EVALUATION OF IMMUNOCONETRACTION PROPERTY OF SPERMATOZOA IN HOUSE RAT (RATTUS RATTUS)

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ABSTRACT

Immunization of house rats with three antigenic preparations having 1, 5 and 10 million spermatozoa, resulted in maximum production of antisperm-antibodies (ASA) after 3rd intradermal injection. The highest value of %ASA was found to be 26.11 ± 0.52 after immunization with 5 million spermatoza at 28 days post immunization (DPI) as compared to other two sperm antigens. While ELISA identified maximally the production of ASA at 21 DPI in the serum of house rats immunized with 5 million sperm. Study of various sperm parameters revealed a significant reduction in sperm concentration (million/ml) from 161.84 ± 18.79 (control) to 11.69 ± 4.09, per cent sperm motility from 88.33 ± 7.26 (control) to 22.33 ± 11.57 and increase in per cent sperm abnormalities from 1.55 ± 0.45 (control) to 68.15 ± 4.19 after immunization with antigen having 5 million spermatozoa., indicated that this antigenic preparation can be used as immunocontraceptive agent for the management of house rat in future.

Key words: Antisperm-antibodies, House rat, Immunocontraception, Sperm parameters.

INTRODUCTION

Commensal rodents, especially house rat, (Rattus rattus) causes extensive losses by feeding and contaminating the food products and also plays a role in spreading several diseases of health importance (Parshad 1999). Chemical control by rodenticides is the most widely used and efficient method of all the available methods for the control of rodent pests, both under agricultural and commensal situations. However, rodenticides which are common in use for rodent control have their own drawbacks like poison aversion, bait shyness, lack of specificity, genetic resistance and risk of poisoning the non-target species (Buckle et al. 1994). Thus, reducing fertility is a more preferable, safe and effective method to control rodents, preferably the commensal species. Recent advances in molecular biology, immunology and especially vaccine technology have brought the development of birth control vaccines technically called immunocontraceptive vaccines to the forefront. Immunocontraception is the most promising method which provides viable and valuable alternative to the presently available methods for fertility control and management of pest vertebrates (Naz et al. 2005, Cooper and Larson 2006 and Busso et al. 2007). A successful contraceptice vaccine stimulate immune response against gametes (sperm or ova) or reproductive hormones and preventing conception by interfering at sperm-oocyte interaction level while not affecting other reproductive functions (Miller 1995). In an effort to identify new contraceptive alternatives, sperm-specific antigens are under the investigations as the basis for immunological regulation of fertility. The spermatozoa have proteins that are unique, specific, immunogenic and accessible to antibodies (Risvanli et al. 2003, Suri 2005, Chen et al. 2009 and Khan et al 2009) which might interfere with sperm functions and sperm-egg binding, thus controlling fertilization.

Though immunocontraceptive methods are currently in experimental phase but their potential for effective control of animals is promising. Therefore, keeping in mind the advantages of immunocontraception and its possible use for the control of house rat, the present study was undertaken.

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**MATERIALS AND METHODS**

Adult house rats were trapped live from poultry farms, grocery shops, godowns, store houses etc. of Ludhiana (Latitude 30°56'N, Longitude 75°52'E) with single and multicatch rat traps. The mature male rats (body weight>100g) were acclimatized in separate cages for 10-15 days under standard hygienic conditions before the commencement of the experiment. During this period rats were fed on 2% plain bait (wheat: sugar: oil in the ratio of 96: 2: 2) and water *ad libitum*. Acclimatized male rats were dissected to collect cauda epididymis. Cauda epididymal fluid was obtained by extirpation of cauda epididymis in normal saline solution (0.9% sodium chloride). Cauda epididymal fluid having spermatozoa and saline was collected with the help of dropper and after measuring its volume, this was stored at -4°C till further use. Stored cauda epididymal fluid was thawed, centrifuged and then supernatant was discarded. The residue having spermatozoa was suspended in known amount of saline solution and the concentration of sperm i.e. number of sperm/ml was recorded with the help of haemocytometer (Salisbury et al. 1978). Thereafter, three types of antigen were prepared, such that type I with 1 million sperms, type II with 5 million sperms and type III with 10 million sperms and then stored at -4°C till their further use. Prepared three types of spermatozoa antigens (1, 5 and 10 million) mixed in Complete Freund's Adjuvant, FCA (1:0.67) were injected intradermally to male rats of groups 1, 2 and 3 (three rats per group) along with three control rats (group IV, injected with normal saline solution) and three vehicle rats (group V, only adjuvant was injected). 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. Blood was collected from tail of immunized, vehicle and control groups of rats at 0, 7, 14, 21, 28 and 35 days post immunization (Fig. 1) and serum was collected.

