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Sexual urge, semen quality and seminal oxidative markers of rabbit bucks of different reproductive stages in response to peak of heat stress in Southwest of Nigeria

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Abstract

Rabbits of three reproductive stages were evaluated for their reproductive superiority during peak thermal discomfort in Ibadan, southwest Nigeria. Within rabbit population in the study area, bucks were categorized into age groups from the parity records of the farm. Pubertal rabbits (4-5 months old), Mature rabbits (7-9 months old), Adult rabbits (above 1-year-old). Animals were housed individually and allotted randomly into experimental units. This investigation was carried out within February and March when the average daily temperature humidity index (THI) is at the peak. Thirty rabbit bucks per age group, housed individually and randomly allotted to experimental units were used for this study. All bucks were made to serve an artificial vagina, libido was evaluated, and semen collected were assessed for semen characteristics, seminal biochemical and oxidative stress indices weekly from the ninth week. The result obtained revealed that mature bucks had significantly ($p<0.05$) highest semen volume, mass activity, motility, sperm concentration, total motile and total live spermatozoa among the three age groups. The effect of heat stress was most severe in pubertal bucks as they recorded the least ($p<0.05$) semen quality among the three age groups. Seminal lipid peroxidation was significantly ($p<0.05$) higher in mature and adult bucks compared with pubertal bucks. Seminal total antioxidant capacity was significantly ($p<0.05$) highest in pubertal bucks but the least value was obtained in mature bucks. It can be concluded that pubertal rabbits possess the best antioxidant stability among the three age group but possessed least semen quality compared with adult and mature buck.

Keywords: Bucks; Ibadan; Sexual maturity; Lipid peroxides; Antioxidant enzyme; heat stress

Le désir sexuelle, qualité du sperme et marqueurs oxydatifs séminals de mâles lapin de différents stages reproducteurs en réponse au maximum de stress thermique dans le sud-ouest du Nigeria

Résumé

Des lapins de trois stages reproducteurs ont été évalués pour leur supériorité reproductrice pendant le maximum de malaise thermique à Ibadan, au sud-ouest du Nigeria. Dans la population de lapins dans la zone d'étude, les mâles ont été classés en groupes d'âge à partir des registres de parité de la ferme. Lapins pubertés (4-5 mois), lapins matures (7-9 mois), lapins adultes (au-dessus de 1 an). Les animaux ont été logés individuellement et attribués au hasard dans des unités expérimentales. Cette enquête a été menée en février et mars, lorsque l'indice quotidien moyen d'humidité de la température (THI) est au sommet. Trente mâles de lapin par groupe d'âge, logés individuellement et aléatoirement attribués à des unités expérimentales ont été utilisés pour cette étude. Tous les mâles ont été faits pour servir un vagina artificiel, la libido a été évaluée, et le sperme recueilli ont été évalués pour des *caractéristiques de sperme, des indices biochimiques et oxydants séminaux de stress hebdomadaires de la neuvième semaine. Le résultat obtenu a révélé que les mâles matures avaient significativement (p<0,05) le volume de sperme le plus élevé, l'activité de masse, la motilité, la concentration de sperme, le motile total et le spermatozoa vivant total parmi les trois groupes d'âge. L'effet du stress thermique a été le plus grave chez les mâles pubertés, car ils ont enregistré la moins de qualité de sperme (p<0,05) parmi les trois groupes d'âge. La peroxydation séminale de lipide était sensiblement (p<0.05) plus élevée dans les mâles mûrs et adultes comparés aux mâles pubertals. La capacité antioxydante totale séminale était significativement (p<0.05) la plus élevée dans les mâles pubertals mais la valeur la moins élevée a été obtenue dans les mâles mûrs. On peut conclure que les lapins pubertés possèdent la meilleure stabilité antioxydante parmi le groupe d'âge trois mais ont possédé la qualité inférieure de sperme comparée à l'argent adulte et mûr.*

