Article

The behavioral assessment and alleviation of pain associated with castration in beef calves treated with flunixin meglumine and caudal lidocaine epidural anesthesia with epinephrine

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Abstract — The objectives of this study were 1) to determine the effects of flunixin megulmine in combination with caudal epidural anesthesia as a postoperative analgesic in beef calves following surgical castration, and 2) to consider stride length and pedometry as potential behavioral assessment tools for detecting postcastration pain. Surgical castration was performed in 101 beef calves randomly assigned to 3 treatment subgroups: 1) castration without anesthesia (SURG); 2) castration following lidocaine with epinephrine caudal epidural anesthesia (SURG + EPI); 3) castration following lidocaine with epinephrine caudal epidural anesthesia and flunixin meglumine (SURG + EPI + F). Several outcomes, including pedometer counts, changes in stride length, subjective visual assessment of pain, instantaneous scan sampling of the calves' postoperative activities, and the amount of movement and vocalization during the castration procedure, were measured to identify and quantify pain. The results indicated that stride length and the number of steps taken by calves after castration appear to be good measures of pain. Significant differences found between treatment groups for stride length and visual assessments suggest that flunixin meglumine can be considered to provide visible pain relief up to 8 hours postcastration.

Résumé — L'évaluation du comportement et le soulagement de la douleur associée à la castration chez des veaux traités avec la méglumine de flunixine et l'anesthésie épidurale caudale à la lidocaïne accompagnée d'épinéphrine. Les objectifs de cette étude étaient de 1) déterminer les effets de la méglumine de flunixine en combinaison avec l'anesthésie caudale épidurale comme analgésie postopératoire chez les veaux après la castration chirurgicale et 2) de considérer la longueur de l'enjambée et la podométrie comme des outils d'évaluation potentiels du comportement pour la détection de la douleur après la castration. La castration chirurgicale a été réalisée chez 101 veaux assignés au hasard à trois sous-groupes de traitement : 1) la castration sans anesthésie (SURG); 2) la castration après anesthésie épidurale caudale avec la lidocaïne combinée à l'épinéphrine (SURG + EPI); 3) la castration après anesthésie épidurale caudale avec la lidocaïne combinée à l'épinéphrine et la méglumine de flunixine (SURG + EPI + F). Plusieurs résultats, incluant les comptes de podomètres, les changements de la longueur de l'enjambée, une évaluation visuelle subjective de la douleur, un échantillon d'observations instantanées des activités postopératoires des veaux et la quantité de mouvements et de vocalisations durant l'intervention de castration, ont été mesurés pour identifier et quantifier la douleur. Les résultats ont indiqué que la longueur de l'enjambée et le nombre de pas effectués par les veaux après la castration semblent être de bonnes méthodes pour mesurer la douleur. Les différences importantes trouvées entre les groupes de traitement pour la longueur de l'enjambée et les évaluations visuelles suggèrent que la méglumine de flunixine peut être considérée comme fournissant un soulagement visible de la douleur jusqu'à 8 heures après la castration.

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Introduction

Castration of male cattle is common practice throughout many parts of the world. Although castration does inflict pain on the animal, slow growth rate, and result in poorer feed efficiency, the benefits of castration, as viewed by the North American cattle industry, outweigh the negative aspects. Castration prevents unwanted pregnancies and results in a lower level of testosterone being present in the animal, which decreases aggression in male animals and makes them less dangerous and easier to handle. The number of carcasses with a high muscle pH

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("dark cutter") is also decreased and overall carcass quality is improved through increased tenderness and palatability of the meat (1). There are several different methods of castration. Stafford and Mellor (2) classify the methods of castration as physical, chemical, or hormonal. Further subdivisions can be made, but castration is primarily achieved by removing the testicles surgically, damaging them irreparably, or causing them to atrophy by stricture of the blood supply.

To minimize the pain experienced by castrated animals, different anesthetics and analgesics may be administered. Lidocaine can be administered either locally into the neck of the scrotum, thereby affecting the cord or testicles (3), or epidurally (4). The volume of anesthetic used locally and the injection site used can change the efficiency of the drug (3). Providing anesthesia by caudal epidural injection is a balance between achieving sufficient loss of nociception to the scrotal area and preventing ataxia in the hind limbs. Both methods of administering lidocaine have been shown to be successful in providing anesthesia. The use of lidocaine for local anesthesia has been shown to reduce acute behavioral and cortisol responses to rubber ring and Burdizzo methods of castration (3,5). Lidocaine given as a caudal epidural has also been shown to be an effective, safe, cheap, and easy method of providing analgesia in cattle (4).

