

Effects of ejaculation frequency on semen characteristics and serum testosterone concentration in Red Sokoto bucks

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Abstract

Low productivity has been identified as a problem of Nigerian indigenous goats. Their productivity can be increased through the employment of artificial insemination whose success also depends on semen quality. Limited reports are available on the effect of frequency of collection on semen quality. In this study, the effects of semen collection frequency on semen parameters and testosterone concentration in Red Sokoto bucks were assessed. Twenty bucks were randomly assigned to four groups (A-D) of five animals each. These were subjected to: once daily semen-collection for seven days (A), twice a week collection for twenty eight days (B), once a week collection for twenty eight days (C) and non-ejaculated control group (D). Semen analysis was done after each collection. Serum testosterone was assayed following blood collection before ejaculation (0hr) and at intervals (1 and 3hrs) post ejaculation. Body weight, height at withers, scrotal length and scrotal circumference were measured weekly throughout the study. Ejaculation at the frequencies in the study did not affect the body weight, height at withers, scrotal circumference and scrotal length ($P>0.05$) across all groups. Semen volume decreased from day1 ($1.34\pm 1.52\text{mL}$) to day7 ($0.24\pm 0.15\text{mL}$) in A, week 1 ($0.84\pm 0.36\text{mL}$) to week 4 ($0.33\pm 0.16\text{mL}$) in B, but increased from week 1 ($0.33\pm 0.13\text{mL}$) to week 4 ($0.73\pm 0.31\text{mL}$) in C. Time-dependent decrease in sperm motility (Day1 to 7 in A:186.45%; Week 1 to 4 in B:32.69%) and percentage sperm liveability occurred, while they were relatively constant in C. Sperm concentration fluctuated in B while progressive decrease and increase were observed in A and C respectively. Morphological sperm abnormalities in A, B and C were 26.8%, 31.93% and 20.55% respectively. Serum testosterone concentration was highest on day 5 in group A, 3hrs post ejaculation at week 2 day 1 in B and 3hrs post ejaculation at week 3 in C. This study revealed that sperm motility, concentration and liveability reduced while sperm abnormalities increased following more frequent ejaculations.

Keywords: Ejaculation, Fertility, Goats, Red Sokoto, Semen, Testosterone

Effets de la fréquence de l'éjaculation sur les caractéristiques du sperme et la concentration sérique de testostérone chez les boucs rouges de Sokoto



Résumé

La faible productivité a été identifiée comme un problème des chèvres indigènes nigérianes. Leur productivité peut être augmentée grâce à l'emploi de l'insémination artificielle dont le succès dépend également de la qualité de la semence. Des rapports limités sont disponibles

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sur l'effet de la fréquence de collecte sur la qualité du sperme. Dans cette étude, les effets de la fréquence de collecte du sperme sur les paramètres du sperme et la concentration de testostérone chez les boucs Red Sokoto ont été évalués. Vingt boucs ont été répartis au hasard dans quatre groupes (A-D) de cinq animaux chacun. Ceux-ci ont été soumis à : une collecte de sperme une fois par jour pendant sept jours (A), une collecte deux fois par semaine pendant vingt-huit jours (B), une collecte une fois par semaine pendant vingt-huit jours (C) et un groupe témoin non éjaculé (D). L'analyse du sperme a été effectuée après chaque prélèvement. La testostérone sérique a été dosée après un prélèvement sanguin avant l'éjaculation (0h) et à intervalles (1 et 3h) après l'éjaculation. Le poids corporel, la hauteur au garrot, la longueur et la circonférence du scrotum ont été mesurés chaque semaine tout au long de l'étude. L'éjaculation aux fréquences de l'étude n'a pas affecté le poids corporel, la hauteur au garrot, la circonférence et la longueur du scrotum ($P > 0,05$) dans tous les groupes. Le volume de sperme a diminué du jour 1 ($1,34 \pm 1,52$ ml) au jour 7 ($0,24 \pm 0,15$ ml) dans A, semaine 1 ($0,84 \pm 0,36$ ml) à la semaine 4 ($0,33 \pm 0,16$ ml) dans B, mais a augmenté à partir de la semaine 1 ($0,33 \pm 0,13$ ml) à la semaine 4 ($0,73 \pm 0,31$ ml) chez C. Une diminution en fonction du temps de la motilité des spermatozoïdes (Jour 1 à 7 dans A : 186,45 % ; Semaine 1 à 4 dans B : 32,69 %) et le pourcentage de viabilité des spermatozoïdes s'est produit, alors qu'ils étaient relativement constante dans C. La concentration de spermatozoïdes a fluctué dans B tandis qu'une diminution et une augmentation progressives ont été observées dans A et C respectivement. Les anomalies morphologiques des spermatozoïdes dans A, B et C étaient respectivement de 26,8 %, 31,93 % et 20,55 %. La concentration sérique de testostérone était la plus élevée au jour 5 dans le groupe A, 3 heures après l'éjaculation à la semaine 2 le jour 1 dans B et 3 heures après l'éjaculation à la semaine 3 dans C. Cette étude a révélé que la motilité, la concentration et la vivabilité des spermatozoïdes diminuaient tandis que les anomalies des spermatozoïdes augmentaient après une fréquence plus élevée. éjaculations.