Mots-clés: mâles de lapin; Ibadan; Maturité sexuelle; Peroxydes lipidiques; Enzyme antioxydante; stress thermique

Introduction

The most obvious limitation to rabbit production in a hot climate area is their susceptibility to heat stress that evokes a series of drastic changes in their biological functions such as oxidative stress which in turn ends with impairment of production and reproduction (Jimoh and Ewuola, 2018). Bucks are one of the founding blocks of reproductive success in intensive rabbit farming, especially when considering the influence of one single male in the fertility and prolificacy of approximately one hundred females through the practice of artificial insemination (Eid, 2008; Jimoh and Ewuola, 2017).

Productivity traits such as kindling rate and litter size appear to be dependent on both semen quality and quantity (Brun *et al*., 2002). However, many environmental factors affect semen parameters such as ambient temperature, age, breed, nutrition, etc (Eid, 2008). The overgeneration of ROS may be detrimental to sperm and has even been associated with male infertility (Jimoh *et al*., 2021). The spermatozoa of vertebrates including rabbits display high rates of metabolic activity and are also rich in polyunsaturated fatty acids (Castellini *et al*., 2006). This renders them particularly susceptible to oxidation by ROS, especially under stress conditions in humans (Aitken *et al*., 1989), domestic birds (Eid *et al*., 2008) and also rabbits (Castellini *et al*., 2000; Jimoh and Ewuola, 2019). ROS can modify the spermatozoon cytoskeleton and axoneme resulting in a reduction of sperm motility (De Lamirande and Gagnon, 1992) and the inhibition of sperm-oocyte fusion (Aitken *et al*., 1989) which leads to reduced fertility (Wishart, 1984; Jimoh *et al.,* 2020). ROS can also attack the DNA within the sperm nucleus and such damage to the genome may be responsible for infertility (Roberts, 1998; Eid, 2008). Seminal plasma affects the viability and membrane integrity of the sperms with regard to its lipid peroxidation level and antioxidant content (Castellini *et al*., 2000) and influence fertility and litter performance in rabbits (Jimoh and Ayedun, 2020). A positive correlation exists between final TBARS and sperm viability indicating that peroxidation could be one of the causes of rabbit sperm deterioration (Eid, 2008; Jimoh, 2020). In addition to reactive oxygen species, glutathione and glutathione-related enzymes are also involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds (Knapen *et al.,* 1999). Cholesterol has been shown to be involved in the process of capacitation in mammalian spermatozoa and an important role in the fluidity and structure of the plasma membrane (Blesbois *et al*., 2000). According to Ansah and Buckland (1982), the correlation between seminal plasma cholesterol and fertility of semen were negative. Proteins and serum replacements have also been reported to affect sperm activity. Anatomically, male animals would continue to produce good quality semen from sexual maturity to old age (Nelson and Mukherjee, 1973). Dowsett and Knott (1996) indicated that most of the semen and sperm characteristics were influenced by age. However, the effects of heat stress on semen quality in rabbit of different physiological ages are unknown. In consequence, the extent to which heat stress induces oxidative stress in aging of rabbit bucks and its performance is unknown (Jimoh 2016). Therefore, the study aimed to determine the sexual urge, semen quality, and seminal oxidative stress biomarkers in bucks at three physiological ages at the peak of heat stress in Southwestern Nigeria