Nonsteroidal anti-inflammatory drugs (NSAIDS), such as ketoprofen, have also been evaluated as analgesics for castration. Ketoprofen has been found to be more effective than local anesthesics in reducing cortisol levels during castration (6,7). However, cortisol levels are associated with stress and fear and not necessarily pain. In a study on the effect of ketoprofen during Burdizzo castration, Ting et al (8) concluded that the use of ketoprofen was more effective than that of lidocaine local anesthesia alone in reducing pain-associated behavioral responses during the first 6 h after treatment, since lidocaine has a shorter acting impact. Ketoprofen has also been shown to alleviate pain as reflected in the reduction in pain-associated behaviors and has been used as an analgesic in many different species. For example, ketoprofen has been shown to reduce vocalizations, movement, and heart rate in cats in the postoperative period (9), reduce numerical pain scores at rest and with movement in dogs (10), and shorten the return of standing and lying down times to normal in cattle (8).

Another NSAID that is used commercially is flunixin meglumine. Similar to ketoprofen, flunixin meglumine is used in a wide variety of species, and is commonly used for treatment of colic pain in horses (11). The pharmacokinetics of flunixin meglumine in the cow differs from that in other species. It has a distribution phase half-life of 0.294 h, an elimination phase half-life of 8–12 h, and a volume of distribution of 1050 mL/kg (12). The distribution phase half-life in cattle is shorter than that in dogs, but similar to that in horses and the elimination phase half-life is considerably longer in dogs than in cattle (12). Flunixin meglumine is labeled for use in cattle for respiratory disease; however, little work has been done to show its effectiveness as an analgesic for postoperative pain control in calves.

The objectives of this study were 1) to determine the effects of flunixin meglumine in combination with caudal epidural anesthesia as a postoperative analgesic in beef calves following surgical castration, and 2) to consider stride length and pedometer counts as potential behavioral assessment tools for detecting postcastration pain.

Materials and methods

This 2-part study was approved by the University of Saskatchewan's Committee on Animal Care and Supply (Protocol # 20060037) and conducted in accordance with Canadian Council on Animal Care guidelines. Sample size was based on the availability of bull calves at both locations and by the number of pedometers available.

Part 1 — Termuende location

Animals

Thirty (30), 3-month-old, Angus-cross bull calves were randomly divided into 2 processing groups because only 15 pedometers were available for use. Castrations were performed on day 0 (Group 1) and day 2 (Group 2) of the trial. Each processing group was randomly divided into 3 treatment subgroups, as described below (n = 5 animals per treatment group per day).

Behavioral assessments

Pedometers (Omron HJ-105 Pedometer, Omron Healthcare, Vernon Hills, Illinois, USA) were placed on the right hind leg of the calf, as previously described (13). The baseline number of steps taken by each calf was determined over the 24-hour period before castration (day -1), from 0900 till 0900 the following day. At castration (day 0), the pedometers were removed, the number of steps on the pedometer recorded, and the pedometers reset and reapplied to the same calf. Pedometers were then removed from the calves 24 h (day 1) following castration and the number of steps taken over this 24-hour period (at approximately 1000 till 1000 the following day, the 1-hour shift in time was due to the time for treatment and castration) was recorded for each animal. The times of day that each calf was handled at baseline, castration, and postcastration were all recorded. The pedometers had been evaluated on both humans and calves of similar age prior to the study to determine the reproducibility of the number of steps taken. The sensitivity of each pedometer was adjusted so that the number of steps recorded on the pedometer was within 5% of the actual number of steps taken.

Part 2 — Goodale location

Animals

Seventy-one (71) Hereford-cross bull calves were divided into 3 groups based on age. The oldest calves (Group 1, n = 24) were randomly divided into 3 treatment subgroups, as described below, and castrated during week 1. Groups 2 (n = 23) and 3 (n = 24) underwent the same randomization and castration in successive weeks (weeks 2 and 3). After the randomization of all calves, each treatment subgroup had 23 or 24 animals.

