Influence of local anaesthesia on pain and distress induced by two bloodless castration methods in young lambs

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Abstract

To assess short- and long-term effects of bloodless castration methods with and without local anaesthesia, behavioural and cortisol responses of lambs were used as indicators of pain and distress. Seventy lambs, aged 2–7 days, were control-handled or castrated by Burdizzo or rubber ring methods with and without local anaesthesia. Either 5 mL of diluted lidocaine (4 mg/kg) or physiological sodium chloride solution was distributed in both spermatic cords and the scrotal neck. The serum cortisol response was monitored for 48 h, and behavioural and clinical traits were followed for three months. Local anaesthesia tended to reduce behavioural and cortisol responses after Burdizzo castration and provided a significant reduction after rubber ring castration. Prolonged pain after rubber ring castration with anaesthesia was not evident. If combined with local anaesthesia, both the rubber ring and the Burdizzo methods are acceptable methods for castration of lambs up to one week of age.
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1. Introduction

Hofmeyr (1987) described several methods for the castration of male ruminants, including surgical removal of the testes, bloodless techniques that interrupt the blood supply to the testes such as elastic rubber rings or Burdizzo castration, and chemical or immunological castration. The method combining rubber ring and Burdizzo castration was first described by Molony et al. (1993). Aside from immunological castration, these methods may induce considerable pain depending on the technique. For example, surgical removal of the testes is reported to be more painful than bloodless castration (Kent et al., 1993; Lester et al., 1996).

The terms "animal pain" and "distress" are often used in the context of castration. Molony and Kent (1997) defined "animal pain" as "an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery". Likewise Mellor and Stafford (1999) used the term "distress" to acknowledge that the experience induced by pain includes interacting emotional and physical facets. Consequently, the use of both terms is indicated when assessing the overall impact of different castration methods on lamb welfare.

It is not possible to measure pain and distress directly, but indirect physiological and behavioural indices may be used as indicators. Activity in afferent nerves (Cottrell and Molony, 1995), activity of the sympathetic nervous system (Peers et al., 2002), activity of the hypothalamo-pituitary-adrenal axis (Mellor and Murray, 1989a,b; Shutt et al., 1988), as well as changes in posture, locomotor activity, and behaviour (Mellor and Murray, 1989b; Molony et al., 1993; Wood et al., 1991) are feasible ways to assess animal pain (Molony and Kent, 1997; Molony et al., 2002). Several authors have used analysis of plasma cortisol concentration and/or observation of behaviour and postures to assess castration pain and distress over the first few hours after castration (Kent et al., 1993; Lester et al., 1991; Mellor and Murray, 1989b; Molony et al., 1993; Molony et al., 2002; Thornton and Waterman-Pearson, 1999).

The consequences of painful practices over the longer term (days to weeks) have not been well investigated in lambs. These consequences might include chronic pain, hyperalgesia, phantom pain, neuropathic pain, or infections (Mellor and Stafford, 1999). Moreover, it is of interest to confirm the results of Kent et al. (2000), who found that local anaesthesia resulted in a reduction of castration associated pain and distress beyond the duration of analgesia.

To minimise pain and distress, sedation and/or local or general anaesthesia as well as nonsteroidal anti-inflammatory drugs (NSAIDs) may be used. Sedatives may be beneficial in reducing handling distress and blunting pain (Grant and Upton, 2001; Mellor and Stafford, 1999). However, sedation alone is not an appropriate anaesthesia for castration of lambs.

General anaesthesia may reduce acute pain and distress, but it is hazardous and does not prevent the occurrence of post-castration pain (Mellor and Stafford, 1999). When injected prior to castration, NSAIDs have significant beneficial effects on the overall cortisol response and on the time spent in abnormal postures caused by Burdizzo castration in lambs (Molony et al., 1997). However, the pre-treatment use of caprofen did not reduce the discomfort scores after rubber ring castration or tail docking (Price and Nolan, 2001; Steiner et al., 2003).

Local anaesthesia reduces physiological and behavioural changes by preventing the afferent impulses from reaching the brain (Cottrell and Molony, 1995; Molony et al., 1997). Depending on the local anaesthetic, volume, and injection site, however, local anaesthesias may vary in efficiency. For rubber ring castration in lambs, injection of local anaesthetic into the testes was less effective than its injection into the neck of the scrotum at the site of the ring (Kent et al., 1998).

Acknowledgement that castration of male lambs interferes with their welfare has led to changes in animal welfare legislation in some countries. For example, since 2001 in Switzerland, male ruminants may only be castrated under local or general anaesthesia. Therefore, the combined Burdizzo and rubber ring castration without additional anaesthesia is not allowed in Switzerland, regardless of its reported pain-reducing effect (Kent et al., 1993, 1995, 1998; Molony et al., 1993; Thornton and Waterman-Pearson, 1999) and reduced healing time (Kent et al., 2000; Sutherland et al., 2000).

