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CHANGES IN SPERMIOGRAMS, BIOCHEMICAL AND PHYSIOLOGICAL INDICES FOLLOWING SUCCESSIVE ELECTROEJACULATION DURING DIFFERENT PERIODS OF THE DAY IN WEST AFRICAN DWARF BUCKS

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ABSTRACT

This study was conducted to investigate the changes that might occur in spermiograms, blood and physiological indices following successive electroejaculation (EE) during different periods of the day. Twenty (20) West African Dwarf (WAD) bucks were grouped into four consisting of five bucks each and ejaculated at 0900, 1200, 0300 and 0600Hrs for 3 successive days in a completely randomized design. The results showed that progressive sperm motility, sperm concentration and mass activity followed similar trend and the values deteriorated with respect to elevated temperatures during semen collection periods ($p<0.05$). Also, primary abnormality increased with respect to elevated temperatures during semen collection periods ($p<0.05$). The results also showed that Testosterone, Na^{+---}, K^{+---}, Glucose Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Aspartate Aminotransferase (AST) were similar irrespective of the periods of semen collection except cortisol that increased with respect to the semen collection periods ($p<0.05$). The rectal temperature and pulse rate were not affected, irrespective of the period of the day bucks were ejaculated. The findings of this study indicate that reduced ejaculate quality probably reflects stress stimuli arising from increased ambient temperature. Physiological traits in WAD goat is probably the adaptive mechanism evolved to cope with stress arising from elevated temperature.

Keywords: Ambient temperature, bucks, spermiograms, stress, successive ejaculation

INTRODUCTION

Handling, collection technique and period of the day semen is collected affect the reproductive potential of animal and semen characteristics in various animal species. Some physiological traits that have direct bearing with quality of ejaculates are known to be affected when bucks are stressed due to handling, method of ejaculation and elevated temperature during the time of collection (Marai *et al.,* 1997). Among all climatic elements, temperature is the most important parameter affecting spermatogenesis. Skinner and Louw (1966) reported that high ambient temperature causes a sharp reduction in semen quality with many abnormal sperm cells. Rectal temperature is one of the physiological parameters that indicate the body response to heat, cold and nutrition stress (Bianca, 1961). Taylor and Bogart (1988) reported that high ambient temperature increased the scrotal temperature in males and consequently a decline in semen quality due to elevated subcutaneous scrotal

temperature. Moreira et al (2001) reported that heat stress caused temporary interruption of sperm production and sperm motility and secondary defects seemed to be the most sensitive criteria in Santa Ines hair rams. Heart Rates during handling increases and animals are easily stressed by routine procedures. At rest, the heart rate can be affected by many factors such as stress, age, sex, size, posture, ingestion of food, emotion, body temperature and environmental factors such as ambient temperature, humidity and air movement (Devries and Housh, 1994). No information is available on the changes in spermiogramic and physiological indices following successive EE in WAD bucks. The objective of this study was therefore to determine the changes that might occur in spermiogramic biochemical and physiological indices following electroejaculation during different periods of the day in WAD bucks.

MATERIALS AND METHODS

The experiment was carried out at the Small Ruminant Unit of Department of Animal Production, University of Ilorin, in the Southern Guinea Savannah ecological zone of Nigeria. Twenty (20) West African Dwarf bucks were selected and managed under semi- intensive system. The bucks aged 11-18months and weighing 10-15 kg were fed with 400g concentrates each and supplemented with Guinea grass. The feed concentrate consisted of maize (30kg), soybeans meal (5g), groundnut cake (10g), wheat offal (25g), dried cassava peel (27g), bone meal (2g), Premix (0.5g), salt (0.5g). The electro ejaculator, a locally made electronic device consisting of a medium sized rectal probe, has a total length of 28cm and diameter of 1.3cm (body) and 1cm (at the tip) connected to an electrical unit which supplies electrical currents of small volts ranging from 0-12 volts with rheostats to control the amplitude of the delivered current and lots of circuiting to prevent accidental electrocution was used to collect the semen. The animals were randomly assigned into four groups comprising of 5 bucks per group and ejaculated at 0900, 1200, 1500 and 1800Hrs for 3 successive days.

