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Effect of Anesthesia on Welfare Aspects of Hair Sheep (*Ovis aries*) During Electro-Ejaculation

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Abstract: Animal welfare concern has been expressed regarding the use of Electro-Ejaculation (EE) as a semen collection technique. In the present experiment, the effect of anesthesia on measures of welfare of hair sheep (*Ovis aries*) during EE was evaluate. Twenty intact F1 Dorper/St. Croix rams aged 12-13 months were randomly assigned to one of 2 groups: Controls (T_0) and animals receiving im injection of xylazine and ketamine (T_1). All animals were electro-ejaculated once. Changes in cortisol concentration, Heart (HR) and Respiratory Rate (RR) were used to quantify the stress response to EE. A peak of >200 beats min^{-1} was observed at the time of EE in both groups. Anesthetized rams in T_1 returned to their basal heart rate within 4 min after this peak, while the heart rate of rams in the T_0 group remained relatively high 30 min after EE. Serum cortisol levels were significantly higher ($p<0.05$) in the T_0 group than in the T_1 rams 20 min after EE (161.57±14.37 and 110.60±11.74 ng mL^{-1} in T_0 and T_1, respectively). No differences in the respiratory rate variable ($p>0.05$) were observed between or within groups. It was concluded that anesthetic treatment during EE improved 2 of 3 measures of welfare (heart rate and cortisol concentration). These findings provide support for the use of anaesthetics when collecting semen in hair sheep by EE.

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

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). This technique has been described as simple and convenient and has been recommended when rams refuse to serve an artificial vagina because of debility or lack of libido (Rasbech, 1993). However, EE sometimes results in collection failures (Cameron, 1977) and has been recently, described as a stressful when used with rams (Ax *et al*., 2000).

In America (Palmer, 2005) and tropical latitudes, EE is still considered an acceptable procedure by most animal welfare committees. However, in several European countries, EE without anesthesia in bulls has been discouraged (Mosure *et al*., 1998).

In previous studies (Orihuela *et al*., 2008), our group demonstrated that EE induced severe stress in tropical hair sheep (*Ovis aries*), supported with data on heart rate and cortisol concentration. We therefore, conducted the following experiment to evaluate the effect of ketamine and xylazine anesthesia on several measures of welfare in hair sheep during EE.

MATERIALS AND METHODS

The study was carried out at the University of Morelos, Mexico 18°37'N 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 20 intact F1 Dorper/St. Croix (hair sheep; *Ovis aries*) rams aged 12-13 months and with and average weight of 68.5±5.6 kg. They were fed 600 g of a commercial concentrate per day with 14% protein (NU3®, Mexico) and 2 kg of fresh Taiwan grass per animal. Mineral salt and water were offered *ad libitum*.

The population was maintained as a single all-male group from weaning at approximately 3 months of age. They were well adapted to human presence and never subjected to EE before.

Anesthetics used consisted of intramuscular injections of ketamine 1 mg kg^{-1}; Ketavet, Revermex) and

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xylazine (0.02 mg kg^{-1}; Rompun, Bayer) (Hugan *et al*., 2001). Serum cortsol concentration, Heart and respiratory rate were measured in all groups.

All treatments were applied in the rams home pen. The animals were restrained using halters along the periphery of their pen (facing outside) and separated 4 m from each neighbor. To avoid variations due to ambient conditions or diurnal rhythms of metabolic variables all recordings started at 09:00 am and each variable was measured by the same person.

A CGS electroejaculator, model 500 M1 (Ratek Instruments Pty Ltd., Thornton Cr, Mitcham, Vic 3132) 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 was 22 cm long and 2.5 cm in diameter, comprising 2 electrodes measuring 100×6 mm 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_1 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.

After the injection of anesthetics, halters were loosened and rams were kept in a recumbent position during collection. In this position, the penis was grasped and held at the end of a calibrated centrifuge tube.

Heart Rate (HR) was measured using a battery operated HR monitor (Polar S610™). HR electrodes were placed over the scapular and heart apex areas 30 min before the onset of the experiment. These areas were previously 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 heart rate transmitter and electrodes from dislocation. Heart rate measures for each animal begun immediately when the probe was inserted and were recorded continuously for approximately 60 min.

Respiration Rate (RR) was measure in all animals 20, 40 and 60 min after the beginning of the experiment, by counting the rate of flank movements (Sevi *et al*., 2001a, b).

A 5 mL blood sample was collected by venipuncture from the jugular vein into vacutainers at: 0 (immediately before inserting the rectal probe), 20 and 70 min after insertion of the probe. The samples were held in an ice bath (no more than 30 min) and then centrifuged (1500 rpm for 15 min) 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).

To compare heart rhythm and blood cortisol concentration among and within treatments, a 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 and T_1)
k = 1-5 (sample points)
μ = Mean of the distribution of Y
α_i = Effects of the 2 treatments (A)
β_k = Effect of time at the various sampling points in the process of repeated measurement of the subjects (B)
$(\alpha\beta)_{ik}$ = The interaction of A and B
$E_{(ik)}$ = The residual error

When significant differences were detected within treatments, multiple comparisons were performed using Fisher's post hoc Protected Least Squares Difference (PLSD) test (Gill, 1978).

RESULTS

A heart rate peak of >200 beats min^{-1} was observed at the time of EE in both groups. Heart rates in anaesthetized rams (T_1) returned to basal levels within 4 min after this peak, while heart rates of (T_0) rams were still relatively high 30 min after EE (Fig. 1).

Serum concentrations of cortisol were significantly higher ($p<0.05$) for animals in T_0 than rams in T_1, 20 min after EE (161.57±14.37 and 110.60±11.74 ng mL^{-1}, respectively) and then decline to pre-EE levels 70 min post-EE (51.23±6.41 and 56.83±8.81 ng mL^{-1} for T_0 and T_1, respectively). No differences ($p>0.05$) were observed between or within groups in the respiratory rate (Table 1).