Calves were identified throughout the experiment by ear tags and large cattle numbers glued across the back behind the shoulder. These back numbers were large enough so that calves could be viewed at a distance and, therefore, remain undisturbed during all observations.

Behavioral assessments

During the castration procedure, the number of calf vocalizations was recorded. The force exerted on the tip table as the calf struggled during the castration procedure was also measured by using an electronic movement measuring device (MMD) (Jack Hanson, College of Engineering, University of Saskatchewan, Saskatoon, Saskatchewan). This machine measures the movements made by the animal for 1 min by analyzing the electronic signals sent to the device through load cells (14). Load cells were located on a suspended electronic scale with the tip table being secured on top. Signals from the load cells and scale were recorded and processed in the manner previously described (15).

Stride length baseline observations were obtained on day -1. Calves were trained to walk through a 9.8-meter-long chute and past a video camera (Panasonic CCTV WV-BL200 Camera; Matsushita Communication Industrial Company, Tokyo, Japan) without stopping. For each processing group, 3 passes through the chute were required before calves had been habituated to the routine and walked freely through the chute with no physical encouragement (calves were castrated in a separate handling facility in an effort to maintain their willingness to pass through the chute to measure stride length). The camera was placed 1.5 m from the side of the chute and approximately in the center of its total length. The camera recorded the calf walking a distance of 3 m along the length of the chute. Stride length was determined by using a 2.4-meter-long measured grid as a background and the footage from the video tape was digitized and analyzed by using a computer software program (Northern Eclipse version 6.0; Empix Imaging, Mississauga, Ontario), as previously described (16). At 4, 8, 12, and 24 h postcastration, calves were again walked through the chute past the video camera and the stride lengths were recorded. The calves were walked through the chute twice at each observation time. The quality of the calf's pass (walk, trot, or run) by the camera was recorded. This helped to insure that at least 1 acceptable pass (a walk by) was obtained at each observation time. However, stride length measurements were disregarded if the calf stopped in the chute or ran past the camera. Stride length observations were averaged for all calves with 2 acceptable passes, whereas the stride length was based on a single measurement for calves with only 1 acceptable pass at an observation time.

A subjective visual assessment of the calves was made when they left the chute by a single observer, a graduate student with an interest in animal behavior. Each calf was categorized as either "in pain" or "not in pain" after being observed for approximately 10–30 s. The criteria used to classify the calves in pain were signs that the calves appeared different from normal calves in respect to body posture (head carriage, tail tuck, and degree of back arch) and locomotion (willingness to move). The observer was blinded to the treatments and to any previous score assigned to a particular calf. A baseline visual assessment was not made prior to castration of the calves. Visual assessments were made at 4, 8, 12, and 24 h postcastration.

During the time intervals 1–4, 5–8, and 9–12 h postcastration, calves were housed in dry lots with their dams. The behavior activity of each calf was recorded by using live observations at 15-min intervals when the calves were loose in the pen. Behaviors recorded included lying down, standing, and nursing.

Parts 1 and 2 — Termuende and Goodale locations

Treatments

Each group of calves was divided into 3 treatment subgroups: 1) surgical castration (**SURG**); 2) surgical castration following lidocaine caudal epidural anesthesia with epinephrine (**SURG + EPI**); 3) surgical castration following flunixin meglumine and lidocaine caudal epidural anesthesia with epinephrine (**SURG + EPI + F**).

Experimental procedures

All calves underwent the same surgical procedure. On day -1, calves were weighed to ensure that the correct dose of lidocaine, flunixin meglumine, or saline was calculated. During the treatment and surgical procedures, calves were restrained on a tip table. Caudal epidural anesthesia in SURG + EPI calves was provided by using 2% lidocaine with epinephrine (Lidocaine HCl 2% with Epinephrine; Bimedia-MTC, Cambridge, Ontario), 0.6 mg/kg BW, into the sacrococcygeal epidural space through a 1 1/2″ 18-G needle. The injection site for the epidural lidocaine was confirmed by moving the tail up and down and identifying the 1st joint space caudal to the sacrum. A drop of lidocaine was put into the hub of a needle placed at the sacrococcygeal site and visualized to ensure that the lidocaine was drawn into the epidural space. The needle was also considered to be properly placed if the lidocaine was injected with no resistance. Flunixin meglumine (Banamine; Schering-Plough Animal Health, Pointe Claire, Quebec), 2.2 mg/kg BW, was provided to the SURG + EPI + F calves by jugular IV injection. All calves not receiving flunixin meglumine (SURG, or SURG + EPI) or lidocaine with epinephrine (SURG) received an equivalent volume of sterile saline (Physiological Saline; Bimedia-MTC) by jugular IV injection or injection into the sacrococcygeal epidural space, respectively. Calves were put through the chute and given their randomly allocated treatments and then released. Immediately following the treatments, calves in the same processing or age group were then put through the chute and castrated. Castration was performed in SURG calves by using a closed surgical method, with 1 incision in the caudoventral part of the scrotum by using a Newberry knife, followed by expression of the testicles and the application of emasculators to crush and cut the spermatic cords. The time of treatment and castration was recorded for each calf.

