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THE PHYSICAL CHARACTERISTICS OF OVINE-CAPRINE EJACULATE MIXTURES AND THE SURVIVAL OF THEIR SPERMATOZOA IN COW'S MILK EXTENDER IN THE HUMID TROPICS.

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Target Audience: Animal scientists, sheep and goat breeders/farmers and veterinarians.

ABSTRACT

Semen samples were obtained twice weekly by electrical stimulation from four bucks and two rams for three months, mixed in equal volumes and evaluated for physical characteristics. Proportions of semen from each species and the ejaculate mixtures were diluted in known volumes of skim milk and the survival of spermatozoa evaluated at 0,1,2 and 4 hours at room temperature. While the seminal characteristics of the ejaculate mixtures were good and highly comparable to those of the species, there was a progressive decline in sperm motility in buck semen (59.5±2.95 to 31.89 2.37%), ram semen (57.00±3.75 to 26.25 ±3.50%) and the mixtures (67.00±1.09 to 34.00±2.19%) from 0h to 4 h in cow's milk extender. Sperm motility in cow's milk extender was similar between the species as well as between the species and mixtures at all times of sampling except at 0h when there was a significant difference ($p<0.05$) between the ejaculate mixtures and ram semen, but not buck semen. These results may be indicative of the possibility of using the same media for sheep and goat semen in the tropics. The results also show that cow's milk may be a good extender for sheep and goat semen in our environment especially when fortified with energy and antibiotics.

Key words: Ejaculate mixtures; semen characteristics; sperm motility; milk extender; buck; ram.

DESCRIPTION OF PROBLEM

Even though Artificial Insemination (AI) is the most important single technique ever devised for the genetic improvement of animals (1), AI in the humid tropics is still only at experimental stages especially in respect of the small ruminants. In Nigeria, sheep and goats with an estimated population of 56.50m (2) appear to be the most important agricultural animals as is the case in most developing countries. Production is usually mainly for self sufficiency in subsistence farming as farmers cannot afford cattle.

The genetic improvement of sheep and goats in the humid tropics will however, require not only a knowledge of their semen physiology as different species but

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also when their ejaculates are mixed. This would provide information as to whether or not both species might require similar or different media when contemplating suitable media for semen extension. Moreover, a knowledge of the motility and survival rate of the spermatozoa of both species separately and when mixed in a relatively cheap and easily available extender like fresh cow's milk could provide good grounds for the development of AI programmes for these species in the humid tropics as well as add knowledge to the parameters to be considered in the evaluation of both ram and buck fertility in this environment. Also, even though there are reports on the ejaculate and seminal plasma characteristics of rams (5,6) and bucks (7,8,9) of tropical origin, bordering on semen physical and biochemical characteristics as well as spermatozoa dimensions, similar reports on the mixtures of the semen of these species are completely lacking. The survival rates of the spermatozoa of these species in cow's milk and perhaps other extenders are also completely unknown. We therefore undertook this study to provide preliminary information on the physical attributes of buck/ram ejaculate mixtures and the survival of their spermatozoa as different species and as a mixtures in cow's milk extender.

MATERIALS AND METHODS

Animals and management

Four adult West African Dwarf (WAD) bucks, 24 -36 months in age and 15-22 kg in weight and two Yankasa rams, 32 and 36 months in age weighing 29.50 and 33.00 kg were used for this study. The humid tropical climate of Ibadan where the study was conducted had already been described (10). All the animals had been used previously for breeding with satisfactory results. Each species was housed separately in a standard goat pen at the Teaching and Research Farm of the University of Ibadan. They were fed a maize-based diet supplemented with forage *ad libitum* with cool clean water applied always.

Semen collection

Semen was collected twice weekly (Tuesdays and Saturdays) between 0800 and 0900h for three months by electrical stimulation.

Semen evaluation

Equal volumes of buck and ram semen were pipetted and pooled immediately after collection and evaluated (as buck semen, ram semen and mixtures) for some physical characteristics of semen by standard methods used in our laboratory and elsewhere (7,9).

Semen extension

Fresh cow's milk was obtained at the dairy section of the Teaching and Research Farm of the University of Ibadan, heated to 95°C for about 10min and cooled to room temperature. It was when dispensed into sterile plastic tubes and centrifuged at 3,000 rpm for 15min. The cream was separated and discarded. A drop of semen from each species and from a mixture of undiluted raw semen of both species was diluted in 0.4ml of the skimmed milk and the motility of the spermatozoa evaluated, at 0,1,2, and 4 hours as already described.

Statistical analysis

The data were analysed using the student's 't' test and the analysis of variance(11),

the method of least significant difference (LSD) was used to determine significant differences between means.

RESULTS AND DISCUSSION

A summary of the physical characteristics of buck and ram ejaculate mixtures is presented in Table 1, while Table 2 shows the survival rates of spermatozoa from buck and ram ejaculates and mixture of their ejaculates in cow's milk extender. Sperm progressive motility was good and characteristic of the spermatozoa of both species separately in their respective seminal plasma. Live sperm and morphologically normal sperm were excellent, while sperm abnormalities were all low and within acceptable limits.

