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Abstract

The objective of the present study was to evaluate the effect of 24 hours fasting prior to semen collection by electroejaculation on behavioral responses, volume of rectal fecal content, bladder size, penis protrusion, erection, ejaculation stimuli, and ejaculate parameters in young Simmental bulls. Twenty-two Simmental beef bulls with an age of 13.2 ± 1.2 months (mean ± SD) were used in a prospective randomized blinded controlled cross-over design with two corrals fasted for 24 hours (n=9; FAS group) and the other three corrals were non-fasted (n= 13;

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 motiliy, and morphology. The behavioral response of the bulls to electroejaculation was not different between CON group and FAS group (3.2 ± 0.5 and 3.0 ± 0.7, respectively; P=0.36). Bladder size was significantly reduced in the FAS group compared with CON group (2.3 ± 0.8 vs. 2.8 ± 0.9, respectively; P=0.02). The volume of feces in the rectum was not different between both groups (CON was 2.3 ± 1.7 and FAS was 3.0 ± 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 ± 3.7, 14.9 ± 3.7, and 20.8 ± 5.8 and for FAS group was 15.0 ± 4.2, 16.6 ± 4.2, and 20.2 ± 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 ± 2.6 ml and 4.1 ± 2.3 ml for CON and FAS groups, respectively (P=0.90). The motility was 1.4 ± 0.7 and 1.4 ± 0.8 for CON and FAS groups, respectively (P=0.72). The concentration of spermatozoa was 336.2 ± 273.1 million and 421.1 ± 300.6 million for CON and FAS groups, respectively (P=0.31). The percentage of normal spermatozoa was 50.9 ± 18.8 and 45.6 ± 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.

Introduction

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

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

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

2.Material and Methods

2.1. Animals

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

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

2.2. Experimental design

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

The order of work was evaluation of volume rectal fecal content, bladder size determination, behavior response to electroejaculation, protrusion, erection, and ejaculates stimuli, and ejaculate parameters. The volume of rectal fecal content prior to semen collection was recorded as: 1-No feces in the rectum; 2-One hand scoop of feces in the rectum;3-Two hand scoop of feces in the rectum; 4-Three to five hand scoops of feces in the rectum; and 5-More than 5 hand scoops of feces in the rectum. The bladder size score was evaluated by per rectum palpation. The scored used was: 1-Small: Bladder completely contracted (example: immediately after urination); 2-Medium: bladder of the hand size; 3-Large: bladder between one and two hands size; and 4-Extra-large: bladder more than 2 hands size. The behavioral response during the electroejaculation was score as: 1-Light, evidence of light/almost undetectable of muscle contractions; 2-Mild, included slight uneasiness and muscle tremors; 3-Moderate: included hunching of back and limb and neck extension; 4-Severe: included marked hunching of the back, limb extension, neck straining, salivation and vocalization; 5-Dangerous, the signs of score 4 plus at this time, bulls which appeared to be in considerable distress or likely to lie down. Semen was collected from each bull by electroejaculation by using an electroejaculator in automatic mode using the same set-up for all the bulls (Pulsator V, Lane Manufacturing, Denver, CO, USA) using a two-electrode rectal probe of 60 mm. The number of stimuli required for obtaining penis protrusion, erection, and ejaculation was recorded from the screen of electroejaculator. In addition, the combined efficiency of penis protrusion, erection, and ejaculation (CE-PPEE) was calculated as the proportion of achievement of penis protrusion, erection and ejaculation from the

total collection for each treatment. Volume of ejaculate was measured in graduated tubes to 0.1 ml at the time of collection. Semen (25µl) was placed in one warm slide and covered by a coverslip to assess progressive sperm motility. A board-certified Theriogenologist analyzed percent of progressive sperm motility by evaluating multiple fields under light microscopy at X400. Motility was scored as: 1- Very good: mass activity characterized by rapid swirling with an individual motility ≥70%; 2- Good: mass activity characterized by slower swirling with an individual motility between 50 and 69%; 3; Fair: mass activity characterized by generalized oscillating and individual motility between 30 and 49%; and 4-Poor: mass activity with sporadic oscillation and individual motility ≤ 29% according to the criteria the Society for Theriogenology [20]. Concentration of spermatozoa in the ejaculate was determined by using NucleoCounter SP-100 previously validated for bull sperm concentration (24). Another aliquot of neat semen (25µl) was diluted with buffer formalin (1.0 mL) and taken back to the lab for the evaluation of sperm morphology. Sperm morphology was analyzed at higher power magnification (X1000) by using contrast phase microscope and 200 hundred sperm cells were evaluated for their morphology according to SFT criteria [20]. Bulls were monitored twice daily for demeanor and appetite during the whole period of investigation and for the following week of the last semen collection. Procedures used in this investigation were approved by Committee for Animal Welfare, Bologna University (Prot. N. 0005783).

