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EFFECTS OF AGE AND COLLECTION TIME ON THE CHARACTERISTICS OF
RAM SEMEN IN AKURE, NIGERIA.

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EFFETS DE L'AGE ET DE L'HEURE DE COLLECTE SUR LES
CARACTERISTIQUES DU SPERME DES BELIERS A AKURE AU NIGERIA

Résumé

Deux groupes de béliers Yankasa âgés de 12 – 14 mois (environ un an) et de 24 – 48 mois (adultes) étaient utilisés pour la présente étude. Les animaux étaient soumis à un système d'élevage semi-intensif. La circonférence scrotale (CS) des béliers était relevée au début et à la fin de l'expérience. Les béliers étaient éjaculés deux fois par jour (7 h – 10 h et 16 h – 19 h) pendant soixante jours à l'aide d'un électro-éjaculateur. Il y avait une pause d'une journée entre chaque jour d'éjaculation. Le sperme était examiné après chaque collecte pour déterminer la concentration de sperme, le taux de motilité du sperme, les spermatozoïdes vivants et le taux de difformité du sperme.

La circonférence scrotale initiale et finale était en moyenne de 22.67 ± 3.48 cm et 25.67 ± 3.84 cm, 27.67 ± 0.33 cm et 29.67 ± 0.33 cm pour les béliers d'un an et les béliers adultes respectivement. La concentration moyenne de sperme, le taux de motilité du sperme, le taux de spermatozoïdes vivants et le taux de difformité du sperme pour les béliers d'un an et les béliers adultes étaient respectivement de $2.07 \pm 0.52 \times 10^9$ /ml et $2.31 \pm 0.41 \times 10^9$ /ml; 72.06 ± 1.64 et 77.68 ± 0.98 ; 79.81 ± 1.72 et 85.03 ± 1 et 8.83 ± 0.82 et 8.47 ± 0.67 . La moindre différence significative de Fisher (LSD) a montré qu'il y avait une différence notable relative à l'âge ($P < 0.05$) en ce qui concerne la concentration de sperme, la motilité progressive et les spermatozoïdes vivants, tandis que le taux de difformité du sperme n'était pas beaucoup influencé par l'âge. Les béliers adultes étaient nettement supérieurs pour ce qui est des caractéristiques du sperme comparé à celles des béliers d'un an. Les caractéristiques du sperme, à savoir : la concentration de sperme, le taux de motilité du sperme, le taux de spermatozoïdes vivants et le taux de difformité du sperme à 7 h – 10 h et 16 h – 19 h étaient en moyenne de $2.28 \pm 0.48 \times 10^9$ /ml et $2.10 \pm 0.46 \times 10^9$ /ml, 77.50 ± 1.23 et 72.24 ± 1.39 ; 85.24 ± 1.20 et 79.60 ± 1.52 ; et 6.94 ± 0.69 et 10.37 ± 0.81 pour les deux périodes respectivement. Les différences entre les deux périodes de collecte étaient significatives ($P < 0.05$) pour toutes les caractéristiques du sperme. Le sperme recueilli à 7 h – 10 h était de loin supérieur quant à leurs caractéristiques par rapport aux éjaculats collectés à 16 h – 19 h. La présente étude révèle que la collecte de sperme devrait être faite dans la matinée (7 h – 10 h) avec les béliers adultes qui seront utilisés pour les programmes d'insémination artificielle.

Summary

Two groups of Yankasa rams, aged 12-14 months (yearlings) and 24-48 months (adults) were used for this study. The animals were kept under semi-intensive management system. Scrotal circumference (SC) of rams were taken at the beginning and end of the experiment. The rams were ejaculated twice per day (7.00-10.00AM and 4.00-7.00PM) for sixty days using an electro-ejaculator. A day was left free in between each ejaculation day. The semen was evaluated after each collection for sperm concentration, percent sperm motility, live spermatozoa and percent sperm abnormalities.

