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Breaking down the Effect of Electro-Ejaculation on the Serum Cortisol Response, Heart and Respiratory Rates in Hair Sheep (*Ovis aries*)

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Abstract: Animal welfare concerns have been expressed regarding the use of Electro-Ejaculation (EE) as a semen collection technique. Furthermore, there is little information on the stress responses of hair sheep, particularly when effects due to physical manipulation and electrical stimulation are disaggregated during the EE process. Therefore in the present experiment, changes in concentration of serum cortisol in serial blood samples, Heart (HR) and Respiratory Rates (RR) rates were used to quantify stress response to EE in hair sheep males. Eighteen intact F1 Dorper/St. Croix rams aged 12-13 months were randomly assigned to one of three groups: a control group where, no stimulus was applied, compared to rams that a rectal probe was inserted without EE (T_1) and males that were electro-ejaculated (T_2). Blood samples were collected by venipuncture at 0 (immediately before inserting the rectal probe) 20, 40, 70 and 100 min after the onset of the experiment. At these same intervals, RR was measured, while HR was recorded continuously. Higher ($p<0.05$) HR was found in T_2 in comparison with T_1 and controls. The highest rate (218 beats min^{-1}) was observed in T_2, 3 min after electrical stimulation was applied, returning to baseline rhythm 45 min after the start of recording. A significant ($p<0.05$) increase in serum cortisol was detected 20 min after stimulation in T_2 when compared with controls and T_1. No difference ($p>0.05$) was found in RR among or within groups. It was concluded that during EE, electricity application induces major changes in HR and cortisol concentration in hair rams, while manipulation, including probe insertion does not contribute significantly to this effect.

Key words: Hair sheep, welfare, electro-ejaculation, cortisol, heart rate

INTRODUCTION

Electro-Ejaculation (EE) has been used for the collection of semen from rams for many years since its introduction in Australia by Gunn (1936) and at that time was described to be simple and convenient. This method has been recommended for rams that refuse to serve the artificial vagina because of debility or lack of libido (Rasbech, 1993). However, EE can induce a great number of collection failures (Cameron, 1977) and has been recently described as a stressful technique on the ram by Ax *et al.* (2000).

In America (Palmer, 2005) and tropical latitudes, EE is still considered an acceptable procedure by most animal welfare committees, even though, it has been banned in several European countries. Consequently, its use has become controversial. The debate has been fueled by a lack of critical evidence to define the reaction of sheep to this Semen collection method as well as some conflicting results. For example, rams subjected to aversion tests did not show aversion to handlers or the restraint facilities when EE was carried out frequently (Cook, 1996). Furthermore, in other studies (Stafford, 1995), EE tended to be slightly less aversive than being restrained for partial shearing.

Information concerning the cortisol stress response to EE in sheep is scarce and inconclusive. Furthermore, no information is available about the relationship of cortisol concentration or other physiological measures and EE in hair sheep. Thus, the present experiment was designed to evaluate blood cortisol concentration, heart and respiratory rates in hair sheep in response to EE, disaggregating effects due to the physical manipulation and the electrical stimulation involved during this semen collection procedure.

MATERIALS AND METHODS

Experimental materials and procedures: The study was carried out at the University of Morelos, Mexico 18°37'N

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and 99°19'W, situated 899 m above sea level and with an average annual rainfall and temperature of 800 mm and 23°C.

The subjects were 18 intact F_1 Doper/St. Croix (hair sheep; *Ovis aries*) rams aged 12-13 months and weighing 68.5±5.6 kg, well adapted to human presence and subjected to EE before. The population was maintained as a single all-male group since weaning, approximately, 3 months of age throughout the duration of this study and fed 600 g of a commercial concentrate per day with 14% protein (NU3®, Mexico) and 3 kg of fresh Taiwan grass per animal, to cover maintenance requirements. Mineral salt and water were offered *ad libitum*.

Rams were assigned randomly to one of three groups: controls, where no stimulus was applied and a second group in which, a rectal probe was inserted into the rectum, held in position for 2 min while, no electricity was applied (T_1) and thirdly, subjected to EE once (T_2), using the same rectal probe, but applying an electrical stimulus as described later.

