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Acute pain and peripheral sensitization following cautery disbudding in 1- and 4-week-old calves

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ABSTRACT

Acute pain and peripheral sensitization development after cautery disbudding was investigated in 33 calves administered preventive multimodal analgesia. The animals were assigned randomly to three groups: 1) Group SH (Control), undergoing sham disbudding at 1 and 4 weeks of age; 2) Group ED (Early Disbudding), undergoing disbudding at 1 week of age and sham disbudding at 4 weeks of age; 3) Group LD (Late Disbudding), undergoing sham disbudding at 1 week of age and disbudding at 4 weeks of age. Physiological parameters (heart rate, respiratory rate, rectal temperature, invasive blood pressure, cortisol, β-endorphin, interleukin-1β, interleukin-6, tumor necrosis factor-α and haptoglobin plasmatic concentration), local variables (tactile sensitivity score, pressure pain thresholds and horn temperature), behavior and pain scores [multidimensional pain scale and visual analogue scale (VAS)] were assessed at baseline and at several pre-determined time points until 24 h after disbudding. Tactile sensitivity score significantly and equally increased in both groups ED and LD and pressure pain thresholds significantly decreased in group LD until 24 h after disbudding compared to group SH. Pain and VAS scores significantly and equally increased in both groups ED and LD until 24 h after disbudding compared to group SH. No significant differences in physiological parameters, behavior and horn temperature were detected among groups. The present study suggests that acute pain and peripheral sensitization develop and do not differ in calves disbudded at 1 week and 4 weeks of age. Moreover, the use of physiological and behavioral parameters as sole indicators of acute pain might lead to improper conclusions and should be reassessed.

1. Introduction

Disbudding is a routine practice in dairy calves up to around 8 weeks of age [1]. It is practiced mainly to improve safety in handling, decrease the risk of injury to both people and other animals, and decrease the incidence of carcass wastage because of bruising [2]. The procedure is inevitably accompanied by stress and acute pain as various degrees of tissue damage are elicited.

Several factors can influence the severity of acute pain following disbudding. Among available techniques, cautery disbudding has been recommended over scoop and chemical methods [3,4]. Various pharmacologic approaches have been used to reduce acute pain elicited from disbudding. There is evidence that the combination of a local anesthetic and a nonsteroidal anti-inflammatory drug (NSAID) is more effective than the use of either drug alone [2,5] and that sedative drugs can improve the welfare of calves during the procedure [2,6,7]. Age of disbudding may also play a role in acute pain development; indeed, there is a widespread assumption that younger animals are less sensitive to pain [3,6,8–12]. However, there is little heterogeneous evidence supporting this theory [13–15], while other studies show that age has no influence on acute pain development in animals undergoing tail docking and castration [16] or cautery disbudding [6].

Peripheral sensitization (namely the increased responsiveness and reduced threshold of nociceptors to stimulation of their receptive fields [17]) has been shown to occur and last at least 75 up to 96 h after cautery disbudding in calves [18,19], despite administration of multimodal analgesia. However, whether the age at which the procedure is performed has an influence on the development of peripheral

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sensitization, to the best of the authors' knowledge, has never been explored.

This study aimed at investigating several markers of acute pain and peripheral sensitization after early (1 week) and late (4 weeks) cautery disbudding in calves administered preventive multimodal analgesia.

Our hypotheses were that 1) despite the use of preventive multimodal analgesia, acute pain and peripheral sensitization occur following cautery disbudding and 2) acute pain and peripheral sensitization develop and do not differ between early (1 week) and late (4 weeks) disbudded calves.

2. Materials and methods

The experiment received ethical approval from the Canton of Fribourg (2014_52_FR) and was carried out as a prospective, controlled, randomized study between January 2015 and January 2016 at the Swiss center for agricultural research Agroscope, Posieux, Switzerland. This study was part of a wider data collection aimed at evaluating the development of chronic pain in calves over a three month period.

2.1. Animals and housing

Thirty-four Holstein bull calves were recruited after birth from their farms of origin. Inclusion criteria were a normal birth weight and an unremarkable clinical exam.

Body weight was measured using an analogue scale (reported in the Results as mean body weight ± SD). Animals that did not maintain normal clinical health or gain weight satisfactorily (0.8 ± 0.2 kg/day in the first 4 weeks) were excluded from the study. Animals that contracted illnesses causing distress and suffering that were not treatable with medical therapy administered over 3 days, were excluded and euthanized.

At arrival to the experimental facility (1 to 3 days after birth), the calves were supplemented with colostrum (Locatim, Biokema AG, Switzerland). During the first four weeks of age, the animals were kept in single boxes (150 × 150 cm) and permanent visual contact among them was guaranteed. Calves were fed twice a day with powdered milk (UFA 207 Plus, UFA SA, Switzerland); hay and water were offered ad libitum. After this period, the calves were kept in small groups in a straw bedded stall with access to an outdoor pen and were fed with hay ad libitum and 8 l/day of powder milk.