Data collection and analysis

Upon application of the electrical stimulations graduated tubes were used to collect the semen. The volume of the semen collected was read directly from graduated collection tube.

Mass activity was determined within one minute of collection in a drop of concentrated semen without coverslip under low magnification (X4). The microscopic wave pattern was observed, ranging from slow to very rapid motion depending on the quality of the semen. The activity was graded as: 0= no mass activity; += slow wave motion; ++= rapid wave motion; +++= very wave motion. Progressive sperm motility was determined with a drop of semen in a drop of Sodium citrate under coverslip at a magnification of X10 as the percentage of sperm moving straight forward over the microscopic field. The concentration was determined by the use of an improved Neubauer haemocytometer. Semen was pipetted to the 0.5 mark on the pipette (using the red blood cell pipette) and this was made up to 1.01 marks on the pipette with normal saline. Normal saline serves both to dilute the semen and fix the spermatozoa present. The pipette was then introduced into pipette shaker and allowed to mix. About 2 to 3 drops of the diluents were discarded from the pipette before it was introduced into the counting chamber of the haemocytometer chamber for counting. The five squares that

formed the diagonal segment of the square were counted. The spermatozoa morphology was determined by staining a drop of semen with a drop of eosin solution. These were gently mixed together and a smear was made on another clean warm slide and air-dried. The slide was observed under a light microscope, (×400 magnifications) for the presence of abnormal sperm cells out of at least 600 sperm cells from several fields of the slides. The number of sperm cells and percentages of abnormal sperm cells were noted and recorded. Sperm cells that absorbed eosin solution were recorded as dead sperm cells.

A 7ml blood sample was collected by vein puncture from the jugular vein into anticoagulant free plastic tube after each of the insertion of the probe at every 3 hours interval for each of the groups. Serum was harvested from the blood sample by centrifuge for 15 minutes. The serum was kept in the refrigerator at -20^{0}C pending biochemical analysis. The analysis was done with Randox® test kit for Sodium, Potassium, Glucose, ALT, AST and ALP; Enzyme immunoassay test kit for Testosterone; and Radio Immunoassay kit for Cortisol.

The rectal temperature of each buck was taken by inserting the bulb of clinical thermometer into the rectum for 1 minute before reading. Respiratory count rates (RCR), Pulse rate (PR) and Heart rate (HR) were determined (Eriksson and Teräväinen, 1989). The RCR was determined using stethoscope to measure the number of echoes produced by lungs per minute. The PR was determined by placing stethoscope near the fore limbs close to the heart to measure the pulsation or sound of the heart produced per minute. The HR was determined using stethoscope to measure the number of sounds produced by the heart per minute. The scrotal temperature of each buck was taken by placing the bulb of the clinical thermometer on the scrotum for 1minute before reading.

Statistical Analysis

Data obtained from each buck were values of the mean for each collection period of the three days observations. Data collected were subjected to analysis of variance in completely randomized design and Duncan Multiple Range Test (Duncan, 1955) to separate the means.

RESULTS

Daily temperatures (^{o}C) at 09.00, 12.00, 15.00 and 18.00Hrs were 26.87 ±0.84, 32.77 ±0.74, 34.45 ±0.69, and 30.43 ±0.61respectively. Table 1 shows the results of the effect of period of ejaculation on sperm characteristics. Semen volume, mass activity, progressive sperm motility and sperm concentration followed similar trend. These parameters declined ($p<0.05$) from 0900h to 1800h and reached lowest value at 1500h when the ambient temperature reached maximum. Morphological abnormalities of the sperm cells increased ($p<0.05$) in response to elevated ambient temperature from 09.00h to 18.00hrs.

The effect of period of ejaculation on some biochemical parameters are presented in Table 2. The results showed that some biochemical parameters such as testosterone, Na^+, K^+, Glucose, ALP, ALT and AST were similar during the different periods of ejaculation. However, the values obtained for cortisol levels differed among the different time of ejaculation ($p<0.05$). The results showed that cortisol levels increased gradually with the times of ejaculation. Irrespective of the period of the day of ejaculation, the results

Table 1: Means (± SD) of semen characteristics of West African Dwarf bucks subjected to successive electroejaculation during different periods of the day