Statistical analyses

All data collected during the study were entered into computerized spreadsheets (Microsoft Excel; Microsoft, Redmond, Washington, USA). Birth dates for the calves were provided by the 2 herd managers. A new continuous variable, called the elapsed time between treatment and castration, was created from the recorded times at treatment and castration. This variable was created because calves were not treated and immediately castrated. Instead, calves were processed in groups and the

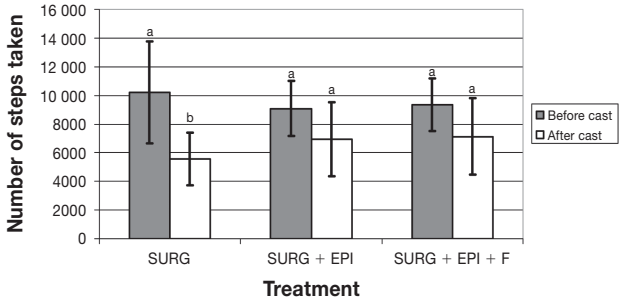

Figure 1. Comparison of the mean number of steps (95% CI) over 24-hour periods before and after castration between treatment subgroups (SURG – surgical castration; SURG + EPI – surgical castration following lidocaine with epinephrine caudal epidural anesthesia; SURG + EPI + F – surgical castration following flunixin meglumine and lidocaine with epinephrine caudal epidural anesthesia). Bars within treatment groups with different letters are statistically different from one another ($P < 0.05$).

elapsed time between treatment and castration was different for each calf. Statistical analyses for this study were completed by using a commercial statistics software program (SAS version 9.1; SAS Institute, Cary, North Carolina, USA) with an alpha value of 0.05. Descriptive statistics were completed first to compare the ages and weight of both treatment and processing groups. The normality of all continuous variables was evaluated with the Anderson-Darling normality test (PROC UNIVARIATE; SAS v.9.1, SAS Institute). Parametric and nonparametric tests were then applied where appropriate to evaluate univariable associations between selected predictors and outcomes in the datasets. In Part 1 of the study, the difference between steps taken by calves before and after castration was calculated by using a one-way analysis of variance (ANOVA) and a Bonferroni *t*-test (PROC ANOVA; SAS v.9.1, SAS Institute) to compare the differences in steps and number of steps taken by treatment subgroup. Paired *t*-tests were used to compare pedometer readings before and after castration regardless of treatment and also within each treatment subgroup (PROC TTEST; SAS v.9.1, SAS Institute).

In the univariable analyses of Part 2, potential confounders were identified. However, before multivariable analyses could be completed, a natural logarithm transformation was necessary to normalize several of the continuous outcome variables. A linear regression model (PROC REG; SAS v.9.1, SAS Institute) was used to model the number of peaks and standard deviation of the analogue signal from the MMD machine. The same regression technique was used to evaluate the percentage of pen observations in which calves were lying down. For all 3 of these models, treatment, processing group, age, weight, and elapsed time between treatment and castration were included as predictor variables. A manual backwards-stepwise procedure was used to determine the reduced final model. Similarly, a logistic regression model (PROC LOGISTIC; SAS v.9.1, SAS Institute) was used to model the proportion of calves that vocalized during the castration procedure. The same predictor variables were initially added to the logistic regression model and were reduced in a similar manner as for the linear regression models above.

