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Physiological and behavioral stress parameters in calves in response to partial scrotal resection, orchidectomy, and Burdizzo castration

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ABSTRACT

Establishing artificial cryptorchids by partial scrotal resection without removing the testicles is a technique for castration of bull calves that recently has gained new interest. In contrast to orchidectomy and Burdizzo castration, the stress response of calves to shortening of the scrotum is unknown. In this study, partial scrotal resection in bull calves was compared with orchidectomy, Burdizzo castration, and controls without intervention (n = 10 per group, ages 56 ± 3 d). Procedures were performed under xylazine sedation and local anesthesia. We hypothesized that partial scrotal resection is least stressful. Salivary cortisol, heart rate, heart rate variability, behavior, and locomotion were analyzed. Cortisol concentration peaked 60 min after start of the procedures. Cortisol release was at least in part xylazine induced and none of the experimental procedures released additional cortisol. Heart rate increased in calves of all groups with initial handling, but immediately after xylazine sedation decreased to 30% below initial values and was not modified by surgical procedures. The heart rate variability variables standard deviation of beat-to-beat interval and root mean square of successive beat-to-beat differences increased when calves were placed on the surgery table but effects were similar in calves submitted to surgeries and control calves. Locomotion increased, whereas lying time decreased in response to all surgeries. Locomotion increase was most pronounced after orchidectomy. Plasma fibrinogen concentrations increased after orchidectomy only. With adequate pain medication, orchidectomy, Burdizzo castration, and partial scrotal resection do not differ with regard to acute stress and, by inference, pain. Partial scrotal resection when carried out under xylazine sedation and local anesthesia thus is an acceptable castration technique in bull calves.
Key words: short scrotum, calf, castration, stress

INTRODUCTION

Castration of bull calves from dairy breeds during the first weeks of life is a standard veterinary procedure with the aim to reduce male behavior and to improve meat quality (Baker and Gonyou, 1986; Faulkner et al., 1992). In addition, utilization of grazing land shared with female cattle is only possible if sexual activity of young males is prevented. Castration is considered a stressor and potentially painful experience for the animal (Mellor et al., 1991; Robertson et al., 1994; Stafford and Mellor, 2005). Animal welfare concerns thus warrant that surgical procedures are performed in a way that causes the least stress and pain. Options for pain mitigation include local anesthesia and the use of analgesics such as nonsteroidal antiinflammatory drugs, opioid drugs, and α_2-adrenergic receptor agonists (Stafford et al., 2002). Pain perception of calves at castration and the subsequent stress response do not only depend on the surgical techniques but also on the animals' age, with a less-pronounced response in younger animals (Robertson et al., 1994; Ting et al., 2005). In addition, castration may have a negative effect on feed intake and growth of calves (Faulkner et al., 1992; Bretschneider, 2005).

Behavioral signs of pain can be difficult to recognize in prey species. Thus, mostly physiological and behavioral stress parameters are determined, assuming that they are closely correlated with the pain experienced by the animal. To choose the least stressful and, by inference, least painful method, castration techniques in cattle have to be compared with regard to animal stress and pain. The most common techniques are surgical removal of the testicles (orchidectomy), bloodless castration by crushing the spermatic cord and thus inhib-

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iting testicular blood supply with a Burdizzo clamp, or inhibition of testicular blood supply with rubber rings placed on the neck of the scrotum (King et al., 1991; Robertson et al., 1994; Stafford et al., 2002; Bretschneider, 2005). Because gonadal steroids stimulate growth and increase feed efficiency, interest exists in males that are infertile but exposed to endogenous testicular steroid hormones. Such a situation occurs naturally in cryptorchids and has been imitated surgically by shortening the scrotum to an extent that the testes are located in a near-inguinal position. Short scrotum bulls, due to the anabolic properties of androgens, are more similar to intact bulls than steers in growth rate, carcass characteristics, and meat tenderness but have the advantage that their infertility simplifies management (Glimp et al., 1971; Albaugh et al., 1975). Although a considerable number of studies have been performed on castration-induced stress in bull calves (reviewed by Stafford et al., 2002; Bretschneider, 2005), the stress response and, by inference, pain perception of calves in response to shortening of the scrotum as a means of castration has not been investigated so far.

Stress caused by castration has been studied using a variety of physiological and behavioral parameters (Faulkner et al., 1992; Fisher et al., 1996; Knight et al., 2000; Ting et al., 2003; Pang et al., 2006; González et al., 2010). Increases in cortisol release have been suggested to correspond with predicted noxious stimuli (Mellor et al., 2000). Because cortisol rapidly diffuses into saliva, salivary cortisol concentrations reliably mirror changes in cortisol concentrations in blood plasma (Kirschbaum, 2000; Peeters et al., 2011) but have rarely been determined in cattle at castration so far (González et al., 2010). The most immediate stress response is an increase in adrenomedullary and sympathetic nervous activity. An acute stress elicits an immediate release of epinephrine and increase in heart rate. Besides heart rate, heart rate variability (**HRV**) is used as an indicator for the response of the autonomic nervous system to stress. Heart rate variability (i.e., short-term fluctuations in heart rate) is essentially based on the antagonistic oscillatory influences of the sympathetic and parasympathetic nervous system on the sinus node of the heart. It thus reflects the prevailing balance of sympathetic and parasympathetic tone. In general, increases in the values of the HRV variables standard deviation of beat-to-beat (**RR**) interval (**SDRR**) and root mean square of successive RR differences (**RMSSD**) reflect a shift toward more sympathetic dominance, whereas reduced values indicate a shift toward parasympathetic dominance (von Borell et al., 2007).

In this study, effects of partial scrotal resection, castration by orchidectomy, and crushing of the spermatic cord with a Burdizzo clamp on the stress response of bull calves were analyzed. All procedures were performed with the animals sedated and under local anesthesia. We hypothesized that shortening of the scrotum is less traumatic and, therefore, less stressful than orchidectomy or Burdizzo castration. As physiological stress parameters, salivary cortisol, heart rate, and HRV were determined and supplemented by observation of the animals' behavior and locomotion recordings with pedometers.

