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Chemical castration in cattle with intratesticular injection of sodium chloride: Effects on stress and inflammatory markers

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ABSTRACT

Intratesticular injection (ITI) of sodium chloride (NaCl) is efficient for chemical castration of young calves, but its effects on calves welfare are unknown. Two experiments were conducted to evaluate the effects of ITI of 20% NaCl on stress and inflammatory markers in calves less than 20 days old and to assess the efficiency of ITI of 30% NaCl in 5 months old calves. In Experiment 1, control calves were only restrained and compared to calves submitted to castration through surgery (SC) and ITI with 20% NaCl (n = 9/group). No differences were observed for the eye corner temperature measured by thermography from 60 s before to 60 s after the procedures ($P > 0.05$). In the SC group, acute serum cortisol levels increased at 30 and 60 min after the procedure, but increased levels in the ITI group occurred only at 30 min ($P < 0.05$). Chronic discomfort markers were measured at 0, 24, 48, 72 and 96 h after the procedures (D0, D1, D2, D3 and D4, respectively). The serum levels of the paraoxonase 1 (PON1) enzyme and cortisol did not differ among groups ($P > 0.05$). Scrotal temperature was higher at D1 in the SC group than for the other groups, but lowest at D4 compared to the control (both $P < 0.05$). In Experiment 2, histological sections of testes were compared after ITI with either 30% NaCl or 30% calcium chloride ($CaCl_2$), to intact calves (control). After 60 days, intact seminiferous tubules and mediastinum were observed after ITI with 30% NaCl, whereas coagulative necrosis, inflammatory infiltration and calcification occurred after ITI with 30% $CaCl_2$. Efficient chemical castration through ITI of 20% NaCl in young calves was followed by slight stress and inflammatory responses compared to surgical castration. However, ITI of 30% NaCl was ineffective for chemical castration of 5 months old calves.

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1. Introduction

Castration of calves is a widespread practice among beef breeders to prevent aggressive behavior, sexual activity and bull breeding [1], with additional benefits for carcass finishing, since decreased circulating testosterone levels prevent the pH increase in the meat [2–4]. Castration can be done using physical, immunological or chemical methods. Physical castration can be performed through either surgery (orchiectomy) or emasculation, using Burdizzo clamps, stenotic elastic rings or strips to interrupt the blood supply to the testicles [5]. Immunological castration is based on reducing testosterone levels through immunocontraception, inducing the production of antibodies against GnRH [6]. Chemical castration can be accomplished by intratesticular injection (ITI) of compounds that induce destruction of testicular cells through caustic or osmotic processes, such as lactic acid [7], $CaCl_2$ [8] and NaCl [9].

Surgical castration is widely used in cattle, even though it is often performed under inadequate conditions, resulting in a

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significant degree of pain and bacterial contamination [10,11]. Besides the pain, calves show various behavioral changes during and after the surgical procedure such as: agitation, leg movements, tail swing, disoriented walk, prostration, reduced interest in their mothers, and decreased feed intake [12]. Such signs of discomfort can be assessed by markers capable of identifying processes of stress, pain and inflammation, such as the serum levels of cortisol [13], the eye corner temperature [14] and acute phase proteins such as the paraoxonase 1 (PON1) enzyme [15].

Currently, consumers are increasingly questioning management practices that may be associated with pain and discomfort on the animals that provide the meat they consume [16,17]. Thus, the use of alternative castration methods that can improve animal welfare is of interest for the cattle industry. The ITI is a minimally invasive chemical castration technique that is efficient when using NaCl on calves that are 30 days old or younger [9] and $CaCl_2$ in 7–8 months old buffalos [8]. However, the effects of ITI with NaCl on the welfare of castrated young calves are still unknown and its effectiveness for chemical castration of older calves has not yet been evaluated. The objectives of this study were to evaluate the effects of ITI with 20% NaCl on markers of stress and inflammatory responses in calves up to two weeks old, and to test the efficiency of 30% NaCl ITI for chemical castration of 5-months old calves.

2. Material and methods

All procedures were approved by the Ethics in Animal Experimentation Committee (CEEA-UFPel; process # 2258).

2.1. Experiment 1

2.1.1. Experimental design

This experiment was conducted with 27 beef calves from 4 to 20 days of age, with average 36 kg of body weight and kept with their mothers in ryegrass pasture with *ad libitum* access to water. All calves were submitted to the same manual restraining procedures (calves were put down on the ground by gently pulling their legs) and randomly assigned to three groups ($n = 9$ each). Calves in the control group were only restrained, calves in the second group were castrated through orchiectomy and those in the third group were submitted to ITI of 20% NaCl.