Table 1. The mean or median age, weight, and time difference between treatment and castration by processing group (weeks 1, 2, and 3) for the 71 calves castrated at the Goodale Research Farm (Part 2)

	Group 1 (week 1)	Group 2 (week 2)	Group 3 (week 3)
Median age (d) (25th and 75th percentile)	95 (93,100)[a]	94 (90,98)[a]	67 (59,78)[b]
Mean weight (kg) (95% CI)	126 (118,134)[a]	130 (121,139)[a]	107 (96,118)[b]
Mean time between treatment and castration (min) (95% CI)	88 (77,98)[a]	39 (34,44)[b]	41 (35,46)[b]

[a,b] Different letter designations within each row represent significant differences ($P < 0.05$); CI — confidence interval.

Two of the outcome variables consisted of repeated measurements of the calves over time. A mixed linear model (PROC MIXED; SAS v.9.1, SAS Institute) was used to analyze the stride length of calves postcastration. Once again, treatment, processing group, age, weight, and elapsed time between treatment and castration were included as predictor variables. However, the baseline average stride length was also included as a covariate in the model. A compound symmetry correlation structure was used in the analyses, so that stride lengths were equally correlated for each individual calf over time.

The visual assessment of pain was the second outcome measure with repeated observations. The odds of a calf being observed as pained postcastration was modeled with a generalized linear model. A multivariable logistic regression model was fit by using a logit link function and a binomial error distribution (PROC GENMOD; SAS v.9.1, SAS Institute). The correlation between successive pain assessments of a calf was accounted for by using generalized estimation equations and a compound symmetry correlation structure.

For each of the multivariable models, the goodness of fit was assessed, and all 2- and 3-way interactions were evaluated for significance. The estimated regression parameters from the logistic regression models were converted to odds ratios. Parameter estimates from the transformed continuous outcomes were then back-transformed for ease of interpretation.

Results

Part 1 — Turmuende location

Data from 3 calves had to be removed from the analyses as their pedometers were lost during the study. Data from a total of 27 calves ($n = 9$ calves per treatment subgroup) was obtained and analyzed.

The mean number of steps taken before castration was the same for all 3 treatment subgroups. When comparing all 27 calves, there was a significant decrease in the mean number of steps taken after castration as compared with before. However, the mean number of steps taken following castration was equivalent for all treatment subgroups. In the SURG calves, there was a significant difference between steps taken before and after castration. No significant difference was seen between steps

Table 2. The effect of treatment on the stride length of beef calves following castration (4, 8, 12, and 24 h), based on mixed model analysis of a randomized clinical trial. Three treatment subgroups were compared (SURG, SURG + EPI, and SURG + EPI + F). Covariates included in the model were the baseline stride length, processing group, time postcastration, and a time by treatment interaction term

Effect	Estimate (cm)	Standard error (cm)	*P*-value
Stride length — Baseline average	1.004	1.000	0.002
Treatment 1 (SURG)	-1.078	1.032	0.012
Treatment 2 (SURG + EPI)	-1.092	1.031	0.009
Treatment 3 (SURG + EPI + F)	Ref	Ref	Ref
Time	-1.019	1.001	< 0.001
Time × Treatment 1	1.004	1.002	0.030
Time × Treatment 2	1.005	1.002	0.005
Time × Treatment 3	Ref	Ref	Ref
Time × Time	1.001	1.000	< 0.001
Processing Group 1	-1.082	1.032	0.003
Processing Group 2	-1.041	1.034	0.160
Processing Group 3	Ref	Ref	Ref
Intercept	118.89	1.092	< 0.001

n — 284 observations from 71 calves.
SURG — surgical castration without anesthesia.
SURG + EPI — surgical castration following lidocaine with epinephrine caudal epidural anesthesia.
SURG + EPI + F — surgical castration following IV flunixin meglumine and lidocaine with epinephrine caudal epidural anesthesia.
Ref — referent level within the variable.

taken before and after castration in SURG + EPI and SURG + EPI + F calves (Figure 1). No differences were seen between processing groups 1 and 2 (on days 1 and 3, respectively.)

Part 2 — Goodale location

The median and quartile ages for all 3 groups are listed in Table 1. Differences among age, weight, and time between treatment and castration were found between processing groups (weeks 1, 2, and 3), but not among subgroups (SURG, SURG + EPI and SURG + EPI + F). Univariable associations were also found among processing group, age, weight, time between treatment and castration, and among several of the investigated outcome measures. So, multivariable analyses were used to control for these confounders.

