

SPERM ANTIBODIES AND REPRODUCTIVE EFFICIENCY IN THE ZEBU CATTLE IN SOUTH-WESTERN NIGERIA

O. Fayemi

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

ABSTRACT

The association of sperm antibodies with infertility in 1346 Zebu cattle, including 435 males and 911 females, was serologically investigated. Sperm antibodies were not detected in yearling animals and very few animals were positive for sperm antibodies below the age of 36 months. The proportion of animals, both males and females, with sperm antibodies increased significantly with age ($P < 0.001$). The proportion of animals positive for sperm antibodies was also significantly related to sex ($P < 0.001$). In the females, the proportion of animals positive for sperm antibodies increased, and was significantly associated, with increased parity ($P < 0.001$). The mean age at first calving and the mean intercalving interval were significantly higher in the group positive for sperm antibodies compared to the negative animals ($P < 0.001$).

Key words: Sperm antibodies, Zebu cattle, infertility

INTRODUCTION

Infertility is a serious problem in animal production and accounts for great economic losses in the livestock industry. Causes of infertility may be infections or non-infections. Infertility investigation is usually requested by cattle farmers when the calf crop is low or when there is regular return to oestrus by cows that had been mated either naturally or by artificial insemination in the herd. In the North-eastern United States of America, 12-19% of dairy cows culled from herds each year were because of infertility or sterility (Thompson and Patterson, 1967). Apart from infectious causes of infertility, there have been some reports that there were immunological causes. For example, antibodies to sperm or egg yolk had been suggested to be possible causes of subfertility in cows (Hunter, 1972; Coulter *et al.*, 1976) and this has stimulated interest in the consideration of immunological infertility investigation. Menge *et al.* (1962) observed that semen treated with immune sera prior to insemination of heifers resulted in fertilization failure and possible early embryonic mortality, while normal sera did not have anti-infertility effect. Menge and Protzman (1967) also demonstrated anti-infertility effect of sperm antibodies in heifers iso-immunized with semen and bred artificially. A high incidence of delayed return to oestrus and very low pregnancy rates had been observed in heifers iso-immunized with semen which also correlated with sperm antibody level (Menge, 1969).

Since most information on sperm antibodies in cattle, had been based on experimental immunization and use of antisera on semen for artificial insemination, this study was designed to investigate whether sperm antibodies are produced by naturally bred Zebu cows. Attempts were also made to see if there is any correlation between such antibodies and the reproductive efficiency of the animals.

MATERIALS AND METHODS

Blood samples were randomly obtained from 1346 cattle including 435 males and 911 females, kept in private and Government farms in the South-western part of Nigeria. These animals varied in age from newborn to >72 months. The animals were of the Zebu breed (White Fulani, Sokoto Gudali, Red Bororo) and some Ndama. Sera were separated from these samples and kept at -20°C until used for assay for sperm antibodies. The recorded age, parity and intercalving intervals of the females were also obtained from the farms as part of the history of each animal.

Preparation of antigens

Sperm antigens were prepared as described previously for the boar (Fayemi, 1988; Fayemi *et al.*, 1992). Briefly, semen samples were collected by artificial vaginal from five bulls. The semen samples were pooled together and centrifuged at 1200g for 5 minutes to separate the sperm cells, which were then washed three times in phosphate buffered saline (PBS)

and resuspended at a concentration of 1×10^6 cells/ml. Smears of this sperm cell suspension were made on slides and fixed with methanol.

Assay of sera from sperm antibodies

The sera were analyzed using the immunoperoxidase assay, previously described for human sperm antibody (Holcberg *et al.*, 1986) and modified for swine sperm antibody (Fayemi, 1988). Briefly, the slides prepared above were incubated with 1% bovine sperm albumin (BSA) for 2 hours at 4°C, washed and then incubated with various dilutions of each serum sample for 1 hour at 37°C. The slides were washed for 15 minutes in PBS before addition of peroxidase conjugated rabbit anti-bovine IgG (Kirkegard and Perry Laboratories, KPL). The slides were then incubated for 45 minutes at 37°C and washed for 15 minutes in PBS. The substrate solution (10 mg 3, 3'-Diaminobenzidine tetrahydrochloride, Polysciences Inc) dissolved in 30 ml of Tris buffer (0.05M, pH 7.6 at 25°C) and 27µl 3% hydrogen peroxide (added before use) was poured on the slides and left for 5 minutes at room temperature before washing in PBS. The slides were then mounted in 10% glycerol in PBS, covered with coverslips and examined under the microscope for dark brownish colouration of the sperm membranes if the serum sample contained sperm antibodies, as demonstrated previously in swine (Fayemi, 1988).