All treatments were applied in the rams' home pen. The animals were restrained at the periphery of the pen facing outside by the use of portable stanchions attached to the floor, separated 4 m from each neighbor. Each stanchion was built with solid lateral panels to avoid visual contact among the animals during treatment. A CGS electrojector, model 500 M1 (Ratek Instruments Pty Ltd., Thornton Cr, Mitcham, Vic 3132. Victoria, Australia) sine-wave equipment was used throughout the experiment, operating at approximately 18 Hz with a fully controlled output voltage from 0-15 V root mean square (RMS, DC equivalent of sinusoidal waveform) that is 43 V peak to peak.

The rectal probe used in T_1 and T_2 was 22 cm long and 2.5 cm in diameter, comprising two electrodes placed longitudinally on the cylinder. The electrodes were separated by an angle of approximately 100° of arc on the body of the probe. The handle was a flexible hose of smaller diameter so that the probe when, fully inserted into the rectum did not cause undue stretching of the anal sphincter. Obstetrical lubricant was applied to the probe and to the anal sphincter before insertion to minimize trauma.

The machine was used on manual control, which allowed the operator to vary the voltage applied to the probe. Electrical stimulation was applied in T_2 for intervals of 3-5 sec and alternated with rest periods of similar duration. With each stimulation, the current was gradually increased until semen was produced. The entire procedure was performed in approximately, 1 min. A mean maximum voltage of 5.0 V produced ejaculation.

Rams were held in a standing position during collection. In this position, the penis was easily grasped and held in the end of a calibrated centrifuge tube.

Heart Rate (HR) was measured using a battery operated HR monitor (Polar S610™. Oulu, Finland). HR electrodes were placed over the scapular and heart apex areas 30 min before the onset of the experiment. These areas were earlier shaved and cleaned with 70% alcohol. To facilitate effective transmission, an ultrasound gel was applied between the electrodes and the skin. Flexible elastic bandage wrap around the thorax protected the HR transmitter and electrodes from dislocation. HR for each animal was recorded continuously for approximately, 60 min, beginning after probe insertion. This delay was thought to provide a sufficient period for the animals to adjust physiologically to the instrumentation and to obtain stable HR.

A 5 mL blood sample was collected by venipuncture from the jugular vein into vacationers at: 0, 20, 40, 70 and 100 min (immediately before inserting the rectal probe) after insertion of the probe. The samples were held in an ice bath (no >30 min) until centrifugation (1500 rpm for 15 min) was achieved to separate serum from plasma. One person collected all samples. Blood serum was stored at -20°C pending analysis.

Cortisol concentrations were determined in duplicate using commercial, coated tube radioimmunoassay kits (Pantex, Santa Monica, CA) according to the method of Jephcott *et al.* (1986). The between-assay coefficient of variation was 3.06%.

Respiratory Rate (RR) was determined by the use of a stethoscope immediately before obtaining the blood samples.

Control observations were collected following the same schedule as treated groups. To avoid variations due to ambient conditions or diurnal rhythms of metabolic variables (Lefcourt *et al.*, 1999) all recordings (except for HR) were taken in the same order (among treatments) from one ram at random within each group, repeating the progression at the established sample times (0, 20, 40, 70 and 100 min) until all animals from the three groups were tested.

Statistical analysis: The area under the HR curves was estimated using the Trapezoidal rule (numerical approximation method proposed by Purves (1992)) and subjected to ANOVA (Analysis of Variance) (SAS, 1985) for comparison among treatments.

To compare HR, blood cortisol concentration and RR among treatments and the sequential data recorded on the same set of animals, repeated measures analysis of variance model was used:

$$Y_{ik} = \mu + \alpha_i + \beta_k + (\alpha\beta)_{ik} + E_{(ik)}$$

where, $n_1 = 6$; $n_2 = 6$; $n_3 = 6$; i = 1, 2, 3: effect of the ith treatment (T_0-T_2); k is 1-5 (sample points), μ is Mean of the distribution of Y, α_i is effects of the two treatments (A), β_k is effect of time at the various sampling points in the process of repeated measurement of the subjects (B), $(\alpha\beta)_{ik}$ is the interaction of A and B and $E_{(ik)}$ is the residual error (SAS, 1985).