Mots clés : Ejaculation, Fertilité, Chèvres, Red Sokoto, Sperme, Testostérone

Introduction

Goats are multi-functional animals that play significant roles in the economy and nutrition of low income earners in many developing countries (Baruwa, 2013). Small ruminants especially goats can efficiently survive on available shrubs and trees in harsh environment where large ruminants' growth are restricted. They fulfill a useful task of supplying a part of human population with milk, meat, hair and leather (Hagan *et al.*, 2012). In Nigeria, goats represent a resource for economic development and livelihood security. Goats have a population of about 22 to 26 million in Nigeria with rough estimates of 6.6 million and 20 million, respectively in southern and northern region of the country (Lawal-Adebowale, 2012). The breeds of

goats in Nigeria are largely indigenous; and the common ones include the West African Dwarf (WAD) of the south, Sahel and Sokoto Red which are common in the north. These breeds of goats have low mortality rates (22% for kids and 14.4% for adults) but low fertility rate (40% twins and triplets birth rates). Improvement in productivity of this animal requires the employment of Assisted Reproductive Technology such as Artificial Insemination which is a biotechnological tool in which semen is collected, evaluated processed, preserved and introduced into the genitalia of an estrus female by use of special equipment to get such females to conceive without having any physical contact with the male. Good semen quality is of utmost importance in achieving successful Artificial

Insemination and this is partly dependent on frequency of ejaculations. A study showed that repeated ejaculations in WAD bucks caused a decrease in volume and semen concentration and an increase in abnormal spermatozoa as frequency of ejaculation increased (Oyeyemi *et al.*, 2000). Also, another study in WAD buck reported that semen volume, sperm concentration, sperm motility score and percentage live sperm cells were higher when semen was collected once per week compared with twice, thrice and four times per week collections. (Iheukwumere and Okere, 1990). To the best of our knowledge, effect of repeated ejaculation on semen quality in Red Sokoto bucks have not been determine to be similar or different to that of WAD bucks as difference may occur due to breed variation. The aim of this study was therefore to determine the effect of ejaculation frequency on the semen characteristics of Red Sokoto bucks and serum testosterone concentration.

Materials and methods

Study location

The study was carried out in the experimental small ruminant unit of the Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun state (Latitude 3° 19.655E and longitude 7° 09.775N).

Experimental animals

Twenty apparently healthy male bucks between the ages of 1-2 years (estimated using dentition) were used. These were sourced from a prominent local market after thorough preliminary physical examinations. These experimental animals were acclimatized for three weeks, during which they were screened for ecto- and endo-parasitic infestations and given treatments were necessary. The animals were allowed to graze in the morning and

they were fed concentrate in the evening. Water was served *ad-libitum*.

Experimental protocol

The animals were randomly assigned into four groups (A to D) of 5 bucks each. Semen was collected by the use of an electro ejaculator (EE). In group A, semen was collected once daily for one week with twenty four hours rest interval. In group B, semen was collected twice a week for 4 weeks, specifically, Mondays and Thursdays. In group C, semen was collected once a week for 4 weeks, with six days rest interval and in group D, only blood was collected for serum once daily for 4 weeks. On the day of semen collection, blood samples were collected at 0, 1 and 3 hours from animals in group A to C, while blood was collected from animals in group D concurrently once for 4 weeks. Serum was extracted from the blood samples collected from the jugular veins of the animals and stored in deep freezer at -20°C for testosterone assay. The animals were weighed weekly using a platform scale (HANA, H-9012) while, height at withers, scrotal length and scrotal circumference were measured in centimetres using a measuring tape weekly.