MATERIALS AND METHODS

Animals

A total of 40 male Fleckvieh (Austrian dairy/dual-purpose Simmental) calves were included into the study over a 5-mo period (October–February). Each month, 8 calves were bought at a livestock market and delivered to the University of Veterinary Science (Vienna, Austria) the same day. Age of the calves on the day of surgery was 56 ± 3 (±SD) days and did not differ significantly between groups. The calves were housed on straw in pairs of 2 in pens measuring 3 × 2.5 m. They were fed 2.5 L of milk replacer (1.25 L twice daily; Kalbi Milch Fit Protect; H. Wilhelm Schaumann GmbH & Co. KG, Brunn, Austria) and concentrates (corn, soybean, and mineral mixture; Kalvicin Pro; H. Wilhelm Schaumann GmbH & Co. KG; 150–300 g twice daily) and had free access to hay and water. The calves had not been handled before except feeding. Calves were allowed to adjust to the stable for 4 d and were made familiar with saliva sampling, heart rate recordings, and pedometers during this time. Before the recordings started, the calves were handled and brushed daily to make them acquainted to handling by humans.

Experimental Design

The 8 calves of each of the 5 lots were assigned randomly to 4 groups (n = 2 each). Calves were castrated either by shortening of the scrotum without removing the testes (partial scrotal resection; **SR** group; n = 10), crushing of the spermatic cords with a Burdizzo clamp (**BZ** group; n = 10), orchidectomy (**OR** group; n = 10), or were left gonad-intact as controls (**CO** group; n = 10). The animals were followed from 2 d before to 8 d after the surgical intervention. All examinations were carried out in the same stable familiar to the calves. The experiment was approved by the competent authorities for animal experimentation in Austria (Federal Ministry for Science and Research, license number BMWF-68.205/0108-II/3b/2011).

Experimental Procedures

Castrations. Calves of all groups including control calves were sedated with xylazine (0.2 mg/kg; Chanazine 2%; Richter Pharma AG, Wels, Austria) intramuscularly while in their pen. Five minutes later they were moved to the surgery room adjacent to the stable, and fixed in right lateral recumbency on a surgery table with ropes on the fore- and hind limbs. Then, the scrotum was cleaned and prepared aseptically. Castration was performed, depending on the experimental group. In calves of the SR group, the testicles were gently pushed proximally toward the inguinal canal within the scrotum and were fixed in this position by clamping the empty distal part of the scrotum with an intestinal clamp. Proximal to the clamp, 10 mL of 2% procaine hydrochloride (VMD nv/sa, Arendonk, Belgium) was injected along the width of the scrotum. Approximately 5 mm distal to the intestinal clamp, the scrotum was cut off with a scalpel and the wound was closed with a stapler (Skin Stapler 6.9 × 3.6 mm; Braun, Maria Enzersdorf, Austria). Chlortetracycline spray (Vana GmbH, Vienna, Austria) was applied onto the closed wound. In calves of the BZ group, 10 mL of 2% procaine hydrochloride were injected into each spermatic cord. Each spermatic cord was then squeezed twice for 1 min with a Burdizzo clamp (450 mm; Henry Schein Medical GmbH, Vienna, Austria). In calves of the OR group, local anesthesia was performed as in the BZ group. In addition, the distal part of the scrotum was anesthetized with 2 mL of 2% procaine hydrochloride. A bilateral skin incision was made at the distal end of the testes and both testicles were exteriorized. A clamp was placed on the spermatic cord which was subsequently ligated with a polyglactin braided suture (Vicryl, metric 6; Ethicon, Portland, OR). Afterward, the cord was cut with an emasculator left in place for 2 min and the testicles were removed. Chlortetracycline spray was applied on the scrotal incision, which was left open to allow drainage. In all 3 surgical groups, time between application of local anesthesia and start of the surgical procedures was 1 min. Animals of the CO group underwent the same preparation procedure, including cleaning of the scrotum, but were then left without further manipulation in lateral position on the surgery table for 10 min.

Surgeries were always started at 1000 h, with 8 calves per day and calves from the 4 groups included in alternating order. Calves were returned to their pens immediately after surgery. Postoperatively, they received penicillin streptomycin (100,000 IU of penicillin and 10 mg of streptomycin per kilogram of BW; Peni-Strepto; Virbac, Vienna, Austria) intramuscularly once daily for 5 consecutive days. All castrations were performed by the same experienced veterinarian.

Clinical Observations and Fibrinogen Determination. Daily physical examination of the calves included evaluation and scoring of general attitude (1 = reduced activity, 2 = bright and alert, 3 = lively and alert, and 4 = increased activity), color of oral mucous membranes and conjunctivae (1 = anemic, 2 = pink, and 3 = hyperemic), respiratory rate, and rectal temperature. Body weight of calves was determined 2 d before and on d 5 and 8 after surgery. For fibrinogen determination, blood was collected from the jugular vein once daily except for the day of surgery. Fibrinogen concentration was determined with a standard autoanalyzer (Cobas 311c; Roche Diagnostics GmbH, Vienna, Austria).

Behavior. Behavior of the calves was directly observed for 2 h in the morning (1000–1200 h) and afternoon (1500–1700 h) on the day before (day −1) and on d 1, 2, and 3 after surgery or CO experiments, respectively. The frequency of eating, drinking, rumination, urination, defecation, vocalization, contact between calves (licking or sniffing at each other), and self-licking and self-scratching was recorded for each calf during the observation period (Table 1). Drinking, urination, defecation, and contact were counted and recorded as events per 2 h of observation. Feeding and rumination behavior was scored from 0 to 3 [feeding: 0 = no interest in hay or straw, 1 = slight interest in hay or straw (i.e., feeding less than 5 out of 15 min), 2 = moderate interest in hay or straw (i.e., feeding 5 to 10 min out of 15 min), and 3 = constant eating or ruminating (i.e., 15 min per 15 min); rumination: 0 = no rumination, 1 = ruminating less than 5 out of 15 min, 2 = ruminating between 5 and 10 min out of 15 min, and 3 = constant ruminating (i.e., 15 min per 15 min)]. The 15-min scores were then averaged for the 2-h observation period. All observations were carried out by the same 2 trained observers, who, due to the nature of the experiment, could not be blinded with regard to experimental groups. Each observer was positioned between 2 adjacent pens and recorded behavior of 4 calves.