The current study was designed to evaluate inexpensive and simple castration methods combined with an effective analgesia, to meet the interests of both farmers and lambs. In addition to control-handling, elastic rubber ring and Burdizzo castration were tested. These two bloodless castration techniques and control-handling were compared with and without local anaesthesia, allowing multiple comparisons. To assess both the short- and long-term consequences of castration and anaesthesia, serum cortisol levels were measured over 48 h, and posture, behaviour, and clinical signs were monitored for a three-month period.

2. Materials and methods

2.1. Animals and housing

Seventy male lambs were housed indoor in straw-bedded pens together with their dam and siblings. White Swiss Mountain and White Swiss Mountain × Charolais lambs were treated at 2–7 days of age. From the day before treatment (day −1) until blood sampling was finished on day 2 after treatment, the animals were kept in pens measuring 1×2 m. Later in the study, lambs and their mothers were kept in large pens in groups of 20 ewes and their offspring. Only lambs not exhibiting any signs of disease, as determined by clinical examination, were included. On day −1, the lambs were weighed, marked to allow identification, and an indwelling catheter (Venflon, 18 G, Becton Dickinson) was introduced into one of the jugular veins to facilitate blood sampling.

2.2. Study design and treatments

Treatment groups included rubber ring castration with (RR+) and without anaesthesia (RR−), Burdizzo castration with (B+) and without anaesthesia (B−), and control-handling with (H+) and without anaesthesia (H−).

The lambs were randomly allocated to one of these six treatment groups by a second person drawing lots from a pot. The pot included one lot for each group. Each time a lot was drawn, it was removed from the pot and not put back until a group of six lambs was assigned to the different treatment groups. No attempt was made to balance the groups for weight of lamb or single and multiple births.

For the treatment groups with anaesthesia, 4 mg/kg bodyweight (BW) lidocaine hydrochloride 2% (Biokema AG) was diluted with physiologic sodium chloride (NaCl) solution to a final volume of 5 mL. After local disinfection of the skin with diluted povidone iodine solution (Betadine, Provet AG), 1.5 mL of the diluted lidocaine were injected into each spermatic cord and 2 mL subcutaneously around the scrotal neck. In lambs that did not receive anaesthesia, 5 mL of 0.9% NaCl were injected as described above.

The operator carried out injections, castration, or control-handling and all following evaluations. The syringes for local injections were prepared by a second person. The operator, therefore, was blinded as to lidocaine or NaCl injection but not as to castration method or control-handling.

Before castration or control-handling, all lambs received a tetanus prophylaxis of 1500 IU of Tetanus-Serum (Veterinaria AG) and the skin of the scrotal neck was disinfected one more time with diluted povidone iodine solution. Castration and control-handling were carried out 5 min after local injection of lidocaine or NaCl.

For rubber ring castration, constricting rings (Provet AG) were applied to the neck of the scrotum with an Elastrator (Provet AG), after ensuring that both testes were situated distal and the teats proximal to the ring. Burdizzo castration was performed by a Burdizzo clamp (23 cm, unused before this experiment, Provet AG). Each spermatic cord and associated scrotal tissue was crushed twice at a distance of 0.5 cm for 30 s each. The second crush was placed distal to the first. Care was taken to ensure that the clamp crush lines did not overlap. Control lambs were manipulated for approximately 2 min by imitating a Burdizzo castration, but were left intact. Time needed for castration was measured from the beginning until completion of castration.

2.3. Assessment of immediate expression of pain during castration

The subjective overall behavioural response (0 = no response, 1 = moderate response, 2 = strong response) of each lamb as well as struggling (0 = no struggling, 1 = struggling with hind limbs, 2 = struggling with hind and/or front limbs, 3 = struggling with the whole body), vocalisation (0 = none, 1 = once, 2 = several times), and lip curling (0 = no, 1 = yes) were noted and the scores added up.

2.4. Blood sampling and serum cortisol assays

Blood samples (2.5 mL) from each lamb were collected in a blood sampling system for serum preparation (Monovette, Sarstedt). They were taken prior to anaesthesia, immediately after anaesthesia, and at 20, 40, 60, 90, 120, 150, 180, 210, 240, 360, 540, 1440, and 2880 min

after castration or control-handling. The blood samples were centrifuged at 2376g for 10 min within 1 h of sampling, and the serum was immediately stored at −20 °C until cortisol analysis. Serum cortisol concentrations were determined using a competitive immunoassay (Immulite, Labor Laupeneck). The area under the curve (AUC) was calculated for cortisol values between −20 and 120 min.

2.5. Recording of behaviour and postures

Active behaviours and postures (Table 1) were recorded according to Molony et al. (2002). Active behaviour and postures were recorded by direct observation from outside the pen without disturbing the lamb. On day −1, each lamb was observed for 10 min to describe the individual baseline values. On days 0, 1, and 2, recordings were done during the 10 min preceding each blood sampling. On days 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, and 30 and within the time periods (days) 36–38, 43–45, 50–52, 58–60, 61–67, 68–74, 75–81, and 82–89, lambs were observed for a period of 10 min. If no change in posture occurred, the actual posture was recorded every 2 min, otherwise the change was noted. The combined indices "total active behaviour" and the "proportion of abnormal postures" were calculated (Table 1).

