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Effect of fasting prior to electroejaculation on behavioral responses and reproductive parameters in young Simmental bulls

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4 1 Effect of fasting prior to electroejaculation on behavioral responses  
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7 2 and reproductive parameters in young Simmental bulls  
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15 5 Juan E. Romano<sup>1a</sup>, Gaetano Mari<sup>2</sup>, Giuseppe Stradaoli<sup>3</sup>, Beatrice Mislei<sup>4</sup>  
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48 18 Abstract  
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50 19 The objective of the present study was to evaluate the effect of 24 hours fasting prior to  
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52 20 semen collection by electroejaculation on behavioral responses, volume of rectal fecal content,  
53  
54 21 bladder size, penis protrusion, erection, ejaculation stimuli, and ejaculate parameters in young  
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56 22 Simmental bulls. Twenty-two Simmental beef bulls with an age of  $13.2 \pm 1.2$  months (mean  $\pm$   
57  
58 23 SD) were used in a prospective randomized blinded controlled cross-over design with two  
59  
60 24 corrals fasted for 24 hours (n=9; FAS group) and the other three corrals were non-fasted (n= 13;  
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CON group). The bulls were maintained under confined conditions without access to pasture. One week later the pen treatments were inverted and semen collected again by the same conditions and team. The behavioral responses, volume of fecal rectal content, bladder size to electroejaculation, as well as number of stimuli required to obtain penis protrusion, erection, and ejaculation were measured. The following ejaculate parameters were measured: volume, concentration, spermatozoa motility, and morphology. The behavioral response of the bulls to electroejaculation was not different between CON group and FAS group ( $3.2 \pm 0.5$  and  $3.0 \pm 0.7$ , respectively;  $P=0.36$ ). Bladder size was significantly reduced in the FAS group compared with CON group ( $2.3 \pm 0.8$  vs.  $2.8 \pm 0.9$ , respectively;  $P=0.02$ ). The volume of feces in the rectum was not different between both groups (CON was  $2.3 \pm 1.7$  and FAS was  $3.0 \pm 1.8$ ;  $P=0.23$ ). The FAS group resulted in a higher proportion of penis protrusion compared with CON group (100% versus 81.8%,  $P=0.10$ ), erection (100% versus 81.8%;  $P=0.10$ ), and ejaculation (100% versus 90.9%;  $P=0.49$ ), respectively. The combined efficiency of penis protrusion, erection, and ejaculation (CE-PPEE) in FAS group was superior than CON group ( $P=0.001$ ) for those parameters. The number of stimuli necessary for penis protrusion, erection, and ejaculation for the CON group was  $13.5 \pm 3.7$ ,  $14.9 \pm 3.7$ , and  $20.8 \pm 5.8$  and for FAS group was  $15.0 \pm 4.2$ ,  $16.6 \pm 4.2$ , and  $20.2 \pm 8.1$ , respectively. The number of stimuli for penis protrusion ( $P=0.09$ ), erection ( $P=0.08$ ), and ejaculation ( $P=0.77$ ) were no different between groups. Ejaculate volume was  $4.0 \pm 2.6$  ml and  $4.1 \pm 2.3$  ml for CON and FAS groups, respectively ( $P=0.90$ ). The motility was  $1.4 \pm 0.7$  and  $1.4 \pm 0.8$  for CON and FAS groups, respectively ( $P=0.72$ ). The concentration of spermatozoa was  $336.2 \pm 273.1$  million and  $421.1 \pm 300.6$  million for CON and FAS groups, respectively ( $P=0.31$ ). The percentage of normal spermatozoa was  $50.9 \pm 18.8$  and  $45.6 \pm 14.3$  for CON and FAS groups, respectively ( $P=0.16$ ). It was concluded that fasting for 24 hours prior semen collection by electroejaculation reduced the bladder size and increased the proportion of bulls with penis protrusion, erection and ejaculation without any difference detected in behavioral responses, volume of rectal fecal content, and ejaculate parameters.

