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Seroprevalence of sperm antibodies in goats

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ABSTRACT

Sera from 235 goats in and around Ibadan (Nigeria) were randomly collected and tested for sperm antibodies by the enzyme-linked immunosorbent assay (ELISA) method. Of the 164 females tested, 25 (15.2%) were positive for sperm antibody while 7 (9.8%) of the 71 males tested were positive. The presence of circulating sperm antibody was significantly associated with age ($P<0.001$) and parity ($P<0.001$). Sex was not significantly associated ($P>0.05$). The study indicates the prevalence of sperm antibodies in the herds tested.

Key words: seroprevalence, sperm, antibodies, goat

Intoduction

Infertility is a serious problem that accounts for major economic losses in the livestock industry. It is a common cause of culling farm animals, including goats (D'ALLAIRE et al., 1987). Infertility in sheep and goats is not uncommon, but comprehensive, accurate and up-to-date information is not available (LAING, 1979).

There are many possible causes of infertility, which may be infectious or non infectious. The major cause in the male is impairment of sperm quality, traditionally evaluated in terms of count, motility and morphology. In the female, infertility may involve a number of factors, including problems of ovulation, obstruction of oviducts, presence of pathological lesions in the uterus and poor quality of cervical mucus (SHULMAN, 1986).

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However, in both sexes a more recently appreciated cause of infertility is an immunological factor associated with the presence of sperm antibodies as a result of auto-immunization in males and isoimmunization in females (JONES, 1980; HANCOCK, 1983).

There were several indications that associated presence of sperm antibodies in serum with infertility in a variety of species, including human (BRONSON et al., 1984), guinea pigs (KATSH, 1959), cattle (MENGE, 1980), rabbits (FAYEMI, 1988) and swine (FAYEMI et al., 1990).

Although the effects of immunologic infertility on livestock production and the attendant economic losses resulting from low breeding efficiency and culling are now well appreciated, there is a paucity of relevant information on goats, and none at all in the area of study. The purpose of this investigation was, therefore, to determine the prevalence of sperm antibodies in randomly selected Nigerian herds of goats, and to correlate findings to age, sex and parity (in the case of females).

Material and methods

Sampling protocol

A total of 235 sera samples were randomly collected from goats in and around Ibadan, ranging from the intensive ILCA (International Livestock Centre for Africa) farm to the semi-intensive, extensive small farms scattered in and around Ibadan. Some samples were collected from the Ibadan abattoir. Sera were stored at –20 °C until used. The history on age, sex and parity (in the case of females) of each animal was obtained. Sera samples were also obtained from virgin goats (3-month-old kids) and were pooled as the negative control.

Experimental procedure

Samples were screened for the presence of sperm antibodies by Enzyme-Linked immunosorbent assay (ELISA) technique. The choice was based on earlier reports (HAREL and NELKEN, 1985; FAYEMI, 1988).

Pooled semen samples from 15 bucks were centrifuged at 1200 g for 5 minutes to separate sperm cells, which were then washed three times in phosphate buffered saline (PBS) pH 7.5 and re- suspended. The cells were frozen-thawed and later sonicated for about 2 minutes with a Labsonic sonicator, model L-2000, and centrifuged twice with PBS at 1500 g for 30 minutes at 4 °C. The optical density (OD) of the second supernatant was determined and adjusted to 0.2 OD (equivalent to 0.15 mg/ml protein) at 405 m to make the sperm antigen.

Polyvinyl micro plates (Falcon 3912 Microtest III, Becton Dickinson and Co., Oxnard, CA. U.S.A.) were coated with 50 μl/well sperm antigen overnight at 4 °C. The fluid was then removed and the antigen fixed to the plates, with the addition of 50 μl/well 0.1% acetone in PBS-Tween 20 (PBS + 0.05% Tween 20, Sigma) for 5 minutes. The plates were then washed three times in PBS-Tween 20 and incubated with 100 μl/well 1% bovine serum albumin (BSA, Sigma) in PBS for 1 hour at 37 °C. Excess BSA was removed by washing three times with PBS-Tween 20 and 50 μl/well of the test sera were added to each well in pairs. The plates were then incubated for 2 hours at 37 °C and washed with PBS-Tween 20. Biotin labelled anti-goat IgG 50 μl/well (Sigma, 1:2000) was added and the plates incubated for 30 minutes at 37 °C in a humid chamber. The plates were then washed and incubated with 50 μl/well Streptoavidin peroxidase (Sigma, 1:400) for 30 minutes at 37 °C, then washed three times with PBS-Tween 20 before adding 50 μl/well of substrate for 20 minutes at room temperature in the dark. The substrate was a mixture of equal volumes of ABTS (2,2'-azino-di-(3-ethyl-benzothiazoline sulfornate)) and Hydrogen peroxide labelled solution A and B, respectively (KPL). The colour intensity was read at 405 m using a micro ELISA reader Model B1-45 (Multiskan plus). The mean OD of negative samples was calculated and any sample with double the mean OD of negative samples was taken as positive (FAYEMI, 1988).