Semen evaluation

Semen volume and color

Ejaculate volume was measured using graduated collecting tube while semen colour was determined by visual observation.

Percentage sperm motility

This was determined using methods described by Chenoweth (2005). A drop of semen diluted with a drop of sodium citrate was placed on a warm glass slide covered with cover slip. This was viewed using X40 objective of light microscope and the percentage active, progressively motile cells were estimated.

Sperm concentration

This was determined using the improved

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Neubauerhemocytometer. A cover slip was charged with the buck's semen diluted with 0.05% formol-saline. The charged hemocytometer was viewed under a microscope at X 40 objective. The sperm heads in the 5 large cells of the hemocytometer were counted.

Percentage sperm liveability

Semen was stained with Eosin-Nigrosin stain. The dead spermatozoa usually stain pinkish or reddish while live spermatozoa remained colourless. Sperm cells were observed under the microscope at x100 magnification. Cells that picked up the stain were counted as dead cells. About 200 cells were counted in four fields and the percentage of those that picked up the stains was noted.

Morphological abnormalities

One drop of the collected semen was added to two drops of eosin-nigrosin stain, mixed thoroughly before a smear was made on a clean glass slide. The slides were examined at X 100. Morphological aberrations were determined from a total count of 100 spermatozoa and the number of various abnormalities was noted. Sperm abnormalities were classified accordingly as described by Chenoweth (2005).

Testosterone concentration

Blood, from where serum was derived, was aseptically collected from the jugular vein of experimental animals. Enzyme Linked Immunosorbent Assay (ELISA) kit (AccuBind® ELISA micro wells; Product Code: 3725-300) was used to assay for serum testosterone.

Data analysis

Data were analyzed using descriptive statistics. Differences between the mean values obtained were tested using ANOVA. Post-hoc test was used to separate means. The level of significance was set at $p < 0.05$. Values were given as means \pm Standard Deviation (SD). SPSS version 16.0 was

used for the analyses.

Results

Weight (Kg)

In groups A and B, the mean weights at the commencement of the study (14.20 ± 1.92 and 16.20 ± 2.28 respectively) dropped slightly by the second week (14.10 ± 1.75 and 16.00 ± 1.22 respectively). It then remained constant till week four. In group C, it was 14.50 ± 1.80 at the start of the study, but increased to 15.00 ± 1.22 by the second week, and remained constant till week four. There was no significant ($P < 0.05$) difference in the weight of Red Sokoto bucks comparing animals within and between groups following frequent ejaculation for one week in group A and four weeks in groups B and C.

Height (cm)

The mean height increased from first week to second week in groups A (53.40 ± 1.67 and 54.70 ± 2.90 respectively) and B (54.20 ± 2.28 and 55.75 ± 2.95 respectively) and remained constant till fourth week. In group C it increased from first week (53.00 ± 3.00) to second (53.50 ± 3.28) and third (53.73 ± 2.81) after which it remained constant till the fourth week. There was no significant difference ($P < 0.05$) in the height of the bucks comparing animals within and between groups

Scrotal length

The mean scrotal length (cm) was 12.20 ± 1.48 at the start of the study in group A and had increased to 12.30 ± 1.54 by the second week. In group B, the mean scrotal length was 12.30 ± 0.97 at the start of the study then dropped to 12.18 ± 0.81 by the 2nd week. The value was 12.22 ± 0.81 by the third week and remained so till week 4. In group C, the scrotal length was 14.00 ± 1.87 at the start of the study and this remained so till the second week. By the 3rd week, the value slightly increased to 14.03 ± 1.88 then to 14.25 ± 1.75 by the 4th week. However, there was no significant ($P < 0.05$) difference

in the scrotal length of the bucks comparing animals within and between groups.