Materials and methods

The research was carried out from February to March (peak of dry season), it has been reported that animals are exposed to the peak of heat stress in the study area during these months when highest Temperature Humidity Index was observed within the study (Jimoh and Ewuola, 2016). The average daily THI obtained in February and March were 29.94±1.56 and 28.59±3.10 at the rabbit unit of the Teaching and Research Farm of University of Ibadan, Ibadan, Nigeria. They are situated in the rainforest agro-ecological zone of Nigeria, between lat. 7˚ 27' 18.74''N and 7˚ 27' 19.17''N and Long. 3˚ 53' 13.98''E and 3˚ 53' 32.69''E. The approval for the present study was obtained from the Institutional Animal Ethics Committee for care and use of animals for research. Before commencement of the trial, animals were confirmed to be of good health status, without abnormalities and conform to the breed and/or age group categorization. Within rabbit population in the study area, bucks were categorized into age groups from the parity records of the farm. Pubertal rabbits were between 4 and 5 months old, Mature rabbits were between 7 and 9 months old, Adult rabbits were above 1year old. Animals were housed individually and allotted randomly into experimental units. The rabbit house has natural lighting program (12D:12L), with the dynamic ventilation system. The experimental units are made of wire mesh double sided boxes of 1.1sq. m. The experimental design was Randomised Complete Block Design RCBD. The animals were fed *ad libitum* with diets containing crude protein 17.05%, digestible energy 2592.06 Kcal/kg, crude fiber 10.02%, Calcium 0.45% and phosphorus 0.21%. Freshwater was made available to the animals always. Other routines and periodic management practices necessary for rabbit production were carried out.

Sexual urge and semen quality assessment

After 9 weeks of exposure of the animals to the average daily THI (29.94 and 28.59) of the prevailing heat stress condition in the study area, bucks were trained to serve an artificial vagina for a week. Two ejaculates per male were collected weekly, with an interval of 3-4 days between successive ejaculation, over a period of 4 weeks from all males. The first ejaculate was used for spermiogram and the second ejaculate was centrifuged at 4000 rpm for 15 min to separate seminal plasma, and stored at -20^{0}C, until further analysis. Briefly, Libido was measured in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck ejaculated, Semen volume from each of the buck was measured using tuberculin syringe to the nearest 0.1ml. For Mass Activity, a drop of fresh semen was placed on a clean glass slide and examined with a microscope under x10 objective lens to determine mass activity. The mass activity was scored subjectively according to the intensity of the wave motion seen in the medium by the collective activities of spermatozoa, from the absence of wave motion (+) to very turbulent motions (+++) scored in percentage. For Sperm motility, a drop of semen with the aid of a micropipette was placed on a pre-warmed microscope slide and a drop of the diluent sodium citrate), was added before it was covered with a glass coverslip and examine at a magnification of ×400. The percentage of progressively motile spermatozoa was estimated and score subjectively between 0 and 100. At least 5 microscopic fields were examined for each semen sample. The sperm concentration was measured by the direct sperm cell count method, using improved Neubauer hemocytometer slide. Formal saline was mixed with semen at v/v dilution. The diluted semen was then charged on each of two ends of the hemocytometer using a micropipette. The charged hemocytometer was placed on the microscope at a magnification of ×400. The concentration of sperm per volume was determined using the formula: $C = 32{,}000 \times N \times D$. Where C = concentration of sperm cell per ml of semen, N = Number of spermatozoa counted, D = Dilution rate.

The structural membrane integrity of spermatozoa is an assay of liveability of spermatozoa. Thus it was determined to live to the dead ratio of sperm cells. It involved adding a drop of the staining solution Nigrosin–Eosin on a clean slide and a drop of undiluted semen, mixed gently to prepare a smear. The slide was air-dried and examined with a microscope at x 400 magnification. The functional membrane integrity was determined in semen samples in a 1:10 dilution of hypo-osmotic solution 75 mOsmol/L. To a warm 1ml swelling solution in a closed Eppendorf tube at 37˚C, 0.1 ml of liquefied semen was added and mixed gently within the tube. The mixture was kept at 37˚C for at least 30 minutes and sperm cells examined at X 400 magnification. Swelling of sperm cell was identified as changes in the shape of the tail. Counting in duplicate the number of swollen cells in a total of 200 spermatozoa counted and expressed as a percentage. The total motile sperm cell was calculated as the product of sperm concentration/ml and percentage motility of semen sample per animal. Total live sperm cell was obtained by a multiple of sperm concentration per ml and percentage livability.

