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Effect of Monoclonal Antibodies to Boar Sperm on Conception Rate and Litter Size

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Target Audience: Veterinary Practitioners, Animal Scientist, Researchers in human and animal reproduction.

Abstract

Two monoclonal anibodies-Hmabs and Tambs-against boar spermatozoa were produced. The Monoclonal antibodies were used to immunize two groups of ten gilt and a third, control groups of ten gilt was injected with phosphate buffered saline (PBS). The Conception Rates(CR) were 100%, 30% and 50% for control, Hmab and Tmab respectively. The litter size were 8.4±1.71,5.0+1.0 and 5.4 ±1.14for the control, Hmab and Tmab group espectively. Immunization with both Hmab and Tmab significantly reduced CR(P<0.001), the reduction in CR was significantly lower than that produced byTmab (P<0.01). The hmab and Tmab significantly reduced litter size (P<0.001). There was no significant difference in litter sizes in the Hmab and Tmab treatments (P>0.05). Monoclonal antibodies to boar sperm may become candidates for contraception for population control in humans.

Key words: Sperm, monoclonal antibodies, conception rate, litter size.

Description of Problem

Sperm antibodies have been associated with infertility in humans (1) and farm animals (2,3,4). The mechanism involved have not been fully explained but the interference with reproductive functions may occur in a variety of ways like causing sperm agglutination (5), inhibition of sperm motility (6,7), inhibition of sperm adherence to and penetration of ova (8) Monoclonal antibodies to numerious sperm surface antigens in laboratory animals and humans, involved in fertilization process had been produced (9,10,11). Monoclonal antibodies to boar spermatozoa was produced and their effect on motility had been demonstrated (4). Furthermore, sperm agglutinating monoclonal antibodies that rapidly and completely agglutinate sperm when mixed with semen have been developed. However, their effect on female reproduction had not been investigated.

The objective of this study was to investigate the effect of immunization of gilts with the monoclonal antibodies, on their reproduction performance.

Materials and Methods

Preparation of Antigens

Five boars of the large white breed aged 15-18 months were ejaculated by the gloved hand technique. The gel free portions of the semen collected were pulled together , centrifuged at 1200g for 5 minutes. The sperm cells were then washed three times in 0.005M phosphate buffered saline (PBS), resuspended at a concentration of 1 x10^9 cells/ml. The cell suspension was sonicated (Sonicator model 380, Heat System Ultrasonic Inc.)and mixed with either complete or incomplete freund's adjuvant at a ratio of2:1(Sperm: Adjuvant, v/v.

Preparation of Monoclonal Antibodies.

This had been described somewhere else(3). Briefly, five mice of the BALB/ C stain were each

given 2mls intraperitoneal injection of the mixture of antigen in complete Freund's adjuvant on Day 0 and 2 mls of the mixture with incomplete adjuvant on Day 14. Intraperitoneal injection of 2mls sonicated sperm without adjuvant was given to each mouse on Day21. The mice were sacrificed on Day 24, the spleens removed, minced and the cells suspended in RPMI - 1640 (Gibco).

The spleen cells were used with myeloma cells Ag. 8.653 in a 2:1 ratio respectively by addition of drops of Polyethylene glycol (PEG) 4000. The fused cells were dispersed into 96 wells plates, after addition of RPMI and peritoneal exudates feeder cells at approximate concentration of $5x10^5$ spleen cells/well. The supernatants from the wells were screened by indirect immunoflourescence to confirm monoclonal antibody (Mab) production. The Mabs were characterized using the immunoperoxidase and protein A agglutination tests and tested with sheep red blood cells, bovine, ovine and equine sperm cells for specificity.

The anti-Head (Hmab) and anti-Tail (Tmabs) produced were stored at - 70°C until used for immunization.

Immunization of Gilts

Thirty gilts of ages between 12 and 15 months, of the large white breed were used. The gilts were kept in the University of Minnesota piggery and fed on commercial feed. The gilts were selected among those synchronized for oestrus and were divided into 3 groups of ten. The first and second groups were injected with Phosphate buffered saline (PBS).

Insemination of Gilts

The 30 gilts were inseminated with frozen semen prepared previously from proven boars 48 hours after injection with monoclonal antibodies and PBS as described above.

Pregnancy tests were carried out 30 days after insemination using vaginal biopsy technique as described previously (13;4). Briefly, vaginal biopsies were taken, using vaginal biopsy instrument, from the anterior vagina. The biopsies were fixed in Bouin's fluid embedded in wax and sections cut and stained on slides. The layers of epithelial cells, 2-3 layers regularly arranged confirmed pregnancy. The conception rate was recorded.

At parturition, the litter sizes were recorded.

Statistical Analysis

The results were analyzed using the Panacea, a University of Minnesota statistical package.

Results and Discussion

Tabe 1 shows the results of pregnancy tests and litter sizes with different treatment groups. The control group had 100% conception rate with a mena litter size of 8.4+1.71. The Hmabs and Tmabs had 30% and 50% conception rates respectively. The Hmabs had a litter size of 5.0±1.00 compared to 5.4±14 for Tmabs treatment. The Hmabs and Tmabs both significantly reduced CR and litter size ($P<0.001$). The reduction in CR with the Hmabs was significantly lower than that of Tmabs ($P<0.01$).

The Hmab and Tmabs significantly reduced the litter size ($P<0.001$). The difference in litter size with Hmabs and Tmabs treatment groups was not significant ($P>0.05$).

The CR was reduced significantly by both Hmab and Tmab ($p<0.001$). The lowering by Hmab may be by the blocking of the acrosome area of the sperm thereby preventing Acrosome reaction and capacitation which are necessary pre-requisites to the fertilization process. Sperm antibodies are known to block capacitating and acrosome reaction by inactivating Hyaluronidase and acrosin involved in sperm penetration of the investments surrounding the egg (1:15;16). The antibody may also be blocking, sperm adherence to ova and acrosome reaction by binding reactive sites on the sperm membrane and consequently preventing fertilization (12;15;17;18;19).

The anti-tail effect can be explained by the fact that the tail is responsible for the motlity of the sperm cells. Progressive motility is a

prerequisite for the sperm cells to travel across the uterus in the oviduct where fertilization takes places. Sperm antibodies have been shown to reduce sperm motility (6:20), which will reduce fertility.

The difference in litter size might have been a result of early embryonic mortality as immune complexes have been shown to cause early embryonic death (21). Early embryonic death may also account for the reduced conception rate because it is generally accepted that reduction of number of embryo to less than four prior to implantation, automatically terminates pregnancy.

The results of this study suggest that monoclonal antibodies to spermatozoa may be candidates for contraception in birth control programmes. Steroid contraceptives are known to have side effects like pulmonary embolism, cerebral thromobosis and tumor of the endometrium (22).

There is a need for more research effort at producing monoclonal antibodies to spermatozoa in the porcine species and studying the possibility of their use in human population control.

Table 1: **Proportion of pregnant gilts and litter size of gilts injected with monoclonal antibodies to boar sperm**

Group	Total	No. Preg.	%Preg.	(CR) Litter Size (Mean ±SD)
Control	10	10	100	8.4±1.71
Hmabs	10	3	30	5.0±1.00
Tmabs	10	5	50	5.4±1.14

Conclusion

1. Monclonal antibodies to boar sperm, Hmab and Tmab, were produced.
2. The monoclonal antibodies, when used to immunize sows, reduced conception rate and litter size
3. The monoclonal antibodies will cause infertility.
4. The monoclonal antibodies may become useful in human population control.

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