Scrotal circumference

In group A, the mean scrotal circumference (cm) was 16.30 ± 2.50 at the start of the study and it had dropped to 16.26 ± 2.50 by the second week. In group B, the scrotal circumference was 17.40 ± 1.52 at the start of the study, the value was 17.42 ± 1.49 by the second week, 17.75 ± 1.30 by the third week and 17.73 ± 1.31 by the 4th week. In group C, the scrotal circumference was 18.25 ± 1.30 at the start, maintained till the 2nd week, then dropped by the 3rd and fourth week to 18.10 ± 1.22 and 18.00 ± 1.16 respectively. There was no significant difference ($P < 0.05$) comparing animals within and between groups.

Semen characteristics

Volume

There was a progressive decline in semen volume in group A from day 1 (1.34 ± 1.52) till day 7 (0.24 ± 0.15) representing 82.09% decrease. In group B, decline was observed from Week (WK)1Day (D) 1 (0.84 ± 0.23) to WK1D2 (0.60 ± 0.51). This was followed by an increase to 0.88 ± 0.58 on WK2D1 and then a sharp decline to 0.32 ± 0.22 on WK2D2. The volume then increased on WK3D1 (0.53 ± 0.08) and dropped to 0.48 ± 0.08 on WK3D2. There was a continued drop in volume at WK4D1 (0.30 ± 0.16) followed by an increase to 0.33 ± 0.16 by WK4D2. The drop in semen volume from WK1D1 (0.84 ± 0.23) to the WK4D2 (0.33 ± 0.16) represents a 60.71% decrease. In group C, there was an increase in semen volume from the first week of collection (0.33 ± 0.13) to the second week (0.75 ± 0.42). The volume dropped to 0.70 ± 0.12 by the third week and increased to 0.73 ± 0.31 by the 4th week. The increase in semen volume from the first week of collection (0.33 ± 0.13) to the fourth week (0.73 ± 0.31) represents 121.21% increase. In group B, there was progressive decline in semen volume from the first week of

collection (0.60 ± 0.51) to the fourth week (0.33 ± 0.16), representing 45% decrease. In group C, there was an increase in semen volume from the first week of collection (0.33 ± 0.13) to the fourth week (0.73 ± 0.31), representing 121.21% increase.

Progressive motility

In group A (once daily semen collection for seven days period), there was a progressive decrease in motility from the first day of collection (88.8 ± 11.17) to the 7th day (31.0 ± 28.81) indicating 186.45% decrease. In group B (twice a week collection for a period of twenty eight days), there was a decline in the mean motility score from WK1D1 (78.00 ± 11.51) to WK2D2 (44.00 ± 34.17) and then a slight increase from WK3D1 (51.25 ± 18.83) to WK4D2 (52.50 ± 10.31). The decline in progressive motility from WK1D1 (78.00 ± 11.51) when compared to WK4D2 (52.50 ± 10.31) represents 32.69% decrease. In group C (once a week collection for a period of twenty eight days), the mean motility score was 76.25 ± 8.93 at week one. There was a slight decrease to 73.75 ± 8.20 by the second week. This increased slightly to 77.50 ± 2.50 at week 3 and decreased to 73.75 ± 2.17 by week 4. There was no significant difference ($P > 0.05$) in the mean motility score when the first week was compared to the fourth week. There was no significant difference ($P > 0.05$) in the mean motility score of group B (59.00 ± 33.43) when compared to group C (76.25 ± 8.93) in the first week but from the second week to the 4th week of the study, group B had a significantly lower ($P < 0.05$) mean motility score when compared with group C.

Concentration

The value of sperm concentration on day 1 (359.80 ± 34.00) reduced by 72.54% when compared to day 7 (98.8 ± 27.65) following frequent ejaculation for a week in group A. In group B, there was a reduction in mean sperm concentration from WK1D1 (243.20 ± 79.62) to WK1D2

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(163.20±138.66). At WK2D1, the concentration increased to 228.00±119.15 and reduced at WK2D2 (89.20±50.13). The concentration increased at WK3D1 (169.00±27.68) and reduced at WK3D2 (137.25±54.98). At WK4D1, the concentration increased (237.25±54.63) then reduced at WK4D2 (158.50±46.03). The reduction in mean semen concentration from WK1D1 (243.20±79.62) to WK4D2 (158.50±46.03) represents a 34.83% decrease. While in group C, there was a progressive rise in mean concentration from the first week (211.50±109.68) to the 4th week (311.50±53.43).

