African Journal of Biomedical Research, Vol. 6 (2003); 137 - 140
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

Full Length Research Article

ASSOCIATION OF TRYPANOSOME INFECTION WITH CIRCULATING ZONA PELLUCIDA (ZP) ANTIBODIES IN WEST AFRICAN DWARF (WAD) GOATS

O. FAYEMI
Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan, Nigeria.

Sera from 967 adult female West African Dwarf (WAD) goats previously screened for Trypanosome infection by some diagnostic laboratories around Ibadan metropolis, in Southwestern part of Nigeria, were assayed for zona pellucida (ZP) antibodies by the enzyme-linked immunoassay (ELISA) technique. Of the 967 female goats, 534 (55.22%) were positive and 433 (44.78%) negative for Trypanosome infection. Out of those that were positive for Trypanosome infection, 346 (64.79%) and 188 (35.21%) were positive and negative for ZP antibodies respectively. These represented 35.78% and 19.44% of the total number of animals screened respectively. The group that was negative for Trypanosome infections had 149 (34.41%) and 284 (65.59%) positive and negative for ZP antibodies, representing 15.41% and 29.37% of the total number of animals screened, respectively. Seropositivity for ZP antibodies was positively correlated with Trypanosome infection (P<O.OO1). There is the possibility of damage to the blood-ovary barrier as a result of trypanosome infection thereby exposing the zona pellucida to the immune system and being recognized as foreign. The possibility of zona pellucida antibodies as a cause of infertility in domestic animals should always be considered in infertility investigations.

Key words: - Trypanosomes, zona pellucida antibodies, and infertility, Goats.

INTRODUCTION

Goats are raised extensively and semi-intensively in Nigeria as a source of animal protein for human nutrition. The West African Dwarf (WAD) breed is noted for its ability to survive the harsh environmental conditions of heat stress and humidity (Devendra and Mcleroy, 1982, Osuagwuh and Akpokodje, 1981) and it is the predominant breed in the humid Southwestern part of the country.

The WAD goats are kept in small flocks, typically numbering 2 — 6 animals, per household as an adjunct to the main business of cropping, thereby contributing significantly to the economy of third world countries (Upton, 1985; Ademosun, 1985). Despite the importance of this species of animal (goat); it has received very little attention (Okere *et. al*, 1982) and there is low productivity, which has been attributed to factors including nutrition, infectious and non-infectious agents (Laing *et al*, 1988, Cullen, 1990). Low productivity can be caused by infertility and immunological reactions had been associated with infertility especially antibodies produced against the *zona pellucida* in humans and animals (Sacco, 1979, Wood *et. al*, 1981 Sacco, *et al*, 1981, Haseguwa, 1995).

Trypanosome infections have been reported to cause infertility due perhaps to ovarian pathology in goats (Ikede and Akpavie, 1981). The ovary produces follicles which contain ova surrounded by zonae pellucidae. The zona pellucida is an acellular glycoprotein which is antigenic (Gupta *et.al*., 1997, Brown *et al*., 1997) but ordinarily will not stimulate antibody production because it is protected by the blood-ovary barrier which when disrupted will lead to antibody production. The ZP antibody production has been associated with infertility. It has not been established if damage to the ovary can lead to damage to the blood-ovary barrier thereby stimulating ZP antibody production. The ZP antibody has not been correlated with Trypanosome infection.

The objective of this study was therefore to check whether there is any correlation between trypanosome infection and ZP antibody production

MATERIALS AND METHODS

Serum samples previously collected and screened for Trypanosome infections by the private veterinary clinics, Government and University Laboratories in the Southwestern part of Nigeria were used for this investigation. The samples were randomly selected from the pool without previous reference to the trypanosome screening results until the results of the ZP antibody assay were ready. The samples were taken in ice-packs and air-freighted to the laboratories in the University of Minnesota, St. Paul. Minnesota, U.S.A for ZP antibodies assay.

Preparation of Antigen

Caprine ovaries were collected from slaughterhouses at goat farms around St. Paul, Minnesota, U.S.A. The follicles were punctured and 1 ml. tuberculin syringes with 26 gauge needles were used to aspirate the follicular fluid into Petri dishes. The ova were collected in 0.5mls 0.1M phosphate buffered saline (PBS) using a stereomicroscope. The ova were transferred into a test tube containing 0.01% w/v sodium citrate in PBS and shaken for 60 seconds to remove the cumulus cells. The *zonae pellucidae* were then separated from the eggs using glass pipettes with bores a little narrower than the diameter of the egg.

