Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls

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Abstract

Electroejaculation is a reliable method of obtaining a semen sample for a bull breeding soundness examination, but is sometimes regarded as painful. Substance P is a neuropeptide involved in the integration of pain, stress, and anxiety. We hypothesized that substance P is a measure of pain in bulls following electroejaculation. The specific objective was to compare vocalization and plasma concentrations of cortisol, progesterone, and substance P immunoreactivity in bulls following electroejaculation. Nine Angus bulls (501.9 ± 14.3 kg) were used. Blood samples were collected at −60, −30, 0, 2, 10, 20, 30, 45, 60, 75, 90, 120 min relative to treatment. At Time 0, bulls were subject to electroejaculation, rectal probe insertion without electroejaculation, or no manipulation. Treatments were administered contemporaneously to three bulls. Treatments were repeated weekly until each bull had received each treatment in a 3 × 3 Latin square design. More bulls (P = 0.0147) in the electroejaculation group vocalized (5 of 9 bulls; 55.6%) when compared to controls (0 of 9 bulls; 0%). Mean plasma cortisol and progesterone concentration following electroejaculation in bulls were higher (P < 0.05) than concentrations in probed and control bulls through the 45 min sample. However, mean plasma substance P concentration following electroejaculation in bulls (77.2 ± 17.2 pg/mL) was not different (P = 0.6264) from probed (79.1 ± 17.2 pg/mL) or control bulls (93.4 ± 17.2 pg/mL). A significant increase in vocalization and plasma cortisol and progesterone concentrations in bulls following electroejaculation was likely owing to acute stress. However, the lack of a difference in plasma concentrations of substance P after electroejaculation was interpreted as a lack of pain associated with nociception.

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1. Introduction

Electroejaculation (EEJ) is an important and routine procedure in food animal veterinary medicine, particularly owing to application in bull breeding soundness evaluation (BSE). Bulls contribute more to the overall reproductive success of the herd than any other individual animal; therefore, male subfertility has significant economic consequences. Evaluation of semen motility and morphology are an integral part of the BSE, and both components are correlated to fertility [1,2]. Therefore, the ability to safely, quickly, and reliably obtain a semen sample is important for practitioners.
Electroejaculation is the most common means of collecting semen for the BSE because of improved reliability in obtaining a sample, ease and safety of application, and increased likelihood of penile protrusion, thereby improving both the ability to observe penile anatomy and the quality of the semen collected [3].

Despite the advantages of using EEJ for semen collection, the application incites criticism for being inhumane and may attract attention as an animal well-being issue. For this study, the term “stress” will be used to indicate “the state of homeostatic imbalance induced by any physical or psychological stressor, involving physiological (neural and endocrine) and behavioral responses that tend to re-establish the homeostasis” [4,5]. The term “pain” will be used to indicate “an aversive feeling or sensation associated with actual or potential tissue damage and resulting in physiologic, neuroendocrine, and behavioral changes that indicate a ‘stress’ response” [6–8]. The main argument against the use of EEJ stems from reports that it is painful in humans [9]. Additionally, animals exposed to this procedure often vocalize, struggle, or attempt to lie down. These behaviors are associated with nociceptive stimuli. As a result, EEJ has been banned or discouraged in some countries [3,10], and research to determine and quantify stress and pain associated with EEJ have been conducted, each method having inherent advantages and disadvantages. These indexes are also important in attempting to provide an objective and effective method to evaluate different approaches to minimizing or alleviating the potential pain associated with EEJ [10,11].

Palmer [3] recently reviewed the animal well-being aspects of EEJ and relevant studies. In brief, attempts to quantify and evaluate nociception have examined aversive behavior [12], heart rate [10], blood cortisol concentrations [11,13], blood progesterone (also secreted by the adrenal glands in response to stress) concentrations [11,13,14], vocalization [11,15], and subjective scoring of escape behaviors [11]. After a thorough review and despite some conflicting results, Palmer concluded that changes in progesterone coupled with ability to elicit vocalization suggested that EEJ may be painful, and some of the variability may be at least partially attributable to differences in stimulation technique [3].