There was no significant difference ($P>0.05$) in the mean sperm concentration comparing groups B and C in the 1st and 2nd week of the study, but at the 3rd and 4th week, the mean sperm concentration was higher significantly ($P<0.05$) in group C compared to group B.

Percentage livability

There was a progressive decline in the percentage live sperm cells from day 1 (76.00±5.48) to day 7 (52.00±8.37), representing 31.58% decrease in the percentage live sperm cells when comparing day 1 to day 7 following one week of group A. In group B, there was reduction in percentage live sperm cells from WK1D1 (81.60±16.44) to WK2D1 (58.00±21.68). This further decreased to WK2D2 (39.00±29.67), then an increase at WK3D1 (51.25±16.72) before a final decline at WK4D1 (47.50±11.46) and an increase by WK4D2 (50.00±3.54). The decrease in percentage live sperm cells from WK1D1 (81.60±16.44) to WK4D2 (50.00±3.54), represents a 38.73%

decrease. For group C, there was a progressive increase in percentage live sperm cells from week 1 (67.50±8.29) to week 4 (75.00±11.18), representing 11.11%. There was no significant difference ($P>0.05$) in the percentage live sperm cells comparing groups B and C in the first to third week of the study, but at 4 weeks, percentage live sperm cells was significantly higher ($P<0.05$) in group C compared to group B.

Sperm morphology

There was a significant difference ($P<0.05$) in the number of abnormalities on day one (15.00±3.08) when compared to day 7 (39.40±7.70) in group A. Rudimentary tail (RT) had the highest percentage (16.84%) of the total abnormalities while distal cytoplasmic droplet (DCD) had the lowest percentage (2.13%). The percentage of total abnormalities was 26.8% (Figure 1). There was a significant difference ($P<0.05$) in the number of abnormalities on week one day one (18.40±2.07) when compared to week four day two (53.40±2.88) in group B. Detached tail (DT) had the highest percentage (34.4%) of the total abnormalities while proximal cytoplasmic droplet (PCD) had the lowest percentage (6%). The percentage of total abnormalities was 31.93% (Figure 2). There was a significant difference ($P<0.05$) in the number of abnormalities in week one (15.60±2.19) when compared to week four (24.00±1.73) in group C. Detached tail (DT) had the highest percentage (16.55%) of the total abnormalities while DCD had the lowest percentage (2.92%). The percentage of total abnormalities was 20.55% (Figure 3).

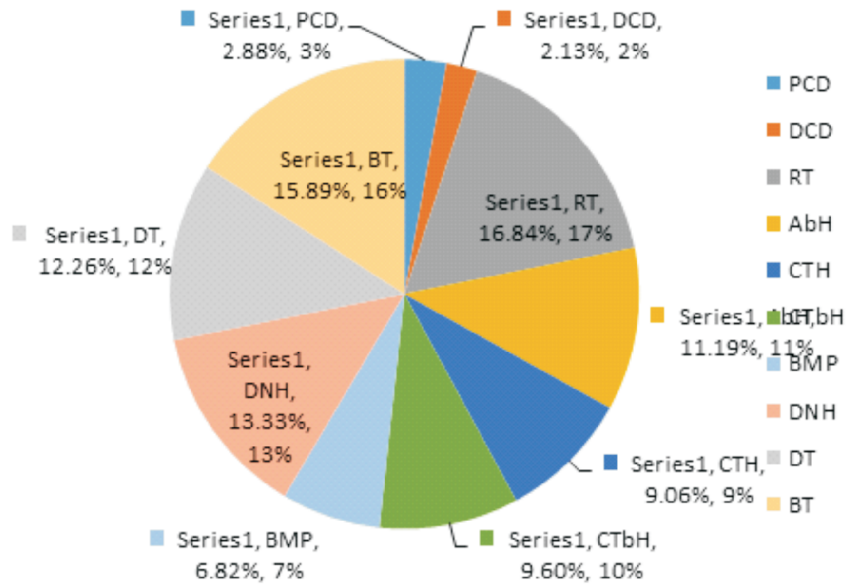


Figure 1: Percentage of abnormalities following semen collection everyday for one week.
 LEGEND: PCD: Proximal Cytoplasmic Droplet; DCD: Distal Cytoplasmic Droplet; RT: Rudimentary Tail; AbH: Abnormal Head; CTH: Tail coiled around head; CTbH: Tail coiled below head; BMP: Bent Midpiece; DNH: Detached Normal Head; DT: Detached Tail; BT: Bent Tail