Locomotion. Locomotion activity and lying time of the calves were recorded with activity, lying, and temperature pedometers (Ingenieurbüro Holz, Falkenhagen, Germany) as described by Brehme et al. (2008) and Rose-Meierhöfer et al. (2010). Recordings were made for 3 h in the morning (0900–1200 h) and afternoon (1400–1700 h) on all days, except the day of surgery. Pedometers were fixed with a hook and loop fastener on the right forelimb of the calves. Locomotion, standing, and lying times were recorded for 15-min intervals.

Table 1. Ethogram of behaviors recorded

Behavior	Definition
Eating	With head lowered to the ground, the calf takes hay or straw into mouth, chews, and swallows
Drinking	Consumption of water from trough
Rumination	Regurgitating and chewing again previously swallowed food
Urination	Excreting urine
Defecation	Excreting fecal matter
Vocalization	Any vocal noise made such as mooing or groaning
Contact between calves (licking and sniffing)	Calves are standing in close contact, licking or sniffing at each other
Self-licking	Calf turns the head back and licks its body with lips or tongue, or both
Self-scratching	Calf raises one hind leg and scratches parts of its body that are within reach of the raised leg

After each 3-h recording period, data were transferred to a personal computer by radio transmission and stored in a Microsoft Access database (Microsoft Corp., Redmond, WA) until analysis.

Cortisol. For cortisol analysis in saliva, samples were collected as described for horses (Schmidt et al., 2010; Erber et al., 2012b), with a cotton swab (Salivette; Sarstedt AG & Co., Nümbrecht-Rommelsdorf, Germany). The Salivette was placed loosely into the mouth of the calf until it was well soaked and was then centrifuged for 10 min at 1,000 × *g*. Saliva was aspirated and frozen at −20°C until analysis. Collection of saliva was very well tolerated by all calves. Samples were collected on 2 d before surgery (d −2 and −1) and on d 1 to 8 after surgery at 0700, 1300, 1900 and 0100 h. On the day of surgery (d 0), saliva was collected 60 and 30 min before castration and 10 min after surgery, and additional samples were taken at 30-min intervals from 30 to 180 min after surgery.

Cortisol concentration was determined by direct enzyme immunoassay without extraction (Palme and Möstl, 1997) and validated for bovine saliva. The antiserum shows cross-reactivity with cortisone and several corticosterone metabolites. Thus, values obtained must be interpreted as cortisol immunoreactivity. The intraassay coefficient of variation was 5.0%, the interassay variation 6.7%, and the minimal detectable concentration 30 pg/mL of saliva.

Heart Rate and HRV. A portable recording system (S810i; Polar, Kempele, Finland) attached to the thorax of the calf with an elastic girth was used for determination of cardiac RR intervals, as described for foals (Erber et al., 2012a,b). The positive electrode was located at the right shoulder and the negative electrode in the middle of the left thorax. The electrodes were secured with a second girth around the calf's thorax, which also contained a pocket for the recording watch. To obtain baseline values, recordings were performed on d 2 before surgery (day −2; 0900–1200 h). Thereafter, recordings were made from d 0 to 8. The duration of the recordings was 3 h (0900–1200 h), with the exception of d 0, when recording time was extended from 0800 to 1600 h. Infrared transmission was used to retrieve the data to a notebook computer.

From the recorded RR intervals, heart rate and HRV were calculated. On d −2, −1, and 1 to 8, 1-min periods at 30-min intervals were analyzed (i.e., 7 × 1 min over 3 h of recording). On d 0, 1-min intervals were analyzed, starting at 60 and 30 min before surgery, at sedation, at moving the calf to the surgery table, at surgery preparation and washing, at local anesthesia application, at skin incision or squeezing of the spermatic cord (BZ group), at cutting of the spermatic cord (OR group) or empty distal part of the scrotum (SR group) or corresponding times in control calves (CO group), and, in addition at 5, 10, 20, 30, 60, 120, and 180 min thereafter.

The Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Kuopio, Finland) was used for HRV analysis. From the recorded RR intervals, the HRV variables SDRR and RMSSD were calculated. To remove trend components, data were detrended and, in addition, an artifact correction was made. Detrending followed the procedure described by Tarvainen et al. (2002) and Schmidt et al. (2010). The Kubios program uses a detrending procedure based on the smoothness priors approach (Tarvainen et al., 2002). The smoothness parameter was set at 500 ms. For artifact correction, the custom filter of the program was set at 0.3, identifying RR intervals differing from the previous interval by more than 30% as artifacts. After abnormal interval removal, the program's algorithm substitutes detected errors with interpolated intervals calculated from differences between the previous and next accepted RR intervals.

Statistical Analysis

Statistical comparisons were made with the SPSS statistics program (SPSS Inc., Chicago, IL). All scored data were analyzed by nonparametric tests with the

Table 2. Body weight (mean ± SEM) on d 2 before and d 5 and 8 after surgeries in calves undergoing resection of the scrotum, Burdizzo castration, orchidectomy, or sham castration (n = 10 per group)

	Day relative to surgery		
Group	d −2	d 5	d 8
Scrotal resection	96 ± 18[a]	100 ± 19[b]	102 ± 19[c]
Burdizzo castration	101 ± 14[a]	103 ± 14[b]	106 ± 15[c]
Orchidectomy	104 ± 15[a]	105 ± 16[b]	107 ± 16[c]
Sham castration	96 ± 12[a]	97 ± 10[b]	100 ± 10[c]

[a–c]Values within a row with different superscript letters differ significantly ($P < 0.01$).

Kruskal-Wallis H test for comparisons between groups and the Friedman test for comparisons over time within groups. Nonscored data were normally distributed (Kolmogorov-Smirnov test). Recorded heart rate, HRV, and cortisol data were calculated as area under the curve for each day. Differences between days were analyzed by ANOVA using a general linear model for repeated measures, with experimental group as between subject factors. In the case of overall significant effects, individual comparisons between groups were made by testing for least significant differences. In addition, for nonscored parameters, comparisons between groups at individual time points were made by one-way ANOVA, followed by Duncan's test in case of overall significant differences. All data given are means ± standard error of the mean. A P-value below 0.05 was considered significant.