Parameters	0900Hr	1200Hr	1500Hr	1800Hr
Semen volume (ml)	0.29 ±0.13a	O.16 ±0.05b	0.11 ±0.21c	0.07 ±0.04c
Mass activity	1.59 ±0.51a	1.59 ±0.52a	1.55 ±0.39b	1.56 ±0.53b
Mass motility (%)	75.18 ±4.92a	71.93 ±4.99b	70.00 ±6.26b	70.60 ±5.83b
Sperm counts (x109)	2.06 ±0.20 a	1.88 ±0.16ab	1.75 ±0.07bc	1.51 ±0.04 c
Live: Dead sperm	7:2	6:3	7:3	7:2
Total abnormalities	4.53 ±2.79d	7.67 ±3.39c	11.46 ±4.30b	14.33 ±6.61a
% abnormalities	6.03	10.81	16.37	20.30

[a, b, c, d]: values along the rows with different superscripts are statistically different ($p<0.05$).

Table 2: Means (± SD) of serum biochemical parameters of West African Dwarf bucks subjected to successive electroejaculation during different periods of the day

Parameters	0900Hr	1200Hr	1500Hr	1800Hr
Testosterone ng/ml	2.71 ± 0.40	3.07 ± 0.51	3.21 ± 0.36	2.94 ±0.40
Cortisol (ng/ml)	3.04 ± 0.69c	3.55 ± 0.79b	3.81 ± 0.74b	4.80 ± 0.80a
Na+ (mg/ml)	132.87± 5.19	131.48± .79	134.37± 5.32	131.70 ± 5.24
K+ (mg/ml)	6.82 ± 0.30	6.91 ± 1.02	6.93 ± 0.88	6.34 ± 1.58
Glucose (mg/L)	2.47 ± 0.80	2.47 ± 0.49	2.77 ± 0.79	2.92 ± 0.63
ALP (IU/L)	224.59 ±61.78	217.52±78.83	202.06±67.28	217.60 ± 74.10
ALT (IU/L)	27.81 ±19.93	28.29 ± 3.27	38.85 ±17.50	38.00 ± 20.71
AST (IU/L)	10.80 ± 5.56	9.70 ± 43.16	13.38 ±60.60	13.11 ± 63.86

[a,b]. means with different superscripts within row differ ($p<0.05$).

Table 3: Means (± SD) of physioclinical parameters of West African Dwarf Bucks subjected to successive electroejaculation during different periods of the day

Parameters	0900Hr	1200Hr	1500Hr	1800Hr
Rectal temperature (OC)	39.87±0.31	40.03 ±0.22	40.04 ±0.68	40.19 ±0.19
Respiratory count rate (min)	89.53 ±18.38b	94.33 ±29.53b	104.67±19.36a	100.44 ±46.21a
Pulse rate (min)	91.30 ±2.95b	93.39 ±14.69b	97.89 ±13.75a	97.03 ±8.40a
Heart rate (min)	124.86 ±17.10c	128.04 ±22.10 b	132.67±15.24 a	128.89 ±16.17b
Scrotal temperature (OC)	36.01 ±0.79	35.82 ±0.73	35.82 ±0.61	35.10 ±0.35

[a,b]. means with different superscripts within row differ ($p<0.05$)

(Table 3) showed that physiological parameters such as scrotal temperature and pulse were similar ($p>0.05$). However, the heart rate, pulse rate and respiratory rate increased with elevated ambient temperature.

DISCUSSION

The decline in sperm characteristics such as mass activity, mass motility and sperm concentration were probably due to the elevated ambient temperature. The proportion of the sperm abnormalities located at different parts of the sperm cells (head, mid-piece and tail) increased concurrently in response to increased ambient temperature. The increase in morphological abnormalities in the samples collected at high ambient temperature probably indicate that the period of collection had deleterious effect on the testes or epididymis such as testicular degeneration. This agreed with the finding of Skinner and Louw (1966) that high ambient temperature causes a sharp reduction in semen quality with many abnormal sperm cells. The rise in primary abnormalities in the present study probably indicates that elevated ambient temperature resulted in the rapid release of immature spermatozoa.