Seminal biochemical indices

Seminal biochemical was determined using the spectrophotometric procedure of Randox commercial assay kits. Determination of seminal total antioxidant activities; The reactive mixture contained 0.5mL of a (10 mmol/L) Na-Benzoate, 0.2mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH=7.4) (prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na2HPO4 (100 mmol/L), then adjusted the pH to 7.4) and 0.2 mL of Fe-EDTA complex (2mmol/L) (prepared freshly by mixing equal volumes of EDTA (2mmol/L) and ammonium ferrous sulfate (2mmol/L), then left to stand at 25˚C for 60 min). Ten microliters of the blood serum were added to the latter reactive mixture and were incubated at 37˚C for 60 min.

Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50mmole/L NaOH) was added and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100˚C for 10 min. Total antioxidant capacity was calculated according to the following formula:

Total antioxidant activities (mmol/L) = $(CUA)(K-A)/(K-UA)$

Where: CUA: Concentration of uric acid (mmol/L); K: Absorbance of control (K1 - K0).

A: Absorbance of the sample (A1 - A0); UA: Absorbance of uric acid solution (UA1 - UA0).

Superoxide dismutase (SOD) activity was estimated by adding 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source and 0.86 ml of distilled water. The reaction is initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance monitored at 420 nm. One unit of SOD is defined as that amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50 % in standard assay system of 3 ml. The specific activity is expressed as units/min/mg protein.

Glutathione peroxidase activity (GPx) was estimated by adding to 0.5 ml 0.4 M buffer pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM GSH, 0.1 ml 0.2 mM H_2O_2 added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as µg of GSH consumed/min/mg protein.

Catalase activity assay system contains 1.9 ml 0.05 M buffer pH 7.0 and 1.0 ml 0.059 M H_2O_2. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm and activity is expressed as nmoles of H_2O_2 decomposed/ min/mg protein. Serum lipid peroxidation assay; the reaction mixture in a total volume of 3.0ml contained 1.0 ml serum, 1.0 ml of TCA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 minutes. The tubes were shifted to the ice bath and then centrifuged at 2500rpm for 10 minutes. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm.

Statistical analysis

Data were subjected to descriptive statistics, analysis of variance of the general linear model procedure to detect significant effects with a confidence level of 95%. Means were separated with New Duncan's multiple range test.

Results

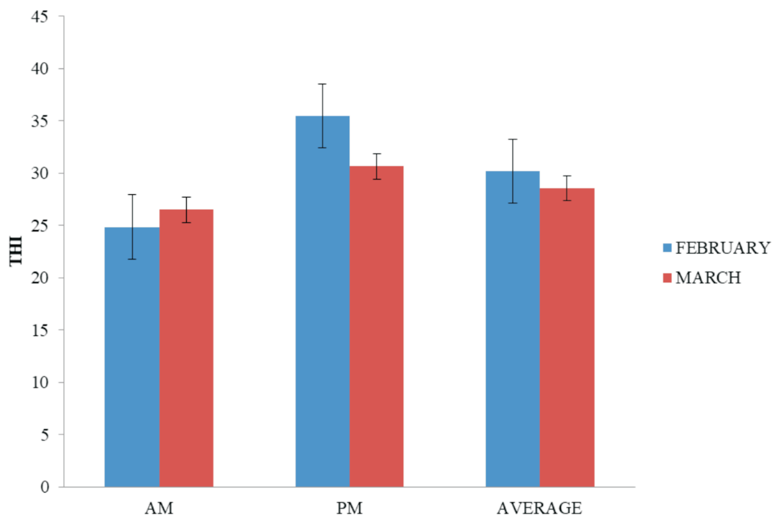

Figure 1: Temperature Humidity Index of Rabbit Pen during heat stress

Temperature humidity index of rabbit pen during during heat stress is presented in Figure 1. In the morning, THI range from 24.86 in February to 26.51 in march. In the evening THI obtained in February and March are 35.02 and 30.66 respectively. The average daily THI obtained in February and March are 29.94 and 28.59 respectively.

