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Assessment of pain responses associated with castration of 10-week-old lambs using the Callicrate 'Wee Bander' compared with a standard elastrator

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Abstract

Castration of ram lambs is a standard procedure to facilitate animal management, however all methods of castration used in the lamb industry are thought to be painful. This experiment compares an alternative method of ring castration (Callicrate Wee Bander) with a traditional method of ring castration (Elastrator). The Wee Bander can be applied with a tighter fit and is thought to result in less pain and therefore would provide a more welfare-friendly alternative for castration. Changes in both behaviour and physiology are widely considered as indicative of pain and we used behavioural and physiological measures to evaluate these two methods of castration. Sixty 10- to 11-week-old lambs were studied in three treatments: Castrated using a traditional elastrator ("Elastrator"); Callicrate WEE Bander ("WEE Bander"); and a sham castration treatment ("Control").

Both castration treatments clearly affected behaviour in the first 2 h after castration with reductions ($P<0.05$) in eating chaff, drinking and grooming, and increases ($P<0.001$) in easing of quarters, foot stamping, restless lying, standing with head down, posture changes, tail wagging and walking. However, no significant ($P>0.05$) differences in behaviour were seen between the two castration treatments during this period. While lambs in both castration treatments had higher ($P<0.05$) plasma cortisol concentrations from 30 to 180 min after treatment than the Control lambs, the lambs in the Wee Bander treatment had higher ($P<0.05$) cortisol concentrations at 30 min after treatment than the Elastrator lambs. While there were no treatment effects on cortisol concentrations at 240 min after treatment, lambs in the Wee Bander treatment had higher ($P<0.05$) cortisol concentrations 7 days after treatment than the Control and Elastrator lambs. These behavioural and physiological results indicate that the Wee Bander does not reduce pain associated with ring castration in 10-11 week old lambs and may even be more painful early after castration than traditional Elastrator rings.

Executive summary

Castration of ram lambs is a standard procedure to facilitate animal management. Ring castration is the most common method of castration of ram lambs, and is considered less painful than surgical castration. However, ring castration still results in acute pain for at least 2 hours after castration. An alternative method of ring castration uses rings that can be adjusted so that a very tight ring can be applied to even small animals. A tighter ring is thought to result in less pain and therefore would provide a more welfare friendly alternative. This project aims to investigate if castration of lambs using the Callicrate 'WEE Bander' results in reduced pain compared to castration using the Elastrator.

A total of 60 lambs were housed in10 pens with 6 lambs per pen and within each pen three treatments were randomly allocated to two of the lambs (randomised block design with pens as blocks and lambs as experimental units).The following three treatments were imposed on lambs (20 per treatment) as follows:

1. Castrated using a traditional elastrator ("Elastrator") - a traditional elastrator (similar to The Original Elastrator Ring Applicator, Heiniger), with the use of "the Original" antiseptic latex rings (green);
2. Callicrate WEE Bander ("WEE Bander") - Callicrate WEE Bander, with the use of Callicrate Wee loops (http://www.nobull.net/bander/WeeBander.html); and
3. Sham castration treatment ("Control") - held by a person in a similar manner to the first two treatments without no castration.

Continuous behaviour observation from the digital video records took place for 2 h after treatment and behaviour indicative of pain was recorded. In addition, instantaneous sampling at 10-min intervals was used 2 to 5 h after treatment and from 0800 to 1600 h on day 2, 3, 6, 9, 13, 20 and 27 to record changes in postures and behaviours. Blood samples were taken immediately prior to treatment and at 30, 60, 120, 180 and 240 min after treatment. Additional blood samples were taken at day 2, 7 and 14 at 1230 h. The blood samples were analysed for cortisol (all samples) and haptoglobin (pre-treatment and days 2, 7 and 14 only). Lambs were weighed prior to treatment, daily in the first week and weekly thereafter at 1000 h. While walking to and from the scales, the gait of the animals was scored for any abnormality. The lesion around the castration-ring area was also scored at the time of weighing. Data were analysed for treatment effects as an analysis of variance by Anova.

In comparison with the Control treatment, both castration treatments clearly affected behaviour in the first 2 h after castration, with most of the recorded behaviours affected ($P<0.05$). However no significant ($P>0.05$) differences in behaviour were seen between the two castration treatments. Behaviour was also affected by treatment in the period of 2 to 5 h after treatment ($P<0.01$), with lambs in the Wee Bander treatment somewhat more affected than lambs in the Elastrator treatment ($P<0.05$). There were no significant differences ($P>0.05$) between treatments on days 2 and 3.