Table 1. The physical characteristics of buck / ram ejaculate mixtures.

Seminal characteristics	means±s.e.m.
Sperm motility (%)	67.00 ± 1.09
Live sperm (%)	94.00 ± 1.30
Dead sperm (%)	6.00 ± 1.30
Normal morphology (%)	90.88 ± 4.29
Abnormal morphology:	
Detached normal heads (%)	7.62 ± 5.17
Simple bent tails(%)	0.50 ± 0.00
Coiled tails (%)	0.50 ± 00
Proximal cytoplasmic droplet (%)	0.50 ± 0.00
Distal cytoplasmic droplet (%)	0.00 ± 0.00
Pear shaped heads (%)	0.00 ± 0.00
Total abnormalities (%)	9.12 ± 4.29

s.e.m: Standard error of mean

The physical characteristics of buck and ram ejaculate mixtures obtained in this study are normal and highly comparable to values obtained for these species and breeds separately by Bitto *et al.* (9). This similarity suggests that the two species may be similar in their responses to chilling/freezing of the semen during storage for artificial insemination as earlier indicated (9). The proportion of live sperm in the mixtures obtained in this study however appears to be higher than values obtained for the respective species and breeds by the same authors (9). It would appear therefore that mixing the ejaculates of bucks and rams in our environment may improve sperm survival, probably by enhancing the buffering capacity of the semen from a combination of the biochemical properties of the seminal plasma of the individual species.

With regard to sperm survival in cow's milk, besides the significant difference ($P<0.05$) between the ejaculate mixtures and ram semen in the initial motility of spermatozoa in cow's milk, there were only similarities between all the ejaculates (ram, buck and mixtures) at the respective times of sampling. There was a progressive decline in sperm progressive motility from 0 to 4 h in both buck and ram ejaculates as well as their mixtures. While the drops in sperm motility from 0 to 1h and 2 to 4 h were not significant, the drops in buck sperm motility from 0

to 2 h and 0 to 4 h were significant ($p<0.05$). A similar trend occurred in ram semen with only similarities in the motility of spermatozoa in milk between 0 and 1h, 1 and 2 h and 2 and 4 h, while significant declines ($P<0.05$) in sperm motility occurred between 0 and 2 h and 0 and 4 h. Sperm motility in the ejaculate mixture, during extension in cow's milk differed significantly ($P<0.05$) between 0 and 1, 0 and 2 h and 0 and 4 h.

Table 2: The survival rates of spermatozoa from buck, ram and buck/ram semen in cow's milk extender at 0, 1, 2 and 4 hours at room temperature (means s.e.m.)

Time (Hours)	sperm motility (%)			Between mixtures and buck	Between mixtures and ram	Between buck and ram
	Buck	Ram	mixtures			
0	59.502 ± 95^{ab}	57.00 ± 3.75^{a}	67.00 ± 1.09^{b}	ns	$p<0.05$	ns
1	45.00±496	50.00±4.96	50.00±2.82	ns	ns	ns
2	38.23±3.45	41.25±2.75	42.00±1.78	ns	ns	ns
4	31.89±2.37	26.25±3.50	38.00±2.19	ns	ns	ns

^{ab}: Values bearing different superscripts in the same row are significantly different ($P<0.05$)

s.e.m: Standard error of mean.

The superiority of the ejaculate mixtures over ram semen in the initial motility of spermatozoa in cow's milk may also be due to a combination of seminal plasma characteristics of both species necessary for sperm survival and motility. The good initial motility of the spermatozoa of bucks and ram semen and their mixtures in cow's milk extender agrees with the earlier reports of Almquist and Wickersham (12) and Emmens and Robin (13) who found cow's milk preparations to compare favourably with conventional citrate media for ram spermatozoa. The progressive decrease in sperm motility from 0 to 4 h at room temperature might be due to a number of factors including a decrease in or depletion of energy sources. Salisbury *et. al.* (14) reported that spermatozoa utilize energy to maintain motility. However, milk is low in hexose although traces of glucose are present. It also contains lecithin and fat decomposition products and is rich in lactose. Spermatozoa cannot utilize lactose, but can metabolise the other substances aerobically and glucose anerobically. Since no additional source of glucose was added to the milk in the present study contrary to the recommendation of Foote (1) for extenders, it is possible that both energy source and energy utilization could have negatively influenced sperm motility. Other factors probably responsible for the decline in motility could be temperature and bacterial growth. The reduction in temperature of the semen mixture in milk could be responsible for the gradual decline in motility since a reduction in temperature reduces the metabolism of sperm cells with a resulting decline in motility. Also, the non addition of antibiotic to the milk in this study may have favoured bacterial activity at room temperature, as it has been shown that bovine semen in extenders containing antibiotics can be

stored for at least 24 h at 28°C (room temperature).

CONCLUSION AND APPLICATIONS

These results demonstrate the feasibility of the use of the same media for the extension and storage of sheep and goat semen for AI in the tropics. The work also shows that cow's milk might be a suitable extender for sheep and goat semen in our environment but may require some glucose ands antibiotics to be effective.

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