Table 1: Libido and semen quality of rabbits at three physiological age during heat stress

	AGE GROUPS			
Parameters	PUBERTAL	MATURE	ADULT	SEM
Libido (reaction time in sec)	8.65	9.22	10.29	0.40
Volume (ml)	0.48[ab]	0.56[a]	0.33[b]	0.04
Mass activity (%)	65.00[b]	81.67[a]	79.33[a]	3.67
Motility (%)	57.50[b]	72.15[a]	74.88[a]	2.93
Sperm concentration (x 10^8/ml)	6.90[b]	9.76[ab]	12.40[a]	0.94
Total motile spermatozoa (x10^8/ml)	3.90[b]	8.07[a]	9.25[a]	0.71
Total live spermatozoa (x10^8/ml)	6.44[b]	9.28[ab]	11.29[a]	0.89

abc: means in the same row with different superscripts are si gnificantly (P<0.05) different. SEM: Standard Error of Mean; Where pubertal rabbits are between 4 and 5 months old, Mature rabbits are between 7 and 9 months old, Adult rabbits are above 1 year old.

Libido and semen quality of rabbits of three physiological age during the peak of heat stress is shown in Table 1. Libido was fastest in pubertal bucks and slowest in the adult during the peak of heat stress. Although there was no significant (p>0.05) difference in libido among the age groups. All semen quality parameters were affected by the physiological ages upon exposure to heat stress. Mature bucks had the significantly (p<0.05) highest semen volume, mass activity, motility, sperm concentration, total motile and total live spermatozoa among the three age groups. The effect of heat stress was most severe in pubertal bucks as they recorded the significantly (p<0.05) least semen quality among the three age groups.

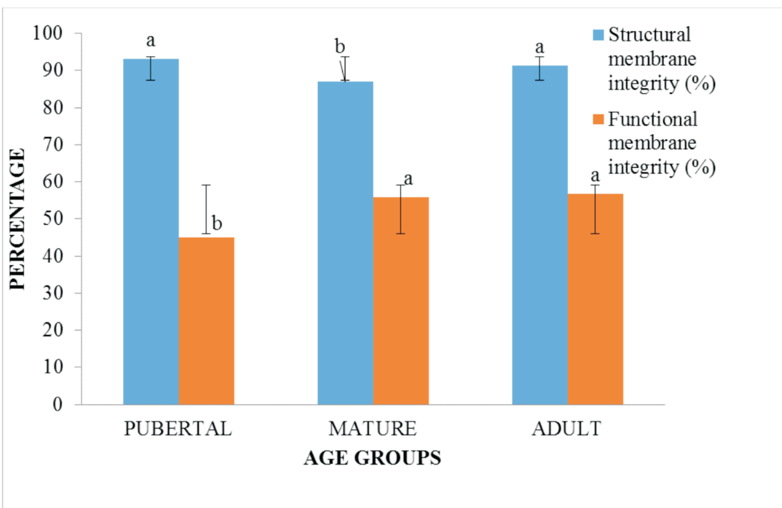

Figure 2: Structural and functional membrane integrity of sperm cells of rabbit buck at three physiological age during heat stress

Structural and functional membrane integrity of sperm cells of rabbit buck of three physiological age during the peak of heat stress is shown in Figure 2. Structural membrane integrity of mature buck spermatozoa during the peak of heat stress was significantly ($p<0.05$) lower than pubertal and adult bucks. However, functional membrane integrity of pubertal bucks spermatozoa during the peak of heat stress was significantly ($p<0.05$) lower than mature and adult bucks spermatozoa.

Seminal biochemistry of bucks of three physiological age during the peak of heat stress is shown in Table 2. Seminal cholesterol, magnesium, and chloride were not statistically influenced by the physiological age of bucks. However, seminal glucose and protein of pubertal bucks were significantly ($p<0.05$) low compared to the other age groups. Contrariwise, Seminal phosphorus, sodium, and potassium of bucks were significantly ($p<0.05$) highest at puberty compared to older age groups.