 Substance P (SP) is an eleven amino acid-peptide neurokinin that plays an important role in the pain response as it increases the excitability of neurons in the dorsal horn of the spinal cord and the integration of pain, stress, and anxiety [16]. It is present in both the central and peripheral nervous systems, where it mediates its effects via binding to the neurokinin-1 receptor. It is released in response to noxious stimuli or stress in proportion to the intensity and frequency of the stimulus [17]. Whereas plasma concentrations of cortisol were not changed in beef calves after castration (compared to sham castration), circulating concentrations of SP were elevated and associated with vocalization [7]. Therefore, it serves as a potential target for objectively and specifically assessing pain.

Production agriculture is faced with the challenge of formulating animal well-being policies relating to routine management practices. To enable the livestock industry to effectively respond to these challenges there is a need for more data on practices commonly used in production settings [18]. The objective of this study was to determine the effect of EEJ on SP as an indicator of pain. Behavioral (vocalization) and hormonal (cortisol and progesterone) responses to EEJ in bulls were also measured. We postulated that SP would be a more specific indicator of pain associated with nociception.

2. Materials and methods

2.1. Bulls

Nine Angus bulls (mean ± SEM 14.15 ± 0.14 mo old and 501.9 ± 14.3 kg body weight) were used in this study. All animal use was approved by the Institutional Animal Care and Use Committee of Berry College (Rome, GA, USA).

2.2. Procedure

To acclimate bulls to standing in the chutes, the bulls were placed in the chutes used for treatment and blood collection for approximately 1 h, starting 1 wk before the first round of treatments. Bulls were fitted with sterile indwelling jugular catheters approximately 24 h before a treatment period. Each bull was restrained in a chute (not a chute used for treatment and blood collection during the experiment) and the area over a jugular vein was clipped and disinfected with 70% isopropyl alcohol and povidone iodine. The catheter site was infiltrated with approximately 2 mL of 2% mepivacaine hydrochloride solution (Carbocaine-V, Pharmacia & Upjohn Company, Division of Pfizer, Inc., New York, NY, USA) before catheter placement. Catheter patency was maintained with heparinized saline (0.9% NaCl) flush solution (10 U of heparin sodium/mL of saline solution).

Bulls were housed in their original groups until treatments were administered. On the day of the study,
bulls were restrained in three chutes within visual and auditory stimulation of each other for the duration of sampling. Treatments administered at time 0 included electroejaculation (EEJ), insertion of a rectal probe without electrical stimulation (probed), or no treatment (control). An unweighted bull electroejaculator rectal probe 90 mm in diameter and 360 mm in length with three ventrally oriented longitudinal electrodes (non-segmented) was used in all cases. In the EEJ treatment, a Lane Pulsator IV (Lane Manufacturing, Denver, CO, USA) electroejaculator utilizing a sine wave pulse at a frequency of 15 Hz was allowed to complete one pre-programmed cycle, regardless of whether or when ejaculation occurred. The pre-programmed cycle consisted of a 214 s period of gradual/incremental increases in voltage from 0 to 13 V and a maximum current of 900 mA, with successive stimulation the intensity of the voltage was steadily increased and held for 1 s, followed by 1 s of rest. Three bulls were treated (one each: EEJ, probed, control) and sampled contemporaneously, and three such groups were done over the course of 1 day. The experiment took place over three consecutive weeks, with each bull receiving each treatment in a 3 × 3 Latin square design with three repetitions. Care was taken to ensure that each bull was in the same group each week and was restrained in the same chute to minimize variance associated with time-of-day and environmental factors with each repetition.

2.3. Sampling

Blood samples were collected in syringes via the jugular catheter at −60, −30, 0, immediately after, 10, 20, 30, 45, 60, 75, 90, 120 min following treatment application. At each time point, 4 mL of blood was collected and immediately transferred to sodium EDTA tubes containing aprotinin, 500 kIU mL⁻¹. Within 10 min after collection, samples were centrifuged for 5 min at 1500g at 4 °C. Plasma was frozen by placing on dry ice and then stored at −80 °C until analyzed. Additional observations during treatment included number of vocalizations [vocalizations were determined for three bulls (one for each treatment group) contemporaneously during EEJ of one bull].