The castration treatments increased ($P<0.01$) cortisol concentrations for at least 2 h after castration (Table 5). In addition, at 30 minutes after castration, cortisol concentrations were significantly ($P<0.05$) higher in lambs in the Wee Bander treatment compared with the Elastrator treatment.

While cortisol in lambs in the Elastrator treatment had returned to values similar to the Control lambs by 3 h after castration, values were still significantly (P<0.01) elevated in the Wee Bander treatment. At day 2 cortisol concentrations were similar in all treatments. At day 7 cortisol concentrations were significantly (P<0.05) higher in lambs in the Wee Bander treatment compared with the Elastrator treatment. There was no significant effect of treatment on haptoglobin concentrations.

While there was minimal weight loss (P<0.05) on the day after treatment compared with the weight prior to castration in lambs castrated with both methods, there was no difference (P>0.05) in weight gain over the whole observation period. From day 14 after treatment, both castration treatments resulted in greater (P<0.05) lesion scores, associated with the slow detachment of the scrotum. However none of the scrotums were completely shed by 28 days after castration with either method. The gait score was normal for nearly all animals and was not affected by treatment (p>0.05).

The behavioural and physiological results of the present experiment indicate that the Wee Bander does not reduce pain associated with ring castration in 10- to 11-week-old lambs and may possibly be more painful early after castration than traditional Elastrator rings.

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1. Background

There is considerable public interest in animal welfare and most people believe that animals, including farm animals, should be not be subjected to pain or severe discomfort (Fraser and Broom, 1990; Coleman, 2008). Some sectors of the general public are increasingly questioning the welfare impact of, and the need for, some husbandry procedures, particularly surgical interventions that are likely to cause pain (Coleman, 2008). Consumer and public attitudes to animal welfare have the potential to dramatically affect, for better or worse, the operations of livestock industries. Failure to assure these stakeholders that welfare standards for farm animals are underpinned by sound scientific evidence will not only risk the practice, but has the potential to adversely affect the profitability and sustainability of the livestock industry.

There are a range of behavioural and physiological changes that are widely considered as indicative of pain in animals. Several behavioural indicators of pain have been described in mammals and they include avoidance and defensive behaviours, vocalizations, behaviours directed towards the painful area, postures and behaviours which reduce stimulation of the painful area and general changes in activity, such as walking, feeding and social interactions (Moloney et al., 1997; Mellor and Stafford, 2000; Benson, 2004; Prunier et al., 2013). Physiological changes indicative of pain include activation of the sympatho-adrenal medullary system and the hypothalmo-pituitary adrenal (HPA) axis (Mellor and Stafford, 2000; Benson, 2004; Hemsworth et al., 2009). The physiological responses are mainly due to two related mechanisms (Prunier et al., 2013). Pain is known to be stressful and stimulates the HPA axis, which can be measured by a rise in corticosteroids. Tissue damage also results in activation of the HPA axis and the immune system (Turnbull et al., 1994; Vukelic et al., 2011; Shi et al., 2003; Mosser and Edwards, 2008) and the release of numerous inflammatory mediators (Shi et al., 2003). While haptoglobin, an acute phase reactant, is not immediately involved in the control of pain it increases in response to inflammation and tissue trauma (Pfeffer and Rogers, 1989).

These changes in response to painful stimuli reflect both negative physical and emotional experiences, but it is well recognised that non-painful but aversive components of a surgical husbandry procedure, including novelty, isolation, restraint and the close presence of humans, may also contribute to these behavioural and physiological changes (Hemsworth et al., 2009). Therefore, while there is debate about the usefulness of behavioural and physiological measures in specifically measuring the level of pain experienced by animals, issues of animal welfare are broader than pain and these behavioural and physiological responses are widely accepted in science as indicators of reduced welfare.