Statistical analysis

The proportion of positive samples were categorized according to age, parity and age at first calving and intercalving intervals of the animals. Logistic regression was used to determine which variables were significantly associated with serological status.

RESULTS

The results of the assay of sperm antibodies in different age groups are shown in Table 1. Sperm antibodies were not detected in the yearlings (0-12 months of age) in both males and females. Very few males carried sperm antibodies below the age of 36 months. A higher proportion of females were tested

positive for the antibodies during this same period. The proportion of animals (both males and females) with sperm antibodies increased significantly with age ($P < 0.001$). The proportion positive was also significantly related to sex ($P < 0.01$) because the proportion of females positive was significantly higher than males in each age group. Total number of males tested was 435, of which only 42 (9.66%) were positive compared to 107 out of 911 (11.75%) for females. There were no significant association between sex and age.

In the females the proportion of cows positive for sperm antibodies increased and was significantly associated with increased parity ($P < 0.001$) (Table 2, Fig. 1). The mean age at 1st calving and the mean intercalving interval were significantly higher in the group positive (58.52 ± 2.46 months and 687.33 ± 64.74 days, respectively) for sperm antibodies compared to the negative animals (46.70 ± 1.10 months and 490.75 ± 33.67 days, respectively, $P < 0.001$).

DISCUSSION

The results of the present study show that there was auto- and iso-immunity to sperm antigens in the bovine animals tested. Sperm antibodies have been demonstrated in the sera in human and animals (Mathur *et al.*, 1986; Fayemi *et al.*, 1992; Waziri and Fayemi, 2000) and associated with infertility (Meinertz *et al.*, 1990). The proportion of animals that were seropositive increased with age both in males and females. The increase in the proportion of seropositive animals with age in the males may be as a result of infection or injuries. Ordinarily, the male does not produce antibodies against sperms because of the effective testis barrier produced by the tight sertoli cell junctions (Dym and Fawcett, 1970), except when they are disrupted especially by infection. Infections like trypanosomes have been associated with testicular pathologies (Ikede and Akpavie, 1982) and these are enzootic in Nigeria. In the cows increased age can result in trauma in the reproductive tract and contact with sperm cells that are foreign, can stimulate immune reaction coupled with the fact that autoimmunity in males had been associated with isoimmunity in females (Mathur *et al.*, 1985).

Table 1: Distribution of age in the bulls and cows tested for sperm antibodies

Age (months)	0-12	13-24	25-36	37-48	49-60	61-72	>72	Total
Total males	36	62	65	125	67	52	28	435
Males positive	0	1	3	11	9	10	8	42
% Male positive	0	1.61	4.62	8.80	13.43	19.23	28.57	9.66
Total females	38	73	187	234	152	138	89	911
Female positive	0	3	15	23	19	25	22	107
% Female positive	0	4.11	8.02	9.83	12.5	18.12	24.72	11.75
Total tested	74	135	252	359	219	190	117	1346
Total positive	0	4	18	34	28	35	30	149
% Total positive	0	2.96	7.14	9.47	12.79	18.42	25.64	11.07

