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Contraception

Contraception 66 (2002) 459–462
Original research article

# Use of cauda epididymis extract as immunocontraceptive

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Received 8 May 2002; received in revised form 22 July 2002; accepted 7 August 2002

**Abstract**

The objective of this study was to evaluate use of a cauda epididymis extract (CEE) as an immunocontraceptive in rats. Twenty-two rats in puberty or 19 rats that gave birth once (primipara) were immunized with intraperitoneal (IP) injection of CEE. Rats in puberty received one or two injections of CEE containing $1.5 \times 10^9$ sperm/mL, while primipara rats received injections of CEE containing $3 \times 10^6$ sperm/mL up to three times. Animals were tested for the presence and concentration of anti-CEE antibody by enzyme-linked immunosorbent assay (ELISA) and monitored for pregnancy following natural insemination. Results revealed that 38 (92.6%) of the 41 rats were positive for anti-CEE antibodies, regardless of animal type or immunization procedure. However, there was no relation between pregnancy rates and concentration of anti-CEE antibody in rats immunized with CEE. These results indicate that immune response against CEE may not play a major role in contraception in rats. 

*Keywords:* Immunocontraception; Cauda epididymis extract; Rat

## 1. Introduction

Control of population growth can be achieved by prevention of pregnancy. Therefore, contraception has been an important area of study in both human and animals. Common contraceptive methods that are being practiced today include surgical methods, such as hysterectomy, ovariohysterectomy, ovariectomy and tubal ligation, and nonsurgical methods, such as hormonal applications. These methods may be difficult in application or costly to control population growth in human and animals. Therefore, alternative methods that are easy to apply, effective, and economically feasible are needed. Immunologic approach has been studied for contraception since the 1930s. Rationale of this approach is to block the reproductive chain through antibodies against gamate-specific antigens [1–5].

Historically, first in 1932, Barkin showed that immunization of women with injections of their husband's sperm resulted in sterility for approximately 1 year. The author further reported that the use of sperm as immunocontraceptive requires high titers of antibodies against sperm in oviduct and uterine fluid. Similar results indicating that antibodies can cause infertility by destroying spermatozoa have been reported by other researchers [6–8].

The objective of the present study was to investigate the effect of antibody concentration against CEE in rats in puberty or primipara rats for preventing pregnancy.

## 2. Materials and methods

### *2.1. Preparation of cauda epididymis extract*

Cauda epididymis from the testes of male rats were surgically removed and macerated manually; 5 to 10 males per batch of cauda epididymis extract (CEE). The maceration was performed in 5 mL of sperm washing solution (HEPES-buffered albumin, 5 mg/mL) within 1 to 2 min of removal. Sperm concentration in the resulting extract (CEE) was determined using a Makler Counting Chamber (Sefimedical Instruments, Haifa, Israel). Decimal dilutions of CEE were made to achieve target sperm concentrations described below.

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0010-7824/02/$ – see front matter 
PII: S0010-7824(02)00406-7

Table 1
Effects of reproductive status antibody concentration against crude extract of cauda epididymis on parturation rates of female rats

| Reproductive status | Numbers of immunizations | Mean A‡ values | Parturient | | Non-parturient | | Total n |
|---|---|---|---|---|---|---|---|
| | | | n | % | n | % | |
| Primipara* | 1 | 0.290 ± 0.030 | 7 | 100.0 | 0 | 0.0 | 7 |
| | 2 | 0.480 ± 0.050 | 4 | 50.0 | 4 | 50.0 | 8 |
| | 3 | 0.560 ± 0.025 | 3 | 42.9 | 4 | 57.1 | 7 |
| Puberty† | 1 | 0.340 ± 0.040 | 3 | 33.3 | 6 | 66.7 | 9 |
| | 2 | 0.510 ± 0.060 | 6 | 60.0 | 4 | 40.0 | 10 |

* Rats that gave birth one.
† Rats that never exposed to sperm previously.
‡ Absorbance value in ELISA.

### 2.2. Immunization procedure and insemination

Twenty-two of one-time parturition rats (primipara) and 19 puberty rats were used. Primipara rats were divided into three groups of 7 or 8 rats. The first group received one IP injection of 0.5 mL CEE containing $3 \times 10^6$ sperm/mL. The second and third groups received two (Day 0 and 10) or three times (Days 0, 10, and 15) the same injection, respectively. Male rats were added to the first, second and third groups after 10, 5, and 5 days after the final injections, respectively, for natural insemination.

Rats in puberty were divided into two groups. The first group of animals received one-time injection of 0.5 mL CEE containing $1.5 \times 10^9$ sperm/mL, while the second group of animals received two (on Days 0 and 14) of the same dose of CEE. Two weeks after the final immunizations, male rats were added to the groups.

Arrangements for all experimental groups were made for a ratio of 1 male/1 female/1 cage and male rats were held in the cages until determination of gestation. Gestations were determined with parturition of rats.

### 2.3. Determination of anti-CEE antibodies

After parturition of female animals, blood samples were taken from all animals, centrifuged at 3000 rpm for 5 min, and the resulting sera were stored at −20°C until analyzed.

