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Effect of honey on West African dwarf buck semen stored under ambient conditions in sodium citrate-glycine and Illinois variable temperature buffers

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ABSTRACT: Two buffers, Illinois Variable Temperature (IVT) and sodium citrate-glycine, each with four levels of honey (0 – 3% by volume) were used to study progressive spermatozoa motility under ambient conditions. It was generally observed that mean percentage motility decreased as the storage period increased. The motility obtained at 1 hr 50 min with 1% and 2% honey and at 2 hr 20 min with 2% honey in sodium attracts glycine used for insemination. Likewise, the result in IVT diluent at 3 hr 15 min with 1% and 2% honey and 3 hr 50 min with 1% honey could also produce good results.

Key Words: Honey; Spermatozoa motility; Insemination.

Introduction

Previous studies have shown that the animal protein intake of the average man in Nigeria is far below the recommended level of 65g per head per day (FAO, 1979). Low productivity due to lack of selection for higher production, poor management practices and unfavourable climatic conditions have been incriminated as part of the factors responsible for the limited supply of animal protein in the tropics (Loosli and Van Blake, 1973).

In the past, efforts made to improve the livestock industry in the tropical and sub-tropical regions by national and international organizations have been mainly concentrated on cattle production (French, 1966; Mackenzie, 1967; Gall, 1980) such that the potential of the small ruminants, particularly goats, were overlooked.

In recent times, an aspect of the physiology of reproduction being applied to farm animals include semen preservation, artificial insemination and oestrous synchronization. These techniques have been used to advantage to increase the efficiency of controlled mating in animals in various parts of the world. However, this practice has not become common in Nigeria due to certain problems which need to be addressed. The main problem is maintaining the quality of semen during storage and transportation, i.e. maintenance of motility and fertilizing capacity of spermatozoa. The present study is aimed at assessing

the capacity of two buffers containing various levels of inclusion of honey in the storage of West African Dwarf (WAD) buck semen under ambient temperature.

Materials and Methods

Two 24- and 48-months old West African Dwarf bucks were used. Both were housed at the Teaching and Research Farm of the University of Ibadan. They were fed *ad libitum* on normal maintenance ration supplemented with cassava peeling with adequate water supply.

Preliminary collection and evaluation of semen characteristics was carried out. The semen samples were collected by electro-ejaculation and evaluation was done on ejaculate colour, volume, mass, activity, initial sperm motility, concentration of sperm cells, live percentages and morphology of sperm cells.

Two types of buffers were used for semen extension: (i) Sodium Citrate Glycine buffer and (ii) Illinois Variable Temperature (IVT) buffer. The selection of these two was based on results obtained from other studies which showed that sperm viability in them is high. Into these buffers were added egg yolk at a constant level and honey at various levels of 0%, 1%, 2% and 3%. the pH of the medium was adjusted to between 6.8 and 7.0 using a pH paper indicator. The composition of the extender is shown in Table 1.

To extend the semen, 0.5 ml of extender was dispensed into small vials using a 1 ml pipette. A 3 ml syringe was used to introduce 0.1 ml of semen into the extender which was kept at a temperature of 37^{o}C. The extended semen was than checked periodically for motility at intervals of 30 min.

The means and standard errors were calculated. The progressive motility was also subjected to 2-way analysis of variance (ANOVA) and Students' t-test (Steel and Torrie, 1960).

Results

The results for semen characteristics and sperm morphology are presented in Tables 2, 3 and 4. The means and standard error of the progressive sperm motility of extended buck semen are also summarised in Table 5.

The percentage progressive sperm motility in semen extended with sodium citrate glycine buffer with zero level of honey decreased from 70.0 to 10.0 at 0 hr to 0.0 at 5 hr at room temperature while that of semen extended in Illinois Variable Temperature buffer (IVT) with zero level of honey significantly reduced the drop in motility with time ($P < 0.05$) (Table 2). However, this effect was lost as the level of honey was increased.

Discussion

The results obtained for the semen characteristics indicate that the semen can be used for artificial insemination. The incidence of bent tails and detached normal heads was higher than other abnormalities probably as a result of handling during staining as well as forceful ejaculation by the use of electro-ejaculator.

Generally, motility and viability of the spermatozoa decreased with increasing period of preservation as shown in Table 5. This is in agreement with the previous reports in the literature (Lovelock, 1953; Normal et al., 1962; Underwood et al., 1982; Udoh, 1987). This could be as a result of rapid energy depletion due to motility (Mann, 1964). The production of lactic acid due to metabolic activity of the sperm cells could also contribute to the osmotic pressure and pH change of the extended semen. This, in turn, affects sperm viability and motility (Lovelock, 1953; Van de Berg and Rose, 1959). Exposure to light could also result in gradual loss of motility due to photo-oxidation.

Table 1: Composition of buffers and extenders.