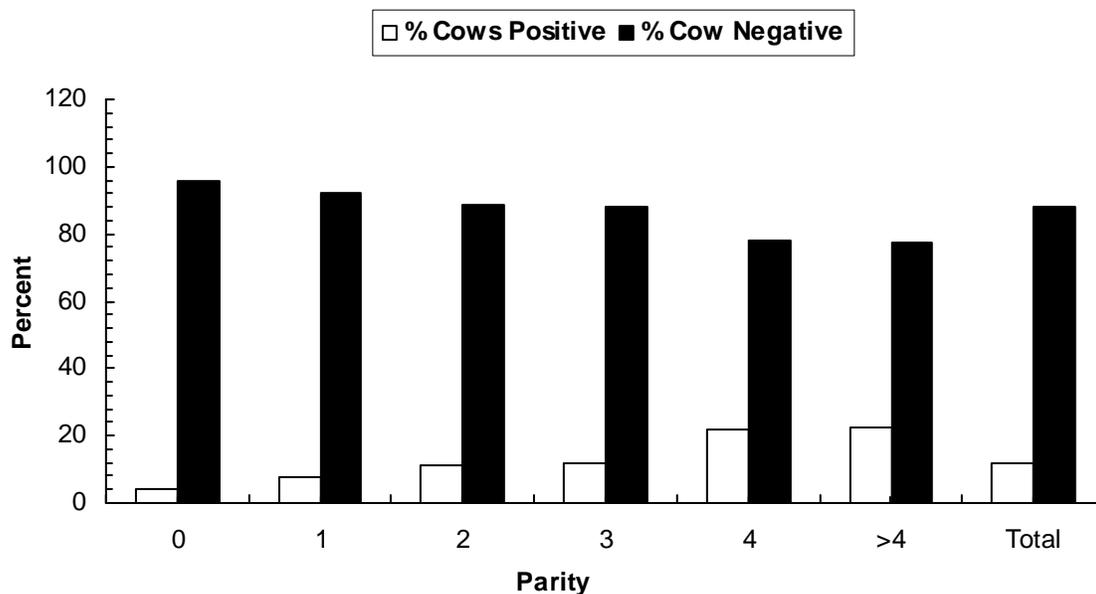


Fig. 1: Sperm antibodies in relation to parity in cows

Table 2: Distribution of parity in the cows tested for sperm antibodies

Parity	0	1	2	3	4	>4	Total
Cow tested	109	195	234	152	123	998	911
Cow positive	4	15	21	18	27	22	107
Cow negative	105	180	213	134	96	76	804

It was observed that higher proportions of males were positive for antibodies than females especially in older animals. This is similar to the finding in humans, where male autoimmunity was more prevalent than female isoimmunity (Mathur *et al.*, 1981).

The proportion of cows positive for sperm antibodies increased with increase in parity. Parturition can induce injury to the reproductive tract especially in cases of dystocia and injury to the reproductive tract may play a role in induction of immunity against sperm (Griffin *et al.*, 1971).

The significant increase in the age at first calving for seropositive animals is explainable by the infertility caused by sperm antibodies. Infertility may be due to sperm agglutination (Boettcher *et al.*, 1977) decreased sperm motility (Mathur *et al.*, 1984), and inhibition of cervical mucus penetration (Hendry *et al.*, 1982; Menge *et al.*, 1982). The infertility may also be a result of inhibition of sperm penetration of ova (Clarke *et al.*, 1995; Castle *et al.*, 1997; D'Cruz *et al.*, 1997). All these probable causes of infertility can also cause increased intercalving interval which in this study was significantly higher in the group that was seropositive to sperm antibodies.

In conclusion, auto- and iso-immunity to sperms in the form of sperm antibodies were detected in the sera of Zebu bulls and cows tested under normal field conditions. It is speculated that sperm antibodies may be one of the causes of infertility in these animals. Further research on the effect of sperm antibodies on fertility in the bovine and other farm animals is considered necessary.

Acknowledgement

The author is grateful to Mr. P.I. Odili for his technical assistance.

REFERENCES

- Boettcher, B., T. Hjort, P. Rumke, S. Shulman and O. E. Vyazov, 1977. Auto- and iso-antibodies to antigens of the human reproductive system. 1. Results of an international comparative study. Clin. Exp. Immunol., 30: 173-180.
- Castle, P. E., K. J. Whaley, T. E. Hoen, T. R. Moench and R. A. Cone, 1997. Contraceptive effect of sperm-agglutinating monoclonal antibodies in rabbits. Biol. Reprod., 56: 153-159.
- Clarke, G. N., D. Y. Liu and H. W. Baker, 1995. Immunoinfertility: a case study with implications for immunocontraception. Arch. Androl., 35: 21-27.
- Coulter, G. H., R. H. Foote, J. J. Schiavo and R. K. Braun, 1976. Antibodies to egg yolk in blood serum of rabbits and cattle and cervical mucus of cattle inseminated artificially. Theriogenology, 60: 585-589.

