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Some Biochemical Characteristics of Spermatozoa and Seminal Plasma of Pubertal West African Dwarf Bucks in Their Native Humid Tropical Environment

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Abstract: Due to the lack of information on biochemical characteristics of spermatozoa and seminal plasma of pubertal bucks in tropical breeds of goats in general and the WAD breed in particular, 6 kid bucks were randomly selected at birth, weaned between 35 and 40 days of life and monitored for puberty. At the attainment of puberty, they were ejaculated twice weekly for 3 months. Semen was separated into spermatozoa and seminal plasma and analyzed for total protein, glucose, cholesterol phospholipids, Lactate Dehydrogenase (LDH) and Acetylcholinesterase activity (AChE). The results in comparison with values obtained from the semen of adult bucks of the same breed under similar conditions of management showed similarities between the age groups ($p>0.05$) in spermatozoal total protein, phospholipids, LDH and AChE, while spermatozoal glucose and cholesterol were depressed ($p<0.001$) in the adult buck. Seminal plasma cholesterol and LDH were likewise similar ($p>0.05$) between pubertal and adult bucks while pubertal buck seminal plasma was inferior to that of the adult in total protein ($p<0.001$), glucose ($p<0.001$), phospholipids ($p<0.05$) and AChE ($p<0.001$). Linear correlations between the biochemical characteristics of pubertal buck spermatozoa and seminal plasma showed significant positive relationships between spermatozoal total protein and cholesterol ($r = 0.36$; $p<0.05$), spermatozoal glucose and LDH ($r = 0.42$; $p<0.05$) and spermatozoal cholesterol with AChE ($r = 00.50$; $P<0.01$). There was a significant negative correlation between spermatozoal glucose and AChE ($r = -0.35$; $p<0.05$). In seminal plasma, total protein was significantly positively related to phospholipids ($r = 0.55$;$p<0.01$) while glucose was significantly positively related to LDH ($r = 0.51$;$p<0.05$). Cholesterol was positively related to LDH ($r = 0.35$; $p<0.05$) but negatively related to LDH ($r = -0.52$, $p<0.05$). These results suggest that differences may exist in the responses of pubertal and adult buck semen to the same extension and storage media in this environment.

Key words: Bucks, pubertal, semen, biochemical, tropics

INTRODUCTION

Even though the West African Dwarf genotype is the most popular and most prolific among the various breeds of goats in the West African sub-region, it has been reported to have a poor potential for growth and milk production (Wilson, 1989; Gall *et al*., 1992) and thus requires improvement. Programmes aimed at improving this breed in its native environment also have limitations especially as Artificial Insemination (AI) is still at experimental stages in many countries in the humid tropics including Nigeria. One of the greatest constraints to the success of (AI in goats in the tropics is the lack of suitable media for semen extension and storage. Egg yolk buffers are still used to extend goat semen despite the many decades of the knowledge that goat semen contains an Egg Yolk Coagulating Enzyme (EYC) which in the presence of calcium causes the release of lysolecithins in quantities that are toxic to spermatozoa (Roy, 1957; Fakuhaara and Nishiokawa, 1973; Corteel, 1992).

The development of extenders for goat semen in the tropics will be dependent on knowledge of the biochemical characteristics of both spermatozoa and seminal plasma in goat ejaculates from puberty to adulthood. Besides the report of Bitto *et al*. (2000) on some biochemical characteristics of buck and ram semen in their native tropical environment, such report are scarce. Knowledge of the biochemical characteristics of the semen of pubertal bucks in the tropics is of great importance as in the absence of planned breeding programmes, pubertal bucks have been known to successfully serve does on range as these animals scavenge for food. Bongso *et al*. (1982) reported that bucks exhibit sexual aggressiveness, penile development

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and permit intromission with good libido under tropical conditions at a very young age. Bitto and Egbunike (2006) also showed sperm production rates, gonadal and extragonadal sperm reserves in bucks of the breed to be sufficient at puberty for use in AI and/or natural service in all seasons of the year. Knowledge of the biochemical characteristics of pubertal buck semen will therefore be a useful tool in the early selection of sires for planned breeding programmes as libido and reaction time of older bucks decline with increasing age (Butswat and Zaharaddeen, 1988). We thus proposed this study to provide information on the total protein, glucose, cholesterol, phospholipids, lactate dehydrogenase and acetylcholinesterase concentrations in spermatozoa and seminal plasma of pubertal WAD bucks born and raised in their native humid tropical environment.

