

## Changes in serum cortisol concentrations in West African Dwarf (WAD) bucks during electroejaculation

Daramola<sup>a\*</sup>, J. O., Adeloye<sup>b</sup>, A. A., Balogun<sup>b</sup>, Ife A., Yousuf<sup>b</sup>, M. B., Olatunde<sup>b</sup>, A. O. and Abiona<sup>a</sup>, J. A.



<sup>a</sup>Department of Animal Physiology, University of Agriculture, Abeokuta, Nigeria



<sup>b</sup>Department of Animal Production, University of Ilorin, Ilorin, Nigeria

Abstract

\*daramolajames2003@yahoo.com

*Changes in the concentration of serum cortisol in blood samples of twenty healthy West African Dwarf (WAD) bucks were determined during electro-ejaculation. The animals were randomly assigned to one of two treatment groups. Group I consisted of bucks that had rectal probe inserted but were not electroejaculated (NEE), and group II consisted of bucks that were electroejaculated (EE) in the morning (0900hr), afternoon (1400hr) and late afternoon (1800hr). The results showed that serum cortisol values found in both groups were similar ( $P > 0.05$ ). Increase in cortisol levels were observed ( $P < 0.05$ ) in the afternoon in both EE and NEE animals in comparison with morning and late afternoon. The findings of this study indicate that the increase observed in cortisol concentrations in the afternoon in both groups reflects stress stimuli due to slight increase in ambient temperature.*

**Keywords:** Ambient temperature; Bucks; Cortisol; Electroejaculation; Stress

### Introduction

Electroejaculation (EE) is a technique widely used to collect semen in ruminants, which produces a stress response with negative effects on animal welfare (Damián and Ungerfel, 2010). Animal welfare concerns have been expressed regarding the use of EE as a semen collection technique (Orihuela *et al.* 2009). There is evidence that stress due to EE, one of the techniques of collection of semen has implication on animal welfare (Damián and Ungerfel, 2010), and semen characteristics in various animal species can be influenced by factors such as techniques of collection and frequency of collection (Hafez, 1980). It has also been reported that during EE, electricity application induces major changes in cortisol concentration in animal (Orihuela *et al.* 2009) due to the stress which the animal is subjected to through the method (Mosure *et al.* 1998; Carter *et al.* 1990). Behavioural patterns due to the effect of electro-ejaculation include animal

vocalizing, struggling, lying down or displaying strong muscular contractions (Mosure *et al.* 1998; Carter *et al.* 1990). Electroejaculation without anesthesia in humans is known to be painful and stressful (Ver Voort, 1987). Therefore, it may be assumed that electro ejaculation is painful to animals, although aversion tests indicated that goats (Carter *et al.* 1990), rams (Cook, 1996) and bulls (Barth and Bowman, 1994) in general did not show aversion to handlers or the restraint facilities when electroejaculation was carried out frequently. Elevated cortisol levels which reflect stress stimuli following successive electroejaculation have been observed on Criollo goats (Ortiz-de-Montellano *et al.* 2007). Electricity application during EE has also been reported to induce major changes in cortisol concentration in hair rams (Orihuela *et al.* 2009), and when rams are electroejaculated, an important stress response including changes in cortisol

concentrations and heart rate has been observed, suggesting that rams were not habituated to EE (Damián and Ungerfel, 2010).

There is little information on the stress responses of WAD bucks, particularly when effects due to electrical stimulation are considered in relation to the welfare implications during the EE process and no information is available about the relationship of cortisol concentration and electroejaculation in WAD bucks. Thus, this study was designed to evaluate changes in blood cortisol concentration in WAD bucks in response to electro-ejaculation.

### Materials and Methods

Twenty healthy WAD bucks aged between 11-18 months and weighing 10-15kg were raised under a semi-intensive method of management in the Southern Guinea Savannah ecological zone of Nigeria. The bucks were fed with concentrate which was supplemented with *Panicum maximum*. The ambient temperatures during the period of the study were 26.6 ° C (morning: 0900hr), 33.1 ° C (afternoon: 0900hr) and 30.7 ° C (late afternoon: 0900hr) with a corresponding relative humidity of 72%, 40% and 52% respectively.

