

## **Transrectal massage of the accessory glands in bulls prior to electroejaculation does not affect interval to penile extension and ejaculation**

Jennifer H. Koziol,<sup>a</sup> George E. Moore<sup>b</sup>

<sup>a</sup>Department of Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN; <sup>b</sup>Department of Veterinary Administration, College of Veterinary Medicine, Purdue University, West Lafayette, IN

### **Abstract**

Transrectal massage of the accessory sex glands for 15-60 seconds in bulls prior to electroejaculation has long been advocated by theriogenologists as this procedure has been anecdotally stated to initiate sexual stimulation in the bull thereby decreasing time to extension of the penis and ejaculation consequently decreasing the amount of electrical stimulation needed for ejaculation. The present study was performed to test the hypothesis that transrectal massage reduces the time for extension of the penis and ejaculation to occur and to determine the optimal length of stimulation between 15 and 60 seconds. One hundred and twenty-two *Bos taurus* bulls ranging in age from 12-14 months-of-age were randomly allocated into four groups: 1) routine examination of accessory sex glands and inguinal rings only; 2) examination of accessory sex glands and inguinal rings followed by 15 seconds of massage of the ampullae, prostate, and urethralis muscle; 3) examination of accessory sex glands and inguinal rings followed by 30 seconds of massage of the ampullae, prostate, and urethralis muscle; 4) examination of accessory sex glands and inguinal rings followed by 60 seconds of massage of the ampullae, prostate, and urethralis muscle. Groups did not differ significantly in mean extension time ( $P=0.76$ ) or mean time to ejaculation ( $P=0.072$ ). In conclusion, transrectal massage of the ampullae, prostate and urethralis muscle for 15-60 seconds prior to electroejaculation did not significantly reduce intervals to penile extension or ejaculation in bulls.

**Keywords:** Bull, bovine, transrectal massage, semen collection, electroejaculation

### **Introduction**

Bull breeding soundness examinations should be an integral part of every cattle operation's management plan as it represents the best currently available predictor of bull breeding potential for pasture breeding. Electroejaculation has long been utilized as a reliable method of collecting semen for performing breeding soundness evaluations. However, this procedure has been discouraged in the UK and banned in several European countries.<sup>1,2</sup> It has been stated that since electroejaculation without anesthesia is painful to humans therefore it is assumed to be painful to bulls.<sup>1-4</sup> However, bulls that were electroejaculated two to three times a week for six weeks did not show aversion to handlers or the restraint over the course of the study.<sup>5</sup> Furthermore, a lack of difference between plasma concentrations of substance P, a specific target for assessing pain, before and after electroejaculation was interpreted as a lack of pain associated with nociception. The authors concluded that the increase in vocalization, plasma cortisol and progesterone in bulls following electroejaculation was likely due to acute stress secondary to being handled and restrained in the chute.<sup>6</sup>

Despite these findings one cannot disagree that the vocalization, muscular contractions, struggling and lying down experienced by some bulls during the electroejaculation process raises welfare concerns. Consequently, multiple studies have attempted to reduce the perceived associated pain and stress from electroejaculation.<sup>5,7</sup> Transrectal massage alone<sup>8</sup> and artificial vaginas<sup>2,9</sup> are not as efficacious as electroejaculation for obtaining a semen sample and drugs used to facilitate electroejaculation have not been efficacious enough to warrant use.<sup>1,10,11</sup>

If electroejaculation of bulls is to remain an accepted procedure, veterinarians must strive to collect semen by using the gentlest methods and least amount of electrical stimulation possible. It has often been stated that transrectal massage of the ampullae, prostate, and urethralis muscle for approximately 10-60 seconds will decrease the duration of the subsequent electroejaculation by sexually exciting the bull aiding in quicker penile extension and ejaculation.<sup>2,10,12,13</sup> However, no field studies have

determined the ideal length of time for transrectal massage before electroejaculation and if transrectal massage truly hastens the onset of penile extension and ejaculation. With the knowledge that the acute stress bulls experience during the electroejaculation process is likely due to handling and restraint suggests that there may be some benefit to decreasing the amount of time the bull is restrained in the chute.<sup>6</sup>

Therefore, the objective of this study was determine if transrectal massage of the ampullae, prostate, and urethralis muscle prior to electroejaculation truly reduces the interval to penile protrusion or collection of the sperm-rich fraction of the ejaculate and if so, what is the ideal duration of transrectal massage.