2.4. Hormone assays

2.4.1. Substance P immunoreactivity

Samples were analyzed for substance-P (SP) concentrations using a validated analytical method and in the same laboratory as previously described [7]. Briefly, substance P was extracted from 0.5 mL of plasma by acidifying with acetic acid and fractionating with reverse-phase solid-phase extraction columns. The peptide was eluted from the column using an organic-aqueous solvent mixture and concentrated by drying under nitrogen. The dried extract was reconstituted and analyzed according to the manufacturer’s instructions in the substance P ELISA kit (Assay Designs, Ann Arbor, MI). Assay performance was monitored using five replicates of bovine plasma samples fortified with 0, 200 or 800 pg/mL of substance P purified standard (Phoenix Pharmaceuticals, Burlingame, CA; Catalog # 061-05). The method was linear across the five replicates at each concentration (R-squared = 0.99) and the coefficient of variation (CV) at each concentration was <15%.

2.4.2. Cortisol

Plasma cortisol concentrations were determined as described [19] by use of a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system (Immulus 1000 cortisol, catalogue No. LKCO1, Diagnostic Products, Corp, Los Angeles, CA, USA). A minimum sample volume of 100 μL was used in each assay well. The reported calibration range for the assay was 28 to 1380 nmol/L, and sensitivity was 5.5 nmol/L.

Cortisol was extracted from pooled bovine plasma by use of diethyl ether to yield cortisol-free plasma to validate the immunoassay. Briefly, blank plasma was fortified with five concentrations of cortisol (obtained from a stock solution of 0.5 g/dL) that spanned the expected analytical range of the assay. Each spiked sample was then analyzed in triplicate. The coefficient of variation for triplicate samples at each spiked concentration was <15%. The linear regression line for the three points at each of the five concentrations had an R-squared value of 0.99.

2.4.3. Progesterone

Plasma progesterone concentrations were determined using the Coat-a-Count progesterone RIA kit (Siemens, Los Angeles, CA, USA). This kit has been previously proven as a reliable method of progesterone quantification in ruminants [20–23]. Limit of detection, intra- and interassay coefficients of variance for the progesterone assay were 0.03 ng/mL, 3.0, and 10.4%, respectively.

2.5. Data analysis

The overall experimental design was a 3 × 3 Latin square repeated three times. Fold responses for plasma concentrations of progesterone and cortisol were calculated relative to plasma concentration at time 0. Data
for plasma concentrations of cortisol, progesterone, and substance P and fold change in progesterone and cortisol were not normally distributed and were Log transformed for analysis. The model included treatment (EEJ, probed or control), sequence (date), and time relative to EEJ as fixed effects, and treatment × sequence, sequence × time, treatment × time, and treatment × sequence interactions, and animal as a random effect using procedures for repeated measures (JMP version 7, SAS Institute, Inc., Cary, NC, USA). Frequency of vocalization in the chute were modeled using a binomial distribution with bulls that vocalized being assigned a score of 1 and bulls that did not vocalize being assigned a score of 0. Data were analyzed using a $\chi^2$ analysis of vocalization score against treatment. Comparison between treatment groups was done using Fisher’s exact test.

### 3. Results

#### 3.1. Substance P immunoreactivity

Mean ± SEM plasma SP concentration following EEJ in bulls (77.2 ± 17.2 pg/mL) was not different from probed (79.1 ± 17.2 pg/mL) or control bulls (93.4 ± 17.2 pg/mL). There was an effect of time ($P = 0.0001$), but no effect of treatment ($P = 0.6264$) and there was no interaction of treatment and time ($P = 0.24$) on plasma SP concentrations (Fig. 1).

#### 3.2. Vocalizations

There was an effect of treatment ($P = 0.0256$) on number of bulls vocalizing. More ($P = 0.0147$) bulls vocalized during EEJ [5 of 9 bulls (55.6%) vocalized from 4 to 28 times] than contemporaneously treated controls (0 of 9 bulls). However, the number of bulls vocalizing during probe insertion [2 of 9 bulls (22.2%) vocalized from 1 to 3 times] was not different from EEJ ($P = 0.9751$) or control ($P = 0.2353$). One of the bulls that vocalized in the probed group also vocalized during EEJ. Mean ± SEM (pooled) vocalization during EEJ in bulls (7.22 ± 1.95) was greater than vocalizations in probed (0.42 ± 1.95) and control bulls (0.00 ± 1.95) during the same time. None of the bulls attempted to lie down during any treatment.