MATERIALS AND METHODS

Animals and management: Six healthy pubertal WAD bucks between 148-156 days in age and weighing between 9.10 and 12.00 kg were used for this study. The animals were obtained as kid bucks born to does at the Physiology unit of the Teaching and Research Farm of the University of Ibadan, Nigeria where the experiment was conducted. The climate and seasons of Ibadan had earlier been described by Egbunike and Steinbach (Egbunike and Steinbach, 1979).

The kid bucks were weaned between 35 and 40 days of age and were housed together in a standard goat pen with concrete floor. They were fed a maize based concentrate ration supplemented with forage *ad libitum* and had access to salt lick and cool clean drinking water and always.

Puberty: The preputial smear technique described by Vandenberg (1971) in male golden hamsters and Egbunike (1979) in boars was used to determine the onset of puberty. The cotton buds used for the preputial smears were the bel de luxe Hartman Ltd. brand.

Semen collection: As soon as the bucks attained puberty, they were ejaculated twice weekly (on Tuesdays and Saturdays) between 0800 and 0900 hours by electroejaculation as already reported (Bitto *et al*., 1988) for 3 months. An electroejaculation probe was specially constructed to fit the relatively small rectum of the pubertal buck. Even though the primary objective of this study was to evaluate the biochemical characteristics of the pubertal WAD buck we similarly harvested semen from two health adult bucks (proven sires) raised under similar conditions of management for a comparison.

Biochemical analysis: Freshly harvested semen samples were immediately evaluated for sperm concentration haemocytometrically as earlier reported (Bitto *et al*., 1988) to provide for a calculation of the concentrations of spermatozoal biochemical characteristics 10^{-6} cells.

The semen samples were immediately after the dilution for concentration, configured at 3000 g at room temperature for 5 min after which the seminal plasma was aspirated and stored at -20°C until analyzed. The pellets (cells) were immediately re-suspended in 1.0 mL. of deionised water and stored under the same conditions as the seminal plasma pending analyses.

Both spermatozoa and seminal plasma were analyzed for total protein, glucose, cholesterol and phospholipids by methods outlined in the Boehringer (Germany) Diagnostica (1979). Lactate dehydrogenase (EC I. I. I. 2.7) concentration was determined by the rapid optimized method of Sigma Chemical Company (1979); while the colorimetric method of Ellman *et al*. (1961) was used in the determination of acetylcholinesterase activity in spermatozoa and seminal plasma.

Statistical analysis: Data were subjected to the student t-test between pubertal and adult buck and linear correlation analysis between biochemical characteristics in pubertal bucks semen (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Where as spermatozoal total protein, phospholipids, LDH and AChE concentrations were similar between pubertal and adult bucks, glucose and cholesterol levels were highly significantly depressed ($p<0.01$) in adult bucks (Table 1). In the seminal plasma, cholesterol and LDH concentrations were similar ($p>0.05$) between the 2 age groups, while pubertal buck seminal plasma had significantly lower ($p<0.01$) levels of total protein, glucose, AChE and phospholipids ($p<0.05$).