## **Materials and methods**

### **Bulls and data collection**

Breeding soundness examinations were performed on performance tested bulls, 12-14 months-of-age, as part of the routine end of test protocol. Bulls were randomly assigned, utilizing a random number generator, to one of the four treatment groups: 1) routine examination of accessory sex glands and inguinal rings only; 2) examination of accessory sex glands and inguinal rings followed by 15 seconds of massage of the ampullae, prostate, and urethralis muscle; 3) examination of accessory sex glands and inguinal rings followed by 30 seconds of massage of the ampullae, prostate, and urethralis muscle; 4) examination of accessory sex glands and inguinal rings followed by 60 seconds of massage of the ampullae, prostate, and urethralis muscle. Bulls were initially assigned into equal sized groups however individuals were lost from each group due to conformational issues or insufficient scrotal circumference.

For semen collection bulls were restrained in an indoor chute system. Their forward and backward movement was limited by the use of a pole placed immediately behind them while their heads remained free. To aid palpation and suitable contact of the electroejaculator electrodes with the rectal mucosa, feces was evacuated manually from the rectum by insertion and removal of the gloved hand. Examination of the accessory sex organs and the inguinal rings was completed according to the guidelines of the Society for Theriogenology for routine breeding soundness examinations. The accessory sex gland examination and transrectal massage was performed by the same veterinarian. Massage consisted of vigorous longitudinal massage over the ampullae, prostate, and urethra. The veterinarian was instructed to start and stop massage by an official time-keeper.

Immediately following the end of the transrectal palpation a lubricated, 64 mm diameter rectal probe with three ventrally oriented electrodes was inserted into the rectum and the process of electroejaculation was accomplished using an automatic setting that provides a series of 40 cycles. The cycle begins at 0.5v and each subsequent cycle increases by 0.5v; each cycle lasts two seconds followed by a two second pause (Electrojac, NeoGen, Lexington, KY). The time to protrusion of the penis (approximately >10 cm of the penis visible) was recorded as well as the time to ejaculation of the sperm-rich fraction. The sperm-rich fraction was defined as a minimum of 0.5 ml of cloudy, white seminal fluid. Once this was achieved electrical stimulation was ceased and the number of stimuli required was recorded. Following the successful collection of a semen sample the rectal probe was removed and cleaned. Disposable rectal sleeves were changed between every bull.

A subjective scoring system was used to determine whether there was a difference in behavioral response to electroejaculation amongst the four treatment groups. Subjective scores were categorized as follows: 0 – no vocalization and no reaction; 1 – one low vocalization or partial collapse; 2 – two or three moderately low bellows, or going down on carpal joints; 3 – four or more loud bellows, or complete collapse.

The seminal characteristics evaluated were: percent progressively motile sperm, and percent live sperm. Percent progressively motile was determined with bright field microscope at 400x magnification after placing a coverslip over a 2-4mm drop of semen on a warmed microscope slide. The percent live sperm was determined by identifying the percentage of sperm that did not take up eosin stain in an eosin-nigrosin smear using bright field microscopy at 1000x.

## Statistical analysis

Statistical analyses comparing the four treatment groups in age, weight, frame size, extension time (seconds), ejaculation time (seconds), and number of electrical stimuli were performed using a one way analysis of variance (ANOVA) with Scheffe's adjustment for pairwise comparisons. Fisher's exact test was used to compare breed and behavior score distributions across groups. A P-value of less than 0.05 was considered statistically significant. Data are reported as mean  $\pm$  standard deviation (SD). Data are graphically displayed in box-and-whisker plots to demonstrate data distribution, including range of observed values. All statistical analyses were performed with commercial software (STATA SE, v.14.2, StataCorp, College Station, TX).