In extensive sheep farming systems in Australia, ram lambs often reach puberty before slaughter. Therefore castration of ram lambs is a standard procedure to facilitate animal management. Ring castration is the most common method of castration of ram lambs, and is considered less painful than surgical castration based on cortisol (Robertson et al., 1994) and behavioural responses (Melches et al., 2007). However, behavioural expression of what is considered to be pain differs between different methods of castration, and the duration and timing of the peak of the cortisol response also depends on the method used (Kent et al., 1993; Grant, 2004), due to the amount of tissue and enervation involved. Castration by elastrator rings leads to chronic ischaemia and tissue necrosis and results in behavioural and physiological changes indicative of acute pain for at least 2 hours (Molony and Kent, 2007) and increased sensitivity to palpation of the region for several weeks (Melches et al., 2007).

There is no standard of design of rubber rings used for castration and pressure exerted by the rings can vary considerably. Kent et al. (1995) found that smaller than standard rings increased severity of acute pain, based on the expression of pain behaviours and an increase in cortisol, but for a shorter duration. In addition, Molony et al. (2012) compared conventional rings with novel smaller rubber rings and found that the smaller rings did not substantially reduce acute pain of castration, based on behavioural responses in the first hour after castration. They concluded that the smaller rings did not apply enough pressure to block nerve conduction. Fern and Harrison (1991), in a study of frog sciatic nerves, concluded that in order to achieve a rapid nerve block by direct nerve compression a pressure of 0.1MPa would need to be achieved to alleviate pain almost instantaneously.

Thus, several studies have used behavioural and physiological indicators to assess pain associated with husbandry procedures in lambs (Melches et al., 2007; Hemsworth et al., 2009).
It appears that achieving adequate tightness to ensure high enough tension to limit nerve conduction is essential to reduce stress and eliminate pain during and following ring castration (Fern and Harrison, 1991).
The Callicrate 'WEE Bander' (http://www.nobull.net/bander/WeeBander.html) was designed to apply high tension banding resulting in complete ligation, stopping blood flow to the area and preventing nerve impulses to block pain while minimizing swelling. This should result in a lower pain response compared with a traditional elastrator banding application.

1.1 Project objectives

The research question addressed in this project is "does castration of lambs using the Callicrate 'WEE Bander' result in reduced pain compared to castration using the Elastrator?"

2. Methodology

2.1 Animals and treatments

Sixty ewes with 6- to 7-week-old ram lambs were transported from a commercial farm to the Werribee DEPI site where the experiment took place. Lambs were familiarised with the indoor housing facility and exposed to hay and chaff at 8 to 9 weeks of age, so they were transitioned to the food prior to weaning. They were given access to the facility every day for several hours during the day with their dams while all pens were open and contained ad lib grass hay and lucerne chaff. The lambs were housed in the indoor facility in their allocated pen one week after weaning, and treatment was applied one week later, when the lambs were 10 to 11 weeks of age.

The following three treatments were imposed on lambs (20 per treatment) while they were individually held by a handler for 30 s as follows:

1. Castrated using a traditional elastrator ("Elastrator") - a traditional elastrator (similar to The Original Elastrator Ring Applicator, Heiniger), with the use of "the Original" antiseptic latex rings (green);
2. Callicrate WEE Bander ("WEE Bander") - Callicrate WEE Bander, with the use of Callicrate Wee loops (http://www.nobull.net/bander/WeeBander.html); and

3. Sham castration treatment ("Control") - held by a person in a similar manner to the first two treatments without no castration.

Lambs were housed in pens of 6 and within each pen the three treatments described above were randomly allocated to two of the lambs (randomised block design with pens as blocks and lambs as experimental units). Treatments were applied on two consecutive days, with lambs in the first five pens treated between 1200-1230 h on one day and lambs treated in the next five pens at the same time the next day. The pens were 4x 5 m with concrete floors covered by straw bedding. Lambs were fed an ad lib mixture of 80% lucerne and 20% oaten chaff. In addition, they had access to ad lib grass hay. Prior to introduction of the lambs to the pens, video cameras were installed to provide overhead views of each pen. Lambs were studied for 4 weeks post-treatment with observations on lambs in the second five pens always following the day after measures were taken in first five pens to ensure that all measurements related to the same time after treatment.

All animal procedures were conducted with prior institutional ethics approval under the requirements of the Victorian Prevention of Cruelty to Animals Act 1986 and in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes.

Photo 1. Applying the Wee Bander treatment

Photo 2. Applying the Elastrator treatment

2.2 Measurements

2.2.1 Behaviour observations

A total of 10 low-light video cameras were used to continuously record the behaviour of lambs in the experiment. For each pen, a camera mounted above the pen provided a view of the entire area available to the lambs. The video cameras were connected to digital video recorders. The behaviour of the lambs in their home pens on days 1 (day of treatment), 2, 3, 6, 9, 13 and 27 post-treatment was collated from the digital video records.