2.6. Assessment of the scrotal region and body-weight measurement

The local effects of the different castration methods and control-handling were assessed by visual inspection and palpation of the scrotal region and measurement of the scrotal circumference (cm). Lambs were captured for a short time after every observation period. Local pain was monitored by assessing the response to palpation of the tissue proximal and distal to the lesions or the rubber ring (0 = no response; 1 = wincing; 2 = struggling with attempts to escape). To evaluate scrotal condition, the tissue proximal and distal to the lesion was inspected. Scrotal conditions were noted as follows: intact skin; scrotum absent with a scab; scrotum hard and dry, beginning to fall off; scrotum dry and firm; scrotum drying; limited swelling of the scrotum; swelling of the surrounding tissues; severe swelling of the surrounding tissue with discoloration; ulcerated lesion with pus or exudate. The body weight (kg) was measured on days −1, 2, 6, 12, 21, 30, and once within days 58–60 and 82–89.

2.7. Slaughter and histological examination of the scrotal tissue

The mean body weight (±SD) at slaughter was 44.9 ± 0.4 kg. The testes of the Burdizzo castrated and control-handled lambs were collected within 10 min

Table 1
Description of active behaviours, postures, and indices recorded during the experiment (based on Molony et al., 2002)

Active behaviours	Description
Foot stamping and kicking	Number of times a front or hind limb was lifted and forcefully placed on the ground while standing or was used to kick while lying
Easing quarters	One action was recorded each time a front or hind limb was moved in a less forceful manner than stamping or kicking or the whole body was shifted or eased without moving from the place of rest
Restlessness	Number of times a lamb stood up and laid down; each unit scored included both the act of rising and lying down
Head turning	Movement of the head beyond the shoulder: including both looking and touching at the source of pain and grooming
Rolling	Lamb rolled from lying on one side to the other without getting up
Vocalisation	Occurrence of each vocal sound was recorded
Postures	*Description*
Normal standing	Standing, walking and playing, eating, or investigating with no abnormalities
Abnormal standing	Standing or walking unsteadily; walking backwards; walking on knees, circling; leaning on a support
Statue standing	Standing still for more then 10 s, usually with the hind limbs slightly apart and positioned further back than normal
Arched back	Standing with the hind limbs slightly apart and positioned further back than normal with the back arched
Normal lying	Ventral (sternal) recumbency with the legs tucked in and the head down, either round to one side or directly in front or head up
Extended hind limbs	Sternal or lateral recumbency with at least one hind limb partially or fully extended with the head up or down
Dog sitting	Ventral recumbency with the lamb keeping the scrotal region off the ground
Indices	*Description*
Total active behaviour	The sum (over a defined period of animal observation time) of foot stamping and kicking, easing quarters, restlessness, head turning, and vocalisation
Proportion of abnormal postures	The number of recorded abnormal postures (abnormal standing, statue standing, standing with an arched back, lying with extended hind limbs, and dog sitting) relative to the total recorded postures

after culling and preserved in formalin. The formalin-fixed samples were embedded in paraffin wax and slides were stained with haematoxylin and eosin for later histological analysis.

2.8. Statistical analysis

Data were stored in Microsoft Excel spreadsheets (www.microsoft.com) and analysed using the commercial statistic program NCSS (2001, Kayswille). Pre-treatment values of cortisol concentration were subtracted from the subsequent sample values to correct for individual animal variation in baseline cortisol levels.

Kruskal–Wallis analysis of variance (ANOVA) on ranks with Bonferroni correction for multiple comparisons was used to assess the association between the treatment groups and the factors immediate expression of pain, cortisol response, AUC of the cortisol response over defined time intervals, proportion of abnormal postures, and scrotum circumference. Spearman rank correlation was used to analyse the correlation between the immediate expression of pain and the time used for castration. Daily weight gain was analysed using a two-way ANOVA with Bonferroni correction for multiple comparisons. The response to local palpation was transformed into two categories (yes/no), and the treatment groups were then compared by the χ^2 test. Wilcoxon signed-rank test was used to compare cortisol measurements within animals between two time points.

Total active behaviour counts showed a skewed distribution. Therefore, the treatment groups were compared by Poisson regression with the frequency (count) data as the outcome and treatment group (baseline = control-handling) and anaesthesia (baseline = yes) as independent variables. The overall level for statistical significance was set at $P < 0.05$.