Calves were allowed to acclimatize to the facility at least 2 days after arrival and were handled several times by the two main investigators (AM and DC) before starting the experiment. The clinical exam was repeated twice daily before final inclusion.

2.2. Experimental design

The calves were randomly assigned to one of the following groups: 1) Group SH (Control) (n = 12), undergoing sham disbudding at 5–7 days (1 week) and 28–30 days (4 weeks) of age; 2) Group ED (Early Disbudding) (n = 11), undergoing disbudding at 1 week and sham disbudding at 4 weeks of age; 3) Group LD (Late Disbudding) (n = 10), undergoing sham disbudding at 1 week of age and disbudding at 4 weeks of age.

The randomization plan was obtained from commercial software available online (www.randomization.com). The experimental session consisted of a three day trial: measurements were performed 24 h before disbudding (baseline), during the day of disbudding and 24 h after disbudding. Two calves were treated at each session; visual and auditory contact between them was guaranteed during the whole procedure.

Each experimental day started between 07:30 and 08:30 h. A total of 30 min of acclimation in the boxes (85.5 × 145 cm) was allowed before starting the experimental protocol. Food was only withheld during data collection.

On the day of disbudding, the skin over the two jugular veins and the two auricular arteries was clipped, cleaned and disinfected; then, a local anesthetic cream (EMLA cream 5%, Astrazeneca, UK) was applied over the clipped area of the ears. Sedation was achieved with 0.1 mg/kg xylazine (Xylasol, Dr. E. Graeub AG, Switzerland) injected into the triceps brachii muscle. Sedation was scored after 10 min [ranging from (0) no sedation to (3) profound sedation]. If the sedation was deemed insufficient (score 0 or 1), an additional 0.04 mg/kg xylazine was administered in the same muscle and the calf was left undisturbed for additional 10 min. A 13 gauge (G) short term venous catheter was then inserted in one jugular vein. A bilateral block of the cornual branch of the maxillary nerve was performed using lidocaine (Lidocain 2%; Streuli Pharma AG, Switzerland), as previously described by Stock et al. [2]. A 22–24 G arterial catheter was placed in one auricular artery. Ten minutes after the lidocaine injection, disbudding was performed using a preheated (for at least 10 min to reach a temperature of approximately 600 °C) electric cautery iron (Lötkolben 230 V, Albert Kerbl GmbH, Germany). The same procedure was followed for the sham session, but the cautery iron was not preheated. The disbudding was always performed by the same experienced investigator (DC). Immediately after the procedure, 0.5 mg/kg meloxicam (Metacam, Boehringer Ingelheim, Germany) was administered intravenously.

2.3. Data collection

The data collection time points are reported in Table 1.

The first blood sample was obtained by venipuncture, while all the others were withdrawn from the venous catheter. Before sampling from the catheter, 5 ml of blood was withdrawn (to prevent dilution). Seven ml of blood were then sampled and transferred to Li-Heparin glass tubes for cortisol and β-endorphin assessment while 0.5 ml were immediately transferred into tubes (RNAprotect Animal Blood Tubes 500 mcl, G Qiagen, Germany) for blood cell RNA extraction to assess mRNA abundance of cytokines and haptoglobin by RT-qPCR. Following blood sampling, the catheter was flushed with 10 ml of saline to prevent clotting. The Li-Heparin tubes were put in ice prior to refrigerated centrifugation. Plasma was then stored at − 20 °C.

2.3.1. Physiological parameters

2.3.1.1. Vital parameters. Heart rate was evaluated via auscultation using a stethoscope; respiratory rate was obtained by counting thoracic movement; rectal temperature was obtained by a digital thermometer; and invasive blood pressure was measured through a transducer zeroed to atmospheric pressure and positioned at the height of the heart and connected to an anesthesia monitor (Datex-Ohmeda S/5™, GE Healthcare Finland Oy, Finland).

2.3.1.2. Plasma cortisol and β-endorphin. Cortisol and β-endorphin quantification was performed using a radioimmunoassay technique as previously described by Thun et al. [20] and Zbinden et al. [21], respectively.

2.3.1.3. Cytokines and haptoglobin. To assess the mRNA abundance of tumor necrosis factor alpha (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and haptoglobin in blood cells, total RNA extraction, reverse transcription and quantitative real-time PCR were performed, as previously described by Zbinden et al. [22]. As housekeeping genes for normalization of the individual results the means of b-GAPDH and b-UBQ were used. The results of mRNA abundance are presented as a log scale (log 2).

2.3.2. Local variables

Local variables, including tactile sensitivity, pressure pain thresholds and horn temperature, were evaluated on gently manually restrained calves, allowed to freely move their head in response to stimulation. Four landmarks around each bud were tested (Fig. 1). Order