The zonae were washed three times in PBS, resuspended at a concentration of 200 zonae/ml and then sonicated 20 strikes with a sonicator model W380 (HeatSystems Inc.). The sonicates were then centrifuged twice using PBS at 1200g for 20 minutes at 4°C. The optical density OD of the second supernatant was estimated and adjusted to 0.2. This was equivalent to 0.15mg/ml protein and was used as the zona pellucida antigen. The antigen was divided into aliquots and stored in the cold room at —196°C until used for the assay of antibodies.

Assay of Zona Pellucida Antibodies

A total of 967 serum samples were assayed for antizona pellucida antibodies using the methods of Henderson *et al*. (1987) with slight modifications. The zonae antigen was dispensed at 50µl well into 96 — well polyvinyl microplates (Falcon 3912 Micro Test III, Becton Dickinson) and left overnight at 4°C. On the second day, the fluid was decanted and the antigen fixed using 50µ 0.1% gluteraldehyde in PBS-Tween 20 (Sigma) for 5 minutes. The plates were then washed three times in PBS before incubating overnight with 100 1% Bovine Serum Albumin (BSA) at 4°C. After this the plates were washed three times again before dispensing 50µ/well of various dilutions of the test and standard negative samples and incubating for 1 hour at 37°C. The standard negative samples being sera taken from 2-week old kids. The plates were then washed in PBS-Tween 20 and incubated for 30 minutes with addition of 5Oµ/well Biotin-labeled rabbit-anti-goat IgG (KPL, 1:4800) at 37°C. This was followed by washing and incubation for 30 minutes with 50µ streptavidin peroxidase (KPL, 1:9600) at 37°C. The plates were then washed three times before adding 50µ/well substrate for 15 minutes in the dark danser. The substrate consisted of equal volumes of 2,2, azino-di (3-ethylbenzthiazoline sulfonate) (ABTS) and hydrogen peroxide (H The optical density was read at 405nm with a micro ELISA reader model MR380 (Dynatech).

The mean OD of the standard negative sera was calculated and used as the benchmark. Any sample with twice the value of the calculated mean was taken as positive.

Correlation with Trypanosome Infection

The results of the selected samples in term of trypanosome infection status were collected from the laboratories of origin and compared with the results of the ZP antibodies assay.

Statistical Analysis of Results

The antibody assay results were correlated with the trypanosome infection status of the samples using the Panacea statistical package, University of Minnesota

RESULTS

Table 1 shows the absolute numbers and proportions of animals that were positive and negative for Trypanosome infection and ZP antibodies. Out of 967 animals tested 534 (55.22%) were positive compared to 433 (44.78%) that were negative for Trypanosome infection.

Those positive for both ZP antibody and Trypanosome infection were 346(35.78% of Total) while those positive for ZP antibodies but negative for trypanosome infection 149(15.41% of Total). The animals that were negative for ZP antibodies but positive for trypanosome infection were 188 (19.44% of Total) while those that were negative for ZP antibodies and negative for trypanosome infection numbered 284 (29.37% of Total). Of the total number of 967, 495 (51.19% of Total) were positive for ZP antibodies compared to 472 (48.8 1% of Total) that were negative for the antibodies.

Table 1:
Proportions of animals positive and negative for Trypanosome infection and *zona pellucida* antibodies.

	Positive for Trypanosome	Negative for Trypanosome	Total
No. Positive for ZP antibodies	346 (35.78%)	149 (15.41%)	495 (51.19%)
No. Negative for ZP antibodies	188 (19.44%)	284 (29.37%)	472 (48.81%)
Total No Tested	534 (55.22%)	433 (44.78%)	967 (100%)

Table 2:
The proportions of Animals positive and negative for zona pellucida antibodies in each of the groups that tested positive and negative for Trypanosome infection

	Positive for Trypanosome	Negative for Trypanosome
No. Positive for ZP antibodies	346(64.79%)	149(34.41%)
No. Negative for ZP antibodies	188(35.21%)	284(65.59%)
Total	534(100%)	433(100%)

Table 3
The proportions of Animals positive and negative for Trypanosome infection in each of the groups that tested positive and negative for zona pellucida (ZP) antibodies.

	Positive for ZP Antibodies	Negative for ZP Antibodies
No. Positive for Trypanosome	346(69.90%)	188(39.83%)
No. Negative for Typanosome	149 (30.10%)	284 (60.17%
Total	495(100%)	472(100%)

Table 2 shows the proportions of animals that were positive and negative for *zona pellucida* antibodies in each of the groups positive and negative for Trypanosome infection. Of the 534 that were positive for trypanosome infection, 346(64.79%) and 188 (35.21%) were positive and negative for ZP antibodies respectively. In the group that were negative for trypanosome infection totaling 433, 149(34.41%) and 284(65.59%) were positive and negative for ZP antibodies respectively.