Table 1: The respiratory rate

	Electro-ejaculated rams			
	Anesthetized animals		Control rams	
Time after stimulus application (min)	Serun cortisol concentration (ng mL^{-1})	Respiratory rate (breaths min^{-1})	Serun cortisol concentration (ng mL^{-1})	Respiratory rate (breaths min^{-1})
0	83.37±22.13	55.20±4.63	91.21±9.84^{a}	60.67±378
20	110.60±11.74^{a}	63.20±15.41	161.57±14.37^{b}	65.33±9.39
40		52.00±9.38		58.00±7.06
70	56.83±8.80^{b}	45.60±6.88	51.23±6.41^{a}	62.67±4.92
100		39.20±7.31		50.67±513

^{a,b}Different letters indicate statistical significance ($p<0.05$) among samples (time) within treatments

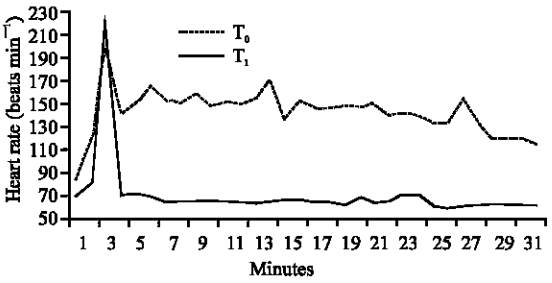

Fig. 1: Mean change in heart rate (beats nute^{-1}) between groups of rams when no anesthetics were used (T_0) and when a combination of xylasine and ketamine treatment (T_1) after electrical stimulation was applied

DISCUSSION

Various anesthetic protocols have been used in attempts to measure and reduce the pain associated with EE in bulls (Palmer, 2005). Caudal epidural anesthesia with 2% lidocaine has been studied extensively (Mosure *et al*., 1998; Falk *et al*., 2001). Unlike other sedatives, lidocaine epidural anesthesia has been found to have no adverse effects on penile protrusion or semen emission (Mosure *et al*., 1998) and elevation in serum cortisol (Falk, 2001) have been reported to be less following EE with lidocaine epidural anesthesia.

Changes in heart rate also tended to be less in bulls administered a lidocaine epidural anesthesia prior to EE than in those receiving no anesthesia (Mosure *et al*., 1998; Falk *et al*., 2001). However, Falk *et al*. (2001) claim that the benefits of lidocaine epidural anesthesia prior to EE do not appear to outweigh the inconvenience associated with administering the anesthetic.

Other treatments, such as narcotics in combination with lidocaine, may be more useful in reducing the pain associated with EE (Falk *et al*., 2001) and should be investigated. Both intravenous xylazine and especially xylazine epidural anesthesia, were also found to be somewhat effective at diminishing heart rate and muscle exertion in bulls (Mosure *et al*., 1998). Unfortunately, ataxia and recumbence limited the practical application of xylaxin treatments (Mosure *et al*., 1998) and further research on bulls was discontinued.

The above findings with bulls are in agreement with our results using hair sheep rams. Using a combination of xylaxin and ketamin, a 35% reduction in cortisol concentration was observed together with a relatively rapid return of heart rate to basal levels suggesting that the anesthetized rams may have experienced less pain/stress in response to EE. In addition, inconveniences of epidural procedure were avoided by the use of im application. Falk *et al*. (2001) found that in bulls cortisol concentrations were significantly elevated above pre-treatment levels 25 min after EE in both lidocaine epidural and non-anesthetized animals, although concentrations tended to be higher in the latter treatment. They concluded that the epidural anesthesia offered a slight reduction in the pain/stress response to EE.

In the present experiment, cortisol concentrations in both groups were raised above 110 ng mL^{-1}. Apple *et al*. (1995) found that in sheep, 6 h of restraint stress caused dark cutting meat and very high (>110 ng mL^{-1}) levels of cortisol. They found that epidural blockage with lidocaine, which prevents the animals from contracting their muscles and straining against the restraint, failed to inhibit glycogen metabolism, indicating that psychological stress is probably a significant factor (Grandin, 1997). Unfortunately, in our experiment muscle contraction was not measured. However, it can be assumed that conscious animals may associate the handling procedures (i.e., probe inserted in the rectum) with EE, which could also increase psychological stress.

Physiological measures of stress such as heart rate have been repeatedly used to assess welfare (Manteca, 1998). Sheep subjected to cold stress exhibited an average heart rate ranging from 72-239 beats min^{-1} depending on the severity of the temperature to which they were exposed (Hays and Webster, 1971). Heart rates of 217 beats min^{-1} were found by Crossley *et al*. (1988) after the administration of 0.5 mg iv. of adrenaline. In the

present experiment, elevations in heart rate during EE for the anesthetized sheep were similar to that of the control animals, reaching levels similar to the stressed situations mentioned above. However, anesthetized animals were back to basal levels almost immediately after electric stimulation, indicating that pain from EE is due to strong nerve stimulation (Mosure *et al*., 1998) and anestethics tended to reduce painful subsequent effects.

Hays and Webster (1971) reported that 15-20 mg kg^{-1} sodium pentobarbitone anesthesia induced a marked tachycardia in sheep. However, this effect was not found with the anesthetic substances used in the present experiment.

CONCLUSION

It was concluded that anesthetic treatment improved the welfare of sheep rams undergoing EE as indicated by 2 indicators of physiological stress (heart rate and cortisol levels). This finding supports the position that EE without anesthesia in these animals is unacceptable.

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