The mean initial and final scrotal circumference were 22.67 ± 3.48 cm and 25.67 ± 3.84 cm; 27.67 ± 0.33 cm and 29.67 ± 0.33 cm for yearling and adult rams respectively. The mean sperm concentration, percent sperm motility, percent live spermatozoa and percent sperm abnormalities for the yearling and adult rams were $2.07 \pm 0.52 \times 10^9$ /ml and $2.31 \pm 0.41 \times 10^9$ /ml; 72.06 ± 1.64 and 77.68 ± 0.98 ; 79.81 ± 1.72 and 85.03 ± 1.00 and 8.83 ± 0.82 and 8.47 ± 0.67 , respectively. The Fisher's Least Significant Difference (LSD) showed that there was significant age difference ($P < 0.05$) in sperm concentration, progressive motility and live spermatozoa while the percent sperm abnormalities was not significantly influenced by age. The adult rams were significantly superior in their semen characteristics compared to those of the yearlings. The mean semen characteristics: sperm concentration, percent sperm motility, percent live-spermatozoa and percent sperm abnormalities at 7.00-10.00AM and 4.00-7.00PM were $2.28 \pm 0.48 \times 10^9$ /ml and $2.10 \pm 0.46 \times 10^9$ /ml; 77.50 ± 1.23 and 72.24 ± 1.39 ; 85.24 ± 1.20 and 79.60 ± 1.52 ; and 6.94 ± 0.69 and 10.37 ± 0.81 for the two periods respectively. Differences between the two periods of collection were significant ($P < 0.05$) for all the semen characteristics. Semen collected at 7.00-10.00AM were significantly superior in their characteristics compared to ejaculates collected at 4.00-7.00PM. This study suggests that semen collection could be done in the morning hours (7.00-10.00AM) from adult rams to be used in artificial insemination programmes.

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Introduction

Fertility of males intended for breeding (natural mating or Artificial insemination) programmes is dependent on age of sexual maturity^{1,2}. At sexual maturity or puberty, spermatozoa production and release are two gradual processes. However, studies have shown that rams could be used in natural breeding programmes as early as 6 months of age with some good fertility results^{3,4}. While some studies have shown high correlations between age at puberty and testicular size^{5,6}, one study with yearling and adult bulls⁷ showed significant differences only in ejaculate volume of the two types of bulls.

Epididymal sperm reserve in yearling Yankasa rams has been estimated as 24.0×10^9 spermatozoa^{8,9}. The increase in total sperm out-put per ejaculate which is dependent on epididymal sperm reserve has been attributed to increase in testicular size^{9,10,11} and since age and testicular size have been highly correlated^{5,6}, the age at which a ram's semen could be collected, could influence the quality of the semen.

Apart from age, some other factors that could influence the quality of semen collected from male animals include management procedures, housing and ambient temperature. The importance of optimum environmental factors such as housing, feeding, health management in enhancing reproductive efficiency in cattle has been stressed¹². Many workers^{13,14,15} have reported seasonal effects on some semen characteristics. One study in a tropical environment has shown that the season of semen collection influences sperm concentration but not progressive motility and semen volume¹⁶.

In the tropics, ambient temperatures are generally above the thermoneutral zone for most tropical animals. These temperatures change with the periods of the day. The effects of such diurnal temperature

changes on semen characteristics are not known. This study was designed to investigate the effects of age of rams and period of semen collection on the characteristics of the semen.

Materials and Methods

The study was carried out at the small ruminant unit of the Federal University of Technology, Akure, Teaching and Research Farm. A total of six Yankasa rams were used for the study. Three were yearlings (12-14 months of age) while the other three were mature adult (above 24 months of age). The animals were managed under a semi-intensive system. They were taken out for grazing (mixture of Guinea and Elephant grasses and some weeds) in the morning after semen collection and brought back in the afternoon for the second semen collection. No supplementary feed was given.