Continuous behaviour observation from the digital video records took place for 2 h after treatment. Behaviour recorded include posture (standing (incl. head down and head leaning against the wall) and lying postures (lateral or on sternum); see Melches et al., 2007; Molony et al., 2012) and specific behaviours (feeding, social interactions, foot stamping, head turning, vocalization) as an indication of pain. From these data, the following was calculated:

Total time spent feeding hay or chaff and lying either lateral or on the sternum, occurrences (bouts) of walking, standing, drinking, standing with head down, head leaning against a wall, rubbing quarters against the wall, easing quarters (stepping/walking on the spot/back and forth), playing, tail wagging, foot stamping, self-biting at treatment area, head shaking, head butting another lamb, grooming, posture changes, restless lying and rolling.

From the digital video recordings of 2 to 5 h after treatment, instantaneous sampling at 10-min intervals was used to record whether or not the above behaviours and postures were shown by each lamb. In addition, from recordings from 0800 to 1600 h on day 2, 3, 6, 9, 13, 20 and 27 instantaneous sampling at 10-min intervals was used to record whether or not the above behaviours and postures were shown by each lamb. Due to the nature of instantaneous sampling low frequency, short-duration behaviours were rarely seen, so only the main behaviours (standing and lying postures, feeding and drinking) are reported. Behaviour was not recorded when people were in the pen to collect blood samples or when lambs were removed during weighing.

2.2.2 Physiological measurements

Blood samples (8 ml) were collected via venipuncture from the jugular vein with an lithium-heparin vacutainer and a 20g needle immediately prior to treatment and at 30, 60, 120, 180 and 240 min after treatment. Additional blood samples were taken at day 2, 7 and 14 at 1230 h. Each lamb was sampled within 2 min of two experimenters entering the pen to avoid an acute stress response to capture and handling influencing the basal levels of cortisol in the blood (i.e. to avoid handling confounding basal measures). Blood samples were analysed for cortisol (all samples) and haptoglobin (pre-treatment and days 2, 7 and 14 only).

Plasma concentrations of cortisol were determined using commercial radioimmunoassay kits (Diasorin Australia Ltd. NSW) at the department of Animal Biology, University of Western Australia. The sensitivity of the assay was 3.5 nmol/L. Mean intra assay variation for low (21.55nmol/L) and high (170.24nmol/L) cortisol plasma samples were 1.6% and 2.49%, respectively. Haptoglobin was assessed using a modification of the technique of Jones and Mould (1984) as previously described (Paull et al. 2009). The intra-plate co-efficients of variation (CV) for quality control samples containing 0.120, 0.059 and 0.029 mg/ml were 3.08, 3.60 and 3.73% respectively.

2.2.3 Physical measurements

Lambs were weighed prior to treatment, daily in the first week and weekly thereafter at 1000 h (days 2, 3, 4, 5, 6, 7, 14, 21, 28). The weighing scales were located in the corridor outside the pens, and lambs were removed as a group and walked along the corridor. While walking to and from the scales, the gait of the animals was scored for any abnormality on a scale from 1 to 4 (1 = sound animal; 2 = asymmetric gait; 3 = obvious difficulty in moving; 4 = unable or refused to move, Hemsworth et al., 2009).

The lesion around the castration-ring area was also scored at the time of weighing (days 3, 7, 10, 14, 17, 21, 24 and 28). The lamb was tipped on their rump by one person while a second person carefully spread the wool spread away from castration ring to inspect the castration site. Lesions were scored on a scale of 0 to 4 (0= no swelling or lesion, 1= lesion <1cm, 2= lesion 1-2 cm, 3= lesion >2 cm, 4= purse off), adapted from Molony et al. (2012).

2.2.4 Statistical analysis

Data were analysed for treatment effects as an analysis of variance by Anova with a block structure of Pen nested within Treatment Day using Genstat.

3. Results

3.1 Behavioural responses

In comparison with the Control treatment, both castration treatments clearly affected behaviour in the first 2 h after castration (Table 1), with most of the recorded behaviours affected. Some of the most notable effects of the two castration treatments were reductions ($P<0.05$) in feeding behaviour of chaff, drinking and grooming, and increases ($P<0.001$) in easing of quarters, foot stamping, restless lying, standing with head down, posture changes, tail wagging and walking. However no significant ($P>0.05$) differences in behaviour were seen between the two castration treatments.