The increase in the cortisol levels agreed with the previous finding that handling including the discomfort generated by the insertion of the probe into the rectum is known to elevate concentration levels of this hormone, and elevated cortisol levels which reflect stress stimuli following elevated ambient temperature have been observed on goats (Daramola *et al.,* 2008). However, the values observed in the present study were within the physiological range of 1-15ng/ml reported for several breeds of goats (Eriksson and Teräväinen, 1989; Mellor *et al.,* 1991), and were similar to observation of Daramola *et al*. (2008) that cortisol values that fall within the baseline values would indicate that the procedure is either low stress or very quick. Properly performed EE would seem not to be stressful while quick procedures would be completed before cortisol levels rise. In addition, several breeds of goats respond differently to the same stressor (Hart *et al.,* 1993), suggesting a different capacity to deal with stress or pain.

The WAD goat is hardy and thrives in harsh conditions of management (Adeloye, 1998). This is largely possible because of the innate adaptogenic power of the animal to react to particular quantum stressors (Adeloye and Daramola, 2004). The terms 'hardiness' and 'adaptability' are attached to the WAD because of its ability to inhabit a harsh environment (Adeloye and Daramola, 2004). This physiological mechanism probably plays an important role in the evolved adaptation of this goat type to pain or stress caused by electroejaculation. Apparently, the higher cortisol levels observed could be a result of stress caused by increase in ambient temperature. The rise in the cortisol levels suggested that the air temperature was at maximum, similar to the reports of Ortiz-de -Montellano *et al.* (2007) and therefore reflects stress stimuli due to elevated ambient temperature.

A major change in the concentration of sodium or potassium ions in the seminal plasma has been reported as an indication of disturbed sperm motility and reduced viability in humans (Skandhan and Mazumdar, 1981). Serum Na^+ in the study fell within the normal values obtained in WAD goats (Daramola *et al.,* 2005). In addition, no marked change in serum Na^+ and K^{+-} concentrations were observed in all the tested groups indicating that electrolyte balance during the experimental period were similar. The study showed that effect of period of electroejaculation on the blood glucose level in the WAD goats was not pronounced and the values obtained were comparable with 3.12 ± 0.25 reported for Iranian goat (Nazifi *et al.,* 2000). Measurement of enzyme activities which leak into extracellular medium through injured sperm membranes had been suggested as a marker to estimate cellular damage occurring during cryopreservation (Graham; Pace, 1967). AAT and AST activities measured were similar at the different periods indicating that membrane damage or altered membrane function did not occur; hence epididymal maturation was not affected by successive and period of collection. In contrast, Strzežek *et al.* (1995) reported the biochemical changes in spermatozoa similar to apoptosis in the somatic cells and that reduced ALT activity in boars as a consequence of frequent ejaculation. The ALP, ALT and AST in this experiment were similar in all the treatment groups and this indicated that the electric stimulation and elevated ambient temperature did not cause any damage or stress to the liver cells (Coles, 1986; Oyeyemi *et al.,* 2000; Daramola *et al.,* 2005).

The results of the rectal temperature obtained in this study compared favourably with the findings of Gamble and Clough (1976) who reported 39.5 ± 0.5^0C. The results showed increase in the respiratory count rate (RCR) with increasing ambient temperature intensity and the values were higher than that recorded by Gamble and Clough, (1976), (10 – 16 beats/minute) and Boden (1999) (20 – 24 beats/minute). Although RCR level can be influenced by age, posture, size, sex and health status of the animals (Gamble and Clough, 1976; Boden, 1999), the animals in this study were apparently healthy and these parameters could not have been influenced by these factors. The pulse rate in this study was higher than that reported by Boden (1999) (70-80 beats/ minute) at rest, but compared favorably with that of Frazer (1986) with average of 90 beats/minute for goats. Also, as the ambient temperature increases, the pulse rate as well as the circulation of blood increases to transfer heat from the interior of the body to the surface. The heart rate values recorded in

this study showed that it increased with increasing intensity of ambient temperature. The ambient temperature probably affected the heart rate and the total cardiovascular response of the animal has an increase of beats/minute and this depends on the magnitude of the temperature rise (Devries and Housh, 1994).

CONCLUSION

The findings of this study indicate that reduced ejaculate quality probably reflects stress stimuli arising from increased ambient temperature. Physiological traits in WAD goat are probably the adaptive mechanism evolved to cope with stress arising from elevated temperature.

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