Ingredients	Buffers	
	Sodium Citrate Glycine	Illinois Variable Temperature (IVT)
Sodium citrate (g)	10.0	20.3
Glycine (g)	3.0	–
Sulphanilamide (g)	–	3.0
Glucose (g)	–	3.0
Sodium bicarbonate (g)	–	2.1
Citric acid (g)	–	1.0
Potassium chloride (g)	–	0.4
Boiled distilled water (ml)	1,000	1,000

Extenders

	0%	1%	2%	3%
Buffer (ml/100ml)	90	89	88	87
Egg yolk (ml/100ml)	10	10	10	10
Honey (ml/100ml)	0	1	2	3
Penicillin (i.u./100ml)	10^5	10^5	10^5	10^5
Streptomycin (ml/100ml)	100	100	100	100

Table 2: Semen characteristics of the West African Dwarf Buck.

Characteristics	Range	Mean ± SEM
Colour	Milky white	
Ejaculate volume (ml)	0.02 – 0.80	0.49 ± 0.33
Mass activity (%)	70.0 – 99.0	67.63 ± 1.21
Motility (initial) (%)	80.0 – 98.0	94.18 ± 0.84
Average live sperm (%)	93.3 – 99.4	97.62 ± 0.34
Concentration (cell/ml x 10^9)	1.20 – 4.50	2.33 ± 0.19
Total sperm number (x 10^9)	0.35 – 2.55	1.13 ± 0.11

Table 3: Range and mean values of sperm morphology.

Morphology	Range	Mean ± SEM
Simple Bent Tail	3 – 30	12.50 ± 1.96
Tail coiled below head	0 – 20	9.54 ± 1.35
Tail coiled round head	0 – 8	3.07 ± 0.59
Distal cytoplasmic droplet	0 – 8	2.2 ± 0.72
Proximal cytoplasmic droplet	0 – 4	2.1 ± 0.25
Detached normal head	4 – 24	11.75 ± 1.35
Percentage abnormal cells	3.50 – 17.75	9.17 ± 0.97
Percentage normal cells	62.25 – 96.50	90.82 ± 0.98

Table 4: Summary of correlation coefficients for buck semen characteristics.

Volumetric Characteristics	Concentration	Total Sperm Number (c)
Volume (ml)	– 0.11	+0.51*
Concentration (c/ml)	–	+0.78**
Percentile characteristics	Motility (%)	Live spermatozoa (%)
Mass activity (%)	– 0.71**	+0.25
Motility (%)	–	+0.55*

*P < 0.05; **P < 0.01

The Illinois Variable Temperature (IVT) buffer, when compared to sodium citrate glycine buffer, proved to be a better diluent probably as a result of the presence of glucose which served as a source of energy, and the presence of bicarbonate which has a high buffering capacity (Kampsohmidt et al., 1951, 1953). The presence of potassium and citric acid may also be a contributory factor in enhancing the capacity of IVT.

The presence of glycine in sodium citrate glycine diluent may be a major factor in lowering the viability of spermatozoa, when compared to IVT. Other workers have reported that incorporation of glycine into diluents should be accompanied by other radical changes like the addition of glycerol (Stower and Bud Hasaein, 1957), or reduction in egg yolk.

Table 5: Mean and SEM of progressive motility of buck semen in sodium citrate glycine and IVT buffers at various levels of honey inclusion and a temperature of 27°C.

Time (hr)	Honey Inclusion Level			
	0%	1%	2%	3%
	(1) Sodium Citrate Glycine Buffer			
0	70.0 ± 10.0	65.0 ± 5.0	72.5 ± 7.5	70.0 ± 5.0
1 hr 50 min	43.0 ± 17.0	50.0 ± 12.0	57.5 ± 2.5	37.5 ± 7.5
2 hr 20 min	33.5 ± 11.5	43.5 ± 13.5	52.5 ± 2.5	33.0 ± 5.0
2 hr 50 min	14.0 ± 4.0	38.0 ± 8.2	40.0 ± 5.0	26.0 ± 4.0
3 hr 20 min	4.0 ± 2.0	25.0 ± 5.0	35.0 ± 5.0	10.0 ± 0.0
4 hr	2.0 ± 0.0	11.0 ± 1.0	31.5 ± 3.5	7.0 ± 1.0
5 hr	0.0	4.0 ± 0.0	18.0 ± 2.0	3.0 ± 1.0
	(**2) Illinois Variable Temperature Buffer**			
0	77.5 ± 7.5	77.5 ± 7.5	80.0 ± 5.1	77.5 ± 7.5
1 hr 40 min	60.0 ± 5.0	70.5 ± 7.5	68.5 ± 6.5	62.0 ± 8.0
2 hr 15 min	57.0 ± 3.0	65.0 ± 10.0	65.0 ± 5.0	57.5 ± 0.5
2 hr 45 min	55.0 ± 5.0	65.0 ± 11.0	65.0 ± 5.0	51.5 ± 11.5
3 hr 15 min	47.5 ± 3.5	54.0 ± 6.0	44.0 ± 4.0	35.0 ± 5.0
4 hr 20 min	38.0 ± 4.0	47.5 ± 2.5	41.0 ± 4.0	25.0 ± 5.0
5 hr	27.0 ± 3.0	33.5 ± 1.5	27.5 ± 2.5	12.0 ± 3.0

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