While spermatozoal protein values obtained in the present study in both pubertal and adult bucks were comparable to corresponding values reported for adult animals of the same breed in an earlier report (Bitto *et al*., 2000). The level of cholesterol in pubertal buck spermatozoa in the present study (being higher than that of the adult) was much higher than values earlier reported in adult bucks of the same breed (Bitto *et al*., 2000). This result suggests the involvement of cholesterol in the process of sperm maturation in agreement with the reports of several workers who generally agreed that a decrease in cholesterol content of spermatozoa occurs during maturation in the epididymis (Scott *et al*., 1963; Quinn and White, 1967; Scott and Dawson, 1968). Even

Table 1: A comparison of biochemical characteristics of pubertal and adult WAD buck semen (means+sem)

Parameter	Pubertal buck	Adult buck	Level of significance
Spermatozoa			
(a) Total protein (g 10^{-6} cells)	1.43+0.09	1.20+0.06	ns
(b) Glucose (mg 10^{-6} cells)	117.0+22.95	51.02+2.40	p<0.001
(c) Cholesterol (mg 10^{-6} cells)	367.0+31.01	226.88+20.19	p<0.001
(d) Phospholipids (μg 10^{-6} cells)	229.33+20.42	208.63+20.42	ns
(e) Lacate dehydrogenase (μ mole/min/10^6 cells)	54.28+9.62	75.52+4.16	ns
(f) Acetylcholinesteras (μ mole/min/10^6 cells)	48.40+7.73	46.89+3.38	ns
Seminal plasma			
(a) Total Protein (g L^{-1})	7.70+0.64	14.87+1.62	p<0.001
(b) Glucose (g L^{-1})	642.94+6.36	699.23+3.81	p<0.001
(c) Cholesterol (mg L^{-1})	2249.64+131.07	2276.97+294.44	Ns
(d) Phospholipids (μg L^{-1})	1746.0+167.58	3518.00+913.83	p<0.05
(e) Lactate dhydrogenase (μmole/min/L)	415.70+67.58	669.12+145.96	Ns
(f) Accetylcholinestyerase (μ mole/min/L)	366.25+39.50	658.25+39.31	p<0.001

sem = standard error of mean, ns = not significant (p>0.05)

Table 2: Relationships between some biochemical characteristics of semen of the pubertal WARD buck

	6	5	4	3	2	1
Spermatozoa						
1. Total protein	-0.07	-0.06	-0.30	0.36*	-0.26	
2. Glucose	-0.35*	0.42*	-0.09	-0.06	-	
3. Cholesterol	0.50*	-0.07	-0.32	-	-	
4. Phospholipids	0.17	0.09	-			
5. LDH	-					
6. AchE						
Seminal plasma						
1. Total protein	0.27	-0.01	-0.55**	0.04	-0.29	
2. Glucose	-0.16	0.51*	-0.01	-0.08	-	
3. Cholesterol	0.52*	-0.35	-0.21	-	-	
4. Phospholipids	0.8	0.219	-			
5. LDH-0.17	-	-				
6. AchE	-					

* = p<0.05; + = p<0.01

though we evaluated cholesterol levels in ejaculated spermatozoa, the much higher cholesterol levels in pubertal bucks than in adult bucks suggests higher proportions of mature spermatozoa in the semen of adult bucks than in pubertal bucks

Phospholipid levels in spermatozoa being similar between the age groups (p>0.05) were comparable to values reported in adult bucks by Bitto *et al.* (2000), thus suggesting a stability of this lipid fraction in spermatozoa of goats from puberty to adulthood. One would therefore expect stability in sperm motility and respiration (Mann, 1964) as well as in the ability of spermatozoa to withstand certain stress conditions (Marin, 1963).