**Keywords:** Bulls, electroejaculation, fasting, behavioral responses, reproductive parameters.

54

## 55 Introduction

56

57           Electroejaculation is a widely used technique of semen collection in beef bulls for  
58 breeding soundness examinations, for non-trained bulls in artificial vagina, and for the diagnosis  
59 and treatment of penile and preputial diseases [1-3]. It is also used in artificial insemination (AI)  
60 centers to collect semen from bulls unwilling or unable to serve an artificial vagina because of  
61 specific pathological conditions or psychological weaknesses [2]. The equipment required for  
62 electroejaculation has advanced and improved noticeably since the first collections in guinea pigs  
63 [4,5], then with the invention of bipolar electrode rectal probe for ruminants [6,7] to the present  
64 equipment computerized and under automatic control [8]. When appropriately used to collect  
65 semen from bulls, electroejaculation can produce consistently satisfactory results [1,2]. Carroll et  
66 al. [1] reported in beef bulls submitted to breeding soundness examination by using  
67 electroejaculation, satisfactory penile protrusion occurred in 95.9 %, with penile erection in 97.7  
68 %, and ejaculation in 96.1 % of 5,397 evaluations performed. Multiple factors have been  
69 associated with effective electroejaculation such as: individual variation, bred, restraint  
70 conditions, equipment and probe used, skill and experience of the person in charge of semen  
71 collection among others [2,9,10]. Wide variation in behavioral response were also detected  
72 [8,11,12]. To the best of our knowledge, one aspect of electroejaculation procedure is that no  
73 information was available on the effect of fasting prior to electroejaculation in bulls. Deprivation  
74 of food and water is used in planned surgery in ruminants because it reduces heart rate and  
75 contents in the digestive system, decreases gas production, and the risk of regurgitation, among  
76 others [13-19]. In our ambulatory food animal practice or under clinic conditions working either  
77 with *Bos Taurus* or *Bos indicus*, one recommendation prior to perform breeding soundness  
78 examination in clinically healthy bulls was complete fasting between 12- and 24-hours. Overall,  
79 using this prerequisite satisfactory result were obtained. Nevertheless, under these non-controlled  
80 situations the true beneficial effect of fasting cannot be critically assessed. Therefore, if the  
81 same bulls randomly assigned and each it is used as control and treatment, maintained under  
82 similar environmental conditions, where semen is collected using the same equipment, and

83 people are blinded to the animal's treatments will produce more objective and reliable answers  
84 about the real effect of fasting on reproductive and non-reproductive parameters.

85 The objective for the present study was to evaluate the effect of 24 hours fasting prior to  
86 semen collection by electroejaculation on behavioral responses, volume of fecal rectal content,  
87 bladder size, penis protrusion, erection, and ejaculation stimuli as well as ejaculate parameters in  
88 young Simmental bulls.

## 90 2. Material and Methods

### 91 2.1. Animals

92 One week prior to the experiment, in fall season, 24 young Simmental bulls were  
93 physically evaluated and submitted to breeding soundness examination. One bull was removed  
94 from this pool due to a previous surgery for an umbilical hernia. A CBC and a urinalysis were  
95 performed in all eligible bulls in order to detect potential subclinical pathological conditions.  
96 Finally, all bulls were declared physically healthy and available for this project. All the bulls  
97 were post-pubertal, with above the minimum requirements on scrotal circumference and motility  
98 according to the Society for Theriogenology [19]. However, most of them presented a  
99 spermogram characteristic of immature bulls [21-22].

100 All the bulls were maintained in different group corrals in a close barn. Each group corral  
101 contained 4 to 6 bulls of the same age. The bulls received a ration of corn silage, mixed hay and  
102 alfalfa with water ad libitum. In addition, each bull received 2.5 kg/day of pellets concentrate  
103 once a day containing 14% of crude protein. The bulls age was  $13.2 \pm 1.2$  (mean  $\pm$ SD; range; 12  
104 to 15) and the weight was  $523.8 \pm 67.4$  kg (409.0 to 630). The body condition score was  $6.1 \pm$   
105  $0.6$  (5.0 – 7.0; 23]. The scrotal circumference was  $35.2 \pm 2.8$  cm (30 to 40). These bulls had  
106 never been collected by electroejaculation.