Table 1. Behaviour (bouts of behaviour) in the first 2 h after castration

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Feeding (chaff)	2.50[a]	5.25[b]	3.90[ab]	2.138	**0.044**
Feeding (hay)	0.1	0.65	0.50	0.990	0.518
Drinking	0[a]	1.25[b]	0[a]	0.738	**0.001**
Easing Quarters	26.4[a]	0.1[b]	26.3[a]	8.41	**<0.001**
Foot Stamping	5.15[a]	0.05[b]	3.40[a]	2.471	**<0.001**
Restless lying	14.1[a]	0.1[b]	16.2[a]	7.37	**<0.001**
Grooming	0.45[a]	2.35[b]	0.15[a]	1.044	**<0.001**
Head butting	1.15	0.25	1.0	1.190	0.274
Standing head down	4.55[a]	0.65[b]	5.15[a]	2.519	**0.001**
Head shake	1.10[ab]	0.15[b]	1.60[a]	1.138	**0.042**
Head leaning	2.25[a]	0.0[b]	2.65[a]	1.453	**0.001**
Lying lateral	19.0[a]	0.3[b]	21.3[a]	5.91	**<0.001**
Lying on sternum	4.00	2.95	2.30	2.979	0.516
Rubbing quarters	1.50[a]	0.0[b]	0.50[b]	0.984	**0.012**
Restless lying	14.1[a]	0.1[b]	16.2[a]	5.59	**<0.001**
Lying rolling	1.00	0.0	1.05	1.345	0.219
Self-biting	0.70	0.35	0.75	0.863	0.600
Posture changes	59.1[a]	21.4[b]	49.1[a]	12.45	**<0.001**
Tail wagging	7.85[a]	0.30[b]	6.30[a]	3.700	**<0.001**
Walking	49.6[a]	23.2[b]	42.5[a]	11.81	**<0.001**

[ab] Values are significantly different (P<0.05) when superscripts differ.

Behaviour was also affected by treatment in the period of 2 to 5 h after treatment. Lambs in the Wee Bander treatment spent more (P<0.05) time lying laterally (Table 2). Both lambs in the Wee Bander and Elastration treatments spent more (P<0.01) time standing inactive than the Control lambs.

Table 2. Behaviour (% of observations in which the behaviour was observed) of lambs 2 to 5 h after castration

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Feeding (hay and chaff) and drinking	55.2	65.0	57.8	10.1	0.138
Feeding chaff	53.5	62.4	57.8	9.93	0.208
Lying lateral	12.7[a]	2.1[b]	7.2[ab]	8.41	**0.048**
Lying on Sternum	13.3	23.5	17.6	9.73	0.114
Standing	18.8[a]	9.3[b]	17.1[a]	6.12	**0.007**

[ab] Values are significantly different (P<0.05) when superscripts differ.

There were no significant differences (P>0.05) between treatments on days 2 and 3 (Table 3 and 4).

Table 3. Behaviour (% of observations in which the behaviour was observed) of lambs between 0800 and 1600 h on day 2 after castration

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Drinking	1.04	0.41	0.73	0.719	0.227
Feeding (chaff)	49.4	56.4	53.1	7.39	0.176
Feeding (hay)	0.42	0.85	0.63	0.872	0.621
Head down	0.11	0	0	0.174	0.375
Head leaning	0	0	0		
Lateral lying	0.83	0.11	0.62	0.96	0.303
Lying on sternum	34.8	32.5	34.3	5.91	0.717
Standing	13.4	9.76	10.6	3.48	0.097

Table 4. Behaviour (% of observations in which the behaviour was observed) of lambs between 0800 and 1600 h on day 3 after castration

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Drinking	0.62	0.9	0.92	0.83	0.692
Feeding (chaff)	53.6	54.9	58.9	5.23	0.119
Feeding (hay)	0.72	1.14	0.21	0.96	0.164
Head down	0	0	0		
Head leaning	0	0	0		
Lateral lying	0.62	0.41	0.73	0.83	0.744
Lying on sternum	34.5	34.0	30.8	4.65	0.244
Standing	9.99	8.64	8.44	2.51	0.412