Table 3 shows that out of the 495 animals that were positive for ZP antibodies, 346 (69.90%) and 149(30.10%) were positive and negative for trypanosome infection respectively. Of the 472 that were negative for ZP antibodies, 188 (39.83%) were positive compared to 284(60.17%) that were negative for trypanosome infection. The proportion of animals positive for trypanosome infection was significantly higher than those negative for the infection (P<0.01).

The proportion of animals that were positive for ZP antibodies was significantly higher than those that were negative for the ZP antibodies (P<0.01). Seropositivity to ZP antibodies was positively correlated with trypanosome infection (P<0.001).

DISCUSSION

The results show that in the group positive for trypanosome infection, a significantly higher proportion was seropositive for ZP antibodies (P<0.001). Also in the group that was negative for trypanosome infection the proportion that was seropositive for ZP antibodies was significantly lower than those that were seronegative to the antibody (P<0.001). Seropositively was positively correlated with trypanosome infection.

The *zona pellucida* has been shown to be antigenic (Subramanian *et al*, 1981; Dunbar, *et al*. 1989, Skinner, *et al*, 1999) but does not ordinarily stimulate production of antibodies because of the blood-ovary-barrier.

Reproductive disorders have been associated with trypanosome infections in man and animals (Apted, 1970), Ruminants infected with *Trypanosoma brucei*, T. congolense or *T. vivaxe* showed that the infection can lead to irregular oestrus, infertility and intrauterine infection with abortion in females (Ikede and Akpavie, 1982). The pathogenesis of infertility in such cases may be connected with damage to the blood-ovary-barrier leading to formation of antibodies to the zonae pellucidae which had been associated with infertility (Wolgemuth *et al*., 1984, Maresh *et al*, 1990, Hazeguwal *et al*, 1995, Kolle *et al*., 1996).

The animals that were negative for trypanosomes when tested but were seropositive for ZP antibodies might have been previously infected since even after successful treatment of animals, parasites usually disappear but infertility may not disappear in Ndama cows 3½ to 16 months after infection (Ige and Amodu, 1975). The correlation of seropositivity for ZP antibodies with trypanosome infection in this study allows the speculation that trypanosome infection could have caused enough damage to the ovaries in the affected animals to the point of exposure of the ZP proteins, secreted by the oocytes as well as granulose cells (Wolgemuth *et al*, 1984, Moresh *et al*, 1990), to the immune system The possibility of ovarian structural damage in trypanosome infection which is enzootic in Nigeria causing infertility should therefore be considered in infertility investigation in farm and domestic animals.

Further studies on the histopathology and electron microscopy of the ovary during the course of trypanosome infection will throw further light to this suspected pathogenesis of infertility.

ACKNOWLEDGEMENT

The author is grateful to Dr. H. S. J of the Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108, U.S.A. in whose laboratory the ZP antibody assay was done.

REFERENCES

Ademosun, A. A. (1985). Contributions of Research to small ruminant production in Nigeria. Proc. Nail. Conf Small Ruminant Prod. NAPRI, Zaria pp. 18 — 33.

Apted, P.I.C. (1970): Clinical manifestations and diagnosis of sleeping sickness. In:
The African Trypanosomiasis. Mulligan, H. W. and Potts, W.H. eds. London. Allen and Unwin Publishers. pp. 667-683.

Brown, R.G., Bowen, W. D, Eddington, J. D. Kimmins, W.C., Mezei, M., Parsons, J.L. and Pohajdak, B. (1997). Evidence for a long-lasting single administration contraceptive vaccine in wild grey seals. J. Reprod. Immunol. 35: 43-51.

Cullen, P.T. (1990). Farm animal health — A practical guide. Pergamon press pp. 42—48.

Devendra, C. and McLeroy, G. B. (1982). Goat and sheep production in the tropics. Payne, W.J.A. ed., Longman, London and New York, Publishers. pp.1-55.

Dunbar, B. S. (1989). Ovarian antigens and infertility. Am. J. Reprod. Immunol. 21: 28-31.

Dunbar, B. S. Avery, S., Lee, V., Prasaci, S. Schwahn, D., Schwoebel, E., Skinner, S. and Wilkins, B. (1994). The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology and developmental expression. In: Immunological control of fertility: from gametes to gonads. Reprod. Fertil. Dev. 6: 59-76.