RESULTS

Clinical Observations and Fibrinogen Concentration

The scored general attitude of the calves at no time differed between groups and did not change over time (d −2 to d 8; median on the day of surgery = 2 and on all other d = 3, range 1–3). Also, the color of the conjunctivae and oral mucosae neither differed between groups nor changed over time (data not shown). Neither for respiratory rate nor for rectal temperature were differences between groups or interactions of group × time significant. Respiratory rate increased on the day of surgery in all groups ($P < 0.001$) and was higher than baseline values (d −2: 34.5 ± 7.2; mean of all groups ± SD) on the day of surgery (38.5 ± 6.2; $P < 0.01$ compared with d −2) and 1 d after surgery (37.7 ± 6.8; $P < 0.05$ compared with d −2). Rectal temperature changed over time ($P < 0.001$), with slightly lower values on the day of surgery and an increase 1 d thereafter (d −2: 39.0 ± 0.5; d 0: 38.8 ± 0.6; d 1: 39.1 ± 0.3; mean of all groups ± SD). Body weight increased ($P < 0.001$) in calves of all groups throughout the study and neither absolute weight nor relative increase differed between groups (Table 2). Plasma fibrinogen concentrations increased in calves within 2 d after surgical orchidectomy (differences over time: $P < 0.001$; interaction of time × group: $P < 0.001$; Figure 1).

Behavior and Locomotion

The time calves spent eating did at no time differ significantly between groups but changed over time and increased on the morning after surgery (d 1) and on d 3 ($P < 0.001$; Figure 2a). For rumination, time was reduced on d 1 (changes over time $P < 0.001$) and differed between groups in the afternoon of d 2 and morning of d 3 ($P < 0.01$; Figure 2b). The time the 2 calves in one pen were licking or sniffing each other did not differ between groups, but for all groups combined increased after surgery ($P < 0.01$; Figure 2c). Self-scratching and self-licking increased after surgery (both $P < 0.001$),

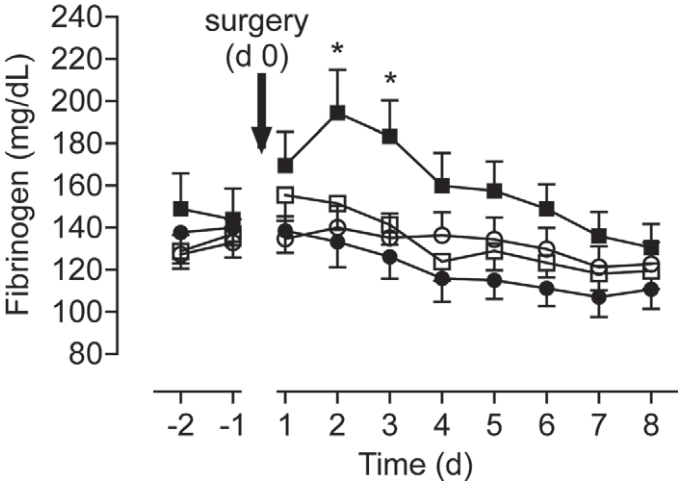

Figure 1. Plasma fibrinogen concentrations (mean ± SEM) from 2 d before to 8 d after surgery in calves undergoing resection of the scrotum (●), Burdizzo castration (○), orchidectomy (■), or left intact as controls (□; n = 10 per group). Error bars are either shown above or below mean values; general linear model ANOVA for repeated measures differences over time: $P < 0.001$; interaction of group × time: $P < 0.001$. *One-way ANOVA with subsequent Duncan's test: group of calves orchidectomized differs from all other groups at indicated time ($P < 0.05$).