3.2 Physiological responses

The castration treatments increased (P<0.01) cortisol concentrations for at least 2 h after castration (Table 5). In addition, at 30 minutes after castration, cortisol concentrations were significantly (P<0.05) higher in lambs in the Wee Bander treatment compared with the Elastrator treatment. While cortisol in lambs in the Elastrator treatment had returned to values similar to the Control lambs by 3 h after castration, values were still significantly (P<0.01) elevated in the Wee Bander treatment. At day 2 cortisol concentrations were similar in all treatments. At day 7 cortisol concentrations were significantly (P<0.05) higher in lambs in the Wee Bander treatment compared with the Elastrator treatment.

There was no significant effect of treatment on haptoglobin concentrations (Table 6). It appears that there was a reduction of haptoglobin over time during the observation period, unrelated to treatment.

Table 5. Cortisol (nmol/l) concentrations on the day of castration and on day 2, 7 and 14 after castration of lambs

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Day 1, Prior	18.7	19.1	19.6	7.91	0.97
30 min	100.8[a]	17.3[c]	72.5[b]	**17.69**	**<0.001**
60 min	117.2[a]	12.6[b]	105.8[a]	18.74	**<0.001**
120 min	68.7[a]	20.4[b]	77.5[a]	18.66	**<0.001**
180 min	33.9[a]	16.0[b]	14.1[b]	**13.85**	**0.002**
240 min	15.7	13.7	14.9	7.29	0.865
Day 2	30.5	22.5	24.2	7.51	0.089
Day 7	21.8[a]	13.4[b]	13.8[b]	**6.09**	**0.012**
Day 14	22.6	17.9	20.6	6.16	0.329

[abc] Values are significantly different (P<0.05) when superscripts differ.

Table 6. Haptoglobin (mg/ml) results concentrations prior to castration and on day 2, 7 and 14 after castration of lambs

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Pre-treatment	0.44	1.37	0.49	0.867	0.063
Day 2	0.30	1.00	0.41	0.716	0.119
Day 7	0.26	0.30	0.28	0.441	0.986
Day 14	0.20	0.14	0.16	0.380	0.937

3.3 Physical responses

The reduction in feeding of chaff observed in the first 2 h after castration, particularly in those lambs castrated with the Wee Bander, was also reflected in the weight gain/loss (P<0.05) on the day after treatment (Day 2) compared with the weight prior to castration on the day before (Table 7). However weight gain thereafter was similar for all treatments, and there was no difference (P>0.05) in weight gain over the whole observation period.

Table 7. Liveweight gain (kg) compared with weight pre-treatment (pre).

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Weight diff D2-pre	-0.89[a]	-0.29[b]	-0.71[ab]	0.432	**0.022**
D3-pre	-0.55	-0.21	-0.47	0.568	0.458
D7-pre	0.01	0.21	0.03	0.491	0.577
D14-pre	1.16	1.53	1.42	0.630	0.484
D21-pre	1.23	1.50	0.81	0.957	0.352
D28-pre	1.98	2.31	2.14	0.766	0.689

[ab] Values are significantly different (P<0.05) when superscripts differ.

From day 14 after treatment, both castration treatments resulted in greater (P<0.05) lesion scores (Table 8). The lesions were mainly associated with the slow detachment of the scrotum, resulting in an area of broken skin around the rubber ring. Sometimes this area could contain small amounts of pus and this was recorded. None of the scrotums were completely shed by 28 days after castration with either method.

The gait score was normal for nearly all animals and was not affected by treatment (p>0.05).

Table 8. Lesion scores on a scale of 1 to 4 on days after castration.

	Wee Bander	Control	Elastrator	Lsd (0.05)	P-value
Day 3	0	0	0		
Day 7	0	0	0		
Day 10	0.10	0.0	0.10	0.164	0.375
Day 14	0.25[ab]	0.0[a]	0.50[b]	0.335	**0.016**
Day 17	1.00[b]	0[a]	1.35[b]	0.646	**<0.001**
Day 21	2.05[b]	0[a]	2.45[b]	0.589	**<0.001**
Day 24	2.35[b]	0[a]	2.55[b]	0.547	**<0.001**
Day 28	2.70[b]	0[a]	2.60[b]	0.502	**<0.001**

[ab] Values are significantly different (P<0.05) when superscripts differ.