Stride length

Eighty-eight percent of the stride length measurements were based on an average of 2 passes made by each calf at each of the time points. The remaining 12% of stride length measurements were based on 1 successful walk by the camera. The mean difference in stride length measured between the 2 successful passes was 12.1 cm [95% (confidence interval) CI: 1.7 cm, 22.6 cm]. The stride lengths measured at baseline and 24 h were both normally distributed. However, at 4, 8, and 12 h postcastration, the stride lengths of the calves were skewed to the right (median to the left of the mean). The results of the mixed linear modeling suggested that stride lengths differed between treatment subgroups, processing groups, and by time (Table 2). More importantly, an interaction was identified between treatment and time. To clarify the time by treatment interaction, the predicted stride length was graphed versus time

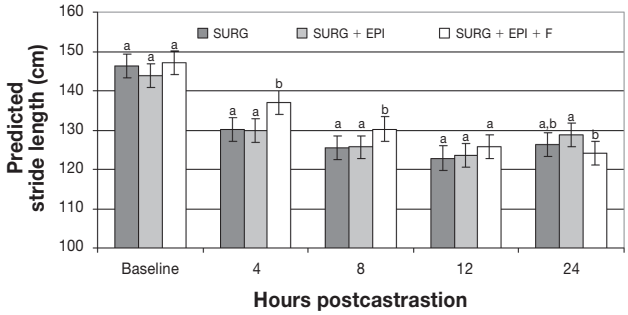

Figure 2. Comparison of the mean predicted stride lengths (95% CI) between treatment groups at baseline, 4, 8, 12, and 24 h postcastration (SURG – surgical castration; SURG + EPI – surgical castration following lidocaine with epinephrine caudal epidural anesthesia; SURG + EPI + F – surgical castration following flunixin meglumine and lidocaine with epinephrine caudal epidural anesthesia). Bars within observation times with different letters are statistically different from one another ($P < 0.05$). The effect of processing group was controlled for in these results.

for the 3 treatment subgroups (Figure 2). Calves in the SURG + EPI + F subgroup had significantly longer stride lengths than calves in the SURG + EPI and SURG subgroups at 4 and 8 h postcastration, but significantly shorter stride lengths at 24 h postcastration (Figure 2).

Visual chute observation

Significant differences were found between the SURG and SURG + EPI + F subgroups at 4 and 8 h postcastration (Table 3). Calves in the SURG subgroup were more likely to be classified as "in pain" than calves in the SURG + EPI + F subgroup (Figure 3). Classifying an animal as "in pain" was also correlated with the age and weight of the animal. For every day that it was older, a calf was 3.7 times more likely to be classified as "in pain" (Table 3).

Vocalization, movement, and behavior observations

There was no significant difference between treatment subgroups for the number of vocalizations, movement of calves restrained on tilt table during castration (as measured from the analog signal output from the load cells), and postcastration behaviors. Only 10 of the 71 calves (14%) vocalized during castration. Differences between processing groups of calves were found when analyzing for movement of calves during castration.

Discussion

The use of behavior to assess pain and determine drug effectiveness presents a challenging task. Pain can be classified as either procedural (occurring during the procedure) or postprocedural. Different methods have been used to quantify behavioral and physical measurements (17). Recording vocalizations (18), measuring exertion force by the animals against the restraint (19), and measuring cortisol levels (20) have all been used to assess pain experienced by animals during painful procedures. Some of these methods are invasive and labor intensive; for example, measuring blood cortisol levels requires multiple samples over time to account for its episodic nature (21), and

Table 3. The odds of a calf being classified in pain during visual observation at 4, 8, 12, and 24 h following castration based on a generalized linear model. Three treatment subgroups were compared (SURG, SURG + EPI, and SURG + EPI + F). Covariates included in the model were age of the calf, and time postcastration

Parameter	OR	95% CI	*P*-value
Treatment 1 (SURG)	6	2, 15	< 0.001
Treatment 2 (SURG + EPI)	3	1, 8	0.035
Treatment 3 (SURG + EPI + F)	Ref	Ref	Ref
Age (for every day older)	3.7	1.00, 1.05	0.032
Time — 4 h Postcastration	62	13, 290	< 0.001
Time — 8 h Postcastration	58	13, 270	< 0.001
Time — 12 h Postcastration	14	3, 68	< 0.001
Time — 24 h Postcastration	Ref	Ref	Ref

n — 284 observations from 71 calves; OR — odds ratio; CI — confidence interval.
SURG — surgical castration without anesthesia.
SURG + EPI — surgical castration following lidocaine with epinephrine caudal epidural anesthesia.
SURG + EPI + F — surgical castration following IV flunixin meglumine and lidocaine with epinephrine caudal epidural anesthesia.
Ref — referent level within the variable.