Gupta, S. K Jethanandani, P. Afzalpurkar, A., Kaul, R. and Santhanam, R. (1997). Prospects of zona pellucida glycoproteins as immunogens for contraceptive vaccine. Human Reprod. Update 3: 311 —324.

Haseguwa, A. Yamasaki, N., Innue, M., Koyama, Rand Isojima, S. (1995). Analysis of an epitope sequence recognized by a monoclonal antibody MAb 5H4 against a porcine zona pellucida glycoprotein (pZP4) that blocks fertilization. J. Reprod. Fertil. 105:295-302.

Henderson, C. J., Holmes, M. J. and Aitken, R. J. (1987). Analysis of the biological properties of antibodies raised against intact and deglycosylated porcine zonae pellucidae. Gamete Res. 16: 323 —341.

Ige, K. and Amodu, A. A. (1975). Tiypanosome infection and infertility in cows. Report of the 14 meeting of International Scientific Council for Trypanosomiasis Research and Control, Dakar, Senegal. PP. 151 — 156.

Ikede, B. 0. and Akpavie, S. 0. (1982). Delay in resolution of trypanosome-induced genital lesions in male rabbits infected with Trypanosome brucei and treated with diminazene aceturate. Res. Vet. Sc. 32: 374 — 376.

Kolle, S., Sinowatz, F. Boie, G., Totzauer, I. Amseigruber, W. and Plendi, J. (1996). Localization of the mRNA encoding the zona protein ZP3 in the porcine ovary, oocyte and embryo by non-radioactive in situ hybridization. Histochem. J. 28: 441 — 447.

Lamg, J. A., Brinley-Morgan, W. J. and Wagner, W. C. (1988). Fertility and Infertility in Veterinary practice. 4 Edition. Saunders, W. B. ed. Balliere Tindall, London. pp. 81 — 90.

Maresh, G. A. Timmons, T. and Dunbar, B. (1990). Effects of extracellular matrix on the expression of specific ovarian proteins of cultured primary ovarian follicles. Biol. Reprod. 43: 965 - 976.

Mutayoba, B. M., Gombe, S., Kaaya, G. P. and Waindi, E. N. (1988). Effect of chronicexperimental Trypanosoma congolense infection on the ovaries, pituitary, thyroid and adrenal glands in female goats. Res. Vet. Sci. 44: 140 - 146.

Okere, C., Chiboka, 0. and Montsina, G. (1982). Effect of frequent ejaculation of WAD goat on semen characteristics. mt. J. Amin. Reprod. Sci. 11: 249 — 258.

Osuagwuh, A. I. A. and Akpokodje, J. U. (1981). The WAD (Fouta D'jallon) goat 1:
cause of early mortality. Tnt. Goat and sheep Res. 1: 303 — 309.

Osuagwuh, A. I. A. and Akpokodje, J. U. (1984). Reproductive performance of the WAD (Fouta D'jallon) Goat. Trop. Anim. Prod. 9: 231 - 238.

Sacco, A. G. (1979). Inhibition of fertility in mice by passive immunization with antibodies to isolated zonae pellucidae. J. Reprod. Fertil. 56: 533 — 537.

Sacco, A. G. , Subramanian, M. C. and Yurewicz, E. C. (1981). Active immunization of mice with porcine zona pellucida: immune response and effect on fertility. J. Exp. Zool. 218: 405 —418.

Skinner, S. M., Schwoebel, E. S., Prasad, S. V., Oguna, M. and Dunbar, B. S. (1999). Mapping of dominant B-cell epitopes of a human zona pellucida protein (ZP1). Biol. Reprod. 61: 1373-1380.

Subramanian, M. G., Yurewicz, E. C. and Sacco, A. G. (1981). Specific radioimmunoassay for the detection of a purified porcine zona pellucida (PPZA). Biol. Reprod. 24: 933 — 943.

Upton, M. (1985). Returns from small ruminant production in Nigeria Nig. Agric. Syst. 17: 65 — 84.

Wolgemuth, D. J., Celenza, J., Bundman, D. S. and Dunbar, B. S. (1984). Formation of the rabbit zona pellucida and its relationships to ovarian follicular development. Dev. Biol. 106: 1 — 14.

Wood, D. M., Liu, C. and Dunbar, B. S. (1981). Effect of alloimmunization and heteroimmunization with zona pellucida on fertility in rabbits. Biol. Reprod.
25: 439 — 450.

Accepted in final form: March 2003