The electro-ejaculator used consisted of a woody rectal probe with a total length of 13cm, a diameter of 1.3cm (body) embedded with electrical element with a resistivity that allows measurable quantity of electrical current of micro-amp connected to step-wise transformer (to control the amplitude of the delivered current), operating at 18Hz with fully controlled output voltage from 0-12V. The bucks were randomly divided into two groups, each consisting of ten (10) bucks. Group I consisted of bucks which had a rectal probe inserted but not electroejaculated (NEE). Group II

consisted of bucks that were electroejaculated (EE) in the morning, afternoon and late afternoon respectively. The probe was lubricated and inserted 7 to 9cm into the rectum such that the electrode lies within the pelvic cavity. Each EE animal received a regimented electroejaculation sequence from a 18Hz AC electrical stimulator. The one day experiment was carried out three times at intervals of 3 hours for each group. Electrical stimulation was applied in EE group at intervals of 3-5 seconds alternated with the rest periods of similar duration. The current was gradually increased until semen was produced. The welfare of the animals was taken into consideration. The technique (voltage and frequency) applied during the experiment resulted in minimum involvement of voluntary muscles (Carter *et al.* 1990). Animals were held in standing position during the treatments. The entire procedure was performed in approximately 1 minute.

### Data collection

Blood sample (5ml) was collected by venipuncture from the jugular vein into anticoagulant free plastic tubes after each application. The samples were centrifuged for 15 minutes to separate the serum from plasma. The blood serum was stored at 20°C till serum cortisol was analyzed using a Radio Immuno Assay kit (IC Biomedicals, Costa Mesa, CA). The assay uses cortisol calibrators in human serum and rabbit antibodies and validated in goats (Sahlu *et al.* 1992).

### Statistical analysis

Data collected were subjected to analysis of variance in a 2 x 3 factorial arrangement (application of EE: NEE and EE; and period of application: morning, afternoon and late afternoon) using the following model:

Table 1: Mean ( $\pm$  SE) changes in concentrations of serum cortisol of WAD bucks electroejaculated at different times of the day.

Period of day	EE (ng/ml)	NEE (ng/ml)
Morning	2.64 $\pm$ 0.19 <sup>b</sup>	2.78 $\pm$ 0.34 <sup>b</sup>
Afternoon	4.26 $\pm$ 0.90 <sup>a</sup>	3.86 $\pm$ 0.80 <sup>a</sup>
Late afternoon	2.34 $\pm$ 0.50 <sup>c</sup>	2.34 $\pm$ 0.24 <sup>c</sup>

<sup>a,b,c</sup> Mean values with different superscripts within rows differ (P<0.05)

$$\hat{Y}_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \Sigma_{ijk}$$

Where  $\hat{Y}_{ijk}$  = level of serum cortisol;

$\mu$  = population mean;

$\alpha_i$  =  $i$ th effect of EE treatment;

$\beta_j$  =  $j$ th effect of time of EE ( $j$  = morning, afternoon and late afternoon);

$\alpha\beta_{ij}$  = interaction effect of EE treatment and time of EE;

and  $\Sigma_{ijk}$  = residual error.

Means were separated using Duncan Multiple Range Test (Duncan, 1955).

### Results and Discussion

The results (Table1) of this study showed that the cortisol levels after the first insertion of the rectal probes and post

insertion in EE and NEE were similar. This suggested that handling, including the discomfort generated by insertion of the rectal probe was not enough to elevate the concentration of this hormone, perhaps due to the habitual handling and human presence that these animals were used to. Carter *et al.* (1990) observed that confident sexually mature active bucks did not resist entering the collection compound. Carter *et al.* (1990) however in the same study, observed individuals that did not adjust to EE and displayed signs of aversion to repetitive EE program. Cortisol levels are highly variable (Grandin, 1997), and absolute comparison between studies may be difficult.