Table 2: Seminal biochemistry of rabbits at three physiological age during heat stress

	AGE GROUPS			
Parameters	PUBERTAL	MATURE	ADULT	SEM
Glucose (mg/dL)	20.74^{b}	23.13^{b}	52.57^{a}	7.09
Protein (mg/dL)	4.86^{b}	11.71^{a}	6.20^{b}	1.99
Cholesterol (mg/dL)	100.27	98.07	100.40	2.15
Magnesium (mg/dL)	1.25	1.24	1.28	0.05
Phosphorus (mg/dL)	9.20^{a}	6.37^{b}	6.6^{b}	0.66
Sodium (mEq/L)	66.74^{a}	34.05^{b}	59.99^{a}	7.51
Potasium (mEq/L)	65.33^{a}	38.21^{b}	39.99^{b}	4.26
Chloride (mEq/L)	85.91	80.72	74.17	9.33

abc: means in the same row with different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean, Where pubertal rabbits are between 4 and 5 months old, Mature rabbits are between 7 and 9 months old, Adult rabbits are above 1 year old.

Seminal oxidative status of bucks of three physiological age groups is presented in Table 3. Seminal lipid peroxidation was significantly ($p<0.05$) higher in mature and adult bucks compared with pubertal bucks. Seminal total antioxidant capacity was significantly ($p<0.05$) highest in pubertal bucks and the significantly ($p<0.05$) least values was obtained in mature bucks. Statistically similar superoxide dismutase activity was observed among the three age groups. Seminal catalase of pubertal and adult bucks was statistically similar and significantly ($p<0.05$) higher than mature bucks. Glutathione peroxidase in pubertal buck semen is significantly ($p<0.05$) higher than mature and adult buck semen.

Table 3: Seminal oxidant and antioxidant status of rabbits at three physiological age during heat stress

	AGE GROUPS			
Parameters	PUBERTAL	MATURE	ADULT	SEM
Lipid peroxidation (MDA/mg protein)	2.77^{b}	3.01^{a}	3.13^{a}	0.37
Total antioxidant capacity (mmol/litre)	0.43^{a}	0.19^{c}	0.35^{b}	0.08
Superoxide dismutase (u/min/mg protein)	0.93	1.05	1.18	0.10
Catalase(nm H_2O_2 /min/mg protein)	152.51^{a}	75.20^{b}	124.46^{a}	17.20
Glutathione peroxidase (µg gsh/min/mg protein)	47.01^{a}	36.87^{b}	33.72^{b}	4.82

abc: means in the same row with different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean, Where p ubertal rabbits are between 4 and 5 months old, Mature rabbits are between 7 and 10 months old, Adult rabbits are above 1 year old.