3. Results

3.1. Immediate expression of pain during castration

The expression of pain during castration was significantly influenced by the castration method. The Burdizzo castrated lambs (B+, B−) had significantly higher median (range) pain scores of 5 (2–7) and 7 (2–8), respectively, compared to rubber ring castrated (RR+: 0 [0–4]; RR−: 1 [0–4]) or control-handled (H+: 1 [0–3]; H−: 3 [0–5]) lambs. The influence of the anaesthesia on the immediate expression of pain was not significant ($P = 0.132$), but the use of lidocaine tended to reduce the immediate pain response during castration. The time needed for castration (B = 273 ± 113 s; RR = 48 ± 17 s; H = 139 ± 37 s) did not influence the immediate expression of pain during castration. The correlation coefficient between the expression of pain during castration and time needed for castration were $r = 0.14$, −0.03, and 0.18 for B, RR, and H, respectively.
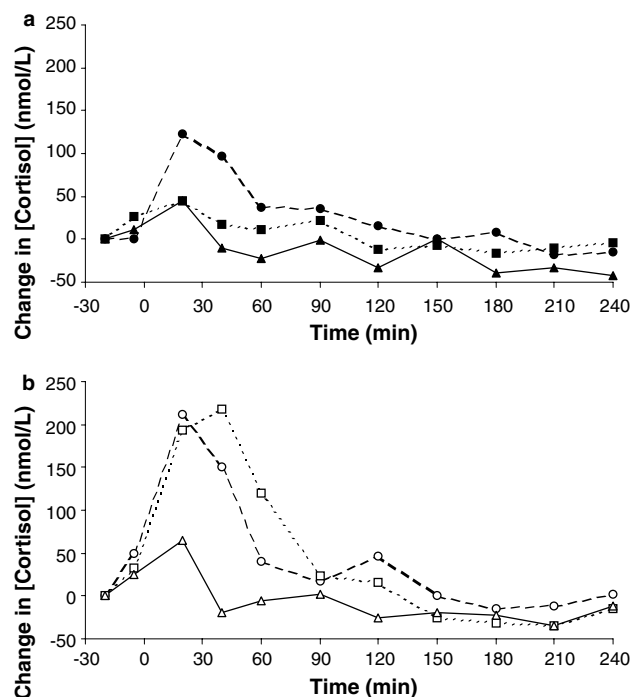
Fig. 1. Changes in the mean serum cortisol response from 20 min before to 4 h after treatment, in response to two different castration methods and control-handling (a) with and (b) without local anaesthesia. Rubber ring castration with local anaesthesia ■, Burdizzo castration with local anaesthesia ●, control-handling with local anaesthesia ▲, rubber ring castration without local anaesthesia □, Burdizzo castration without local anaesthesia ○, control-handling without local anaesthesia △.

3.2. Cortisol

There were no significant between-group differences in the baseline serum cortisol concentrations (i.e., before

castration). Fig. 1 shows the mean serum cortisol concentration for each time point and each treatment group. Lambs of groups B+, B−, and RR− exhibited rises in serum cortisol concentrations in response to treatment ($P < 0.01$). In all castration groups, serum cortisol concentrations returned to pre-treatment values or below within 2 h after castration.

The peak cortisol value on the day of castration was higher after castration without anaesthesia (B−, RR−) when compared to the control groups (H− and H+). Local anaesthesia significantly reduced the mean peak cortisol level after castration by rubber ring ($P < 0.01$); a strong trend towards lower mean peak cortisol concentration ($P = 0.0516$) was evident in lambs of group B+ as compared to B−.

The AUC (calculated from −20 to 120 min) was significantly influenced by castration and anaesthesia method (Table 2). The AUC was significantly smaller for H− and H+ than for B− and RR−. Local anaesthesia significantly reduced the AUC after rubber ring castration but not after Burdizzo castration.

3.3. Behaviour and postures

3.3.1. Total active behaviour

Table 2 shows the mean incidences of active behaviour for different treatment groups for the observable 50 min during the first 2 h after treatment. Castration by rubber ring without local anaesthesia caused most total active behaviour among the different treatment groups H−, B−, and RR−. Foot stamping and kicking or restlessness made up the main part of the total active behaviour after rubber ring castration. Lambs in the RR− group showed a 10.8 times higher rate ratio for total active behaviour during the first 2 h after castration when

Table 2
The effect of castration or control-handling with and without local anaesthesia on the serum cortisol response and the mean incidence (±SD) of active behaviour and abnormal postures for the observable 50 min in the first 120 min after treatment