2.3. Statistical analysis.

The continuous variables were analyzed by "t" student test for paired samples. The dichotomous outcomes were analyzed by McNemar test. A difference was considered significant at $P \leq 0.05$. A software program was used [25].

Results

From the 23 bulls available, one bull presented pneumonia 4 days after the first semen collection. This bull was treated and removed from the experiment. Therefore, 22 matches comparisons were available for statistical analysis. The behavioral response of the bulls to electroejaculation

was not different between FAS and CON groups (3.2 ± 0.5 and 3.0 ± 0.7, respectively; P=0.36). The bladder size was smaller in the FAS group compared with the CON group (2.3 ± 0.8 vs. 2.8 ± 0.9, respectively; P=0.02). The volume of feces in the rectum was not different between groups; CON was 2.3 ± 1.7 and FAS 3.0 ± 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 needed for penis protrusion, erection, and ejaculation for the CON group was 13.5 ± 3.7, 14.9 ± 3.7, and 20.8 ± 5.8 and for the FAS group was 15.0 ± 4.2, 16.6 ± 4.2, and 20.2 ± 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 ± 2.6 ml and 4.1 ± 2.3 ml for CON and FAS groups, respectively (P=0.90). The motility was 1.4 ± 0.7 and 1.4 ± 0.8 for CON and FAS groups, respectively (P=0.72). The concentration of spermatozoa was 336.2 ± 273.1 million and 421.1 ± 300.6 million for CON and FAS groups, respectively (P=0.31). The percentage of normal spermatozoa was 50.9 ± 18.8 and 45.6 ± 14.3 for CON and FAS groups, respectively (P=0.16). No changes in behavior or appetite during the experimental period and for the following week of the last semen collection was identified.

Discussion

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

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

potential beneficial effect of fasting that was failed to be significant to the limited power of the present study. Investigations including more animals to confirm or correct the present findings will be required.

The effect of fasting produced a considerable reduction in bladder size. The decrease in bladder volume has several potential positive effects for semen collection such as improved contact between the rectal probe and the accessory sexual glands, and a decrease in the risk of urospermia. A full bladder size may increase the chances of urine in the ejaculate during the process of electroejaculation. In a previous study, it was showed that almost of 50% of the bulls' electroejaculated presented partial retrograde ejaculation; therefore, part of the semen was sent backward into the bladder rather forward to the terminal urethra [26]. Unfortunately, in this above-mentioned study no information was provided if the bulls were fasted prior to electrostimulation. Urospermia has a negative effect not only in the semen parameters but also could affect the freezability of the semen sample [27,28]. This is an area that needs further investigation.

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

No differences were noticed in the stimuli required to produce penis protrusion, erection, and ejaculation between both groups. However, in the FAS group 100% of penis protrusion, erection, and ejaculation while on the CON group 81.8% of penis protrusion and erection and 90.9% of ejaculation was obtained. The overall efficiency of PPEE for FAS group was higher compared with CON group. The reason of this is unknown, however, the fasting showed a positive effect in increasing the proportion of penis protrusion with erection and ejaculation. Moreover, the proportion of penis protrusion, erection, and ejaculation agrees with previous

studies that obtained more than 90% of satisfactory responses using electroejaculators by manual mode [1,10,11,29].

In the current study, no changes in any of the 4 ejaculate parameters that were evaluated: volume, concentration, spermatozoa motility and morphology between groups were detected. In regards to the last parameter, the percentage of normal sperm morphology was below from the minimum required [70%] for the Society for Theriogenology. The main reason was that most of the bulls were young; from the 22 bulls used in the present study, 10 were 12 months old, 9 were 14 months old, and only 3 were 15 months old. Even though, all the bulls were post-pubertal and most of them presented a spermiogram characteristic of immature bulls [21,22].

It is necessary to comment that the present investigation exhibited multiple weaknesses including the limited number of bulls, only young, just one breed, and that they were maintained in a confined system with a feeding management that only provided total mixed ration during all the process. It is necessary to reinvestigate the effect of fasting in bulls using other breeds and ages as well as in bulls maintained in a pastoral system.

From this investigation, it was concluded that fasting for 24 hours prior to 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.

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Competing Interests

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

References

1- Carroll, E. J, Ball l. Scott JA. Breeding Soundness in Bulls-A summary of 10,940 Examinations. J Amer Vet Med Assoc 1963; 142:1105-11.

2- Ball L, Furman JW. Electroejaculation of the bull. Bov Pract 1972; 7:46-8.

