

## ASSOCIATION OF TRYPANOSOME INFECTION WITH SPERM ANTIBODIES PRODUCTION IN RED SOKOTO (MARADI) GOATS

O. FAYEMI

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

### ABSTRACT

A total of 1021 randomly selected serum samples of adult male goats that had been screened for trypanosome infection were assayed for sperm antibodies using the immunoperoxidase staining technique. The result of the trypanosome screening revealed that 586(57.39%) goats were positive for trypanosome infection, while 435(42.61%) were negative. The assay for sperm antibodies showed that 482(47.21%) animals were positive, while 539(52.79%) were negative. In the group that was positive for trypanosome infection, 364(62.12%) animals were positive, whereas 222(37.88%) were negative for sperm antibodies ( $P<0.001$ ). The group that was negative for trypanosome infection, had a significantly lower number and proportion 118(27.13%) of positive compared to 317(72.87%) negative for sperm antibodies. Out of a total 482 goats that were positive for sperm antibodies, a significantly higher number, 364(75.52%), were positive than 118(24.48%) that were negative for trypanosome infection ( $P<0.001$ ). In the group that was found negative for sperm antibodies, a significantly lower proportion, 222(41.19%), was positive compared to 317(58.81%) that were negative for trypanosome infection ( $P<0.001$ ). Seropositivity to sperm antibodies was positively correlated to trypanosome infection ( $P<0.001$ ). Further work on the pathogenesis of sperm antibody production in trypanosome infection is advocated.

**Key words:** Trypanosome infection, sperm antibodies, goats.

### INTRODUCTION

Goats are among the important domestic farm animals in the world (Lloyd, 1982). They are extensively kept in West Africa and found in all parts of Nigeria (Obudu *et al.*, 1995) and contribute immensely to the supply of animal protein in human nutrition.

Infertility is one of the most serious problems in animal production, resulting in culling of farm animals including goats. Infertility associated with sperm antibodies has been reported in humans and animals (Fayemi *et al.*, 1992; Kamada *et al.*, 1999; Waziri and Fayemi, 2000). Although autoimmunity to sperm has been recorded in goats (Waziri and Fayemi, 2000), its pathogenesis has not been clearly elucidated.

Testicular lesions like orchitis and testicular degeneration have been reported in *Trypanosoma vivax* and *T. brucei* infections in mice (Anosa and Kaneko, 1984), sheep and goats (Anosa and Isoun, 1980) and cattle (Isoun *et al.*, 1976). The objective of this study was to investigate whether there is any correlation between trypanosome infection and sperm antibody production in goats.

### MATERIALS AND METHODS

Serum samples collected from adult male goats (bucks) of the Red Sokoto (Maradi) breed at diagnostic laboratories in the subhumid south western parts of Nigeria were used for this study. The sera were stored in deep freezer at  $-20^{\circ}\text{C}$  until used for sperm antibody

screening. The samples were randomly picked and the screening for trypanosome infection was only carried out after the screening for sperm antibodies.

Sera from 1021 bucks were tested for sperm antibodies using the immunoperoxidase staining technique previously described by Holcberg *et al.* (1986) and modified by Fayemi (1988). Briefly, semen samples collected from these bucks were pooled together, centrifuged and washed three times in 0.1M phosphate buffered saline (PBS). The washed sperm cells, suspended in PBS were used to make smears on slides and fixed with methanol. The slides were incubated for 2 hours at  $4^{\circ}\text{C}$  with 1% bovine serum albumin (BSA), washed and incubated with various dilutions of the test sera in 0.005M PBS, and the controls for 1 hour at  $37^{\circ}\text{C}$ . The control sera were obtained from 2-week old male kids (young goats). The slides were then washed for 15 minutes in PBS, followed by incubation with peroxidase-conjugated rabbit anti-goat IgG (Sigma, 1:1200) for 45 minutes. The slides were washed in PBS for 15 minutes before incubating with substrate for 5 minutes at room temperature. The substrate solution was 10 mg 3, 3 Diaminobenzidine tetrahydrochloride (Polysciences, Inc) dissolved in 30 ml Tris buffer (0.05M, pH 7.6 at  $25^{\circ}\text{C}$ ) and 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The slides were washed in PBS, mounted in 10% glycerol in PBS, covered with cover slip and examined under a light microscope. Slides showing dark-brown staining of the sperm membrane were considered positive for sperm

antibodies. The results of the trypanosome screening were compared to the results of the sperm antibody assay.