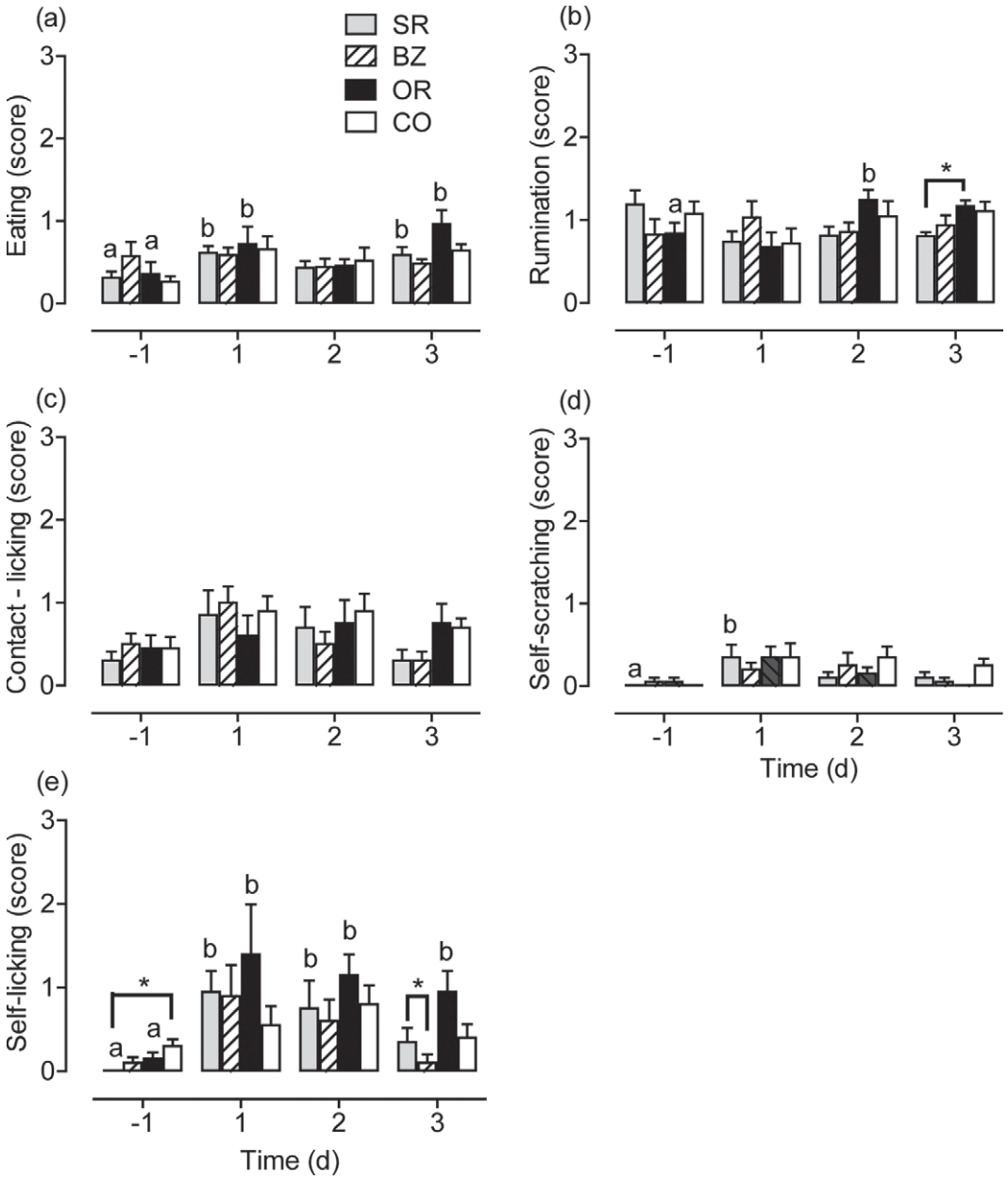

Figure 2. Scored behavior parameters [(a) eating time, (b) rumination, (c) licking of other calves, (d) self-scratching, and (e) self-licking] on the day before and on d 1, 2, and 3 after surgery in calves undergoing partial resection of the scrotum (SR), Burdizzo castration (BZ), orchidectomy (OR), or left intact as controls (CO; n = 10 per group). Values are means ± SEM. *Significant differences between groups at respective time point ($P < 0.05$); a,b values marked b differ significantly ($P < 0.05$) from the baseline value for respective group on d −1 (marked a).

but differed between groups only in the morning of d 3 ($P < 0.05$ and $P < 0.01$, respectively), with no consistent increase in a particular group (Figures 2d and e).

Locomotion time (number of steps determined by activity, lying, and temperature pedometers) was most pronounced in calves after orchidectomy (OR group; interaction of time × group: $P < 0.01$). Locomotion time and lying time changed significantly over time (all $P < 0.001$), with an increase in locomotion time and a decrease in lying time in response to all surgical procedures (Figures 3a and b).

Heart Rate and HRV

Heart rate recorded for 3 h each morning on the days before and after surgery did at no time differ between groups. Heart rate decreased on the days after surgery ($P < 0.001$; Figure 4a). On the day of surgery, heart

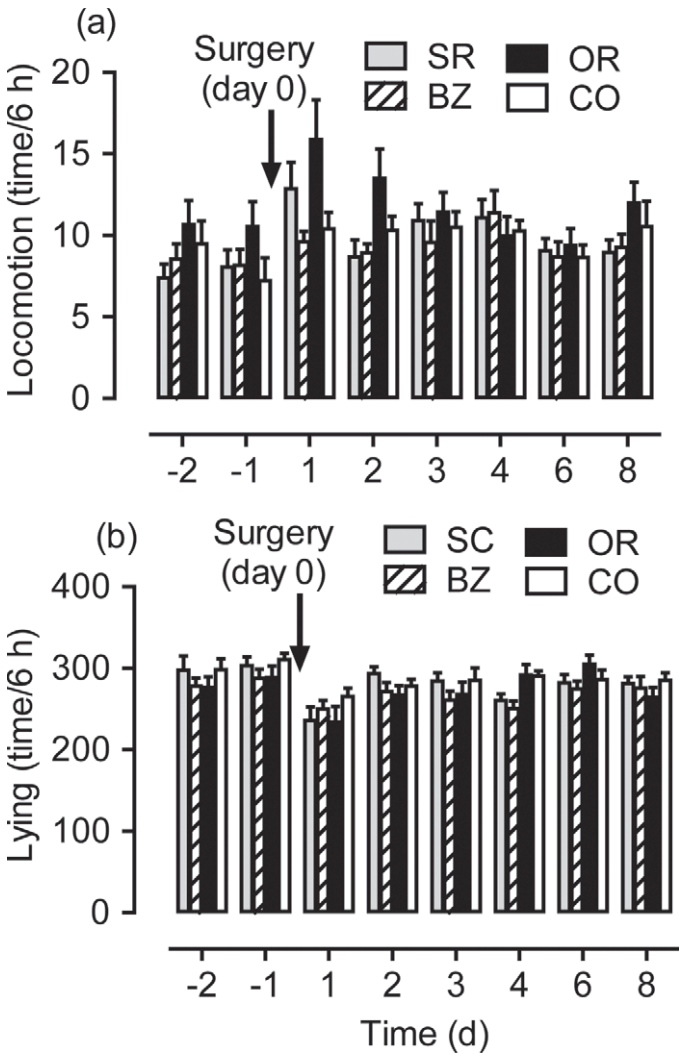

Figure 3. (a) Locomotion and (b) lying time recorded for 6 h per day from 2 d before to 8 d after surgery in calves undergoing resection of the scrotum (SR), Burdizzo castration (BZ), orchidectomy (OR), or left intact as controls (CO; n = 10 per group). Values are means and SEM. (a) Locomotion time: changes over time: $P < 0.001$; interaction of time × group: $P < 0.05$; (b) lying time: changes over time: $P < 0.001$ (general linear model ANOVA for repeated measures).

rate increased in calves of all groups with initial preparation for the experimental procedures and decreased after xylazine sedation to values below the initial baseline ($P < 0.001$; Figure 4b).

The HRV variables SDRR and RMSSD differed between groups, with higher values for the SR group than for calves in the OR group or the BZ group ($P < 0.05$; Figures 4c and e). Both SDRR (Figures 4c and d) and RMSSD (Figures 4e and f) changed over time, with a decrease on d 1 and 2 after surgery. The HRV variables did not differ between experimental groups at surgery, but on the day of surgery increased when the calves were placed on the surgery table and remained elevated during the experimental procedures for approximately 10 min ($P < 0.001$; Figures 4d and f).