Treatment group	H−	H+	B−	B+	RR−	RR+
N	10	10	10	15	10	15
Behaviour						
Foot stamping and kicking	0.0 ± 0.0^{RR-}	0.0 ± 0.0^{RR-}	0.6 ± 1.3^{RR-}	0.4 ± 0.7^{RR-}	11.2 ± 8.5	0.0 ± 0.0^{RR-}
Easing quarters	1.6 ± 2.2	1.6 ± 1.4	3.7 ± 4.0	2.5 ± 3.0	2.6 ± 3.5	1.1 ± 1.6
Restlessness	0.3 ± 0.5^{RR-}	0.3 ± 0.5^{RR-}	1.5 ± 1.8	1.5 ± 3.1^{RR-}	9.6 ± 5.2	0.3 ± 0.6^{RR-}
Head turning	0.9 ± 1.7	0.9 ± 2.5	0.7 ± 1.1	0.5 ± 1.1	0.9 ± 1.9	0.7 ± 1.2
Vocalisation	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.5	0.1 ± 0.3	0.3 ± 0.8
Abnormal postures						
Abnormal standing	0.0 ± 0.0^{RR-}	0.0 ± 0.0^{RR-}	0.6 ± 1.0^{RR-}	0.3 ± 0.8^{RR-}	4.5 ± 3.5	0.1 ± 0.4^{RR-}
Statue standing	1.2 ± 2.0	0.7 ± 1.5	0.9 ± 1.3	1.3 ± 2.3	2.4 ± 3.6	0.1 ± 0.3
Arched back	3.1 ± 6.6	0.9 ± 1.3	2.3 ± 2.6	2.5 ± 3.6	5.4 ± 4.2	1.7 ± 2.3
Extended hind limbs	2.7 ± 6.2$^{RR-, B-}$	5.4 ± 7.4^{RR-}	12.5 ± 7.4	9.4 ± 8.1^{RR-}	24.6 ± 6.5	6.8 ± 5.6^{RR-}
Dog sitting	6.1 ± 3.8	1.6 ± 2.6	5.3 ± 6.0	1.4 ± 2.1	2.2 ± 3.1	2.7 ± 4.2
Serum cortisol						
AUC (nmol/L min)	1092 ± 7715	−54 ± 9593	10989 ± 5637$^{H+, H-}$	6948 ± 6067	13324 ± 6617$^{H+, H-}$	2634 ± 5450^{RR-}

The serum cortisol area under the curve (AUC) was calculated over the time period 20–120 min. Superscripts H−, H+, B−, B+, RR− and RR+ indicate statistically significant differences to the respective groups.

Table 3
Rate ratio and 95% confidence limit for total active behaviour for castration methods without (−) anaesthesia relative to castration methods with (+) anaesthesia, for control-handling (H+: n = 10), Burdizzo (B+: n = 15), and rubber ring castration (RR+: n = 15)

Time period	H− (n = 10)	B− (n = 10)	RR− (n = 10)
0–2 h	1.0 (0.6–1.7)	1.3 (0.9–1.8)	10.8 (7.6–15.3)*
2.5–9 h	1.1 (0.7–1.9)	2.3 (1.5–3.4)*	1.4 (0.95–2.1)
1–6 d	1.5 (1.0–2.1)*	2.1 (1.6–2.9)*	1.7 (1.2–2.2)*
9–90 d	1.2 (0.9–1.7)	1.1 (0.8–1.4)	0.5 (0.4–0.8)*

Asterisks indicate statistically significant differences within type of treatment; h = hours after castration; d = days after castration.

compared to RR+ lambs (Table 3). Lambs in the B− group showed significantly more total active behaviour between 2.5 and 9 h after castration when compared to the control group (H−). Rubber ring castration with anaesthesia (RR+) reduced the total active behaviour to the control level (H+), whereas B+ lambs still showed a 1.8 times higher rate ratio of total active behaviour than H+ animals during the first 2 h after castration.

From days 1 to 6 after treatment, lambs castrated without local anaesthesia (B−, RR−) exhibited a significantly higher rate of total active behaviour than control lambs (H−). After this time there were no significant differences in total active behaviour among groups without local anaesthesia. From days 1 to 6 after treatment, lambs treated without local anaesthesia (B−, RR−, and H−) exhibited significantly more total active behaviour than lambs of the B+, RR+, and H+ groups. After day 9, RR− lambs showed significantly less total active behaviour than their reference group RR+ (Table 3).

3.3.2. Proportion of abnormal postures

The proportion of recorded postures that were abnormal was significantly greater after RR− castration, than after H+, H−, B+, and RR+ during the first 2 h (Table 4). Also, the B− group had a higher proportion of abnormal postures than H+. For the remainder of the castration day, the proportion of abnormal postures after B− and RR− castration was significantly higher than after H−. After day 0, the proportion of abnormal postures did not differ among groups.

3.4. Scrotal condition and swelling

Burdizzo castration resulted in moderate to severe local swelling over an average (±SD) of 16.4 ± 6.1 days.

This swelling was not influenced by the injected fluid (NaCl or lidocaine). The average maximum scrotal circumference (±SD) was 13.1 ± 1.8 cm, measured on day 2 after Burdizzo castration. This is significantly greater than after control-handling or rubber ring castration. At no time was purulent or serous secretion visible after Burdizzo castration.

After rubber ring castration, the scrotal tissue and its contents began to dry up and fell off after an average of 24.7 ± 4.4 days. Three of 25 RR lambs showed some purulent secretion proximal to the site of the rubber ring. Purulent secretion started in two cases on day 9 and in one case on day 12 after castration over a maximum period of 3–6 consecutive days. The remaining 22 lambs showed no secretion.

3.5. Response to local palpation

Before castration, no significant between-group differences were found in response to local palpation. Compared to RR−, lambs castrated by the B− method showed an increased responsiveness to local palpation at 40, 60, and 120 min after castration (Fig. 2). The response to local palpation during the first 2 h after castration was significantly higher for B− lambs than for H+, H−, or RR+ lambs. During the following six days, all castrated lambs (B+, B−, RR+, and RR−) showed significantly higher response to palpation than control lambs (H+, H−). After day 9, only RR− animals showed more response to palpation than control lambs (H+, H−).