The means were compared for significance by Tukey's test (Snedecor and Cochran, 1989) at $p<0.05$. Heart rate values corresponding to the established sample points (0, 20, 40. 70 and 100 min) of the other 2 variables (RR and cortisol) were estimated from the continuous data registered.

RESULTS AND DISCUSSION

Heart rate: Higher ($p<0.05$) HR was found in T_2 in comparison with T_1 and controls. The highest rate (mean value = 218 beats min^{-1}) was observed in T_2, 3 min after electrical stimulation was applied, returning to baseline rhythm 45 min later. No difference ($p>0.05$) was found between HR of animals in controls and T_1. However, sporadic peaks were observed in both groups during the experiment (Fig. 1).

Cortisol concentration: A significant ($p<0.05$) increase in serum cortisol was detected 20 min after stimulation in T_2 when compared with controls and T_1. In addition, no difference was found in serum cortisol over time following EE for the controls and T_1 treatments (Fig. 2).

Respiration rate: No difference ($p>0.05$) was found in respiratory rate among or within groups. However, a non significant ($p>0.05$) trend for higher RR (78.67±10.41 breaths min^{-1}) was found in T_2, 20 min after the onset of the experiment, in comparison with controls and T_1 (54.67±4.46 and 65.33±9.39, respectively; Fig. 2).

Heart rate: Heart rate changes associated with EE have been assumed to be due to a combination of both muscle contraction and pain (Mosure *et al*., 1998). Strong muscular contractions should be regarded as painful (Carter *et al*., 1990) when, applying EE. Consequently, HR can be a useful measure of welfare (Manteca, 1998).

In the present experiment, changes due to the physical insertion of the probe were distinguished from changes caused by electrical stimulation by measuring HR levels of the same animals before (0 min) and after stimulation and comparing these results with levels in T_1 and control treatments. The fact that no differences were found in HR levels between controls and T_1 suggest that the electrical stimulation is responsible for the increase in HR.

Heart rate levels in controls and before stimulation in T_1 and T_2 were similar and comparable to those reported by Lumbers and Yu (1999) in calm animals, while increments of 283% observed in T_2 were only comparable with those data observed by Crossley *et al*. (1988) after IV injection of 0.5 mg of adrenaline or by Hays and Webster (1971) with sheep under cold stress (-20°C).

The relatively long intervals before HR returned to baseline levels in rams subjected to EE also supports the idea that animals in this group were under severe physical stress. In comparison, a similar study, bull's HR returned to baseline levels within 2 min of cessation of electrical stimulation (in the context of EE), while mean changes in HR ranged from 9.2-21.8 beats min^{-1} (Palmer, 2005).

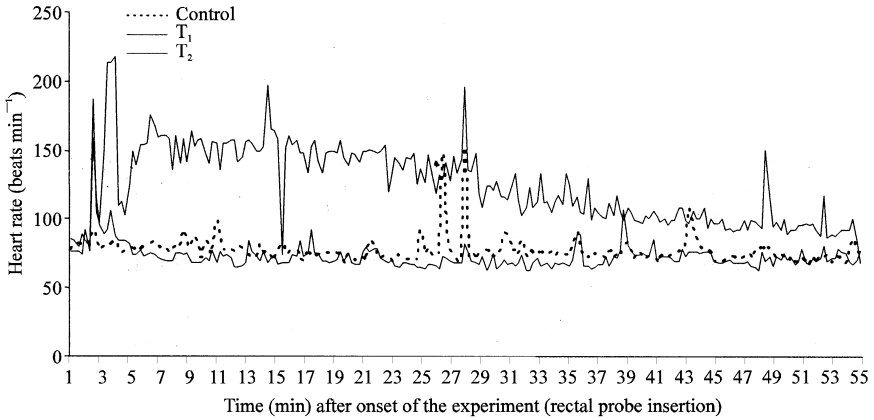

Fig. 1: Profiles of mean heart rate min^{-1} among groups of rams when no probe was inserted (Control), a rectal probe was inserted (T_1) and when a probe was inserted and electrical stimulation applied (T_2)