## Results

A total of 122 bulls completed the study and included the following breeds: Angus (89), Red Angus (2), Charolais (4), Hereford (7), Simmental (3), Simmental-Angus (16) and Simmental-cross (1). In the test groups of 0, 15, 30, and 60 seconds of massage, there were 26, 31, 31, and 34 bulls, respectively. Age variation among the four treatment groups, from  $12.8 \pm 0.9$  months to  $13.1 \pm 0.7$  months, did not significantly differ ( $P=0.525$ ). Weight and frame size did not significantly differ among groups ( $P=0.972$  and  $0.505$ , respectively). Breed distribution across groups was not significantly different ( $P=0.896$ ).

Every bull extended the penis and ejaculated on the first attempt of electroejaculation, bulls were only recollected if poor semen motility or morphology were noted. Time to protrusion of the penis averaged  $21.0 \pm 8.4$  seconds across all 4 groups, and group means ranged from  $20.1 \pm 6.5$  to  $21.7 \pm 8.4$  seconds. Groups did not differ significantly in mean extension time ( $P=0.76$ ).

Time to ejaculation averaged  $43.7 \pm 13.2$  seconds across all groups, and groups did not significantly differ in mean time to ejaculation ( $P=0.072$ ; Figure). Mean times (seconds) to ejaculation were  $48.7 \pm 15.8$  (0 second massage),  $40.1 \pm 13.4$  (15 second massage),  $41.8 \pm 10.6$  (30 second massage), and  $44.9 \pm 12.1$  (60 second massage). Mean time to ejaculation also did not differ if bull with 0 seconds massage where compared to all other group ( $\geq 15$  second massage).

The number of stimuli required for ejaculation averaged  $11.0 \pm 2.5$  across all groups, and group mean number of stimuli ranged from  $10.5 \pm 2.8$  to  $11.3 \pm 2.3$ . The number of stimuli required for ejaculation did not differ significantly among groups ( $P=0.63$ ). Behavior scores ( $P=0.55$ ; Table), percent progressively motile sperm and percent live sperm did not differ among groups.

## Discussion

This study could not demonstrate that transrectal massage of the ampullae, prostate, and urethralis muscle for a period of time between 15-60 seconds prior to electroejaculation significantly reduced the time to extension of the penis and ejaculation in bulls. Group mean times in this study differed by less than 10 seconds.

Most veterinarians can agree that bulls often show some signs of sexual stimulation during the act of transrectal massage with the relaxation of the prepuce, pulsing of the urethralis muscle and emission of pre-seminal fluids. However, contrary to popular belief our study demonstrates that transrectal massage of the accessory sex glands and urethralis muscle for 15-60 seconds prior to electroejaculation does not significantly decrease the number of stimuli nor the time to extension of the penis and electroejaculation in the bull. Furthermore, there is no change in behavioral scores amongst the groups. Similarly, in a previous study, transrectal massage of the prostate, ampullae, and urethralis muscle for 2 minutes prior to electroejaculation did not significantly decrease interval from onset of stimulation to ejaculation.<sup>10</sup>

Perhaps this lack of reduction in time and number of stimuli needed to reach electroejaculation can be attributed to the lack of oxytocin release secondary to transrectal massage. Oxytocin has long been implicated in the process of sperm emission and ejaculation in multiple species.<sup>14-17</sup> Sharma and Hays determined that the action of manually massaging the accessory sex glands per rectum for five minutes resulted in an increase in oxytocin one to four minutes after the end of the manual stimulation or six to nine minutes after the start. Due to the prolonged nature of the rise in oxytocin following transrectal

massage it is likely that bulls within this study did not realize an increase in endogenous oxytocin until after the electroejaculation process was completed.