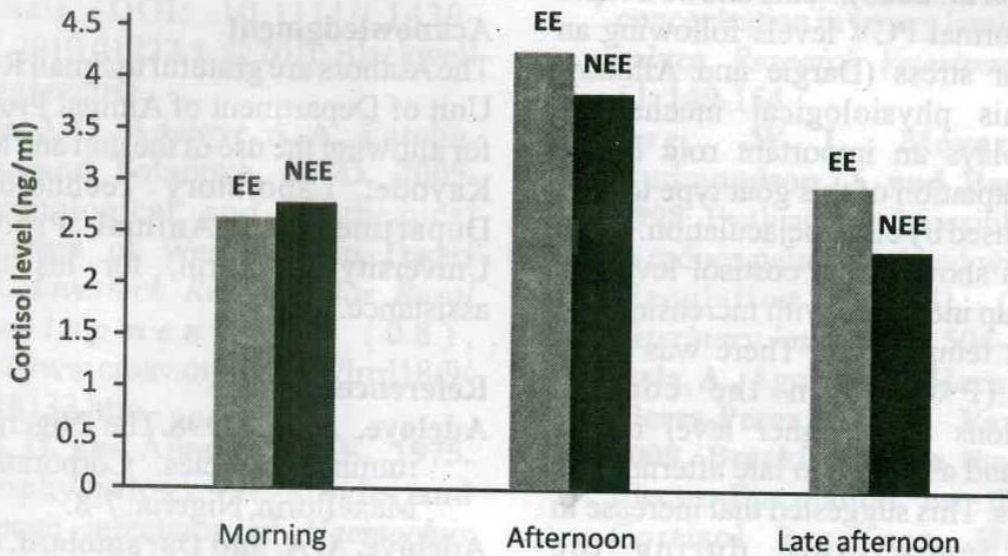


Figure 1: Effect of ejaculation time on serum cortisol levels

Although cortisol levels increased to a mean of 4.26ng/ml in EE compared with 3.86ng/ml in NEE, all values were within the physiological range of 1-15ng/ml reported for several breeds of goats (Eriksson and Teravaine, 1989; Mellor, 1991), and would indicate that the procedure was either low stress or very quick. Properly performed EE would seem to be less stressful while quick procedures would be completed before cortisol levels rise and this is in line with report of Lay *et al.* (1992) that it takes 10-20 minutes for cortisol concentration to peak in circulation after a stressor is imposed. Moreover, 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 WAD goat has a tendency for compensatory accelerated production (CAP) of PCV in case of infection or stress (Daramola *et al.* 2005). This allows a rapid return to normal PCV levels following an infection or stress (Dargie and Allonby, 1975). This physiological mechanism probably plays an important role in the evolved adaptation of this goat type to pain or stress caused by electroejaculation.

The results showed that cortisol levels in the EE group increased with increasing rise in ambient temperature. There was sharp increase ( $P < 0.05$ ) in the cortisol concentrations to a higher level in the afternoon and a decline in late afternoon in both groups. This suggested that increase in ambient temperature during the experimental period triggered the rise in concentration of the hormone. Obviously, the increase in cortisol concentrations

measured after ejaculation could not have been influenced by stress due to electroejaculation, since cortisol release observed was higher only in the afternoon in both groups when ambient temperature increased but did not differ from cortisol release observed in the morning and late afternoon. Apparently, the higher cortisol levels observed after the first insertion and ejaculation could be a result of stress caused by slight increase in ambient temperature. In contrast, Ortiz-de-Montellano *et al.* (2007) in a similar study with Criollo goats however attributed increase in serum cortisol concentration after EE to acute stress response due to EE application. The rise in the cortisol levels in the afternoon in this present study suggested that the air temperature was at maximum.

### Conclusion

The findings of this study indicate that the increase observed in cortisol concentrations in the afternoon in both groups reflects stress stimuli due to elevated ambient temperature and not necessarily due to the application of EE.

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