### Statistical analysis

The results of the trypanosome screening test and sperm antibody assay were analysed by the logistic regression analysis, using the Panacea package, University of Minnesota, USA (Steel and Torrie, 1990).

## RESULTS

In this study, 1021 serum samples were screened for trypanosome infection and sperm antibodies. Of the 1021 goats, 586(57.39%) were positive for trypanosome infection, while 435(42.62%) samples were negative. The sperm antibody assay showed that 482(47.21%) samples were positive, while 539(52.79%) were negative. Out of the 586 goats that were positive for trypanosome infection, 364(62.12%) were positive for sperm antibodies compared to 222(37.88%) that were negative, whereas in the group of 435 goats that was negative for trypanosome infection, 118(27.13%) were positive for sperm antibodies compared to 317(72.87%) that were negative. In the group that were positive for sperm antibodies (482 goats), 364(75.52%) were positive for trypanosome infection compared to 118(24.48%) that were negative. Out of 539 goats that were negative for sperm antibodies, 222(41.19%) were positive for trypanosome infection compared to 317(58.81%) that were negative.

These results show that the proportion of goats positive for trypanosome infection was significantly higher than those negative ( $P < 0.01$ ) and the proportion of goats that were positive for sperm antibodies was lower than those negative ( $P < 0.05$ ). In the group positive for trypanosome infection, the proportion of goats that were positive for sperm antibodies was significantly higher than those negative for these antibodies ( $P < 0.001$ ). The animals that were negative for trypanosome infection had significantly lower proportion positive for sperm antibodies ( $P < 0.001$ ). In the group that were positive for sperm antibodies, a significantly higher proportion was positive for trypanosome infection compared to those negative for the infection ( $P < 0.001$ ). In contrast in the animals negative for sperm antibodies, significantly lower proportion was positive for trypanosome infection than those negative ( $P < 0.001$ ). Seropositivity to sperm antibodies was significantly correlated to trypanosome infection ( $P < 0.001$ ).

## DISCUSSION

Results of the present study show that a significantly higher proportion of goats screened were positive for trypanosome infection. This is not unexpected in an environment where the disease is enzootic. The difference in proportion was not deliberate because the results of the trypanosome

screening were not obtained until after the sperm antibodies screening was conducted.

A significantly higher proportion of goats positive for trypanosome infection were seropositive for sperm antibodies ( $P < 0.001$ ) and there were significantly more negative cases among those that were negative for trypanosomosis ( $P < 0.001$ ). This was confirmed by the fact that there was a significant correlation between trypanosome infection and sperm antibody production ( $P < 0.001$ ). This correlation was also evident from the results in the group that was positive for sperm antibodies, where a significantly higher proportion had trypanosome infection compared to those that were not showing trypanosome in the blood ( $P < 0.001$ ). Also goats that were negative for sperm antibodies had significantly lower proportion carrying the trypanosomes infection in the blood ( $P < 0.001$ ).

Sperm cells are highly immunogenic and express numerous sperm-specific antigens that first appear at puberty and can stimulate both auto- and allo-immune responses, leading to immunological infertility (Millette and Bellve, 1977). Since in the later stages of meiosis and subsequent spermiation, sperms are isolated within the lumen of the seminiferous tubules by a blood-testis barrier produced by the tight junctions between Sertoli cells (Tung, 1980), they are not normally exposed to the immune system. This blood-testis-barrier can be disrupted by genital infections (Witkin and Toth, 1983) and, therefore, predisposes to sperm antibody formation.