Data was subjected to Analysis of variance (ANOVA), Chi-square and simple correlation (LOVEDAY, 1980).

Results

The age distribution of 235 goats tested for sperm antibodies is shown in Table 1. Of the 164 females and 71 males tested 25 (15.2%) and 7 (9.8%) were positive for sperm antibody, respectively. The proportion of goats positive for the sperm antibody increased significantly with age ($P<0.001$). However, the proportion of positive results was not significantly associated with sex ($P>0.05$). Also, there was no significant interaction between age and sex ($P>0.05$).

The distribution of number of parity in female goats tested for sperm antibodies is shown in Table 2. The proportion of female goats that were positive for sperm antibodies increased significantly with parity ($P<0.001$).

Table 1. Distribution of age in male and female goats tested for sperm antibody

	Age (months)						
	0-6	7-12	13-18	19-24	25-30	>30	Total
Males tested	2	6	13	23	15	12	71
No. positive	0	0	1	2	1	3	7
No. negative	2	6	12	21	14	9	64
% positive	0	0	7.6	8.6	6.7	25	9.8
Females tested	1	4	28	47	11	73	164
No. positive	0	0	1	4	1	19	25
No. negative	1	4	27	43	10	54	139
% positive	0	0	3.6	8.6	9.1	26	15.5
Total	3	10	41	70	26	85	235

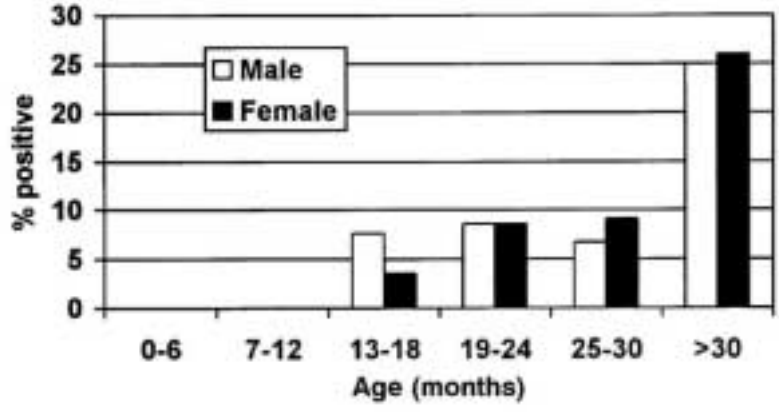

Fig. 1. Proportion of male and female goats positive for sperm antibody in relation to age

Table 2. Distribution of parity number in female goats tested for sperm antibody

	Parity					
	0	1	2	3	>3	Total
Females tested	38	34	28	38	26	164
No. positive	2	2	2	7	12	25
No. negative	36	32	26	31	14	139
% positive	5.2	5.9	7.1	18.4	46.2	15.2

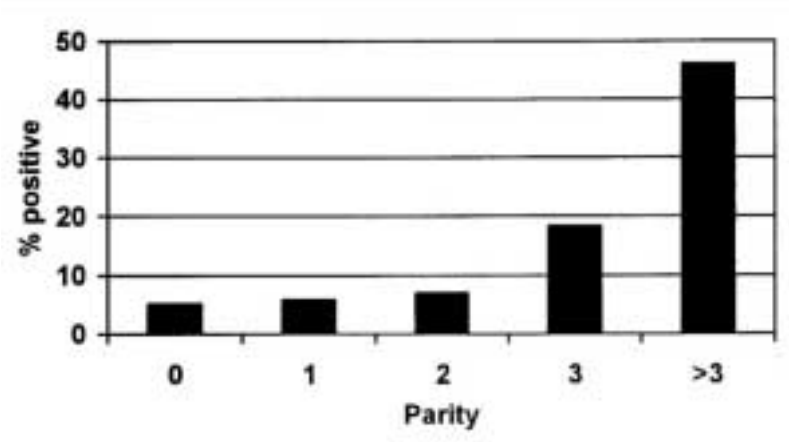

Fig. 2. Proportion of female goats positive for sperm antibody in relation to parity