### 108 2.2. Experimental design

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4 109 Each bull was confined in a cattle crush with only neck restraint without any limitations  
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6 110 in the side to side movement. No restraint belt was used under the abdomen of any of the bulls.  
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8 111 The bulls were used in a prospective randomized blinded controlled cross-over design in which 2  
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10 112 bull corral groups (n=9; treated group; FAS) were fasted for 24 hours and the other 3 bull corrals  
11  
12 113 groups (n=13; control group; CON) were not. The FAS group had no available food nor water 24  
13  
14 114 hours prior to semen collection. The evaluations were performed in the morning starting at 8:00  
15  
16 115 am. One week later the treatments were inverted and semen was collected again by the identical  
17  
18 116 team group using the same equipment and conditions. The semen collection team was aware of  
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20 117 the project but did not know to which treatment group the bull belongs at the time of  
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22 118 electroejaculation. All the information about the bull treatments was provided at the end of the  
23  
24 119 experiment.

25 120 The order of work was evaluation of volume rectal fecal content, bladder size  
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27 121 determination, behavior response to electroejaculation, protrusion, erection, and ejaculates  
28  
29 122 stimuli, and ejaculate parameters. The volume of rectal fecal content prior to semen collection  
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31 123 was recorded as: 1-No feces in the rectum; 2-One hand scoop of feces in the rectum;3-Two hand  
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33 124 scoop of feces in the rectum; 4-Three to five hand scoops of feces in the rectum; and 5-More  
34  
35 125 than 5 hand scoops of feces in the rectum. The bladder size score was evaluated by per rectum  
36  
37 126 palpation. The scored used was: 1-Small: Bladder completely contracted (example: immediately  
38  
39 127 after urination); 2-Medium: bladder of the hand size; 3-Large: bladder between one and two  
40  
41 128 hands size; and 4-Extra-large: bladder more than 2 hands size. The behavioral response during  
42  
43 129 the electroejaculation was score as: 1-Light, evidence of light/almost undetectable of muscle  
44  
45 130 contractions; 2-Mild, included slight uneasiness and muscle tremors; 3-Moderate: included  
46  
47 131 hunching of back and limb and neck extension; 4-Severe: included marked hunching of the back,  
48  
49 132 limb extension, neck straining, salivation and vocalization; 5-Dangerous, the signs of score 4  
50  
51 133 plus at this time, bulls which appeared to be in considerable distress or likely to lie down. Semen  
52  
53 134 was collected from each bull by electroejaculation by using an electroejaculator in automatic  
54  
55 135 mode using the same set-up for all the bulls (Pulsator V, Lane Manufacturing, Denver, CO,  
56  
57 136 USA) using a two-electrode rectal probe of 60 mm. The number of stimuli required for obtaining  
58  
59 137 penis protrusion, erection, and ejaculation was recorded from the screen of electroejaculator. In  
60  
61 138 addition, the combined efficiency of penis protrusion, erection, and ejaculation (CE-PPEE) was  
62  
63 139 calculated as the proportion of achievement of penis protrusion, erection and ejaculation from the  
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4 140 total collection for each treatment. Volume of ejaculate was measured in graduated tubes to 0.1  
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6 141 ml at the time of collection. Semen (25 $\mu$ l) was placed in one warm slide and covered by a  
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8 142 coverslip to assess progressive sperm motility. A board-certified Theriogenologist analyzed  
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10 143 percent of progressive sperm motility by evaluating multiple fields under light microscopy at  
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12 144 X400. Motility was scored as: 1- Very good: mass activity characterized by rapid swirling with  
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14 145 an individual motility  $\geq 70\%$ ; 2- Good: mass activity characterized by slower swirling with an  
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16 146 individual motility between 50 and 69%; 3; Fair: mass activity characterized by generalized  
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18 147 oscillating and individual motility between 30 and 49%; and 4-Poor: mass activity with sporadic  
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20 148 oscillation and individual motility  $\leq 29\%$  according to the criteria the Society for  
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22 149 Theriogenology [20]. Concentration of spermatozoa in the ejaculate was determined by using  
23  
24 150 NucleoCounter SP-100 previously validated for bull sperm concentration (24). Another aliquot  
25  
26 151 of neat semen (25 $\mu$ l) was diluted with buffer formalin (1.0 mL) and taken back to the lab for the  
27  
28 152 evaluation of sperm morphology. Sperm morphology was analyzed at higher power  
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30 153 magnification (X1000) by using contrast phase microscope and 200 hundred sperm cells were  
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32 154 evaluated for their morphology according to SFT criteria [20]. Bulls were monitored twice daily  
33  
34 155 for demeanor and appetite during the whole period of investigation and for the following week of  
35  
36 156 the last semen collection. Procedures used in this investigation were approved by Committee for  
37  
38 157 Animal Welfare, Bologna University (Prot. N. 0005783).