Trypanosome infections, especially *T. congolense* and *T. vivax*, have been shown to be associated with testicular lesions viz severe degeneration, necrosis and focal calcification of the seminiferous tubules in rams (Edeghere and Falope, 1985). *Trypanosoma vivax* caused testicular degeneration, testicular atrophy and total disappearance of spermatozoa in very severe cases in rams, goats (Anosa and Isoun, 1980) and bulls (Isoun *et al.*, 1976). These testicular lesions can lead to disruption of the blood-testis-barrier, resulting in production of sperm antibodies.

The presence of sperm antibodies in animals that did not show infection with trypanosomes in the blood may be a result of damage due to other infections. It is also possible that these animals were infected with trypanosomes in the past but did not carry the organisms in the blood at the time of screening. According to Ikede and Akpavie (1982), damage done to the reproductive organs of *T. brucei* infected male rabbits within two to three weeks of infection was only partially resolved four to five months after the elimination of the parasites by chemotherapy.

Sperm antibodies have been associated with infertility in human and animals (Menge *et al.*, 1982; Fayemi *et al.*, 1992; Clarke *et al.*, 1995; Kamada *et al.*, 1999; Waziri and Fayemi, 2000;) and their consideration in infertility investigation has become important. Since this study has demonstrated a possible correlation of serum sperm antibodies with trypanosome infection, further work on the

pathogenesis of sperm antibody production in trypanosomiasis is suggested.

#### Acknowledgement

The author is grateful to Dr. Han Soo Joo for providing the laboratory facilities for this study.

#### REFERENCES

- Anosa, V. O. and J. J. Kaneko, 1984. Pathogenesis of *Trypanosoma brucei* infection in Deer mice (*Peromyscus maniculatus*). Light and electron microscopic study of testicular lesions. *Vet. Pathol.*, 21: 238-246.
- Anosa, V. O. and T. T. Isoun, 1980. Further observations on the testicular pathology in *Trypanosoma vivax* infection of sheep and goats. *Res. Vet. Sci.*, 28: 151-160.
- Clarke, G. N., D. Y. Liu and H. W. Baker, 1995. Immunoinfertility: a case study with implications for immunocontraception. *Arch. Androl.*, 35: 21-27.
- Edeghere, H. and O. O. Falope, 1985. Genital lesions in rams experimentally infected with single and mixed *Trypanosoma brucei*, *T. congolense* and *T. vivax*. *Proc. Natl. Conf. Small Ruminants Prod. NAPRI, Zaria, Nigeria*, pp: 264.
- 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.
- 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.
- 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.
- Isoun, T. T., J. U. Akpokodje and V. O. Anosa, 1976. Testicular lesions in the White Fulani (Bunaji) cattle experimentally infected with *Trypanosoma vivax*: a preliminary report. *J. Nig. Vet. Med. Assoc.*, 4: 107-108.
- Kamada, M., S. Yamamoto, M. Takikawa, K. Kunimi, M. Maegawa, S. Futaki, Y. Ohmoto, T. Aono and S. S. Koide, 1999. Identification of the human sperm protein that interacts with sperm-immobilizing antibodies in the sera of infertile women. *Fertil. Steril.*, 72: 691-695.
- Lloyd, S., 1982. Goat medicine and surgery. *British Vet. J.*, 138: 70-85.
- Menge, A. C., N. E. Medley, 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.
- Millette, C. F. and A. R. Bellve, 1977. Temporal expression of membrane antigens during mouse spermatogenesis. *J. Cell Biol.*, 74: 86-97.
- Obudu, C. E., O. S. Adedeji and E. B. Otesile, 1995. Incidence and causes of mortality in goats on the University of Ibadan Teaching and Research Farm: A retrospective study. *Israeli J. Vet. Med.*, 50: 29-33.
- Steel, R. G. D. and J. H. Torrie, 1990. Principles and Procedures of Statistics. 2<sup>nd</sup> Ed., McGraw Hill Book Co. Inc., New York, USA.
- 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, USA, pp: 33-91.
- Waziri, M. A. and O. E. Fayemi, 2000. Seroprevalence of sperm antibodies in goats. *Vet. Arhiv*, 70: 95-102.
- Witkin, S. S. and A. Toth, 1983. Relationship between genital tract infections and sperm antibodies in seminal fluid and infertility. *Fertil. Steril.*, 40: 805-808.