#### 3.3. Cortisol

Mean ± SEM plasma cortisol concentration before electroejaculation in bulls (48.44 ± 10.84 nmol/L) was not different from the probed (29.36 ± 8.05 nmol/L) or control bulls (45.41 ± 13.52 nmol/L). Mean cortisol concentration determined for 2 h following treatment was 18.34 ± 5.18, 27.00 ± 6.04, and 62.74 ± 8.07 nmol/L for control, probed, and EEJ bulls, respectively. There was an effect ($P = 0.0013$) of treatment on plasma cortisol concentrations with concentrations in EEJ bulls being greater than in probed or control bulls. There was an interaction of treatment and time ($P < 0.0001$) on plasma cortisol, such that concentrations following EEJ were greater than concentrations in control and probed bulls beginning at 10 and 20 min, respectively, and continuing through the 45 min sample (Fig. 2). There was also an effect ($P = 0.005$) of treatment and an interaction ($P < 0.0001$) of treatment and time on fold-change in plasma cortisol concentrations (Fig. 2). The mean fold-change in plasma cortisol concentration was greater in EEJ bulls than control bulls and not different in probed bulls. The fold-changes in plasma cortisol concentration at the 10 min sample were greater for probed and EEJ bulls than the control bulls, and remained higher in the EEJ bulls.

![Fig. 1. Mean ± SEM plasma SP concentrations in bulls (n = 9 bulls/group) following control (black squares), probe (white circles), or electroejaculation (EEJ; white triangles). Time of EEJ or probe insertion was designated as time 0. No effects were significant.](image-url)
compared to the control bulls through the 45 min sample (Fig. 2).

3.4. Progesterone

Mean ± SEM plasma progesterone concentration before EEJ in bulls (0.70 ± 0.12 nmol/L) was not different from the probed (0.54 ± 0.06 nmol/L) or control bulls (0.61 ± 0.09 nmol/L). There was an effect (P = 0.0012) of treatment on mean plasma progesterone concentrations. Mean ± SEM (pooled) plasma progesterone concentration was greater in EEJ bulls (0.95 ± 0.14 nmol/L) than in probed (0.35 ± 0.14 nmol/L) and control bulls (0.29 ± 0.14 nmol/L). Moreover, there was an interaction (P < 0.0001) of treatment and time on plasma progesterone concentrations such that concentrations following EEJ were greater than concentrations in probed and control bulls beginning at 10 min and going through the 45 and 60 min sample for probed and control bulls, respectively (Fig. 3). There was an effect (P = 0.0005) of treatment such that the mean fold-change in plasma progesterone concentration was greater in EEJ bulls than control and probed bulls. There was also an interaction (P < 0.0001) of treatment and time on fold-change in plasma progesterone concentrations. Fold-changes in plasma progesterone concentration at the 10 min sample were greater for EEJ bulls than the control and probed bulls, and remained higher in the EEJ bulls through the 30 and 45 min samples for the probed and control bulls, respectively (Fig. 3). Ratios of peak concentrations of cortisol to progesterone after control, probed, or EEJ were 99:1, 88:1, and 47:1, respectively.

4. Discussion

The study reported here was conducted to compare the effect of EEJ in bulls on plasma concentrations of SP, cortisol, and progesterone and on vocalization. Our
supposition was that an increase in plasma SP concentration may be a more accurate indicator of nociception associated with EEJ than would increases in vocalization, plasma cortisol, and progesterone concentration. Overall, greater vocalizations and higher mean cortisol and progesterone concentrations were detected following EEJ in bulls. In contrast, plasma SP concentrations in EEJ, probed, and control bulls were not different over the course of the study. To our knowledge, this is the first report in which investigators have evaluated the use of SP concentrations to quantify nociceptive responses in bulls after EEJ.

Although much research justifies the use of a bull BSE to increase reproductive success and ultimately the profitability of the cow-calf herd [24,25], criticism has arisen in its use, owing to concerns about EEJ as an animal well-being issue [3]. In a review of several studies, it was concluded that EEJ induces physiological, neuroendocrine, and behavioral changes that may indicate a stress response associated with pain [3]. Electroejaculation is reported to be painful in humans; it is primarily used in individuals with spinal cord injuries but requires the use of a spinal or general anesthesia in patients who retain sensation [9,26,27]. This has led to concern over whether the procedure is painful in animals. Research has focused on various methods to evaluate a pain response and possibly reduce it; veterinary medicine recognizes the value of EEJ as a diagnostic tool, but it is important that veterinarians remain mindful of animal well-being aspects and work to employ this technique in the most humane manner possible.

