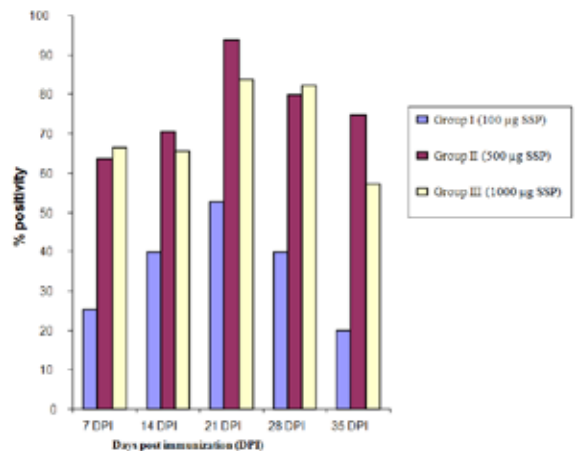


Fig. 1 Comparative level of % positivity of raised antisperm antibodies in response to different concentrations (µg) of sonicated sperm proteins (SSP) in R. rattus.

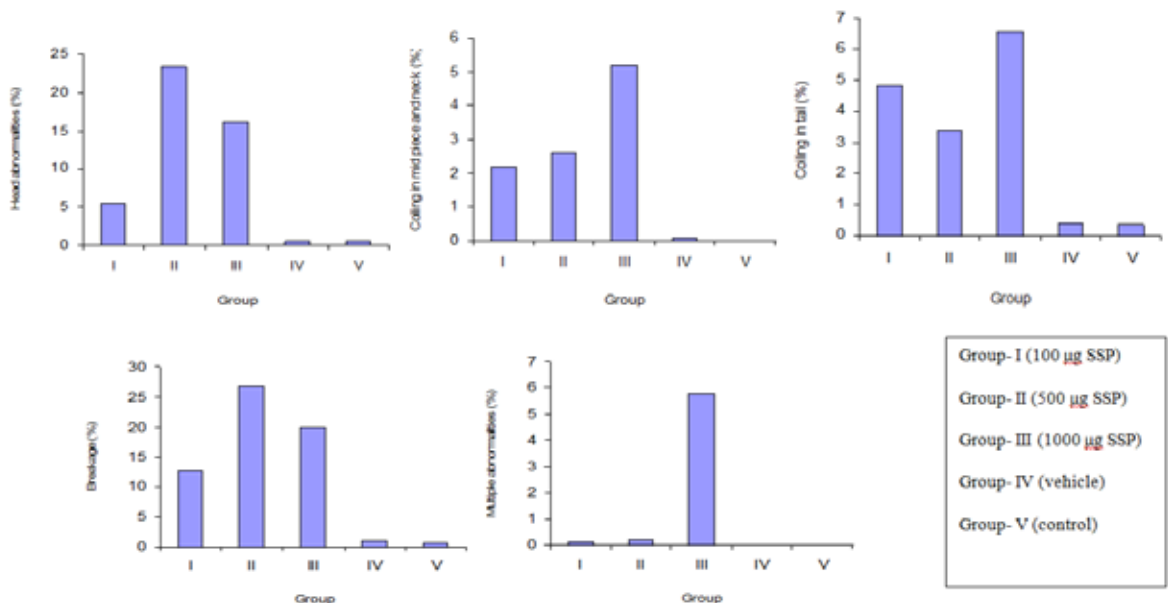


Fig. 2 Effect on sperm morphology of R. rattus immunized with different concentrations (µg) of sonicated sperm proteins (SSP).

Table 1: Effect on sperm parameters of *R. rattus* immunized with different concentrations (μg) of sonicated sperm proteins (SSP).

Group	Concentration of SSP (μg)	Sperm concentration (million/ml)	Sperm motility (%)	Sperm viability (%)	Sperm abnormalities (%)
I	100	18.11 \pm 5.59 ^a	68.33 \pm 8.819 ^a	63.33 \pm 1.67	25.03 \pm 5.90 ^a
II	500	16.40 \pm 0.90 ^a	46.67 \pm 1.67 ^a	71.67 \pm 14.81	67.81 \pm 8.31 ^a
III	1000	20.35 \pm 3.57 ^a	65.00 \pm 0.00 ^a	58.33 \pm 8.33	42.39 \pm 12.39 ^a
IV	Vehicle	163.57 \pm 11.81	71.66 \pm 4.41	75.00 \pm 2.89	2.17 \pm 0.25
V	Control	161.84 \pm 18.79	88.33 \pm 7.26	83.33 \pm 7.26	1.55 \pm 0.45
CD at 5% level		32.65	18.18	NS	22.61

All values are mean \pm S.E.

- a refers to the significant difference in the values at 5% level among different groups.

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