

## EFFECT OF IMMUNIZATION OF RABBIT WITH ZONA PELLUCIDA ANTIGEN ON CONCEPTION RATE AND LITTER SIZE

O. Fayemi

*Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan, Nigeria*

### ABSTRACT

Twenty mature female rabbits were divided into two equal groups. The first group was immunized with zona pellucida (ZP) antigen and the second group was injected with phosphate buffered saline (PBS) at the corresponding time of immunization (control group). When bred by male rabbits, the conception rate in the immunized group (30%) was significantly lower ( $P < 0.001$ ) than 100% recorded for the unimmunized (control) group. The litter size was  $1.67 \pm 0.50$  for the immunized group and was significantly lower than  $7.3 \pm 0.82$  for the control group ( $P < 0.001$ ). It is concluded that ZP antigens may become better candidates for contraception than steroids.

**Key words:** Rabbit, immunization, zona pellucida antigen, conception rate.

### INTRODUCTION

The zona pellucida (ZP), an extracellular glycoprotein matrix, is formed during mammalian ovarian follicular development (Maresh *et al.*, 1990). The ZP is involved in many critical stages of the fertilization process because the capacitated spermatozoon binds to the ZP before acrosome reaction (Dunbar *et al.*, 1994).

The ZP antigens have been known to stimulate antibody production which results in infertility in humans and animals (Gwatkin and Williams, 1978; Subramanian *et al.*, 1981; Franken *et al.*, 1990). This has stimulated interest in the possibility of utilizing ZP antigens from different species as targets for immunocontraception (Gupta *et al.*, 1997; Kirkpatrick *et al.*, 1997).

Heteroimmunization of rabbits with porcine ZP induced serum antibodies that cross-reacted with rabbit zonae, leading to ovarian degeneration and endocrine dysfunction (Skinner *et al.*, 1984) but immunization of rats and mice with porcine ZP did not affect fertility (Drell *et al.*, 1984). The objective of this study was to alloimmunize rabbits with ZP antigens and monitor its effects on conception rate and litter size in rabbits.

### MATERIALS AND METHODS

#### Preparation of immunogen

Ovaries were collected from slaughtered rabbits from local rabbitries in St. Paul and Minneapolis, Minnesota, USA. The mature follicles were punctured and aspirated with 1 ml tuberculin syringe and 26 gauge

needle. Ova were collected, using a stereomicroscope, in 0.5 ml 0.1M phosphate buffered saline (PBS). The ova were then transferred into test tubes containing 0.01% sodium citrate in PBS to remove cumulus cells before separating the zonae pellucidae with glass pipettes. The zonae were made into a suspension at a concentration of 200 zonae/ml after washing in PBS. The suspension was then sonicated using a sonicator model W380 (Heat Systems Inc.) and sonicates were mixed with equal volumes of either complete or incomplete Freund's adjuvant for immunization.

#### Immunization and breeding of does

Twenty mature fertile does (7–8 months old) were randomly divided into two groups (A and B), with ten animals in each group. Does of Group A were immunized with 2 ml of a mixture of sonicated ZP and complete Freund's adjuvant intramuscularly on day 0, 2 ml of a mixture of ZP sonicate and incomplete Freund's adjuvant on days 14 and 21. Animals of Group B were injected intramuscularly with 2 ml PBS on days 0, 14 and 21. All the does of both groups were bred by proven bucks (male rabbits) on days 28 and 29 of the experiment.

#### Serology

Sera were prepared by collecting 5 ml blood intracardially from each doe and screened for ZP antibodies using the enzyme linked immunosorbent assay (Henderson *et al.*, 1987). Pregnancy detection in experimental does was done using the vaginal smear and constant weighing. The does were monitored throughout the gestation period until parturition.

### Statistical analysis

The results were analyzed by logistic regression method, using Panacea statistical programme, University of Minnesota, USA.

## RESULTS AND DISCUSSION

All of immunized does were seropositive for ZP antibodies, while all the non-immunized does were seronegative. The conception rate was 100% in the control group compared to 30% in the does immunized with ZP antigens ( $P < 0.001$ ). The litter size of  $1.67 \pm 0.58$ , recorded for the immunized group, was also significantly lower than  $7.3 \pm 0.82$  observed in the control group ( $P < 0.001$ ). These results show that alloimmunization of female rabbits with ZP antigens provoked ZP antibody production which significantly reduced conception rate and litter size.