Cortisol

Salivary cortisol concentrations, neither on the day of surgery nor on any other day differed significantly between experimental groups. On the day of surgery, concentrations of cortisol in saliva increased in all groups and reached a peak at 60 min after the start of surgical procedures ($P < 0.001$). Throughout the total study period, cortisol concentrations showed a significant decrease over time ($P < 0.001$; Figure 5a and b).

DISCUSSION

Male calves can be castrated by different techniques. Shortening of the scrotum and thus producing artificial cryptorchids by partial scrotal resection is a technique that recently has regained new interest. In contrast to surgical orchidectomy, Burdizzo castration, or the use of rubber rings, shortening of the scrotum to the best of our knowledge has not been studied with regard to the stress response and, by inference, pain perception of the animals.

An acute stress response could be demonstrated in calves submitted to surgery but also in control calves that were only handled, without intervention on the testes. This response did not differ between sedated calves castrated under local anesthesia and sedated control calves. Xylazine sedation combined with local anesthesia thus controlled any surgery-induced acute pain. The stress response of male calves to Burdizzo castration and orchidectomy has been analyzed in several studies (King et al., 1991; Mellor et al., 1991; Faulkner et al. 1992; Earley and Crowe, 2002; Stafford et al., 2002; Thüer et al., 2007; González et al., 2010; Baldridge et al., 2011) but no such information on partial scrotal resection has been available so far. Our results demonstrate that partial scrotal resection with adequate pain medication, in calves of about 2 mo of age, is not more stressful than Burdizzo castration and orchidectomy and thus, under animal welfare aspects, is an acceptable technique to achieve infertility in male dairy calves.

Animal welfare legislation in many countries requires anesthesia during potentially painful veterinary interventions. It is thus common to carry out surgical procedures in cattle with sedation and local anesthesia. Although, until recently, castration in male calves has often been performed without medication for pain control (Stafford et al., 2000; Coetzee et al., 2010b), an experimental group without pain medication would have been unacceptable to the animal welfare and eth-

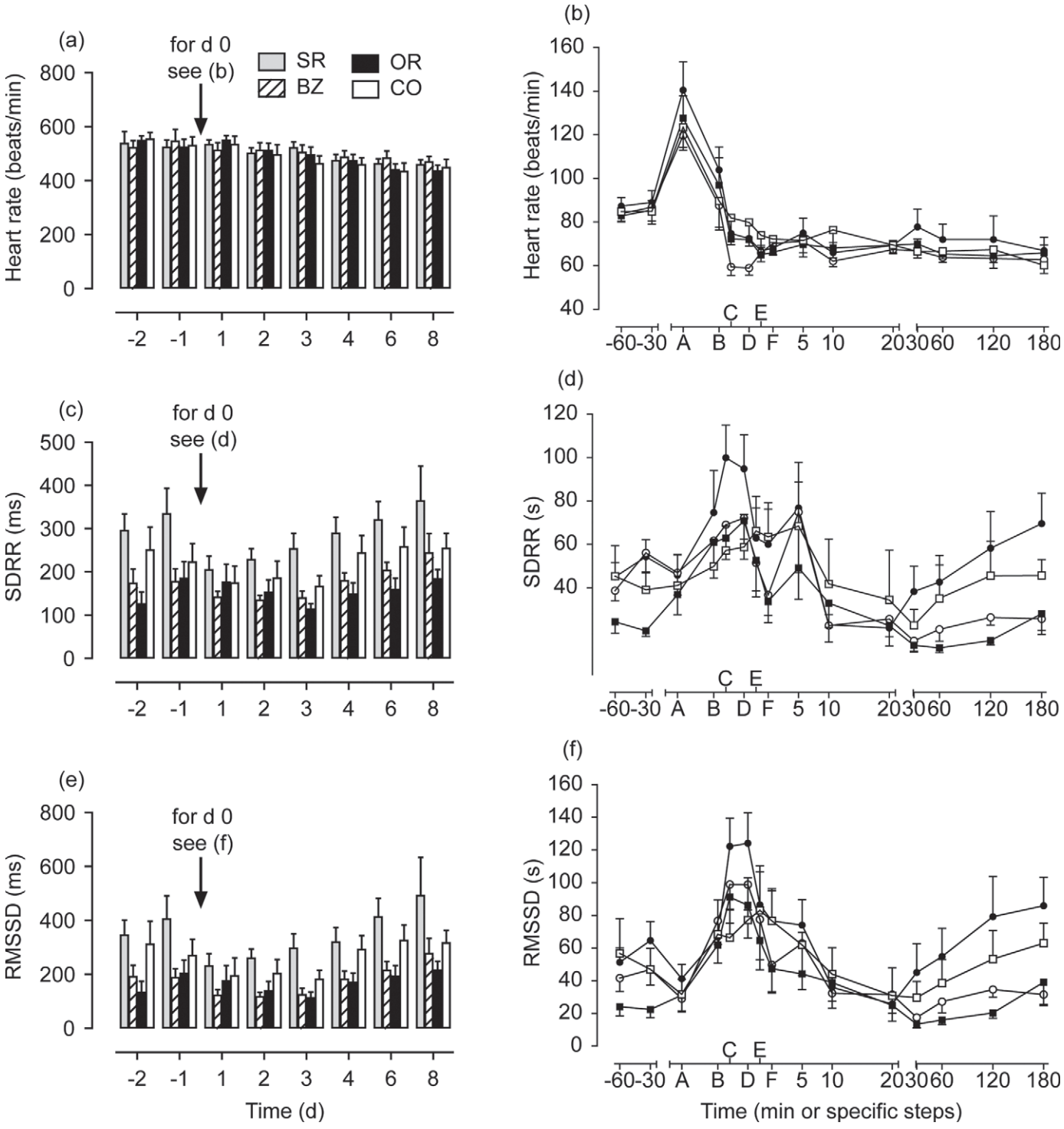

Figure 4. Heart rate (a and b) and the heart rate variability (HRV) variables standard deviation of beat-to-beat interval (SDRR; c and d), and root mean square of successive beat-to-beat differences (RMSSD; e and f) on (a, c, and e) 2 d before and 8 d after surgery calculated as area under the curve (AUC) for each day, and on (b, d, and f) the day of surgery in calves undergoing resection of the scrotum (SR; ●), Burdizzo castration (BZ; ○), orchidectomy (OR; ■), or left intact as controls (CO; □; n = 10 per group). Values are means and SEM (error bars either shown above or below). Specific steps at surgery: A = xylazine sedation, B = positioning on surgery table, C = washing of surgical field, D = local anesthesia, and E and F = begin and end of surgery, respectively (or corresponding time in control animals). (a and b) Heart rate changes over time: $P < 0.001$; (c and e) SDRR and RMSSD changes over time: $P < 0.01$; differences between groups: SR versus BZ and OR: $P < 0.01$; (d and f) SDRR and RMSDD changes over time: $P < 0.001$ (general linear model ANOVA for repeated measures).

