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## **Stress Levels in Bulls during and after Electroejaculation**

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### **Abstract**

The Animal Welfare Society has expressed its concern on the use of electroejaculator as a semen collection device. The Society believes that electroejaculation will incur pain to the animal and thus causing stress. Thus, this will compromise the quality of semen collected from the animal. A study was conducted to observe if bulls were stressed when the electroejaculation technique was applied for semen collection. A serum blood sample was collected before, immediately after and after semen collection using the EE technique. Changes in serum cortisol concentrations in serial blood samples were used to quantify stress response in the bulls. Eight bulls aged from 3 to 8 years and weighing between 320 and 830 kg were randomly assigned to one of the two treatments. The first treatment group consisted of bulls that were inserted with a probe and given electrical stimuli (ES) while the second treatment group comprised of bulls that were inserted with a rectal probe but without electrical stimulus (WES). Blood samples were collected by venipuncture at rest (first before the rectal probe was inserted), immediately after ejaculation, 10 and 15 min postejaculation. There was no significant difference ( $p \geq 0.05$ ) between the group with electrical stimulus and the group without electrical stimulus. However, there are significant differences between bulls in each group. The use of probe with electrical stimuli did not significantly increase serum cortisol concentrations.

**Keywords:** Electroejaculation, semen, bull, electrical stimuli

### **Introduction**

Electroejaculation (EE) is one of the two common methods used to obtain semen from bulls and goats. The procedure is generally used for breeding programmes and research purposes. This method has been used in rams that lacked libido. However, this technique can cause pain and stress to the animals (Mosure *et al.*, 1998).

Stress can be defined into three different ways; first definition is a constraining or impelling force, it is an effort or demand on energy and third definition it is a force exerted on a body. Exposing the animals to stress can induce changes in physiological and immunological behavior. In fact, prolonged stress reduces productivity and performance of animals. Serum cortisol concentration has been used as an indicator for stress. Thus, when an animal is in stress, the cortisol level in the body is increased. Pain is often associated with stress, thus when an animal is in pain it is under stressed. Pain caused by restraining and handling, palpation *per rectum*, and venipuncture leads to elevation of serum cortisol concentrations. During EE, the clinical signs that indicate an animal is in pain are vocalization, struggling, lying down, generalized muscular contraction, and spasms of the hind limb.

Currently, there is no information on the relationship between stress in by EE and cortisol concentrations in bulls. Therefore, the objective of this study was to determine the concentration of serum cortisol before, during, and after EE in breeding bulls.

## **Materials and Methods**

### ***Animals***

The animals used were 8 bulls from 5 different breeds (KK-X, Braford-X, Friesian-X, Draughtmaster-X, and Brangus) aged from 2 to 8 years and weighing between 320 and 830 kg. All bulls were raised under free grazing system and fed with commercial concentrates daily consisting mainly of palm kernel cake (PKC).

### ***Electroejaculator Set***

The rectal probe used in EE was 47.7 cm long and 10.0 cm in diameter. The electrodes were separated by an angle of arc on the body of the probe. The handle was smaller in diameter so the probe when fully inserted into the rectum did not cause stretching of the anal sphincter.

### ***Electrical control panel***

The machine was used on manual control, which allowed the operator to control the amount of voltage from 0 to 15 volts that was channelled to the probe. In electroejaculation, multiple electrical stimuli were applied for an interval of 3 to 5 sec until ejaculation of semen.

### ***Blood Sampling***

An approximate 5 mL blood sample was collected by venipuncture from the jugular vein into a plain tube using an 18-gauge venojet needle. After the collection, the

blood samples were kept in ice and immediately transported to the laboratory. The samples were then centrifuged at 5000 rpm for 15 min. Serum was aspirated after centrifugation and stored below -20°C pending analysis.

### ***Experimental design***

In this study, 8 bulls were divided equally into two groups. The treatment group received the probe with electrical stimuli (ES) while the control group received the probe without electrical stimuli (WES). For the treatment group, electrical stimuli were continued until ejaculation occurred, while for the control group, the probe was inserted into the rectum for 10 min without electrical stimulus.

Blood samples were collected from the treatment group at four different occasions. First, at the end of the 15 min after the bull entered the crush, one immediately after ejaculation, followed by 10 and 15 min after ejaculation. On the other hand, for the control group, the blood samples were collected first at the end of the 15-min resting period, and second immediately after removal of the probe, 10 min after it had been in the rectum, the third and fourth samples were collected 10 and 15 min later.

### ***GammaCoat Cortisol Radioimmunoassay Kit***

For the quantitative determination of serum cortisol concentration, the cortisol was measured using GammaCoat™ Cortisol Radioimmunoassay Kit produced by DiaSorin Company. Serum samples were thawed at room temperature and 10 µL of serum sample was added into each GammaCoat tube. Subsequently, 1.0 mL of tracer-buffer reagent was added into each tube and mixed gently with a vortex mixer. The tubes were incubated in a dryer oven at 37°C for 45 min. After that, all tubes were decanted, and put into an inverted position for 3 to 5 minutes to allow tubes to drain. Finally, all tubes were placed in 1470 Automatic Gamma Counter for cortisol concentration measurements.

### ***Statistical analysis***

Data were analyzed using Repeated Measures Analysis of Variance of the SPSS Version 16.0 to measure differences of serum cortisol concentration between and within ES and WES groups over time.

## **Result and Discussion**

The mean serum cortisol concentrations at rest before the treatment were higher in electrical stimuli (ES) bulls than the without electrical stimulus (WES) bulls. After receiving the electrical stimuli from the electroejaculator, all ES bulls showed drastic increase in mean serum cortisol concentration level from rest to immediately after ejaculation. Subsequently, the concentration of serum cortisol

declined over time. However, an increased serum cortisol concentration was observed in the WES group at 10 min after the probe was inserted into the rectum. In addition, the concentration of serum cortisol continued to increase until the end of predetermined time 15 min after probe was inserted.

There was no significant difference ( $P > 0.05$ ) between the ES group and the WES group at four different occasions. There are significant differences among bulls in each group.

### **Conclusion**

The present study proved that the use of probe with electrical stimulation did not significantly increase cortisol concentration.

### **References**

- Mosure, W. L., Meyer, R.A., Gudmundson, J. and Barth, A.D. (1998). Evaluation of possible methods to reduce pain associated with electroejaculation in bulls. *Can Vet J* **39**: 504 - 506.