The zona pullucida, a glycoprotein surrounding the ovum, is known to be antigenic, especially in rabbits (Kerr *et al.*, 1999). As the ZP is involved in the process of fertilization, antibodies against ZP have been shown to cause infertility (Subramanian *et al.*, 1981). The reduction in conception rate in immunized rabbits seems to be due to fertilization failure due to inability of capacitated spermatozoa to bind to the ZP as a result of antibody antigen binding (Skinner *et al.*, 1999). The observation that some immunized does were pregnant but had reduced litter size could have been due to the fact that although fertilization may take place despite antibody formation, immune complexes formation may lead to early embryonic death (Witkin, 1986).

The overall results of this study confirm suggestions that ZP antigens may become candidates for non-steroidal contraception. Steroidal contraceptives have been shown to have side effects like coronary thrombosis, myocardial infarction and mammary carcinoma (Tatum, 1984, Tavassoli, 1997). It is suggested that more efforts should be made in the area of research into the usefulness of the zona pellucida as contraceptives.

## REFERENCES

- Drell, D., D., Wood, D. Bundman and B. S. Dunbar, 1984. Comparison of the immunological response in rats and rabbits to porcine zona pellucida. *Biol. Reprod.*, 30: 445-457.
- Dunbar, B. S., S. Avery, V. Lee, S. Prasad, D. Schwahn, E. Schwoebel, S. Skinner and B. Wilkins, 1994. The mammalian zona pellucida: Its biochemistry, immunochemistry, molecular biology and developmental expression. *Reprod. Fertil. Dev.*, 6: 59-76.
- Franken, D. R., T. F. Krager, R. Menkveld, S. Oehninger, C. C. Coddington and G. D. Hodgen, 1990. Hemizona assay and teratozoospermia: increasing sperm insemination concentrations to enhance zona pellucida binding. *Fertil. Steril.*, 54: 497-503.
- Gupta, S. K., M. Sharma, A. K. Behera, R. Bisht and R. Kaul, 1997. Sequence of complementary deoxyribonucleic acid encoding bonnet monkey (*Macaca radiata*) zona pellucida glycoprotein – ZP1 and its high level expression in *Escherichia coli*. *Biol. Reprod.*, 57: 532-538.
- Gwatkin, R. B. L. and D. T. Williams, 1978. Immunization of female rabbits with heat-solubilized bovine zonae: Production of anti-zona antibody and inhibition of fertility. *Gamete Res.*, 1: 19-26.
- Henderson, C, M. J. Holme and J. R. Aitken, 1987. Analysis of the biological properties of antibodies raised against intact and deglycosylated porcine zonae pulluciade. *Gamete Res.*, 16: 323-341.
- Kerr, P. J., R. J. Jackson, A. J. Robinson, J. Swan, L. Silvers, N. French, H. Clarke, D. F. Hall and M. K. Holland, 1999. Infertility in male rabbits (*Oryctolagus cuniculus*) alloimmunized with the rabbit zona pellucida protein ZPB either as a purified recombinant protein or expressed by recombinant Myxoma virus. *Biol. Reprod.*, 61: 606-613.
- Kirkpatrick, J. F., J. W. Turner, I. K. Liu, R. Fayrer-Hoskin and A. T. Rutberg, 1997. Case studies in immunocontraception: wild and feral equids and white-tailed deer. *Reprod. Fertil. Dev.*, 9: 105-110.
- Maresh, G. A., T. Timons and B. Dunbar, 1990. Effects of extracellular matrix on the expression of specific ovarian proteins of cultured primary ovarian follicles. *Biol. Reprod.*, 43: 965-976.
- Skinner, S. M., E. S. Schwoebel, S. V. Prasad, M. Oguna and B. S. Dunbar, 1999. Mapping dominant B-cell epitopes of a human zona pellucida protein (ZP1). *Biol. Reprod.*, 61: 1373-1380.
- Subramanian, M. G., E. C. Yurewicz and A. G. Sacco, 1981. Specific radioimmunoassay for the detection of a purified porcine zona pellucida antigen (PPZA). *Biol. Reprod.*, 24: 933-943.
- Tatum, H. J., 1984. Contraception and family planning. In: *Current Obstetrics and Gynaecologic Diagnosis and Treatment*. 5<sup>th</sup> Ed. Benson, R.C. (ed.) Lange Medical Publications, California, USA, pp: 525-555.
- Tavassoli, F. A., 1997. The influence of endogenous and oxogenous reproductive hormones on the mammary glands with emphasis on experimental studies in rhesus monkeys. *Verhandlungen der Deutschen Gesellschaft fur Pathologie*, 81: 514-520.
- Witkin, S. S., 1986. Circulating immune complexes and immunological infertility. *Estra dala rivista*, EOS, 6: 86-88.