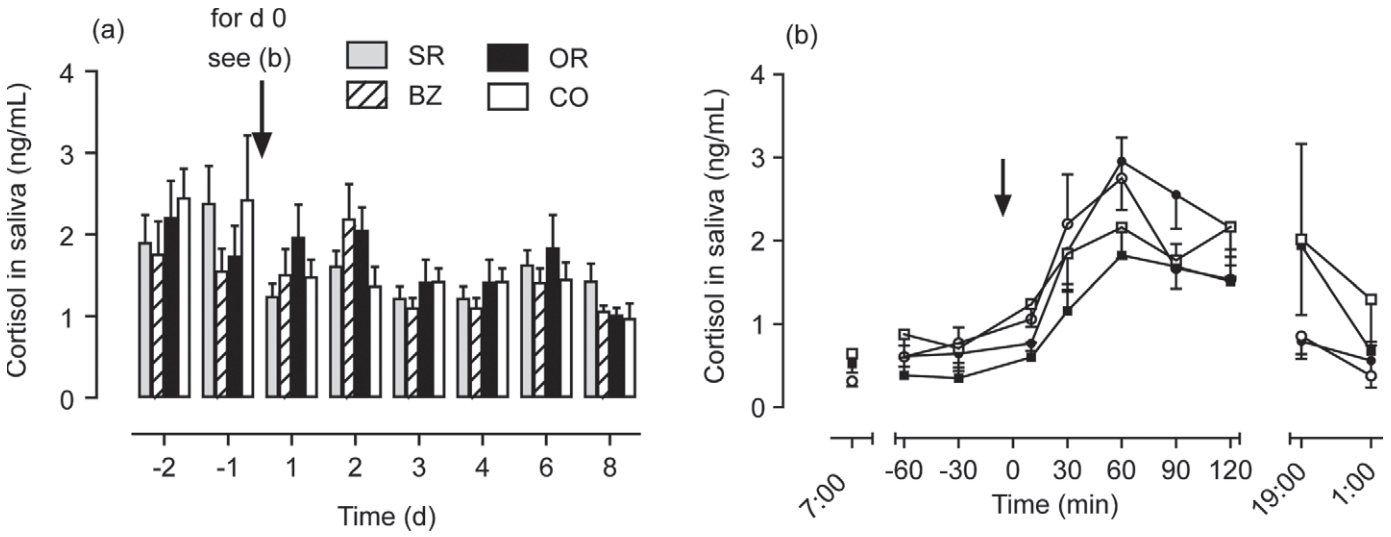

Figure 5. Cortisol concentrations in saliva on (a) 2 d before and 8 d after surgery calculated as area under the curve (AUC) for each day and (b) the day of surgery in calves undergoing resection of the scrotum (●; SR), Burdizzo castration (○; BZ), orchidectomy (■; OR) or left intact as controls (□; CO; n = 10 per group). Values are means and SEM (error bars either shown above or below). Changes over time in (a) and (b): $P < 0.001$ (general linear model ANOVA for repeated measures).

ics committee at the authors' institution. Our results thus do not allow comparisons between castration procedures without effects of analgesic and sedative drugs. The experimental setup pays careful attention to animal welfare, but does not allow differentiating between effects of local anesthesia and general analgesia. Thus, with regard to the animals' immediate responses during surgeries, differences in the stress response between groups are masked by the pain medication protocol.

Surgical castration (Ting et al., 2005; Pang et al., 2006) and other surgical interventions in calves, such as herniorrhaphy (Peiró et al., 2009), cause an increase in plasma fibrinogen concentrations. In our study, fibrinogen concentrations increased only in calves after orchidectomy, but not in calves of the other groups. Pang et al. (2006) found increased fibrinogen concentrations also in response to Burdizzo castration, but calves in that study were between 5 and 6 mo old. With increasing age, not only the stress response to Burdizzo castration increases (King et al., 1991; Ting et al., 2005), but also more pronounced tissue damage, resulting in higher postsurgery fibrinogen concentrations, is to be expected. The lack of such an increase after Burdizzo castration and after partial scrotal resection in the current study indicates that these 2 procedures at 2 mo of age are less traumatic than orchidectomy.

Self-licking and self-scratching increased in calves on the days after surgery but without consistent differences between groups. With effects of sedation and local anesthesia no longer present 1 d after surgery, a certain unease and pain is the most likely cause of this behavior (Faulkner et al., 1992; Fisher et al., 1996; Ting et al., 2003). Locomotion time and time spent standing increased on the days after surgery, whereas lying time was reduced in all 3 groups undergoing surgeries, but not in control calves. Using accelerometers, an increase in the percentage of time spent standing and, thus, a decrease in lying time after surgical castration has been described previously in older beef calves castrated without local anesthesia or systemic analgesia (White et al., 2008) and, based on behavior observations, also in calves after band castration under xylazine sedation and flunixin meglumine treatment (González et al., 2010). Significant time × group interactions indicate the most pronounced increase in locomotion in orchidectomized calves, which is in agreement with orchidectomy being more traumatic than the other procedures investigated.

Heart rate increased in calves of all groups with initial preparation for surgery. This apparently is an effect of handling, irrespective of the surgical or control procedures that followed. Immediately after sedation, heart rate in calves of all groups, including control calves, decreased to approximately 30% below the initial baseline. The α_2-agonist xylazine causes bradycardia in cattle (Scholtysik et al., 1998) and, thus, a decrease in heart rate was to be expected in our study. Throughout the study, heart rate did not differ between groups. The expected bradycardia after xylazine sedation was, thus, not modified by the surgical procedures, indicating that potential acute effects of castration on heart rate were effectively blocked by the medication in our study.