Then collected serum was kept at 56°C for 30 minutes to inactivate the complement and blood parameters such as total proteins (Lowry *et al.* 1951), immunoglobulins (Ig 45% ammonium sulphate precipitation method) and circulating immune complexes (CIC, 7.5% polyethylene glycol precipitation method) were estimated biochemically in triplicate. Anti-sperm antibodies (ASA) raised against these antigens was 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 motility (%), sperm viability (%) and sperm concentration (%) were determined by the methods of Salisbury *et al.* 1978. Sperm morphology/abnormalities and weights of various parts of male reproductive system were also determined from the rats of immunized, vehicle and control groups. The permission from ‘Institutional Animal Ethics Committee’ vide letter no.VPS/2008/874-885, dated 14.5.08 was taken before starting the experimentation on house rats.

**RESULTS AND DISCUSSION**

(I) Study of Immunological parameters:

a) Progressive changes in Ig level in the serum of immunized *R. rattus*:

In group 1 rats, which were immunized with antigen type I (having 1 million spermatozoa) Ig level was 13.79±0.54 before immunization and it showed non significant increase at all the intervals till 35 DPI. Ig level in the serum of group 2 rats immunized with type II antigen (5 million spermatozoa) showed a significant increase at 14 DPI in the value of Ig from 12.20±0.98 % of total proteins at 0 DPI to 15.27±1.24 % of total proteins at 14 DPI. Then Ig level showed a trend of regular increase from 14 to 35 DPI where it was found to be 26.15±0.21 % of total proteins. In the group 3 rats which were immunized with type III antigen (having 10 million spermatozoa), maximum Ig level (22.35±1.78 % of total proteins) was also observed at 28 DPI, but thereafter this level showed a significant decrease (17.17±0.54 % of total proteins) at 35 DPI. However, in case of rats of vehicle and control groups, no significant change in serum Ig level was observed from 0 DPI to 35 DPI (Table 1). This comparative study of Ig level of groups immunized with different
TABLE 1: Progressive changes in the level of immunoglobulins (Ig) in the serum of male *Rattus rattus* immunized with different concentrations (million) of spermatozoa.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Type of Antigen</th>
<th>Concentration of spermatozoa as antigen (million)</th>
<th>Ig level (% of total proteins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>1</td>
<td>13.79±0.54 (0 DPI)</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>5</td>
<td>12.20±0.98 (7 DPI)</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>10</td>
<td>13.29±0.96 (14 DPI)</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td></td>
<td>12.83±1.54 (21 DPI)</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td></td>
<td>11.69±2.20 (28 DPI)</td>
</tr>
</tbody>
</table>

All values are mean ±S.E.

Ig: Immunoglobulins, DPI: Days Post Immunization
- a refers to the significant difference in the values at 5% level from 0 DPI (as control) till 35 DPI of treatment within that group.
- b refers to the significant difference in the values at 5% level among different groups.

CD at 5% level

<table>
<thead>
<tr>
<th>0 DPI</th>
<th>7 DPI</th>
<th>14 DPI</th>
<th>21 DPI</th>
<th>28 DPI</th>
<th>35 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>2.39</td>
<td>2.79</td>
<td>2.79</td>
</tr>
</tbody>
</table>

[Further text...]

- Determination of CIC level in the serum of *Rattus rattus*:

- Progressive changes in CIC level in the serum of *Rattus rattus*.

- Progression of CIC level in the serum of *Rattus rattus*.
TABLE 2: Progressive changes in the level of circulating immune complexes (CIC) in the serum of male *Rattus rattus* immunized with different concentrations (million) of spermatozoa.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Type of Antigen</th>
<th>Concentration of spermatozoa as antigen (million)</th>
<th>0 DPI</th>
<th>7 DPI</th>
<th>14 DPI</th>
<th>21 DPI</th>
<th>28 DPI</th>
<th>35 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>1</td>
<td>1.55±0.30</td>
<td>2.04±0.48</td>
<td>2.23±0.18</td>
<td>2.88±0.18 a</td>
<td>4.92±0.27 ab</td>
<td>5.11±0.11 ab</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5</td>
<td>2.11±0.144</td>
<td>2.51±0.13</td>
<td>3.11±0.26 ab</td>
<td>3.33±0.09 ab</td>
<td>5.29±0.13 ab</td>
<td>5.80±0.08 ab</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>10</td>
<td>1.86±0.13</td>
<td>2.70±0.20 a</td>
<td>2.57±0.25 a</td>
<td>3.57±0.29 a</td>
<td>3.44±0.04 a</td>
<td>3.61±0.04 a</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>—</td>
<td>2.47±0.18</td>
<td>2.37±0.114</td>
<td>2.48±0.16</td>
<td>2.1±0.04</td>
<td>2.62±0.12</td>
<td>2.72±0.08</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>—</td>
<td>2.03±0.16</td>
<td>2.16±0.27</td>
<td>2.14±0.15</td>
<td>2.45±0.34</td>
<td>2.35±0.51</td>
<td>1.99±0.16</td>
</tr>
<tr>
<td></td>
<td>CD at 5% level</td>
<td>NS</td>
<td>NS</td>
<td>0.64</td>
<td>0.68</td>
<td>0.86</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ±S.E.
CIC: Circulating Immune Complexes, DPI: Days Post Immunization.
- a refers to the significant difference in the values at 5% level from 0 DPI (as control) till 35 DPI of treatment within that group.
- b refers to the significant difference in the values at 5% level among different groups.