### 38 158 2.3. Statistical analysis.

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41 159 The continuous variables were analyzed by “t” student test for paired samples. The  
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43 160 dichotomous outcomes were analyzed by McNemar test. A difference was considered significant  
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45 161 at  $P \leq 0.05$ . A software program was used [25].  
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## 48 49 50 163 Results

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55 165 From the 23 bulls available, one bull presented pneumonia 4 days after the first semen collection.  
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57 166 This bull was treated and removed from the experiment. Therefore, 22 matches comparisons  
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59 167 were available for statistical analysis. The behavioral response of the bulls to electroejaculation  
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4 168 was not different between FAS and CON groups ( $3.2 \pm 0.5$  and  $3.0 \pm 0.7$ , respectively;  $P=0.36$ ).  
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6 169 The bladder size was smaller in the FAS group compared with the CON group ( $2.3 \pm 0.8$  vs.  $2.8$   
7  
8 170  $\pm 0.9$ , respectively;  $P=0.02$ ). The volume of feces in the rectum was not different between  
9  
10 171 groups; CON was  $2.3 \pm 1.7$  and FAS  $3.0 \pm 1.8$  ( $P=0.23$ ). The FAS group resulted in a higher  
11  
12 172 proportion of penis protrusion compared with CON group (100% versus 81.8%,  $P=0.10$ ),  
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14 173 erection (100% versus 81.8%;  $P=0.10$ ), and ejaculation (100% versus 90.9%;  $P=0.49$ ),  
15  
16 174 respectively. The combined efficiency of penis protrusion, erection, and ejaculation (CE-PPEE)  
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18 175 in FAS group was superior than CON group ( $P=0.001$ ) for those parameters. The number of  
19  
20 176 stimuli needed for penis protrusion, erection, and ejaculation for the CON group was  $13.5 \pm 3.7$ ,  
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22 177  $14.9 \pm 3.7$ , and  $20.8 \pm 5.8$  and for the FAS group was  $15.0 \pm 4.2$ ,  $16.6 \pm 4.2$ , and  $20.2 \pm 8.1$ ,  
23  
24 178 respectively. The number of stimuli for penis protrusion ( $P=0.09$ ), erection ( $P=0.08$ ), and  
25  
26 179 ejaculation ( $P=0.77$ ) were no different between groups Ejaculate volume was  $4.0 \pm 2.6$  ml and  
27  
28 180  $4.1 \pm 2.3$  ml for CON and FAS groups, respectively ( $P=0.90$ ). The motility was  $1.4 \pm 0.7$  and  $1.4$   
29  
30 181  $\pm 0.8$  for CON and FAS groups, respectively ( $P=0.72$ ). The concentration of spermatozoa was  
31  
32 182  $336.2 \pm 273.1$  million and  $421.1 \pm 300.6$  million for CON and FAS groups, respectively  
33  
34 183 ( $P=0.31$ ). The percentage of normal spermatozoa was  $50.9 \pm 18.8$  and  $45.6 \pm 14.3$  for CON and  
35  
36 184 FAS groups, respectively ( $P=0.16$ ). No changes in behavior or appetite during the experimental  
37  
38 185 period and for the following week of the last semen collection was identified.  
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## 41 187 Discussion

42 188  
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45 189 In the present investigation, no adverse effects were noted during and after semen  
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47 190 collection by electroejaculation in any of the bulls. All bulls remained in a healthy condition  
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49 191 during and after the experiment. The electroejaculation did not affect the wellbeing of the bulls.