Both HRV variables determined; that is, SDRR and RMSSD, did not decrease when calves were handled for surgery, but increased after sedation and when the calves were placed on the surgery table and remained elevated for a time period of approximately 10 min. This increase occurred also in control calves and did not differ between groups. Heart rate variability represents the balance of the sympathetic and parasympathetic branch of the autonomous nervous system, with a decrease indicating sympathetic dominance and an increase indicating reduced sympathetic or increased parasympathetic tone, or both (von Borell et al., 2007). A stress response should have been associated with a decrease in HRV, as has been shown in different animal species, including cattle submitted to anthropogenic stressors (Mohr et al., 2002; Schmidt et al., 2010; Stewart et al., 2010; von Lewinski et al., 2013). The HRV increase in the current study indicates reduced sympathetic or increased parasympathetic activity, or both. An increase in RMSSD in response to surgical castration in calves has been reported previously and the authors suggested that visceral pain when the spermatic cords were torn may be associated with high parasympathetic activity (Stewart et al., 2010). This does, however, not explain increased HRV in calves at scrotal shortening and in control calves.

Effects of xylazine on HRV in cattle or other domestic animals, to the best of our knowledge, have not been published. In rats, the α_2-agonist clonidine potentiates parasympathetic actions on the heart (Toader et al., 2008). An increase in both HRV variables analyzed in response to xylazine in calves may be due to direct or indirect sympatholytic effects. As for changes in heart rate, the effects of xylazine on HRV were found to the same extent in castrated and control calves and, thus, were not overridden by the different surgical procedures. The dose of xylazine used is thus sufficient to block acute effects of castration on sympathoadrenal stress responses. Xylazine-induced bradycardia persisted for at least 3 h, whereas the increase in HRV lasted for less than 30 min. With regard to HRV, this is in agreement with studies on the sedative effect of xylazine in calves, reaching a maximum at 10 min after treatment and decreasing rapidly thereafter (Scholtysik et al., 1998). With the half-life of xylazine approximating 13 min (Coetzee et al., 2010a) and a relatively high xylazine dose used in our study, sedative and analgesic effects can be expected to last longer than the changes in HRV observed.

On the 2 d following surgery, values for both SDRR and RMSSD were reduced, indicating a transient reduction in vagal tone in calves of all groups due to some degree of postoperative stress. Both HRV variables were higher in calves submitted to partial resection of the scrotum than in calves after orchidectomy or Burdizzo castration, indicating that partial resection of the scrotum is associated with less postoperative stress than these 2 accepted and well-studied castration procedures.

Concentrations of cortisol in saliva increased in response to surgery and also in control calves and did not differ between groups. Xylazine is known to stimulate cortisol release to a certain degree and, therefore, cortisol should not be used as the sole indicator of stress in calves under sedative effects of xylazine (Stafford et al., 2003; Stilwell et al., 2010). However, in previous studies, although xylazine itself stimulated cortisol release, it did not mask different effects of the surgical procedures under investigation. In contrast to our results, calves sedated with xylazine and dehorned released more cortisol than xylazine-sedated calves which were sham dehorned (Stafford et al., 2003). Low doses of xylazine either administered alone or in combination with ketamine before castration reduced cortisol release for 60 min compared with control calves pretreated with saline before castration (Coetzee et al., 2010a). Also, a combination of xylazine and flunixin meglumine reduced salivary cortisol at castration compared with controls without pain medication (González et al., 2010), whereas in another study, butorphanol plus xylazine did not alter stress indicators in castrated bull calves (Faulkner et al., 1992). The pronounced xylazine-induced cortisol release in our study may be due to the higher dose of xylazine than that used in other studies. Salivary cortisol concentration in our calves peaked at 60 min after preparation for surgery. This is in agreement with previous studies, finding peak cortisol concentrations at 30 min after surgical castration (Stafford et al., 2002; Baldridge et al., 2011), with the slight delay in our study being most likely due to diffusion time of cortisol into saliva. Although cortisol release in our study is at least in part xylazine induced, it should still be considered that cortisol release is not necessarily attributable only to pain but may also increase when the animal is distressed (e.g., by fixation on the surgery table during sham castrations). Lateral recumbency has been shown as a stressor by itself in adult cows at claw trimming (Rizk et al., 2012). It thus cannot be excluded that handling of the calves and positioning on the surgery table induced an already maximal cortisol response. Therefore, the similar response in calves that underwent castration and in control calves does not prove that castration is no major stressor.

In addition to xylazine, local anesthesia was used in all calves except controls. Local anesthesia has been shown to eliminate the acute pain caused by rubber-ring castration, but needs to be combined with a systemic analgesic at Burdizzo or surgical castrations (Earley

and Crowe, 2002; Thüer et al., 2007). On the other hand, when used alone, the nonsteroidal antiinflammatory drug ketoprofen reduces the cortisol response to Burdizzo or surgical castration but may need to be accompanied by local anesthesia to fully eliminate pain perception during castration (Stafford et al., 2002; Stafford and Mellor, 2005). Xylazine combined with local anesthesia reduced cortisol release in calves at dehorning to levels no longer different from nontreated controls (Stafford et al., 2003). The difference from control calves in our study may be due to the higher xylazine dose used at castration. The lack of a difference in cortisol release between castrated and control calves in our study indicates that with the pretreatment used, none of the experimental procedures was perceived by the calves as a pronounced stressor causing an additional cortisol release.

CONCLUSIONS

The acute stress response did not differ between sedated calves castrated with 3 different techniques under local anesthesia and sedated control calves. Thus, pharmacological methods are available to largely eliminate the acute pain experienced by calves during and following castration. With adequate pretreatment, surgical orchidectomy, Burdizzo castration, and partial scrotal resection without removal of the testes did not differ with regard to stress and, by inference, pain at surgery. Partial scrotal resection thus can be considered an alternative to surgical or Burdizzo castration. With regard to the postoperative period, only a few differences between groups existed but increased locomotion and higher plasma fibrinogen concentrations after surgical orchidectomy may indicate that this castration technique is more traumatic than the other 2 procedures investigated.

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