The first response to palpation was evident within 38 ± 31 min after B− and 103 ± 65 min after B+ castration (Table 5). Local anaesthesia tended to result in a delayed onset of increased responsiveness to local

Table 4
Group average and standard deviation of proportion of abnormal postures during the respective observation period after castration of lambs with the Burdizzo (B) method, the rubber ring (RR) method, or after handling of a control group (H), both with (+) and without (−) local anaesthesia

Time period	H− (n = 10)	H+ (n = 10)	B− (n = 10)	B+ (n = 15)	RR− (n = 10)	RR+ (n = 15)
2 h	0.38 ± 0.30	0.25 ± 0.23	0.67 ± 0.27[H+]	0.44 ± 0.28	0.89 ± 0.09[H+, H−, B+, RR+]	0.36 ± 0.21
2.5–9 h	0.24 ± 0.16	0.33 ± 0.24	0.57 ± 0.21[H−]	0.47 ± 0.21	0.56 ± 0.21[H−]	0.39 ± 0.21
1–6 d	0.20 ± 0.22	0.26 ± 0.17	0.22 ± 0.17	0.13 ± 0.12	0.29 ± 0.24	0.20 ± 0.14
9–90 d	0.07 ± 0.05	0.09 ± 0.09	0.06 ± 0.05	0.06 ± 0.03	0.05 ± 0.07	0.06 ± 0.05

Superscripts H−, H+, B−, B+, RR− and RR+ indicate statistically significant differences to the respective groups; h = hours after castration; d = days after castration.
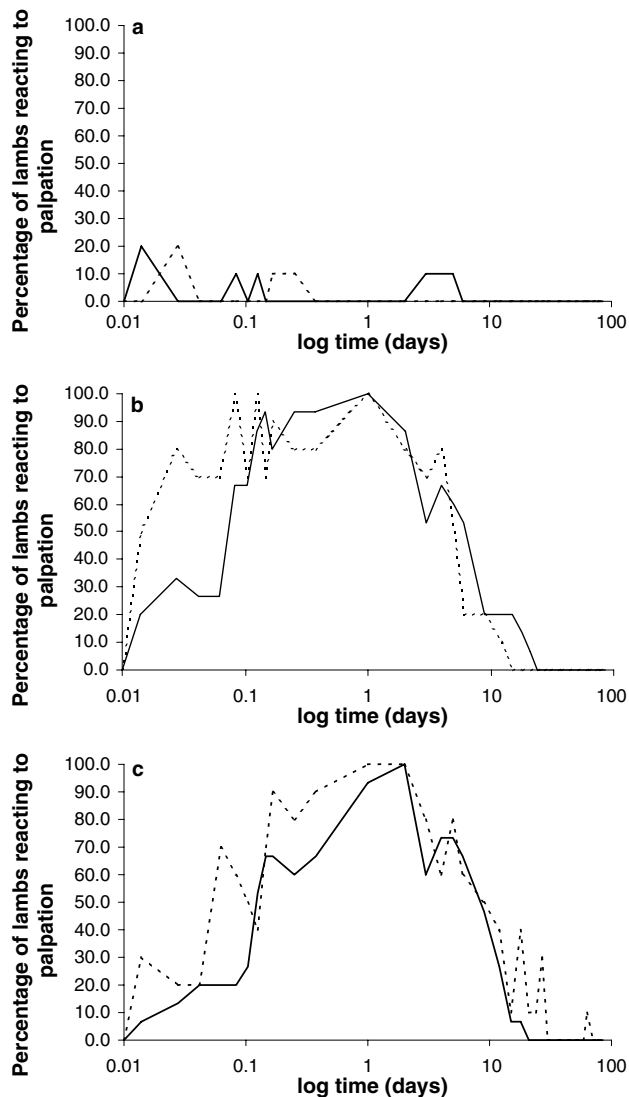
Fig. 2. Percentage of animals eliciting signs of pain at local palpation at different time points during the three months of observation for (a) control-handling, (b) Burdizzo castration, and (c) rubber ring castration with (——) and without (- - - - - -) local anaesthesia.

palpation ($P = 0.0125$), although this can only be considered a trend due to the conservative Bonferroni multiple comparison correction. The last day a painful response to palpation could be evoked was 7 ± 6 days after B+ and 5 ± 3 days after B− castration. Rubber ring castration provoked the first response to local palpation after 403 ± 504 min and 76 ± 65 min for the

treatments with and without local anaesthesia, respectively. Local anaesthesia had a strong but not significant (after Bonferroni correction) effect on delay of the onset of response to palpation ($P = 0.0119$). The first response to palpation was significantly earlier for B− than for RR+. The last occasion that a response to palpation was observed was 8 ± 4 days after RR+ and 15 ± 10 days after RR− castration.