Discussion

Rabbit semen characteristics vary from one season to another (Marai *et al.* 2002). Several studies have found variations of rabbit sperm parameters depending on the environmental conditions (Casares-Crespo *et al*., 2016). Theau-Clement *et al.* (2015) observed that except for pH, all rabbit semen characteristics were influenced by the season, being sperm production higher in autumn. In addition, Schneidgenova *et al*. (2011) showed the seasonal e ect on the fertility traits of rabbit ejaculates and concluded that is necessary to evaluate the semen quality of bucks throughout the year. This variation could be due to di erences in seminal plasma or sperm membrane composition (Lavara *et al*., 2013). The wild rabbit is a seasonal breeder with the peak of reproductive activity occurring in the spring and early summer (Casares-Crespo *et al.,* 2016). However, domestication of rabbits, genetic selection, and environmental control have resulted in sexual activity throughout the year, with periods of reduced fertility, often noticed as a result of high summer temperatures (Casares-Crespo *et al*., 2016). Sexual maturity occurs approximately at 3-5 months depending on the breed and semen quality generally decreases in older rabbit bucks (Gogol *et al*., 2002). The result of this study corroborates Akpa *et al*. (2013) that semen volume and sperm concentration were higher in older red Sokoto bucks than pubertal goats. Decreased sperm chromatin stability was found in ejaculates taken from male rabbits less than 5 months and more than 20 months of age (Gogol *et al*., 2002). This could indicate that mature rabbits have preferred mating partners during heat stress than other age groups. Similar to this result that mature bucks had the highest semen volume, mass activity, motility, sperm concentration, total motile and total live spermatozoa among the three age groups. Changes in chromatin structure suggested a relatively high stability of sperm chromatin of rabbit between 6 and 16 months of age. (Gogol *et al*., 2002). The report of Habeeb *et al*. (1992) corroborates these findings that ejaculate volume, sperm motility, number of motile sperm per ejaculate, sperm-cell concentration and total sperm-output of the buck rabbits were affected by heat stress. The temporary sterility in buck rabbits may be produced by high temperature. Temperatures above 18 ^oC and incidence of infertility in male rabbits were found to be significantly correlated (Sammoggia, 1977) Seminal plasma is a promising source for the study of potential reproductive biomarkers, because it is a complex mixture of secretions from testis, epididymis and male accessory sex glands (Gonzalez-Cadavid *et al*. 2014). Species of mammals di er with the presence and size of accessory sexual glands, which obviously lead to variations in their relative contribution to semen composition and volume, particularly seminal plasma (Rodriguez-Martinez *et al.* 2011). Seminal fluid is very complex and plays an important role in the fertilizing ability of sperm (La Falci *et al*., 2002). In rabbits, seminal plasma has a positive e ect in maintaining sperm motility and viability during in vitro storage (Castellini *et al.,* 2000). This e ect has been associated with the antioxidant properties of seminal plasma (Arruda-Alencar *et al*., 2012). Casares-Crespo *et al*. (2016) reported possible existence of seasonal variations in the seminal plasma protein composition in rabbits. The result obtained in this work revealed seminal phosphorus, sodium, and potassium of bucks were highest at puberty compared to older age groups. Contrarily, Akpa (2013) reported that semen cation concentrations (Sodium, potassium calcium and phosphorus) was relatively higher in the older bucks 21 – 24 months than the younger bucks (9 – 16 months of age) except for sodium ion (Na+) which was also high in younger bucks of 9 – 16 month as observed in red Sokoto goat in Nigeria. This indicates species difference in seminal cation activity in male animals which could be due to the size of the accessory gland. This could account for the low seminal glucose and protein in pubertal bucks as compared with other bucks in this study.

Accumulated oxidative damage to mitochondria DNA, proteins and lipids may result in mutation, inactivation or loss of mitochondria DNA, synthesis of abnormal proteins, accumulation of oxidized dysfunctional proteins and changes in membrane lipid composition (Tarin, 1996). All these effects may inhibit mitochondrial replication, reduce the efficiency of the electron transport chain, increase the likelihood of ROS formation, inactivate key metabolic enzymes and increase membrane permeability, resulting in a reduction in the number of functionally-intact mitochondria and hence in ATP concentrations (Tarin, 1996). Increased production of ROS by defective mitochondria may decrease intracellular glutathione (GSH)/glutathione disulphide (GSSG) ratio (Tarin, 1996). Which in turn, may cause impairment of Ca^{2+} transport and subsequent perturbation of intracellular Ca^{2+} homeostasis, resulting in a sustained increase in cytosolic Ca^{2+} concentration (Beatrice *et al.,* 1984).

Conclusion

This study revealed that adult and mature bucks possess better semen quality at peak of heat stress compared to pubertal buck. Thus, they are better utilized for reproduction at periods of heat stress. However, pubertal bucks possess better oxidative stability than mature and adult bucks but this could not enhance its semen quality. This indicates that pubertal rabbit bucks possess high demand for antioxidant contributions to initiate reproductive features at puberty

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