50  
51 192 The behavioral responses of the young bulls to electroejaculation can be considered  
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53 193 satisfactory based on the mean response was moderated (score 3) in both groups. No significant  
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55 194 differences were detected between fasting and non-fasting bulls. Nevertheless, the detailed  
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57 195 analysis of data showed that the same bulls when they were fasted all the scores were between 1,  
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59 196 2, 3 and 4 and in the non-fasting conditions the scores were all 3, 4, and 5. This suggests a

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4 197 potential beneficial effect of fasting that was failed to be significant to the limited power of the  
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6 198 present study. Investigations including more animals to confirm or correct the present findings  
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8 199 will be required.  
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11 200 The effect of fasting produced a considerable reduction in bladder size. The decrease in  
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13 201 bladder volume has several potential positive effects for semen collection such as improved  
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15 202 contact between the rectal probe and the accessory sexual glands, and a decrease in the risk of  
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17 203 urospermia. A full bladder size may increase the chances of urine in the ejaculate during the  
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19 204 process of electroejaculation. In a previous study, it was showed that almost of 50% of the bulls'  
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21 205 electroejaculated presented partial retrograde ejaculation; therefore, part of the semen was sent  
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23 206 backward into the bladder rather forward to the terminal urethra [26]. Unfortunately, in this  
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25 207 above-mentioned study no information was provided if the bulls were fasted prior to  
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27 208 electrostimulation. Urospermia has a negative effect not only in the semen parameters but also  
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29 209 could affect the freezability of the semen sample [27,28]. This is an area that needs further  
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31 210 investigation.

32 211 No reduction in the rectal fecal content was observed by fasting for 24 hours. This  
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34 212 finding agrees with multiple independent studies which have shown the need of at least two days  
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36 213 of fasting in order to notice a reduction of feces in the rectum [13-15]. The effect of fasting has  
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38 214 been shown not only to reduce rumen and reticular content but also diminish the frequency and  
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40 215 amplitude of rumen contractions as decline the ruminal flora and pH as well [17] as well as  
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42 216 reduced the heart rate [19]. One day of feed and water deprivation in healthy animals stimulated  
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44 217 mobilization of body nutrients and breakdown of fat reserves, elicited neuroendocrine and acute-  
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46 218 phase protein responses, and resulted in loss of body weight, however, these effects were quickly  
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48 219 reversed after feeding [16-18].

49 220 No differences were noticed in the stimuli required to produce penis protrusion, erection,  
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51 221 and ejaculation between both groups. However, in the FAS group 100% of penis protrusion,  
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53 222 erection, and ejaculation while on the CON group 81.8% of penis protrusion and erection and  
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55 223 90.9% of ejaculation was obtained. The overall efficiency of PPEE for FAS group was higher  
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57 224 compared with CON group. The reason of this is unknown, however, the fasting showed a  
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59 225 positive effect in increasing the proportion of penis protrusion with erection and ejaculation.  
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61 226 Moreover, the proportion of penis protrusion, erection, and ejaculation agrees with previous  
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4 227 studies that obtained more than 90% of satisfactory responses using electroejaculators by manual  
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6 228 mode [1,10,11,29].  
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8  
9 229 In the current study, no changes in any of the 4 ejaculate parameters that were evaluated:  
10 230 volume, concentration, spermatozoa motility and morphology between groups were detected. In  
11 231 regards to the last parameter, the percentage of normal sperm morphology was below from the  
12 232 minimum required [70%] for the Society for Theriogenology. The main reason was that most of  
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14 233 the bulls were young; from the 22 bulls used in the present study, 10 were 12 months old, 9 were  
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16 234 14 months old, and only 3 were 15 months old. Even though, all the bulls were post-pubertal and  
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18 235 most of them presented a spermogram characteristic of immature bulls [21,22].  
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22 236 It is necessary to comment that the present investigation exhibited multiple weaknesses  
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24 237 including the limited number of bulls, only young, just one breed, and that they were maintained  
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26 238 in a confined system with a feeding management that only provided total mixed ration during all  
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28 239 the process. It is necessary to reinvestigate the effect of fasting in bulls using other breeds and  
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30 240 ages as well as in bulls maintained in a pastoral system.  
31

32 241 From this investigation, it was concluded that fasting for 24 hours prior to semen  
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34 242 collection by electroejaculation reduced the bladder size and increased the proportion of bulls  
35  
36 243 with penis protrusion, erection and ejaculation without any difference detected in behavioral  
37  
38 244 responses, volume of rectal fecal content, and ejaculate parameters.  
39

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45  
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47  
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59

#### 59 255 Competing Interests

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4 256 All authors declare that there is no conflict of interest that could be perceived as  
5  
6 257 prejudicing the impartiality of the research reported.  
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