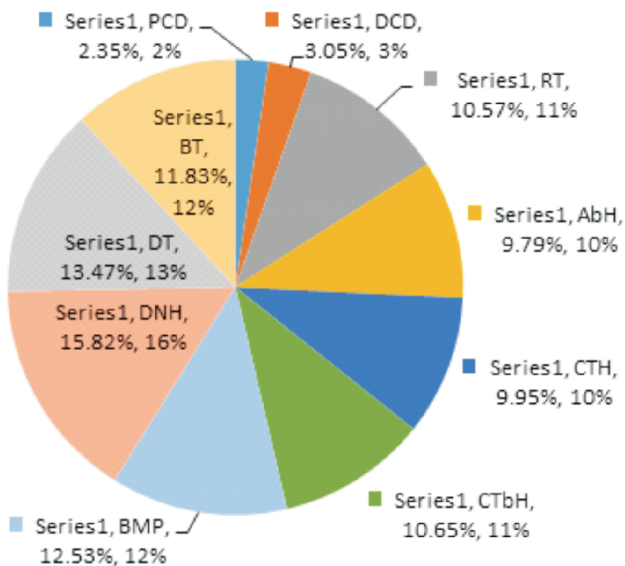


Figure 2: Percentage of abnormalities following semen collection twice a week for four weeks.
 LEGEND: PCD: Proximal Cytoplasmic Droplet, DCD: Distal Cytoplasmic Droplet, RT: Rudimentary Tail, AbH: Abnormal Head, CTH: Tail coiled around head, CTbH: Tail coiled below head, BMP: Bent Midpiece, DNH: Detached Normal Head, DT: Detached Tail, BT: Bent Tail, *WK-Week, D-Day.*

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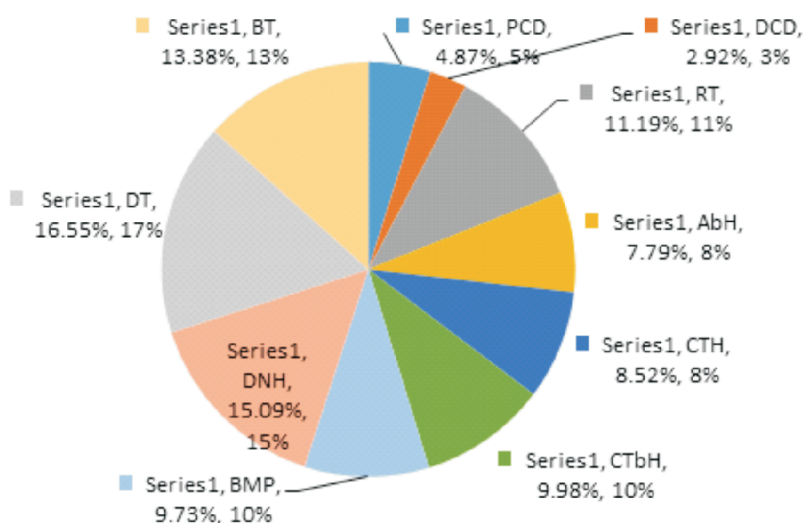


Figure 3: Semen abnormalities following semen collection once a week for four weeks

LEGEND: PCD: Proximal Cytoplasmic Droplet, DCD: Distal Cytoplasmic Droplet, RT: Rudimentary Tail, AbH: Abnormal Head, CTH: Tail coiled around head, CTbH: Tail coiled below head, BMP: Bent Midpiece, DNH: Detached Normal Head, DT: Detached Tail, BT: Bent Tail. Values with different superscripts are significant at $P < 0.05$

Serum testosterone

Serum testosterone concentrations were fluctuating in control (group D) and all other groups [group A (once daily semen collection for a seven days period), group B (twice a week collection for a period of twenty eight days) and group C (once a week collection for a period of twenty eight days)] in the study irrespective of the time of ejaculation.