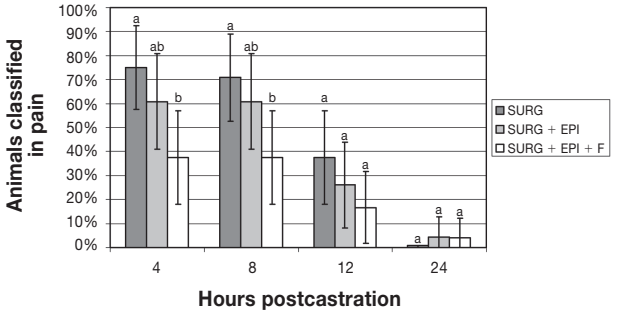

Figure 3. Comparison between treatment subgroups of the proportion of animals (95% CI) classified in pain at 4, 8, 12, and 24 h postcastration (SURG – surgical castration; SURG + EPI – surgical castration following lidocaine with epinephrine caudal epidural anesthesia; SURG + EPI + F – surgical castration following flunixin meglumine and lidocaine with epinephrine caudal epidural anesthesia). Bars within observation times with different letters are statistically different from one another ($P < 0.05$).

such requirements limit the number of methods that can be used to assess pain during experiments. Attempts have been made to quantify the postoperative pain experienced by animals by measuring changes in posture (standing, lying down) and movement (walking, trotting), using continuous (22) or multiple point in time visual observations (8). These behavioral observations may lack sensitivity in detecting treatment differences because of large individual variations or because many behaviors are socially facilitated. For example, a calf may stand up or walk because other calves in the herd are standing up or walking. In addition, prey species are generally stoic in response to pain, since there is little biological advantage for them to advertise they are injured or in a weaker state. Thus there are inherent difficulties in assessing and quantifying pain in cattle, which may explain why a variety of measurements have been used by us and others to assess pain.

Although some of the traditional behavioral measurements described above were included in this study, 2 relatively newer techniques of pain detection in calves were included; stride length and pedometer counts. Peham et al (23) used stride length measurements in horses as a means of assessing the influence of pain following orthopedic surgery. At the onset of our trials, we hypothesized that calves having undergone castration would show some variation in stride length due to pain. Likewise, we believed that pedometers might be useful to detect treatment differences since they had previously been used on cattle to detect estrus (24), monitor calves' response to weaning treatments (13), and determine the distance traveled by cattle on different grazing systems (25).

Pedometers have also been used to assess weaning stress in calves (13), but not as a means of assessing pain in cattle. The results indicate that calves having undergone a painful procedure, like castration, have decreased activity and take fewer steps. The failure to detect differences between treatment subgroups may be attributed to the small sample size in each processing group. We estimated that the sample size required to make the differences that we found significant would be approximately 30 calves per treatment subgroup. There was also wide variation between calves in the number of steps taken per day. For example, one calf recorded a baseline of 2800 steps in 24 h, while another calf in the same treatment subgroup logged 19 600 steps over the same time period. This variation between calves contributed to the nonsignificant difference in the number of steps taken between treatment subgroups.

Calves with a higher baseline average stride length maintained a longer stride length postcastration. Stride length decreased and then increased again after castration, consistent with a quadratic time variable (Table 2). The significant differences between treatment groups indicate that stride length can be used as a method to detect post-castration pain in calves. The stride length decreased in all groups after castration, which suggests that castration altered the animal's locomotion, likely because of pain. Calves in the SURG + EPI + F subgroup showed the least amount of decreased stride length among the 3 treatment subgroups at 4 and 8 h postcastration, which implied that flunixin meglumine is an effective analgesic during this time period. It was anticipated from previous clinical experience that the analgesic effect of flunixin meglumine would last for at least 8 h and our results support this claim. The significantly decreased stride length of calves in the SURG + EPI + F subgroup compared with calves in the SURG and SURG + EPI subgroups at 24 h postcastration suggests that the effect of flunixin meglumine had worn off by this time (Figure 2). We hypothesize that the inflammation and pain associated with castration may be delayed in calves given a single treatment of flunixin meglumine. If the calves had been observed for 48 h postcastration, a more accurate time line for the effects of flunixin meglumine might have been provided and the level of pain the calves experienced after the drug had worn off determined.