3.6. Body weight

On day −1 the mean weight was 6.9 ± 1.0 kg. There were no significant differences of body weight among treatment groups on day −1 except the control groups H− and H+. Daily weight gain was not significantly influenced by castration method or by local anaesthesia (Table 6).

3.7. Castration success

3.7.1. Clinical assessment

All Burdizzo castrated lambs had an undersized scrotum with hard and small testes compared to controls. After rubber ring castration, all lambs lost their scrotum and testes. Therefore, the clinical success is judged to be 100% for both techniques.

3.8. Histology

Thirty of 50 testes from lambs of the groups B+ and B− were available for histological evaluation. In all cases the remaining testicular tissue was rather small. The sex cords contained fewer cells than normal. Mostly, they belonged to the Sertoli cell group, and intact spermatogonia were rare. Isolated Type I spermatocytes appeared only in few preparations. In none of the examined slices of the Burdizzo group were elongated spermatids or sperms found. The connective tissue surrounding the sex cords was broadened by fibrocytes, many macrophages containing haemosiderin, or sites with dystrophic calcification. This was verified with special iron and Kossa staining. Leydig cells were less frequent than normal. In 13 animals of the control groups (H+ and H−), spermatogenesis and sperms were visible as an indicator of early fertility. In the remaining seven control lambs, large quantities of sex cords with supporting cells and spermatogonia were visible.

Table 5

Group average time and standard deviation of the first and last painful response (R) to local palpation after castration, for lambs of the Burdizzo (B), rubber ring (RR), or control group (H), both with (+) and without (−) local anaesthesia

Parameter	H− ($n = 10$)	H+ ($n = 10$)	B− ($n = 10$)	B+ ($n = 15$)	RR− ($n = 10$)	RR+ ($n = 15$)
First R (min)	115 ± 67	277 ± 1239	38 ± 31	103 ± 65	76 ± 65	403 ± 504[B−]
Last R (days)	0.1 ± 0.1	1.6 ± 2.3	5.3 ± 3.2	7.3 ± 6.0	14.5 ± 10.4[H+, H−]	8.3 ± 4.4[H+,H−]

Superscripts H−, H+, B−, B+, RR− and RR+ indicate statistically significant differences to the respective groups.

Table 6
Body weight on day −1 and average daily weight gain (g) with standard deviation after control-handling (H), Burdizzo (B), or rubber ring- (RR) castration, both with (+) and without (−) local anaesthesia

Time period	H− ($n = 10$)	H+ ($n = 10$)	B− ($n = 10$)	B+ ($n = 13–15$)	RR− ($n = 10$)	RR+ ($n = 15$)
−1 d	6.1 ± 0.7[H+]	7.6 ± 0.9	6.8 ± 1.1	6.8 ± 1.0	6.9 ± 0.8	7.1 ± 1.0
−1 to 6 d	350 ± 92	371 ± 88	307 ± 151	343 ± 102	329 ± 113	324 ± 97
6–12 d	292 ± 153	350 ± 141	283 ± 172	294 ± 172	242 ± 100	356 ± 217
12–30 d	274 ± 65	436 ± 109	315 ± 106	386 ± 88	306 ± 146	345 ± 93
1–30 d	286 ± 44[H+]	390 ± 76	297 ± 90	346 ± 69	289 ± 98	331 ± 70
30–60 d	292 ± 75	327 ± 61	302 ± 56	313 ± 129	323 ± 111	332 ± 93
60–90 d	283 ± 152	313 ± 116	267 ± 77	231 ± 124	183 ± 91	258 ± 98

Superscripts H−, H+, B−, B+, RR−, RR+ indicate statistically significant differences to the respective groups; d = days after castration.

4. Discussion

The immediate pain response to Burdizzo castration was only partially reduced by local anaesthesia. The benefit of local anaesthesia was evident during the first few hours after both Burdizzo and rubber ring castration, as measured by cortisol levels, active behaviour, and proportion of abnormal postures. The positive effect was slightly more evident for the rubber ring method. There was no evidence of any long-term pain after both the Burdizzo and rubber ring methods when combined with local anaesthesia.

4.1. Acute pain

Local anaesthesia did not significantly reduce the immediate expression of pain, cortisol levels, or behavioural response during the first 120 min after Burdizzo castration. These findings are consistent with previous studies in older lambs (Dinnis et al., 1997, 1999; Molony et al., 1997). Because the time needed for castration did not significantly influence the immediate pain response, local tissue damage was considered the main source of the respective cortisol response and behaviour of distress and pain. Closure of the jaws of the Burdizzo clamp damages skin, subcutaneous tissue, and spermatic cord, including the pampiniform plexus. The afferent activity in the spermatic nerves increases and evokes visceral pain due to the compression of the mechanoreceptors in the pampiniform plexus (Cottrell and Molony, 1995).