In group A, the serum testosterone concentration before ejaculation on the first day (2.88 ± 1.41) was higher than group D (1.15 ± 0.54). This however dropped on day 2 (0.35 ± 0.24) and day 3 (0.32 ± 0.21). There was an increase (0.87 ± 0.71) on day 4 (but with lower concentration compared with control) and a steady drop from day 5 (0.63 ± 0.35) to day 7 (0.34 ± 0.15). At one hour post ejaculation, the serum testosterone level was higher when compared to control on the first, second and fifth day. There were two serum testosterone peaks on day 2 (3.07 ± 1.32) and day 5 (3.14 ± 2.08). At three hours post

ejaculation; the serum testosterone level was higher on day one (1.22 ± 0.99) when compared to control. However, there was a steady drop below the control from day one (1.22 ± 0.99) to day 7 (0.28 ± 0.12).

In group B, pre ejaculation serum testosterone concentration was low when compared to control (group D) all through the study. There was an increase from week one day one (0.35 ± 0.20) to week two day one (0.54 ± 0.24), then a decrease on week two day two (0.30 ± 0.22). There was a decrease from week three day one (0.59 ± 0.21) to week four day two (0.46 ± 0.14). At one hour post ejaculation, serum testosterone was at low on week 1 day one (0.50 ± 0.35) and lower than control (1.5 ± 0.54). Gradual rise was then observed till it peaks (3.04 ± 1.34) at week1 day2, dropped to 0.61 ± 0.39 at week 3 day 1 and rose to 1.79 ± 1.34 at week 3 day 2 and subsequently 3.13 ± 2.37 at week 4 day 2. At three hours post ejaculation, serum testosterone concentration was lower at week 1 day 1 (0.70 ± 0.53) when compared

to control (1.15 ± 0.54). It was wave-like thereafter with peaks at week 2 day1 (3.99 ± 1.56), week3 day1 (3.30 ± 1.5) and week 4 day 1 (2.58 ± 2.37).

In group C, pre ejaculation, serum testosterone value was higher at week one (1.05 ± 0.57) when compared to control (0.74 ± 0.23). There was an increase in value from week 2 (0.88 ± 0.42) to week 3 (4.53 ± 3.10) and a decrease from week 3 (4.53 ± 3.10) to week 4 (0.72 ± 0.20). One hour post ejaculation revealed serum testosterone level higher at week one (0.98 ± 0.63) when compared to control (0.74 ± 0.23). There was a steady decrease from week 2 (2.74 ± 1.21) to week 4 (0.64 ± 0.22). At three hours post ejaculation, serum testosterone value was higher at week one (2.74 ± 1.04) when compared to control (0.74 ± 0.23). There was an increase in value from week 2 (0.76 ± 0.37) to week 3 (6.25 ± 5.75), and a decrease in week 4 (1.12 ± 0.99).

Discussion

Scrotal parameters like the scrotal length and scrotal circumference have been used as indices for sperm production (Camela *et al.*, 2019) where a decrease in both parameters could indicate testicular degeneration and its attendant poor semen quality (Barth, 2017). In this study there was no significant difference ($P < 0.05$) in the scrotal length and scrotal circumference across groups indicating that four weeks of sustained electro-ejaculations at selected time intervals had no effects on scrotal circumference and length in Red Sokoto bucks indicating absence of testicular degeneration or other form of pathological injuries. This appeared consistent with the submission of Oyeyemi *et al.* (2000) that frequent ejaculation (as high as twice daily ejaculation for 21 days) had no effect on live weight and height at withers in WAD. However, frequent ejaculation did have effect on semen in terms of volume output with the exception of once a week