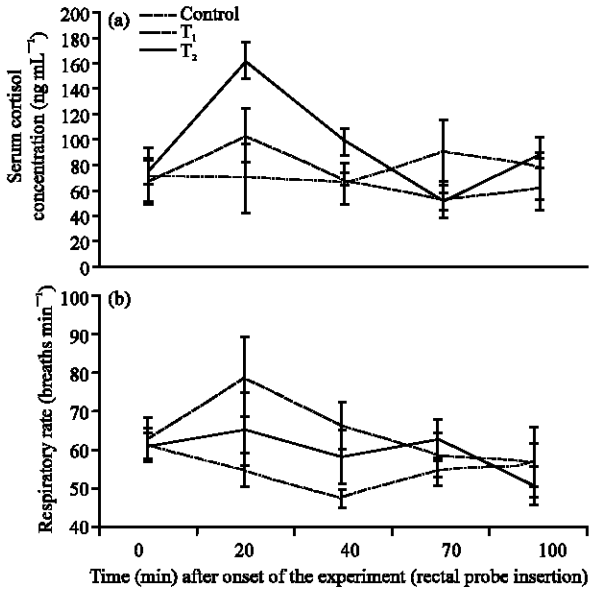

Fig. 2: Profiles of mean serum cortisol concentration, a): and respiratory rates and b): among groups of rams when no probe was inserted (Control), a rectal probe was inserted (T_1) and when a probe was inserted and electrical stimulation applied (T_2)

Cortisol concentration: Cortisol is often used in stress and welfare assessments (Cook and Jacobson, 1995; Cook, 1996; Molony and Kent, 1997). However, it is important to consider that the nature of the aversive stimulus may influence the animal's reaction to it. For instance, whereas, anxiety is generally believed to cause an increase in levels of glucocorticoid (Mason, 1971), pain does not reliably result in such an increase (Rushen, 1986a; Bateson, 1991). Furthermore, differences among species and even among breeds (Ortiz-de-Montellano *et al*., 2007) should be taken into account. For example, profiles of mean cortisol concentration were similar between groups of bulls when a rectal probe was inserted and when a probe was inserted and electrical stimulation applied (Welsh and Johnson, 1981). Since in the present study, a significant difference was observed between treatments.

Respiration rate: In the present experiment, no effect was detected in RR among treatments, perhaps because the first sample after the stimulus was taken 20 min later. Based on the non significant increase found 20 min after EE, it could be possible that RR increased immediately after the treatment was applied and decreased before this period.

The fact that all animals in the present experiment were well adapted to human presence and subjected to EE before, could be a reason for the lack of differences between T_1 and T_0, a degree of habitation to the handling procedure might happen. Different results could be expected from naive rams. Aversion-learning techniques and preference testing, however are not without problems and these have been summarized by Rushen (1986b) and Fraser and Matthews (1977).

Individual differences and breed differences in learning and memory ability could also be mistaken for individual or breed differences in reaction to the treatment (Rushen, 1990). Observations by Carter *et al.* (1990) indicate that confident, sexually active bucks do not resist entering the collection compound and show no signs of aversion to a repetitive EE program. However, individuals that they identified with temperament difficulties did not adjust to EE and displayed signs of aversion to repeated treatments.

Increases in respiration rate and cardiac function allow the animal to meet physical or emotional challenges by its effects on metabolic rate (Hemsworth and Barnett, 2000). These two rates increase as an immediate response to a stressor.

Lack of effect in HR and RR together with a moderate increase in serum cortisol levels suggests that inserting a rectal probe into the rectum and holding it in position for 2 min (T_1) does not significantly affect the animals. Nevertheless, the combination of high HR for long periods together with a significant increase in cortisol concentration and a non significant rise in HR in the T_2 (EE) treatment suggests that electric stimulation associated with the EE process induces considerable stress in hair sheep.

CONCLUSION

It was concluded that during EE, electricity application induces major changes in HR and cortisol concentration in hair rams, while manipulation, including probe insertion does not contribute significantly to this effect.

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