Semen collection was done by electro-ejaculation every other day for a period of sixty days covering the spermatogenic cycle¹⁷. The scrotal circumference was also measured at the beginning and end of the experiment using a measuring tape. The rams were ejaculated twice, that is in the morning (7.00-10.00AM) and afternoon (4.00-7.00PM) on the day of semen collection. The semen was collected into graduated test-tubes in a flask containing warm water at 37°C. The semen was taken to the laboratory in this flask. Semen was preserved in Cornell University Extender (CUE) and refrigerated for three days. In the artificial insemination (AI) laboratory, the semen samples were analysed for the following characteristics: semen volume, sperm concentration, sperm motility, live-dead ratio and sperm abnormalities.

Semen volume was measured directly from the graduated test-tube but only used in concentration calculation due to established inconsistency of volume in the

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electro-ejaculation method of collection. Sperm concentration was measured with an haemocytometer using the steps outlined by Bearden and Fuguay¹⁷ and by Sorensen¹⁸. Sperm motility was estimated by observing the movement of the spermatozoa under an X40 objective microscope¹⁸. Semen smears stained with eosin-nigrosin¹⁷ were made for live-dead ratio and sperm abnormalities determinations. The percentage of normal morphology was determined by using the uncleared slides⁹, under the microscope using X40 objective.

All parametric data, were subjected to analysis of variance (ANOVA) using MINITAB (V.10.1, Minitab Inc. USA) statistical package while comparisons between means were by Fisher's Least Significant Difference (LSD)¹⁹.

Results

The mean scrotal circumferences (SC) of the yearling and adult rams at the beginning and end of the experiment were 22.67 ± 3.48 cm and 27.67 ± 0.33 cm for the yearling and 25.67 ± 3.84 cm and 29.67 ± 0.33 cm for adult rams respectively. The change in SC were 3.00 cm and 2.00 cm for the yearling and adult rams respectively for the period of study. Tables 1 and 2 show the effects of age and period of collection on semen characteristics: concentration, motility, live-spermatozoa and sperm abnormalities.

Sperm concentration

The mean sperm concentration by age were $2.18 \pm 0.53 \times 10^9$ /ml versus $2.39 \pm 0.42 \times 10^9$ /ml during the morning collection while the afternoon collection with corresponding age groups were $2.00 \pm 0.52 \times 10^9$ /ml versus $2.24 \pm 0.41 \times 10^9$ /ml. Age and period of collection significantly ($P < 0.05$) influenced the concentration of ejaculates from rams used in this study. The overall mean ejaculates from yearling rams were significantly ($P < 0.05$) lower than the ejaculates from

the adult rams. In addition, ejaculates during the morning collection from yearling and adult rams had significantly ($P < 0.05$) higher sperm concentration than ejaculates collected in the afternoon period. No interaction was observed between age and period of collection (Table 1).

Progressive motility

The mean progressive sperm motility in yearling and adult rams were $73.96 \pm 1.66\%$ versus $81.04 \pm 0.80\%$ during the morning collection while in the afternoon collection, the corresponding motility in the yearlings and adults were $70.16 \pm 1.62\%$ versus $74.32 \pm 1.15\%$. Age and period of collection significantly ($P < 0.05$) influenced the progressive motility of the sperm from the rams used in this study. The overall mean motility of ejaculate from yearling rams were significantly ($P < 0.05$) lower than the motility of ejaculate from the adult rams. In addition the motility of the ejaculates during the morning collection from yearling and adult rams had significantly ($P < 0.05$) higher progressive motility than ejaculates collected in the afternoon period. No interaction was observed between age and period of collection.

Live-spermatozoa

The mean live-spermatozoa by age were $81.90 \pm 1.67\%$ versus $88.58 \pm 0.73\%$ during the morning collection while in the afternoon collection, the corresponding live spermatozoa in yearling and adult rams ejaculate were $77.72 \pm 1.77\%$ versus $81.48 \pm 1.27\%$ respectively. Age and period of collection significantly ($P < 0.05$) influenced the live-spermatozoa in the ejaculates from the rams used in this study. The overall mean live spermatozoa in ejaculates from yearling rams were significantly ($P < 0.05$) lower than the mean live spermatozoa in ejaculates from the adult rams. In addition, live spermatozoa in ejaculates during the morning collection from yearling and adult rams had significantly