- D'Cruz, O. J. H., Lambert and G. G. Jr. Hass, 1997. Expression of CD15 (Lewisx) antigen on human sperm and its role in sperm-egg interaction. *Amer. J. Reprod. Immunol.*, 37: 172-183.
- Dym, M. and D. W. Fawcett, 1970. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod.*, 3: 308-326.
- Fayemi, O. E., 1988. Studies on boar infertility associated with sperm antibodies. PhD Thesis, Univ. Minnesota, USA.
- Fayemi, O. E., R. B. Morrioso and H. S. Joo, 1992. Seroprevalence of sperm antibodies in selected Minnesota swine breeding herds. *Anim. Reprod. Sci.*, 27: 341-345.
- Griffin, J. F. F., W. R. Nunn and P. J. Hartigan, 1971. An immune response to egg-yolk semen diluent in dairy cows. *J. Reprod. Fertil.*, 25: 193-199.
- Hendry, W. F., J. Stedronska and R. A. Lake, 1982. Mixed erythrocyte-spermatozoa antiglobulin reaction (MAR test) for IgA antisperm antibodies in subfertile males. *Fertil. Steril.*, 37: 108-112.
- Holcberg, G., D. Kleinman, I. Saror, G. Potashnik and V. Inslev, 1986. Immunoperoxidase assay for the detection of specific IgG and IgA antibodies to human spermatozoa in infertile women. *Int. J. Fertil.*, 31: 28-39.
- Hunter, A. G., 1972. Immunological aspects of reproduction associated with repeat breedings. *Proc. 4th Tech. Conf. Anim. Reprod. Artif. Insem.*, Natl. Assoc. Anim. Breeders, USA, pp: 2-7.
- Ikede, B. O. and S. O. Akpavie, 1982. Delay in resolution of trypanosome-induced genital lesions in male rabbits infected with *Trypanosoma brucei* and treated with diminazene aceturate. *Res. Vet. Sci.*, 32: 374-376.
- Mathur, S., E. R. Baker, H. O. Williamson, F. C. Derick, K. J. Teague and H. H. Fudenberg, 1981. Clinical significance of sperm antibodies in infertility. *Fertil. Steril.*, 36: 486-494.
- Mathur, S., H. O. Williamson, M. E. Baker, P. F. Rust, G. L. Holtz and H. H. Fudenberg, 1984. Sperm motility on postcoital testing correlates with male autoimmunity to sperm. *Fertil. Steril.*, 41: 81-87.
- Mathur, S., H. O. Williamson, E. R. Baker, P. F. Rust, G. L. Holtz and H. H. Fudenberg, 1985. Females' isoimmunity to sperm is associated with sperm autoimmunity in their husbands. *J. Clin. Immunol.*, 5: 166-171.
- Mathur, S., M. Baraber, M. Carlton, J. Zeigler and H. O. Williamson, 1986. Motion characteristics of spermatozoa from men with cytotoxic sperm antibodies. *Amer. J. Reprod., Immunol. Microbiol.*, 12: 87-90.
- Meinertz, H., L. Linnet, P. Fogh-Andersen and T. Hjort, 1990. Antisperm antibodies and fertility after vasectomy: a follow-up study of 216 men. *Fertil. Steril.*, 54: 315-321.
- Menge, A. C. and W. P. Protzman, 1967. Origin of the antigens in rabbit semen which induce infertility antibodies. *J. Reprod. Fertil.*, 13: 31-40.
- Menge, A. C., 1969. Early embryo mortality in heifers isoimmunized with semen and conceptus. *J. Reprod. Fertil.*, 18: 67-74.
- Menge, A. C., W. H. Stone, W. J. Tyler and L. E. Casida, 1962. Immunologic studies on fertility. IV. Fertility of cattle and rabbits inseminated with semen treated with antibodies produced against semen, spermatozoa and erythrocytes. *J. Reprod. Fertil.*, 3: 331-341.
- Menge, A. C., N. E. Medle, C. M. Mangione and J. W. Dietrich, 1982. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil. Steril.*, 38: 439-446.
- Thompson, N. R. and W. H. Patterson, 1967. Cow turnover in Virginia dairy herds. *J. Dairy Sci.*, 50: 610.
- Tung, K. S. K., 1980. Autoimmunity of the testis. In: *Immunological Aspects of Fertility Regulation*. Dhindsa, D. S. and G. F. B. Schumacher, (eds.). Elsevier/North Holland, New York, pp: 33-91.
- Waziri, M. A. and O. E. Fayemi, 2000. Seroprevalence of sperm antibodies in goats. *Veterinarski Arhiv*, 70: 95-102.