Substance P is a neuropeptide with an essential role in the response to pain and the integration of pain, stress, and anxiety [16]. It is released in response to noxious stimuli or stress in proportion to the intensity and frequency of the stimulus [17] and in the absence of SP (SP knockout mice or natural lack of SP in African naked mole-rats) some animals can selectively reduce the response to painful stimuli [28–30]. In the present study, there was no significant increase in plasma SP concentrations in bulls following EEJ or probe insertion. In human patients, plasma concentrations of SP have been used to evaluate nociception and the efficacy
of therapeutics for pain relief [31–33]. Although the physiological role of SP in pain has been well-established and is gaining attention in the study of nociception, its application to veterinary medicine and animal well-being has emerged more recently. In a study of cats with chronic gingivitis and periodontitis, there was an increase in concentrations of SP following dental scaling while cortisol concentrations decreased post-handling [34]. In a study of castration in calves, there was an increase in plasma concentrations of SP after castration that was not observed in sham castrated calves. However, there was no difference in cortisol concentrations between the groups such that cortisol increased equally for castrated and sham castrated calves [7]. Substance P is a more specific indicator for nociception and assessment of plasma SP concentrations may help to discriminate between stressful events that cause a transient increase in plasma cortisol and progesterone concentrations and more painful/ nociceptive events that culminate in a prolonged increase in plasma SP concentrations. Therefore, whereas EEJ may be stressful, the lack of a difference in plasma concentrations of SP after EEJ suggested that it may not be a painful procedure.

Research to evaluate animal well-being and nociceptive response to a particular stimulus in farm animals has been predicated on assessment of the hypothalamic-pituitary-adrenal (HPA) axis by measuring the acute cortisol response [35–40]. The mean plasma cortisol concentration in bulls 20 to 45 min following EEJ in our study was statistically greater than the control and probed bulls during the same time. Although probe insertion did not change mean plasma cortisol concentration in bulls in this study, it resulted in an increase in cortisol concentration fold-change 10 min after insertion that was greater than the controls and not different from EEJ bulls. Therefore, while probe insertion may induce some distress, based upon the acute cortisol response, EEJ may be more stressful. The usefulness of acute cortisol response to particular stimuli in the evaluation of nociception is complicated by several factors. Cortisol concentration changes diurnally in the bovine [41] and relatively non-invasive procedures, such as venipuncture and intramuscular injection can increase cortisol concentrations [42]. At the same time, increased concentrations have a so-called ceiling effect; there is a maximum level of response-reachable by the stress of handling alone-such that overall increase in cortisol concentration may not be proportionate to the degree of pain/stress experienced. For example, cortisol did not increase significantly for castration compared to sham castration in beef calves [7], and tail-docking plus bilateral castration, bilateral castration, and unilaterial castration in lambs all resulted in similar cortisol responses [43]. In effect, the so-called “ceiling” may be reached by handling alone or by very noxious stimuli, and interpretation may be difficult.

Efforts to determine response of cortisol to EEJ have failed to yield consistent results [11,13,15]. One early study reported no effect of EEJ on plasma cortisol concentrations in bulls compared to rectal probe insertion without stimulation [13]. However, later studies found that cortisol concentrations were significantly higher in bulls following EEJ as compared to control animals without probe insertion [11]. Results of studies in small ruminants also conflict with one another. One study reported that EEJ did not result in change in plasma cortisol concentration or aversion behavior in rams [44]. However, others reported a significant increase in plasma cortisol concentration post-EEJ in goats [45] and sheep [46,47]. Unlike other studies [11,13,15] where smaller rectal probes (≤75 mm) were used to evaluate pain and stress associated with EEJ in bulls, in our study a larger probe (90 mm) was used to ensure better contact with the rectal mucosa and enhance response to electrical stimulation [48].

The usefulness of acute cortisol response as an indicator of stress and possibly nociception following EEJ may be limited by observations that cortisol increases in response to physical activity and with other sexual behaviors. Steers had a 2-fold increase in plasma cortisol concentration during treadmill exercise [49]. Moreover, plasma cortisol concentrations were approximately 5-fold greater in boars and bulls, and 2-fold greater in stallions following sexual activity [50,51]. Bulls in the previous experiment [50] had increased plasma cortisol concentration during mounting and servicing activities, similar to that observed during EEJ in previous studies [13]. Bulls in the EEJ group in the present study also had an increase (fold-change) in plasma cortisol similar to that measured in the bulls during normal mounting and servicing activities [50]. Increases in cortisol following EEJ in bulls could signify activation of the HPA axis because of stress secondary to nociception, or it could simply be part of the normal ejaculatory process and/or response to physical activity. These findings highlighted the necessity for further research and considerations before final interpretation of the results from our experiment.