TABLE 3: Effect on sperm parameters of *Rattus rattus* immunized with different concentrations (million) of spermatozoa.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Type of Antigen</th>
<th>Concentration of sperm as antigen (million)</th>
<th>Sperm concentration (million/ml)</th>
<th>Sperm motility (%)</th>
<th>Sperm viability (%)</th>
<th>Sperm abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>1</td>
<td>22.39±8.89 a</td>
<td>65.00±10.00</td>
<td>43.33±18.23</td>
<td>46.59±19.37 a</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5</td>
<td>11.61±4.09 a</td>
<td>22.33±11.57 a</td>
<td>55.00±15.00</td>
<td>68.15±4.19 a</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>10</td>
<td>11.89±1.81 a</td>
<td>63.33±19.22</td>
<td>53.33±16.67</td>
<td>42.06±20.61 a</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>—</td>
<td>163.57±11.81</td>
<td>71.66±4.41</td>
<td>75.00±2.89</td>
<td>2.17±0.25</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>—</td>
<td>161.84±18.79</td>
<td>88.33±7.26</td>
<td>83.33±7.26</td>
<td>1.55±0.45</td>
</tr>
<tr>
<td></td>
<td>CD at 5% level</td>
<td>34.27</td>
<td>36.98</td>
<td>NS</td>
<td>40.29</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ±S.E.
- a refers to the significant difference in the values at 5% level among different groups.
TABLE 4: Effect on weights of male reproductive and accessory sex organs of Rattus rattus immunized with different concentrations (million) of spermatozoa.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Type of Antigen</th>
<th>Concentration of sperm as antigen (million)</th>
<th>Body weight (g)</th>
<th>Testes (g/100g body wt)</th>
<th>Epididymis (g/100g body wt)</th>
<th>Vas deferens (g/100g body wt)</th>
<th>Seminal vesicle (g/100g body wt)</th>
<th>Prostate gland (g/100g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>1</td>
<td>153.33±8.92</td>
<td>0.788±0.050</td>
<td>0.337±0.053</td>
<td>0.061±0.013</td>
<td>0.492±0.051</td>
<td>0.137±0.018</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5</td>
<td>178.33±8.82</td>
<td>0.679±0.095</td>
<td>0.372±0.043</td>
<td>0.05±0.004</td>
<td>0.234±0.063 *</td>
<td>0.057±0.013 *</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>10</td>
<td>158.33±13.33</td>
<td>0.756±0.051</td>
<td>0.287±0.032</td>
<td>0.052±0.016</td>
<td>0.365±0.075</td>
<td>0.119±0.015</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>—</td>
<td>155.00±15.28</td>
<td>0.805±0.090</td>
<td>0.375±0.052</td>
<td>0.065±0.004</td>
<td>0.487±0.059</td>
<td>0.156±0.025</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>—</td>
<td>176.67±7.26</td>
<td>0.703±0.049</td>
<td>0.363±0.046</td>
<td>0.079±0.004</td>
<td>0.544±0.027</td>
<td>0.157±0.024</td>
</tr>
</tbody>
</table>

All values are mean ± S.E.

* a refers to the significant difference in the values at 5% level among different groups.
22.39±8.89, 11.61±4.09 and 11.89±1.81 in the epididymal fluid of house rats immunized with 1, 5 and 10 millions of spermatozoa as antigen respectively, from 161.84±18.79 millions/ml of control house rats. However, the values of sperm concentration between vehicle and control was found to be non-significant (Table 3). Fertilizing ability of the sperm can be interpreted in terms of their density/concentration. Significantly lower values of sperm concentration in the epididymal fluid of house rats immunized with types II and III antigens having 5 and 10 millions spermatozoa may have a direct correlation with the fertility level of the immunized rats, as the rats having sperm concentration less than 20 millions per ml are found to be infertile (Irvine and Aitken 1994 and Naz et al. 1995).

b) Effect on sperm motility:

No significant change in percent sperm motility was observed between vehicle and control group rats and also in the groups 1 and 3 immunized with 1 and 10 millions sperms. However, a significant decrease in percent sperm motility (22.33±11.57) was detected in the epididymal fluid of house rats immunized with 5 million sperms (type II antigen), as compared to that of control rats i.e. 88.33±7.26 (Table 3). Previously studies have clearly indicated that the raised antisperm antibodies bind on the tail region of sperm (Zouari and Almeida 1993) and the sperm no longer exhibits forward progressions, but instead becomes stationary and develops shaking motility pattern (Alexander 1984), resulting in severe reduction in fertility rate (Chen et al. 2009). Also, sperm motility less than 50% could lead to infertility (Irvine and Aitken 1994).

c) Effect on sperm viability:

No effect on sperm viability i.e. number of live/dead sperms was observed after immunization with 1, 5 and 10 million sperms as antigens, as the values of percent sperm viability of all the immunized rats was found to be non-significantly different from that of the control and vehicle house rats (Table 3). The present results are in accordance with that of study of Ellerman et al. (1998) indicating no difference in the number of viable sperms in the epididymal fluid of immunized and control rats. But it is not an indicative sign that antigen showing no change in viability of sperms can be considered as ineffective, as immunization resulting in no difference in percent viability has been found to inhibit sperm-egg fusion in vitro (Ellerman et al. 1998).

d) Effect on sperm morphology/sperm abnormalities:

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 in case of the rats immunized with antigen types I, II and III having 1, 5 and 10 millions spermatozoa respectively, significantly higher values of sperm abnormality were observed. Maximum percent sperm abnormalities were found to be 68.15±4.19 in the rats of group 2 which was attained in the rats after immunization with 5 million spermatozoa as compared to the other two groups 1 and 3 (Table 3). The effect of immunization on sperm morphology of house rats was also studied in terms of abnormalities in various regions of spermatozoa e.g. head, neck, mid piece and tail along with the observation of percent breakage and multiple abnormalities (Plate I). Results indicated significantly higher percent of total abnormalities (68.15±4.19) in rats of group 2 after immunization with 5 million spermatozoa as compared to that of control and other two immunized groups (group 1 and 3). When head, neck, mid piece and tail abnormalities in all the immunized rats was compared with that of control, no significant difference was observed. The important observation was, the significant difference in % breakage, which was found to be 19.73±5.06, 20.26±3.90 and 19.01±7.78 in rats of group 1, 2 and 3 respectively as compared to 0.62±0.29 % of the control rats. Multiple abnormalities (%) were also found in all the immunized rats as compared to that of control (Fig. 3). The dismantling/breakage/abnormality in various parts of the sperm may be associated with
FIG. 2: Comparative level of % positivity titre of raised antisperm antibodies in response to different concentrations (million) of spermatozoa in Rattus rattus.
reduction in fertility (Ryder et al. 1990). Overall sperm abnormalities more than 30% can lead to the infertility status of the animal (Irvine and Aitken 1994).

e) Effect on weights of male reproductive and accessory sex organs:

When the weights of male reproductive organs i.e. testes, epididymis and vas deferens of all the rats immunized with different concentrations of spermatozoa (1, 5 and 10 million) were compared with that of control house rats, non-significant difference was observed. However, the weights of accessory sex organs (seminal vesicle and prostate glands) of the rats of group 2 immunized with type II antigen (5 million spermatozoa) decreased significantly as that of control rats (group 4), as the weight of seminal vesicle (g/100g body weight) was found to be reduced to 0.234±0.063 from that of 0.544±0.027 in control rats and the weight of prostate gland (g/100g BW) was found to be reduced to 0.057±0.013 as compared to that of 0.157±0.024 in control rats (Table 4). These two accessory organs provide secretion to the semen and play a role in the motility of sperms, thus reduction in their weights will have direct impact on their secretory function which results in decreasing the motility of the sperms of the rats immunized with 5 million of spermatozoa as mentioned in the (Table 3). Also significant reduction in the weights of the seminal vesicle and prostate glands can be correlated with the fertilizing ability of the animal, as it is a known fact that alteration in the structural and functional integrity of reproductive and accessory organs influence their internal milieu which in turn reduce cauda epididymis sperm motility and result in a severe reduction in fertility rate (Kaur and Parshad 1994).

The present study concluded that out of the three tested antigens types having spermatozoa as antigen (1, 5, 10 million), the antigen type II having spermatozoa 5 million/ml was found to have the maximum effect in terms of significant production of antisperm antibodies, reduction in sperm concentration and sperm motility, increase in sperm abnormalities.
and reduction in weights of accessory glands. Thus immunization with 5 million spermatozoa as antigen indicate the efficacy of this concentration to be used for target as immunococeptive agent.

ACKNOWLEDGEMENT

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