Immune response of animals to CEE injections was determined in serum samples by ELISA. As an antigen, cauda epididymis extracts obtained from male rats were diluted with phosphate buffered solution (PBS) and washed by centrifugation at 2000 rpm five times. The pellet was diluted to $3 \times 10^6$ sperm/mL in phosphate buffered solution (PBS) containing 0.25% glutaraldehyde [9]. With the exception of the first four wells, 100-μL aliquots of this dilution were added to the wells of a 96-well microtitre plate. The resulting plates were incubated at 37°C overnight and washed with PBS-Tween 20 solution before addition of 100 μL PBS containing 10% horse serum (Sigma, Co., St. Louis, MO, USA) to each well. After incubation and washing of the resulting plates, 100 μL of diluted test serum samples (1:25 in PBS) were added to wells. After incubations and washings, peroxidase enzyme conjugated goat anti-rat IgG (1:5000 in PBS, Sigma, Co., St. Louis, MO, USA) was added to the wells and incubation was repeated. After washings, 100 μL of a substrate solution (0.1 M citrate-phosphate buffer containing 1 mg/mL O-phenylendiamine and 0.003% $H_2O_2$) was added into the wells and incubated for color development in a dark room for 15 min. The reaction was stopped by adding 1 M $H_2SO_4$ to the wells. Finally, absorbance for each well was determined at 450 nm using an ELISA reader (Medispec, ESR 200, Awareness Technology Inc., Palm City, FL, USA). For negative control, sera of five of virgin rats that had been immunized with sperm washing medium were used. The mean absorbance value of negative sera +3 SD was considered as the cutoff value point for positive response.

## 3. Results

In ELISA results, mean absorbance value of negative sera was shown to be $A_{450}$: 0.120. Furthermore, cutoff value point between negative and positive sera was determined to be $A_{450}$: 0.245.

Results showed that 38 (92.6%) of the 41 rats, regardless of any grouping, were positive for anti-CCE antibodies. Two of antibody-negative rats were primipara Group 1, the other one was in the puberty Group 1. According to $A_{450}$ in ELISA, the highest immune responses were observed in animals in primipara Group 3.

Parturition occurred in 23 (56.09%) of 41 rats. Parturition rate in primipara Group 3 that produced the highest immune response was 42.9%. The lowest parturition rate occurred in puberty Group 1. Animals in this group had much lower antibody titres than primipara Group 3 and puberty Group 2 (Table 1).

There were no macroscopic abnormalities observed in immunized female rats, although no histopathological examination was pursued.

## 4. Discussion

Generally, the aim of immunocontraception is to block of sperm and egg launching by specific antibodies. A number of studies have been conducted over the years to develop new immunocontraceptive methods [6,10,11–14]. For example, Fayrer et al. [11] reported that immunizations with purified zona pellucida glycoprotein caused irreversible immunosterilization in cats and dogs. Likewise, Bandivdekar et al. [13] reported that immunization of female or male rats with purified proteins of testis, epididymis, and prostate resulted in a reduced fertility rate in females (10%), while remaining the same in males (90%). Similarly, Carballada and Esponda [14] immunized fertile female rats with seminal plasma and examined the effect on fertility. They found an infertility rate of 26% in rats. Antibodies produced against seminal plasma in serum were detected by ELISA and Western immunoblot. However, no relationship was established between antibodies and infertility. Although it has been determined that oviduct fluid contains more antibody than blood serum, it is suspected that these antibodies might have a negative effect on spermatozoa, ovum and embryo, which results in infertility. In another study, Moore et al. [6] reported a sterility rate of 90% in mice and rats in a study that used monoclonal antibodies that blocked sperm from fertilization to the eggs of mice and rats.

Although data on effectiveness of immunocontraception varied among studies, all the above studies, in general, indicate that vaccine-based methods might have promising results in reducing pregnancy rate. However, it is noted that protein purifications or obtaining monoclonal antibodies involves complex procedures and might be cost effective. Immunization of female rats with crude extract of cauda epididymis prepared with simple laboratory equipment and essentially at no cost could have been a valid alternative.

Higher antibody titers against target immunogens of interest such as sperm antigens have been reported to cause lower pregnancy rates in various species, indicating a positive correlation between antibody level and the occurrence of contraception. Naz [10] reported that high titers of antibodies against sperm antigens in the genital canal could cause reversible infertility. Similarly, Ellerman et al. [15] studied the relationship between fertility rate and antibody titers against epididymal proteins that were administered to male rats and found that fertility was reduced. Surprisingly, our results indicated that there was no meaningful correlation between the titers of anti-CEE antibodies and pregnancy rates of rats. It should be noted that the antibody response in the present study was detected against CEE rather than sperm. Because the CEE was a crude extract and a mixture of sperm and a number of other proteins that exits in cauda epididymis, it can be concluded that the immune response was also mixed. Such a mixed response may seem advantageous since it can block the sperm via multiple antibodies. However, composition of crude CEE may also vary from batch to batch resulting in greater variations in the responses.

In conclusion, our results showed that the level of anti-CEE antibodies did not provide meaningful information on understanding the mechanism of contraception we observed. Furthermore, under the conditions described herein, effectiveness of injection with CEE for prevention of pregnancy may vary in different reproductive status of rats. Despite the limited data, our results suggest that reproductive status may have an important effect on the success of CEE method. For example, the lowest pregnancy rate in all groups was found in the rats at puberty injected once, even with relatively low titers of anti-CEE antibody. In contrast, the highest pregnancy was observed in primipara rats that were injected once. One possible explanation for this observation could be the differences in the concentration of sperm in CEE used for injecting primipara rats and rats at puberty. Nevertheless, future studies are warranted to investigate the effect of reproductive status on the success of immunocontraception.

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