The ability to visually classify the calves as "in pain" was correlated to the length of time after castration. Calves were more likely to be classified as "in pain" sooner rather than later after castration (Table 3). This finding was expected, but unfortunately the observer of the calves was aware of this progression in time. Increasing age also increased the odds of being classified as being "in pain" (Table 3). This suggests that castration

should be done when calves are as young as possible. Or it could suggest that it is easier to assess pain in older calves. We believe that older calves did experience more pain, due to the likelihood of there being more tissue trauma in older (larger) calves and, therefore, more inflammation. Visually scoring the level of pain was a subjective process and perhaps should have been video recorded and viewed in a random playback method to help validate the technique. However, the visual pain assessment results, when taken in conjunction with the data obtained from the stride length measurements, helped to support the conclusion that flunixin meglumine in association with caudal epidural anesthesia provided an analgesic effect for up to 8 h postcastration.

It is interesting to note that the number of calves that vocalized during the castration procedure was small (14%). Previous studies have used vocalization as an indicator of pain (26–28), signifying that it can be an effective pain measure. Watts and Stookey (27) suggested that when the effects of severe stressors are being investigated, vocalization measurements can prove useful. In the present study, the small number of vocalizations may indicate that castration is a less severe stressor than branding, though it is difficult to compare our results with those from studies on branding where different aged animals and different breeds of cattle were used (27).

Our results suggest that instantaneous scan sampling at 15-min intervals was too crude a measure to detect postcastration differences between treatment subgroups. However, in the present study, we repeatedly separated calves from their mothers at timed intervals in order to obtain stride length measurements. This likely disrupted their behavior patterns and may have masked changes in behavioral activities detected by our visual observation.

We anticipated that the calves receiving a lidocaine caudal epidural would feel less pain and, therefore, struggle less during castration on the tilt table (as measured via the load cells). The results contradict this hypothesis, suggesting that struggling due to restraint may have masked the pain experiences or that the lidocaine with epinephrine caudal epidurals were ineffective. However, after administration of the epidural, many of the calves were unable to move their tail suggesting that the epidural placement was correct. Anatomical differences may account for some of the differences between calves. There are 3 nerves that act as sensory innervations to the scrotum; the ventral branches of the first 2 lumbar nerves, the genitofemoral nerve, and the pudendal nerve (29). Differences can be found between animals and between animals of different ages as to the exact location and amount of testicular innervation (30). In the present study, not all of the nerves may have been affected by the lidocaine, so it is possible that some calves still had feeling in the scrotum or spermatic chord. Another point is that some calves appeared ataxic and were unable to move their tail, but still struggled during the surgical procedure. This suggests that calves could have been struggling against the restraint (tip table) rather than because of the pain. Maximal caudal epidural anesthesia using 2% lidocaine with epinephrine generally lasts for 30 to 150 min (31). Differences in the processing groups of calves (age, weight, and the time lapse between injection and castration) may be the explanation for some of the associations between subgroups of calves and exertion force outcomes (peaks and standard deviation) obtained. We were unable to standardize and evaluate the precision and accuracy of the MMD machine prior to its use. The validity of the MMD measurements are in question, given that the time between treatment and castration was not associated with the number of calf struggles (number of peaks in the analog signal) and was negatively correlated with the standard deviation of the signal (exertion force of calves).

Stride length and the number of steps taken by calves after castration appear to be useful measures of the postprocedural pain calves may experience. Based on stride length measurements and visual assessments, flunixin meglumine in combination with lidocaine with epinephrine caudal epidural anesthesia can be considered to provide effective pain control for at least 8 to 12 h postcastration.

Authors' contributions

Dr. Currah was involved with the design of the study, acquisition and analysis of the data, and wrote the manuscript. Dr. Hendrick secured the funding and assisted with the design of the study, analysis of the data, and editing of the manuscript. Dr. Stookey assisted with the design of the study, facilitated the collection of the data, and assisted with the editing of the manuscript.

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