3- Chenoweth PJ, McPherson FJ. Bull breeding soundness, semen evaluation and cattle productivity. Anim Reprod Sci 2016; 169:32-6.

4- Battelli, F. Une Methode pour obtenir l' Emission Complete du Liquide des Vesicules Seminales chez le Cobaye. Soc. de Physique et d' Hist. nat. de Geneve, Comp. Rend., 1922; 39: 73-4.

5- Moore CR, Gallagher TF. Seminal-vesicle and prostate function as testis-hormone indicator; the electroejaculation test. Amer J Anat 1930:45-39-9.

6- Lapalud M, Cassou R. Nouveau proceed de récolte du sperme para electrode bipolaire rectal unique. Comp Rend Acad Agr France. 1945; 31:37-8.

7- Thibault C, Lapaud C, Ortavant R. L'Electro-ejaculation chez le taureau, techqniques e resultats. Comp Rend Acad Agr France. 1948:226:2006-8

8- Whitlock BK, Coffman EA, Coetzee JF, Daniel JA. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology 2012; 78:737-46.

9- Chenoweth PJ, Osborn HG. Breed differences in the response of young beef bulls to electroejaculation. Aust Vet J 1978;54;333-7.

10- Hill HJ, Scott FS, Homan N, Gassner FW. Electroejaculation in the bull. J Amer Vet Med Assoc 1956; 128:375-80.

11- Falk AJ, Waldner CL, Cotter BS, Gudmundson J, Barth AD. Effects of epidural lidocaine anesthesia on bulls during electroejaculation. Can Vet J 2001; 42:116-20.

12- Etson CJ, Waldner CJ, Barth AD. Evaluation of a segmented rectal probe and caudal epidural anesthesia for electroejaculation of bulls. Can Vet J 2004; 45:235-40.

13- Blaxter KL, Wainman FW. The fasting metabolism of cattle. Br J Nutr 1966; 20:103-11.

14- Atterbury JT, Johnson HD. Effects of environmental temperature, controlled feeding and fasting on rumen motility. J Anim Sci 1969; 29:734-7.

15- Galyean ML, Lee RW, Hubbert ME. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J Anim Sci 1981; 53:7-18.

16- Phillips WA, Juniewicz PE, VonTungeln DL. The effect of fasting, transit plus fasting, and administration of adrenocorticotropic hormone on the source and amount of weight lost by feeder steers of different ages. J Anim Sci 1991; 69:2342-8.

17- Loerch SC, Fluharty FL. Physiological changes and digestive capabilities of newly received feedlot cattle. J Anim Sci 1999; 77:1113-9.

18- Marques RS, Cooke RF, Francisco CL, Bohnert DW. Effects of twenty-four transport or twenty-four-hour feed and water deprivation on physiologic and performance responses of feeder cattle. J Anim Sci 2012; 90:5040-6.

19- Clabough DL, Swanson CR. Heart rate spectral analysis of fasting-induced bradycardia of cattle. Am J Physiol 1989;257: R1303-6.

20- Hopkins FM, Spitzer JC. The new Society for Theriogenology Breeding Soundness evaluation system. Vet Clin of North Amer: Food Animal Practice. 1997; 13:283-93.

21- Lunstra, DD. and Echternkamp, S. E. Puberty in beef bulls: Acrosome morphology and semen quality in bulls of different breeds. J Anim Sci 1982; 55:638-48.

22- Spitzer JC, Hopkins FM. Breeding soundness evaluation of yearling bulls. Vet Clin of North Amer: Food Animal Practice. 1997; 13: 295-304.

23- Richards, M. W., J. C. Spitzer, and M. B. Warner. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J Anim Sci 1986; 62:300−6.

24- Anzar M, Kroetsch T, Buhr MM. Comparison of Different Methods for Assessment of Sperm Concentration and Membrane Integrity with Bull Semen. J Androl 2009; 30:661–8.

25- Minitab 17. Minitab Inc: State College, PA

26- Dooley MP, Pineda MH, Maurer RR, Lunstra DD. Evidence for retrograde flow of spermatozoa into the urinary bladder of bulls during electroejaculation. Theriogenology 1985; 26:101-9.

27- Griggers S, Paccamonti DL, Thompson RA, Eilts BE. The effects of ph, osmolarity and urine contamination on equine spermatozoal motility. Theriogenology 2001; 56:613-22.

28- Ellerbrock RE, Honorato J, Curcio BR, Stewart JL, Souza JAT, Love CC, Lima FS, Canisso IF. Effect of urine contamination on stallion semen freezing ability. Theriogenology. 2018; 117:1-6.

29- Furman JW, L. Ball L,and G. E. Seidel GE Jr. Electroejaculation of bulls using pulse waves of variable frequency and length. J Anim Sci 1975;40;665-70.