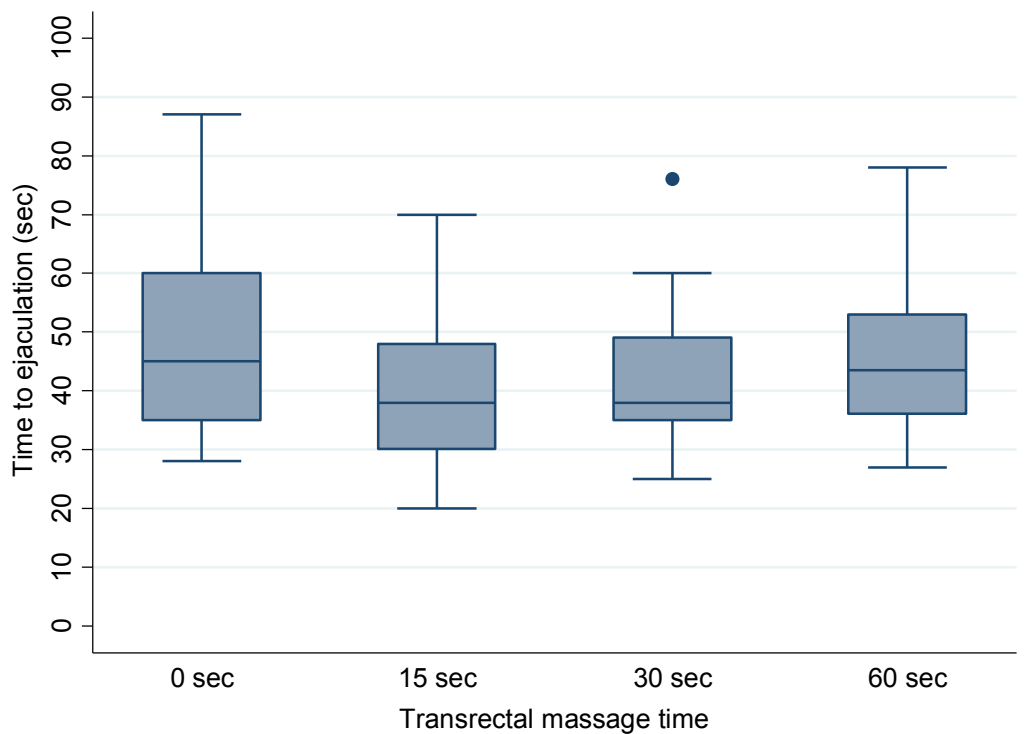
It was stated by Palmer et al. that an average of 67.5 seconds of transrectal massage directed specifically at the ampullae hastened the time to ejaculation by perhaps inducing a greater endogenous oxytocin release than transrectal massage over the prostate ampullae, and urethralis muscle.<sup>10</sup> Research suggests that while electroejaculation may not be a painful procedure the procedure combined with handling and restraint may be stressful to animals.<sup>6</sup> This would suggest that a shorter time of electroejaculation and quicker release from restraint would decrease the stress level of the animal consequently increasing the overall well-being of the animal. Despite the statistical insignificance of varying times of transrectal massage prior to electroejaculation, 15 seconds of massage decreased the time to ejaculation by eight and one half seconds allowing for an average reduction of the number of stimuli needed to collect an adequate semen sample for analysis by two. While not substantial, 15 seconds of manual stimulation, shorter than the anecdotally described 30-60 seconds, reduces the amount of time and number of stimuli needed to collect a semen sample for breeding soundness examination.

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**Figure.** Box-and whisker plots of time to ejaculation in 122 twelve-to-fourteen month old bulls following transrectal massage of accessory sex glands for 0 (n=26), 15 (n=31), 30 (n=31), or 60 seconds (n=34) prior to electroejaculation. Outlier (●) indicates value >1.5xIQR beyond the 75<sup>th</sup> percentile.

**Table.** Behavior score distribution by group and total, represented by both number of bulls per group that received each score (top number) and percentage of each group per score. No bull in this study received a behavior score of 3.

	Behavior Score		
	0	1	2
Group 1 0 seconds	25 96.2%	1 3.8%	0 0%
Group 2 15 seconds	27 87.1%	2 6.5%	2 6.4%
Group 3 30 seconds	26 83.9%	4 12.9%	1 3.2%
Group 4 60 seconds	32 94.2%	2 5.8%	0 0%
Total	110 90.2%	9 7.4%	3 2.4%