Table 1: Summary of Analysis of Variance

SOURCE OF VARIANCE	DEGREE OF FREEDOM df	MEAN SQUARES			
		Concentration	Motility	Live Spermatozoa	Abnormalities
PERIOD OF COLLECTION					
(MORNING OR AFTERNOON)	1	22500.0***	1909.7***	2193.1***	814.04***
AGE OF RAM	1	39027.0***	2181.8***	1878.3***	9.06
PERIOD X AGE	1	551.0	147.8	147.8	13.04
ERROR	272	1533.0	126.3	138.8	38.91

***P<0.001

(P<0.05) higher live-spermatozoa than in ejaculates collected in the afternoon period. No interaction was observed between age and period of collection.

Sperm abnormality

The mean sperm abnormality by age were $7.33 \pm 0.80\%$ versus 6.54 ± 0.57 during the morning collection while in the afternoon collection, the corresponding percent sperm abnormalities in yearling and adult ejaculates age groups were $10.33 \pm 0.84\%$ versus $10.41 \pm 0.77\%$. The percent sperm abnormality was not influenced by age, although it was higher in yearlings. The overall mean percent abnormalities in ejaculates from yearling rams were slightly higher than in ejaculate from adult rams. In addition, ejaculates during the morning collection from the yearling and adult rams had significantly (P<0.05) lower sperm abnormalities than in ejaculates collected in the afternoon period. No interaction was observed between age and period of collection.

Discussion

It was evident from the semen characteristics data presented in Table 1 that the age of the animal significantly affects all the semen characteristics under study. The higher sperm concentration, percent motility and live-spermatozoa observed in the adult rams were in line with other studies^{6,10,13}. However, the percent sperm abnormalities were higher in the yearling, albeit not significant. These significant effects of semen characteristics in relation to age is attributable to the larger scrotal circumference of sexual maturity in the adult rams. Semen characteristics increase appreciably with increase in scrotal size^{2,20}. Lower sperm production in yearling rams as compared with older rams had been reported⁹. This was corroborated by another work⁹ which showed significant differences between yearling and adult ram semen in ejaculate volume, sperm concentration, sperm motility and total sperm output per ejaculate except for

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percent morphologically normal sperm. Unlike most of these workers, another report⁷ revealed that there was no significant change in sperm concentration, sperm number per ejaculate and sperm motility for the yearling and mature bulls with the exception of ejaculate volume. The observed higher gain in scrotal circumference in the yearling rams than in the adult though not significant, is expected. This can be attributed to the fact that the yearling rams are still in the early stage of growth during which the growth of various body parts are occurring at a faster rate²¹. The observed smaller rate of change in scrotal size of the adult

rams is in line with other reports and also in line with the assertion that as organisms mature in age, there is progressive decline in vital capacities, terminating in death and that this process occurs at all levels from macromolecules to the intact animal following cessation of growth.

Semen production efficiency was highest in the adult ram. This is similar to the results of other workers including Colas¹³ Schinckel et al²², Pisselet et al²³ and Haley et al²⁴ which have all shown that sperm production in rams is significantly correlated with testis weight and the area.

Table 2: Effect of age and period of collection on some semen characteristics ($\bar{X} \pm \text{Se}$)