Surgical castration was performed as described elsewhere [9]. Antisepsis of the scrotum was performed with 2% iodine-ethanol solution and 2% chlorhexidine digluconate solution (both from Rioquimica®, São José do Rio Preto-SP, Brazil). Local anesthesia was conducted with 5 ml 2% lidocaine. The orchiectomy was conducted after incision of the scrotum, removal of both testicles and section of the spermatic cords.

The ITI was conducted by dissolving NaCl (Synth®, Diadema, SP, Brazil) [9] and lidocaine [8] in ultrapure water. The final solution with 20% NaCl and 2% lidocaine was sterilized by 0.22 μm filtration and kept in sterile vials at 5 °C. After scrotum antisepsis, each testicle was immobilized and the solution was injected with a 21 G1½ needle, at its distal end. The injected volume (1.5–4.0 ml) varied according to the size of each testicle, as long as the gonad presented firm consistency. For all calves, the ITI was performed by the same technician, using the same criteria to define the injected volume.

2.1.2. Acute discomfort markers

Blood samples were collected from calves of the three groups, at the following time points: during the procedure; 30 min after; and 60 min after. Samples were collected through puncture of the jugular vein with a 21G needle connected to a vacuum collection system (BD Vacutainer®), into 10 ml tubes without anticoagulant. Immediately after collection, samples were centrifuged ($1500 \times g$ for 10 min). Serum samples were subsequently stored in liquid nitrogen.

Cortisol levels were quantified by the electrochemiluminescence assay Cortisol II Cobas (Roche Diagnostics, Mannheim, Germany; REF 06687733), in a commercial laboratory, with intra and inter-assay coefficient of variability lower than 10%.

The eye corner temperature was determined by thermography to identify changes in temperature caused by reduced blood flow in the eye corner due to vasoconstriction of the sympathetic nervous in response to pain [18]. Thermography was conducted at the following time points: before (−60 s and −30 s); during (0); and after the procedures (30 s and 60 s). Thermographic images were obtained with the thermograph FLIR® E25 and analyzed by the FLIR® software (FLIR QuickReport™ PC software).

2.1.3. Chronic discomfort markers

Blood samples were collected from the time of the procedures up to four days after, as described above. Chronic serum cortisol levels were determined as described above for acute cortisol. Scrotal thermography was conducted as described above for eye corner thermography.

The quantification of serum PON1 was performed as described elsewhere [15,19]. Briefly, samples were previously diluted in a 1:3 ratio and mixed with a working solution (3.3 μL of the diluted sample in 500 μL of working solution). The working solution consisted of 20 mM Tris/HCl buffer; 1.0 mM $CaCl_2$; and 4.0 mM phenylacetate. The reading was performed in a Cirrus 80ST spectrophotometer, at 270 nm wavelength for 60 s. Enzyme activity was determined by the following formula: Δ Absorbance x 115×3. The activity of PON1 was expressed in U/L.

Scrotal temperature and serum levels of PON1 were determined at the time of the procedures (D0); after 24 h (D1); 48 h (D2); 72 h (D3); and 96 h (D4). Chronic serum cortisol levels were determined at D0, D2 and D4.

2.2. Experiment 2

Nine calves aging between 120 and 150 days and average live weight of 125 kg (from 119 to 137 kg) were randomly assigned to three groups ($n = 3$ each): in two groups, calves were submitted to ITI with either 30% NaCl or 30% $CaCl_2$; whereas calves in the control group were only restrained. The restraining procedures for calves in all three groups were the same described for Experiment 1. Both hypertonic solutions were prepared using 2% lidocaine (20 mg/ml) in saline, filtered in 0.22 μm filter and stored in sterile vials at 5 °C until use. After scrotum antisepsis, each testicle was immobilized and the solution was injected with a 21 G1½ needle, at its distal end. The injected volume varied from a minimum of 4.0 ml to a maximum of 8.0 ml, which was defined considering the size of each testicle, as long as the gonad presented firm consistency. As occurred in Experiment 1, that procedure was always conducted by the same technician. The group submitted to ITI with $CaCl_2$ was considered as a positive control, since such chemical is known to be effective on promoting sterility in older animals [8].

Sixty days after the procedures, calves from all three groups were surgically castrated, as described for Experiment 1. Immediately after castration, samples of testicular parenchyma were placed in 10% formalin buffered solution. Thereafter, fragments were removed from that solution, dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections of 5 μm were obtained using an automatic microtome (RM2245, Leica Biosystems, San Diego, CA, USA), stained with hematoxylin and eosin (HE) and blindly evaluated by an experienced veterinary pathologist. Testicle samples from calves of the control group were considered as

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