ejaculation where an astronomical increase was recorded. Working with WAD, Iheukwumere (2008) recorded similar finding. This drastic decrease in semen volume due to frequent ejaculation might have resulted from the excessive physiological demand without enough time for recuperation. Moyorga-Torres *et al.* (2015) reported a statistical correlation between length of abstinence and semen volume. The drastic increase observed in weekly collection showed that adequate rest in between ejaculation is important for volume accumulation. The decrease in progressive motility in daily collection observed in this study could be due to the excessive demand for release of spermatozoa possibly leading to release of immature cells. There are slight fluctuations in motility in other collection frequencies but it appeared relatively stable especially in weekly collection. In twice a week collection, where there was moderate demand, there was stable decrease in motility and subsequent recuperation. In once a week collection there was an initial increase in motility followed by slight decrease due to depletion but subsequent increase after rest in between collections. The sperm concentration profiles of reduction by 72.54% comparing day 7 and day 1 following daily semen collection for one week, intermittent weekly rise and drop in biweekly collection for four weeks and a steady increase and subsequent stable concentration from week one to four in weekly collection for four weeks, lend credence to the fact that a sexual rest period is required for restoration of sperm concentration in animals. Yotov *et al.* (2011), working with Plevin Blackhead rams, found that the rest period of 8 hours was sufficient for partial restoration of semen volume and sperm concentration. In biweekly semen collection, there was an effect of day of collection on sperm concentration. There was a decrease in

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concentration on days 1 as opposed to days 2. This could be as a result of a compensatory release of spermatozoa. Iheukwumere *et al.* (1990) reported that the day of collection affected the proportion of motile sperm cells and cells with normal morphology but did not affect volume, with the highest values observed on days 1 compared to days 2.

There was a decrease in the percentage live spermatozoa when semen was collected daily for a week, although this was not statistically significant ($P>0.05$). There was a decrease in percentage live spermatozoa following ejaculation twice a week. The percentage live spermatozoa increased following ejaculation once a week. The percentage live spermatozoa following semen collection for four weeks was significantly ($P<0.05$) higher in the once a week group than that of the twice a week group. There was a decrease in the percentage live sperm cells with increase in frequency of ejaculation. This is in accordance with Iheukwumere (2008), and it has been noted that the accessory sex glands secretions are produced more abundantly than sperm rich semen during high frequencies of ejaculation (Orguer and Signoret, 1984). It was reported by Umesiobi and Iloeje (1999) that semen from bucks with high frequency of ejaculation tends to have lower proportion of live spermatozoa. Weekly collection had a lower percentage of abnormal sperm cells than the other groups. The presence of 11% or more of head, mid-piece or tail abnormalities and 18% or more of total abnormalities of spermatozoa are associated with reduced fertility in ruminants (Sarder, 2004). A higher percentage of the abnormalities recorded in this study were tertiary abnormalities (detached normal head, detached tail and bent tail) and this could have been as a result of poor handling following frequent ejaculation. The high number of abnormal

sperm cells following frequent ejaculation can be attributed to the distortion and subsequent damage to the neck of spermatozoa which is reported to be the most fragile part of the spermatozoa (Iheukwumere and Okere (1990). There was an increase in primary abnormalities such as cytoplasmic droplets, this could be as a result of release of immature spermatozoa as frequency of ejaculation increased, with subsequent reduction in sperm concentration as observed in daily collection, moderate reduction in twice a week and increased in once a week. There seem to be fluctuations in the serum testosterone concentrations across all groups. This can be due to the pulsatile secretion of luteinizing hormone (LH) by the pituitary and the subsequent pulse like release of testosterone (Agarwal *et al.*, 1983; Rekwot *et al.*, 1987). When semen was collected twice a week for four weeks, at 3 hours post ejaculation, the serum testosterone level increased as the sperm concentration increased from week 2 day 2. This could be as a result of increased need for testosterone to aid sperm production and compensate for the frequency of ejaculation. The testosterone concentrations of red Sokoto bucks showed fluctuations with one to three distinct episodic peaks at 30 minutes interval for 6 hours (Aduli *et al.*, 2003). The exact significance of these episodic peaks is not clear but may be related to the sexual and behavioral states of the animals or adjustments to photoperiodicity, temperature and postural states of the animals (Sanwal *et al.*, 1974).

Conclusion

A higher frequency of ejaculation caused deterioration in semen quality and an increase in semen abnormalities. A better semen quality with low percentage of abnormalities was obtained when semen was collected once a week as compared to

daily or twice a week collection. It was observed that as semen concentration increased, serum testosterone concentration also increased. Therefore, for high productivity of Red Sokoto buck, once in a week semen collection for 4 weeks for the purpose of artificial insemination is recommended. Twice a week collection could be explored but with close monitoring of the viability of the semen being produced.

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