SEMEN CHARACTERISTICS	PERIOD OF COLLECTION	AGE		MEAN $\pm$ SEM
		YEARLING	ADULT	
SPERM	7.00-10.0 (A.M).	2.18 $\pm$ 0.53 ^a	2.39 $\pm$ 0.42 ^c	2.28 $\pm$ 0.48 ^a
CONCENTRATION $\times 10^9/\text{ml}$	4.00-7.00 (P.M).	2.00 $\pm$ 0.52 ^b	2.24 $\pm$ 0.41 ^c	2.10 $\pm$ 0.46
	MEAN $\pm$ S.E.M	2.07 $\pm$ 0.52	2.31 $\pm$ 0.41	4.39 $\pm$ 0.91
PROGRESSIVE	7.00-10.00 (A.M).	73.96 $\pm$ 1.66 ^a	81.04 $\pm$ 0.80 ^c	77.50 $\pm$ 1.23 ^a
MOTILITY (%)	4.00-7.00 (P.M)	70.16 $\pm$ 1.62 ^b	74.32 $\pm$ 1.15 ^d	72.24 $\pm$ 1.39
	MEAN $\pm$ S.E.M	72.06 $\pm$ 1.64	77.68 $\pm$ 0.98	149.74 $\pm$ 2.62
LIVE SPERMATOZOA	7.00-10.00 (A.M)	81.90 $\pm$ 1.67 ^a	88.58 $\pm$ 0.73 ^c	85.24 $\pm$ 1.20 ^a
(%)	4.00-7.00 (P.M)	77.72 $\pm$ 1.77 ^b	81.48 $\pm$ 1.27 ^d	79.60 $\pm$ 1.52
	MEAN $\pm$ S.E.M	79.81 $\pm$ 1.72	85.03 $\pm$ 1.00	164.84 $\pm$ 2.72
SPERM ABNORMALITY	7.00-10.00 (A.M).	7.33 $\pm$ 0.80 ^a	6.54 $\pm$ 0.57 ^a	6.94 $\pm$ 0.69 ^a
(%)	4.00-7.00 (P.M)	10.33 $\pm$ 0.84 ^b	10.41 $\pm$ 0.77 ^b	10.37 $\pm$ 0.81
	MEAN $\pm$ S.E.M	8.83 $\pm$ 0.82	8.47 $\pm$ 0.67	17.31 $\pm$ 1.50

Means along the same column with different superscripts differ significantly ($P < 0.05$)
^aOverall mean of each semen characteristics during morning collections were significantly ($P < 0.05$) superior.

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^{25, 26} It has been shown that animals with small testes and thus small scrotal circumferences are often not as fertile as others with higher scrotal circumferences.²⁸ Therefore, from these results it could be said that the small scrotal circumferences of the yearling rams could be responsible for their poorer semen characteristics when compared to those of the adult rams.

The period of collection effects were significantly different ($P < 0.05$) for all the sperm characteristics studied. The observed differences in semen characteristics (concentration, percent motility, percent live-spermatozoa and percent sperm abnormalities) in relation to the period of collection in both the yearling and adult rams could be due to the effects of environmental factors on sperm production and sperm metabolic activities.^{29, 30, 31} The spermatozoa concentration, percent motility, percent live-spermatozoa and sperm abnormalities were highest in the morning collections. The superior spermatozoan characteristics in the morning ejaculates can not be easily explained. However, this superiority could be attributable to a number of factors of the environment. First, in the morning collections the animals have just passed through the cool night and this probably explains the superior spermatozoan characteristics in the morning collections. Whereas in the afternoon collections in addition to probable uncontrolled sexual encounters, high ambient temperatures could exert stress on the animal which could adversely affect sperm qualities. This is in line with the report of some workers which have shown that increased ambient temperature decreases sperm production and modifies the characteristics of the

sperm.^{29, 30, 31} However, the degree of change in semen characteristics is dependent on the duration and intensity of the thermal stress.²⁹

Additionally, the high ambient temperatures when the afternoon collections were made could have caused increased sperm activity and faster rate of sperm metabolism leading to depletion of nutrients in the semen, sperm stress and gradual death. This could explain the higher number of sperm abnormalities in the afternoon collections. Rams develop a variety of severe sperm cell deformities when exposed to high ambient temperatures.²⁹ This finding is also in line with another work which showed that scrotal heating in rams adversely affected semen characteristics, fecundity and embryonic mortality.³²

It has been shown in this study that the ages of rams and periods of semen collection significantly affect most of the semen characteristics in rams. The semen characteristics were better in adult than in yearling rams and that semen collected in the morning were significantly superior in their characteristics than semen collected in the afternoon in both ages. It is therefore suggested that semen collection be done in the morning hours when the weather is cool and should be restricted to the adult rams. However, yearling rams could be used on the basis of their sperm quality and output per ejaculate.⁹ Further studies are suggested to establish the ideal time of the day for semen collection in rams under tropical conditions.

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