Even though there were no reports in the literature with which we could directly compare spermatozoal concentrations of glucose, LDH and AChE, the significantly higher level of glucose in pubertal buck spermatozoa than in the adult suggests the availability of more glucose to be metabolized by spermatozoa for maintaining motility and viability at puberty than at adulthood. As fructose is a more important sugar (Evans and Maxwell, 1987) in the metabolism of spermatozoa, it does appear that as the buck matures in age, glucose levels in spermatozoa drop with a corresponding increase in fructose levels. This is a subject for further investigations. It is however, known that under anaerobic conditions, spermatozoa break down glucose, fructose or mannose to lactic acid (Garner and Hafez, 1980). Carbon dioxide (CO_2) and water are also produced (Evans and Maxwell, 1987). Whether the increase in the volume of semen and concentration of spermatozoa that accompany adulthood would produce an increased concentration of CO_2 which would inhibit sperm motility and as such less glucose would become available for metabolism with age, or whether fructose, begin a main component of acrosomal carbohydrate increases in concentration with age for both structural and metabolic uses becoming the principal seminal sugar can not be explained from the results of the present study. It is clear from our results however, that in the formulation of extenders for the semen of these animals less glucose would need to be added to diluents for pubertal semen than for adult semen.

The similarities between the two age groups in both spermatozoal and seminal plasma LDH might imply similarities between the groups in metabolic processes supplying energy for sperm motility and survival in agreement with the reports of Storey and Kayne (1977), Gerez de Burgos *et al.* (1978) and Burgos *et al.* (1979).

With regard to seminal plasma, the significantly higher total protein values in adult bucks than in pubertal bucks suggests a higher secretory capacity of the adult buck vesicular glands and other accessory sex organs than in the pubertal buck.

The superiority of the adult buck seminal plasma to pubertal buck seminal plasma in glucose concentration on the other hand might imply that the decrease in glucose concentration in spermatozoa that accompanied the increase in age of the animal earlier discussed was accompanied by a corresponding increase in seminal plasma glucose as a source of energy for spermatozoa. Further work is required to confirm these observations and the mechanisms involved. The significantly higher ($p<0.05$) phospholipid levels in adult than in pubertal buck seminal plasma suggests higher demands for this lipid fraction at adulthood for possible roles in the protection of the cell from stressful conditions (Poulos *et al*., 1973) and in the supply of energy for sperm physiological processes (Daarin-Banneth *et al*., 1974; White, 1980).

AchE activity was similar between the age groups in spermatozoa but significantly higher in adult buck seminal plasma. Being that AChE is believed to be localized in the sperm tail where it is believed to control the coordination of the propagations necessary for sperm motility (Sekine, 1951; Nelson, 1972; McGrady and Nelson, 1967). The higher activity of AChE in the seminal plasma of the adult buck may thus affect sperm motility with higher values in adults than in pubertal buck semen.

The correlation matrix between biochemical characteristics in pubertal bucks Table 2) showed spermatozoal total protean to be significantly positively related to cholesterol ($r = 0.36$; $p<0.05$) while spermatozoal glucose concentration was significantly positively related to LDH ($r = 0.42$; $p<0.05$) but significantly negatively related to AChE ($r = -0.35$; $p<0.05$). Spermatozoal cholesterol was also significantly positively related.

Seminal plasma total protein concentration was similarly significantly positively correlated to phospholipids ($r = 0.55$; $p<0.01$), while seminal plasma glucose was related to LDH ($r = 0.51$; $p<0.05$). Also, while cholesterol in seminal plasma was significantly positively related to LDH ($r = 0.35$; $p<0.05$), it was significantly negatively related to AchE ($r = -0.52$, $p<0.05$).

The significant relationship between spermatozoa total protein and cholesterol obtained in the present study is in agreement with the earlier of Bitto *et al.* (2000) in buck of the same breed and Yankassa rams. The report of Bitto *et al.* (2000) was however limited in scope and did not cover glucose LDH and AChE in both spermatozoa and seminal plasma.

While the significant relationships between spermatozoal biochemical characteristics could serve as a basis for the prediction of nutrient availability to cells, the resistance of cells to could hock and stress as wells as the motility of cells; the relationships in seminal plasma may be useful in the formulation of buffers and extenders for the semen of these animals in their native environment.

CONCLUSION

The results of this study suggest that even though the semen of both adult and pubertal WAD bucks might be extended and stored in the same media, differences may exist in the storage ability of spermatozoa and in their responses to freezing and thawing for Artificial Insemination (AI).

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