Discussion

It is generally recognised that the presence of sperm antibodies may lead to involuntary infertility (BEER and NEAVES, 1978; MUMFORD, 1979). Sperm antibodies in the blood sera and/or reproductive tract fluids are known to interfere with the reproductive process in man (MATHUR et al., 1984) and in domestic animals (MENGE, 1980; AWADI et al., 1984).

The lower percentage of male autoimmunity compared to female isoimmunity observed in this study were similar to those reported by FAYEMI (1988) in swine. However, this does not agree with the findings of MATHUR et al. (1981) who observed higher percentage of male

autoimmunity than female isoimmunity to sperms in humans. This may be due to the fact that, in animals, fewer males are kept for long in herds for economic reasons, and a sizeable number of them fall within the 0-24-month age group (Table 1.), a situation that does not occur in humans.

Normally, male animals do not produce antibodies to their own sperm because of the blood-testis-barrier. Infringement of this barrier through injuries or infections could lead to autoimmunity (MATHUR et al., 1985), although there was no recorded evidence of testicular injury or infection in the animals tested. However, this cannot be ruled out, in view of the fact that the animals were exposed to a variety of predisposing factors such as, trauma and inclement weather with extreme temperature and infections, which could affect the integrity of the basement membrane. Enzootic trypanosomiasis infection is also known to affect the testes (IKEDE, 1983). Autoimmunity might also arise as a result of prolonged overuse of the few males usually kept for breeding purposes, due to frequent ejaculation over an extended period of time, as observed with rabbit sperm donors (WITCHER et al., 1987). This situation may be compounded when there is leakage of sperm antigens from the male tract as a result of injury to or infection of the testes.

Isoimmunity to sperm in women has been associated with sperm autoimmunity in their husbands (MATHUR et al., 1985). This suggests that a few autoimmune male goats unknowingly kept for breeding purposes could easily be a source of female isoimmunity for the whole herd.

The prevalence of sperm antibody in relation to age distribution observed in this study is consistent with the reports of FAYEMI (1988), in swine. The significant influence of age on the prevalence of sperm antibody may be attributable to the increasing chances of sperm antigen leakage from the male reproductive system, as well as to the continuous exposure of the female reproductive tract to millions of sperm antigens with advancing age. Injuries to and infections of the reproductive system are common contributing factors in both sexes (BEER and NEAVES, 1978).

Although, isoimmunity has not been reported to be dependent on parity, the proportion of females positive for sperm antibody increased significantly with the parity number in this study. This might be associated with the fact that each succeeding pregnancy and parturition invariably subjects the reproductive tract to greater chances of injuries and infections, thus making the affected animals more vulnerable to isoimmunity.

In conclusion, the study indicated that both, autoimmunity and isoimmunity to caprine sperm exists in the herds of goats tested, and that

there was a significant influence of age and parity (in the case of females) on the seroprevalence of the sperm antibody.

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SAŽETAK

Serumi 235 koza iz Ibadana (Nigeria) i njegove okolice su nasumično prikupljeni i istraženi na prisutnost protutijela za spermije pomoću ELISA postupka. Na protutijela za spermije bilo je pozitivno 25 (15,2%) od 164 pretraženih ženki i 7 (9,8%) od 71 pretraženih mužjaka. Pristnost protutijela za spermije u krvi je bilo značajno povezano s dobi ($P<0,001$) i brojem porođaja ($P<0,001$). Spol istraženih životinja nije imao značajnu vezu ($P>0,05$) s prisutnosti ovih protutijela u krvi. Istraživanje pokazuje proširenost protutijela za spermije u pretraženim stadima.

Ključne riječi: serološko istraživanje, spermiji, protutijela, koza