The most effective shielding of the central nervous system against nociceptive signals is the interruption of the peripheral nerve conduction by regional anaesthesia (Wiebalck and Zenz, 1997). Injecting lidocaine into the spermatic cords and the subcutaneous tissue of the scrotal neck should thus prevent somatic and visceral pain. In this and other studies, the efficiency of the anaesthesia could be proved for rubber ring castrated lambs (Dinnis et al., 1997; Wood et al., 1991). The poor anaesthetic effect evident during Burdizzo castration, however, might be due to the rather short-time period (5 min) between lidocaine injection and castration (Kent et al.,

1998; Sutherland et al., 1999). Because local anaesthesia did reduce the postcastration pain in Burdizzo castrated lambs as well, an extended period of 10 min or more between local anaesthesia and castration might improve the anaesthetic effect for the immediate pain and distress during Burdizzo castration.

The beneficial effect of local anaesthesia was mainly evident in a reduction of cortisol concentrations and behavioural responses during the first few hours after rubber ring castration; in that group the cortisol values did not rise above the values of the control groups. Within the Burdizzo castrated groups, local anaesthesia kept cortisol concentrations at an intermediate level. The slightly better effect of the anaesthesia combined with rubber ring castration might be due to the continuously exerted pressure of the ring on the skin and all subjacent tissues including blood and lymphatic vessels. As a direct effect, the evacuation of the anaesthetic is diminished and it remains at the intended site longer, as proposed by Wood et al. (1991).

Lidocaine is degraded in the liver (Miller, 2000), so the early loss of anaesthetic potency in local tissue as a consequence of local degradation is not likely to occur. The trend of a delayed response to local palpation after rubber ring castration compared to the Burdizzo method supports this idea.

4.2. Chronic pain

There are no established indices for quantitative measurement of long-term pain in lambs. Thus, we used the same behavioural changes as for the short-term observations. The risk of missing signs of chronic pain due to the short observation periods was reduced by the short observation intervals until day 30. The between-group differences suggest the reliability of the method used, although the methodology for chronic pain detection certainly needs further investigation.

Signs of long-term post castration pain or distress were evident in lambs castrated without local anaesthesia. Behavioural responses in lambs castrated with local anaesthesia were reduced up to one week compared to

those castrated without anaesthesia. Thus, the effect of anaesthesia exceeded by far the expected 2 h (half-life of lidocaine in sheep is <1 h; Papich, 1996). These results support the findings of Kent et al. (2000), and they may be explained by the prevention of secondary hyperalgesia. In humans long-lasting and intensive nociceptive signals lead to secondary hyperalgesia, with the effect that even the slightest stimulus is experienced as painful. By blocking the afferent signals from reaching the brain, secondary hyperalgesia can be prevented (Wiebalck and Zenz, 1997).

Although increased response to local palpation in the castrated lambs lasted longer than one week, this could be expected. Wound healing time after rubber ring castration exceeded three weeks, whereas other authors found even longer healing times (Kent et al., 2000, 2004; Sutherland et al., 2000). The extent of inflammation, healing time, and consequential duration of local pain is correlated with the amount of tissue involved (Cotran, 1999). Therefore, it is important that the least traumatic castration technique be chosen and that the person carrying out the procedure is experienced. A comparative study on lamb castration found that post-operative pain increased if Burdizzo castration was performed by inexperienced students as compared with experienced veterinary surgeons (Steiner et al., 2003).

Since Burdizzo castration caused marked swelling followed by atrophy, whereas rubber ring castration led to ischemic necrosis and sometimes purulent secretion, these techniques are difficult to compare in terms of tissue damage. An indirect trait that may be used to compare the long-term effects of the two techniques is the daily weight gain. Because castration pain and distress should affect animal behaviour, they may also affect food intake with consequent differences in daily weight gain. However, neither treatment nor anaesthesia significantly affected the daily weight gain. The lack of significance of this trait in lambs might be explained by the fact that milk is a high-quality feed, and small changes in food intake affected by chronic pain may be compensated; Molony et al. (1995) found an even less pronounced effect in calves. Therefore, changes in daily weight gain seem to be a poor indicator for the existence of chronic pain in nursing young.

Molony et al. (1993) found that age had little effect on the behavioural response after rubber ring castration and tail docking without local anaesthesia. Sutherland et al. (1999) found an effect of local anaesthesia after rubber ring castration on cortisol concentration in 6-week-old lambs. Dinnis et al. (1997, 1999) found local anaesthesia to be partially effective in 4- to 6-week-old lambs after Burdizzo castration. Chronic pain suffered by older lambs after castration has received less attention. Except for the study of Molony et al. (1993), lambs were followed only for short periods. Long-term studies are warranted to describe the long-term effects of Burdizzo castration after local anaesthesia in older lambs.

5. Conclusions

Combined scrotal neck and spermatic cord injection of lidocaine is strongly recommended to reduce pain and distress caused by rubber ring and Burdizzo castration in lambs less than one week of age. By preventing secondary hyperalgesia, the benefits of pre-treatment with local anaesthesia exceed the effective period of lidocaine in Burdizzo and rubber ring castration. Except for the immediate pain response after Burdizzo castration, there were only slight differences in the overall cortisol and behavioural responses between the two castration methods, when preceded by local anaesthesia.

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