Progesterone is also secreted by the adrenal glands as part of the HPA stress response. In the present study, stress-induced concentrations of plasma cortisol and
progesterone had similar profiles, but greater magnitudes than those previously reported following EEJ in bulls [11,15]. In another study, EEJ and probe insertion without EEJ stimulated similar increases in cortisol and progesterone [13]. In our study, although probe insertion without EEJ stimulated a fold-change in cortisol, it had no effect on progesterone concentration (mean or fold-change). Adrenocorticotropic hormone (ACTH) causes an increase in both cortisol and progesterone secretion in cattle [14,52–54]. Even at a low dosage of ACTH, plasma progesterone concentrations in ovariectomized cows were commensurate with the luteal phase of the estrous cycle [52–54]. The adrenal cortex can secrete a considerable amount of progesterone in response to ACTH, and is supported by the finding of a significant correlation \( r = 0.7–0.8 \) between plasma cortisol and progesterone after ACTH challenges in ovariectomized dairy cows [53,54]. Moreover, plasma progesterone concentrations are a physiological biomarker of stress in cattle, with an apparent adrenal source [13,40,55,56]. However, adrenal progesterone secretion is not a specific indicator of pain. To the contrary, the increase in progesterone secretion occurs because of rate-limiting enzymes (e.g., 11β-hydroxylase) responsible for the conversion of progesterone to cortisol [57]. This would result in an initial increase in cortisol secretion with stress, and secretion of precursor progesterone as enzymes converting progesterone to cortisol were overwhelmed. This idea is supported by the ratios of cortisol to progesterone at the peak response to stress in the present study. These indicate a greater proportional secretion of progesterone with greater stress of EEJ than with the stress of probe insertion or restraint alone. Although adrenal secretion of progesterone is not a specific indicator of pain, plasma progesterone concentrations and the ratio of plasma cortisol to progesterone could reflect the degree of stress experienced by an animal.

Vocalization is an easily observable behavioral indicator of distress and pain [58]. It is considered a strong indicator of acute pain and is immediately observable, making it useful in determining the most painful components of individual procedures [58]. Observations of behavioral responses in the experiment reported here suggest that EEJ induced distress or pain in bulls, because significantly more bulls expressed vocalizations during EEJ than control treatment. However, it should be noted that EEJ was associated with vocalization in only 5 of the 9 bulls used in this study. Observation of vocalization in abattoirs has been successfully used as a reliable method to determine animal well-being (both pain and fear) [59]. Some reports suggest that vocalization may be a reasonable indicator of pain associated with EEJ [3]. In an experiment to evaluate the effect of lidocaine epidural anesthesia on behavioral response of bulls to EEJ, most bulls did not vocalize during either the conventional EEJ (74.2%) or lidocaine epidural EEJ (93.5%). Although fewer bulls vocalized during EEJ after lidocaine epidural anesthesia than during conventional EEJ, the difference was not statistically significant [11]. In a later study, none of the bulls vocalized when conventional EEJ was used [15]. Moreover, whereas none of the bulls in this or an earlier study [15] attempted to lie down during any treatment, 39% of bulls in another experiment [11] either lay down, or attempted to do so, during conventional EEJ. The lack of agreement in behavioral responses between this and other studies of EEJ in bulls may be due to differences in EEJ techniques employed, behavioral responses determined and techniques used to measure those responses, and/or the significant variability between and among animals in behavioral responses to EEJ [11,15]. The apparent lack of agreement in behavioral responses to EEJ emphasized the need for research into repeatable physiological and behavioral measures of pain and distress for determining animal well-being.

This is the first report in which investigators have determined plasma SP concentrations to quantify nociceptive responses in bulls after EEJ. We were unable to detect an increase in plasma SP response associated with EEJ. However, a significant increase in vocalization, and plasma concentrations of cortisol and progesterone was detected after EEJ. Although these results suggest that EEJ in bulls may be acutely stressful, based upon the lack of a difference in plasma concentrations of SP, the stress was not associated with nociception. These results have important implications for animal well-being issues and help address criticisms for the justification of electroejaculation as a part of the bull BSE.

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