Photo 3. Typical lesion 4 weeks after castration

4. Discussion / Conclusion

While all methods of castration appear to be painful, the use of rubber rings is considered preferable to knife castration (Mellor and Stefford, 2000; Mellema et al., 2006, Melches et al., 2007, Paull et al., 2009). However, rubber ring castration has been found to result in significant pain as indicated by pain behaviour and posture changes (Grant, 2004; Lomax et al., 2010) as well as increases in cortisol concentrations (Paull et al., 2009). Rubber ring castration occludes blood supply to the scrotum which results in degeneration of its tissues. The pain associated with this procedure is thought to originate in pain receptors in the testis and the spermatic cord (Cottrell and Molony, 1995).The Callicrate Wee Bander rings were designed with a built-in tension lock ring and it is claimed that the rings can be tightened sufficiently to cause a complete ligation that blocks blood supply and the pain response, with the aim of providing a more welfare friendly alternative to traditional ring castration (http://www.callicratebanders.com/bander/WEE-Bander.html). In theory, smaller rubber rings could block transmission of nerve cells in a similar manner to that of the Burdizzo clamp (in combination with rubber rings), which has been shown to result in reduced and shorter pain responses after castration, although not all behavioural and physiological indicators of pain were reduced (Kent et al., 1993; Kent et al., 1995; Mellor and Stafford, 2000).

The behavioural and physiological changes observed after castration with the Elastrator and the Callicrate WEE Bander observed in the present experiment indicate that the Wee Bander did not reduce pain in 10- to 11-week-old lambs in comparison to the traditional Elastrator rings. While there were no behavioural differences between the two castration treatments in the first 2 h after castration, both castration treatments reduced feeding, drinking and grooming and increased foot stamping, restless lying, standing with head down, posture changes, tail wagging and walking. Lambs in both castration treatments had elevated cortisol concentrations up to 180 min after castration, but the lambs in the Wee Bander treatment had higher cortisol concentrations at 30 min after treatment than the Elastrator lambs. These behavioural and physiological results indicate that the Wee Bander does not reduce pain associated with ring castration in 10- to 11-week-old lambs.

Many of the behaviours observed in the castration treatments but rarely seen in the control lambs are indicative of pain (Molony et al., 2002). Activities, such as posture changes, rolling, foot stamping, kicking and easing quarters as well as lying laterally or in abnormal positions, have been found to be related to pain in lambs in response to castration and/or tail docking. Many of these behaviours were increased in lambs castrated with either method of castration in the present experiment, indicating that both methods were similarly painful. Even in the period of 2 to 5 h after castration, differences in behaviour between the control treatment and the castration treatments are observed, indicating that pain lasts beyond 2 h after castration.

While the present experiment provided no convincing behavioural and physiological evidence that the Callicrate WEE Bander reduced pain in comparison to the Elastrator, the experiment provides some limited evidence that castration with the Wee Bander may be more painful than castration with the Elastrator. In the first 2 h after castration lambs castrated with the Wee Bander but not the Elastrator spent less time eating chaff compared with the control lambs. Similarly, lambs castrated with the Wee Bander but not the Elastrator spent more time lying laterally in the period 2-5 h after castration compared with the control lambs. Active pain behaviours, such as standing with head down, head leaning against a wall, rubbing quarters against the wall, easing quarters, tail wagging, foot stamping, head shaking, restless lying and rolling, may indicate unavoidable pain, whereas behaviours such as lying laterally and standing immobile may indicate pain on movement and are likely performed to avoid aggravating a painful area and can perhaps be seen as indicators of lower levels of pain (Kent et al., 1995).

The cortisol data indicates that castration with either method results in pain for at least 2 h after castration. Furthermore, as seen in general with the behavioural changes, the Wee Bander castration method resulted in a greater cortisol response 30 minutes after castration than the traditional Elastrator rings. While the cortisol concentrations in the group castrated with the Elastrator rings had returned to concentrations similar to the control group 3 h after castration, the cortisol concentrations in the group castrated with the Wee Bander were still elevated, and did not return to similar concentrations as in the other two treatments until 4 h after castration. This suggests that castration using the Wee Bander is more painful immediately after castration and that the pain response lasts for longer than castration using the traditional Elastrator rings.

While it is possible that inflammatory mediators such as interleukins may be responsible for the increase in cortisol, rather than pain, a tight rubber ring should prevent the release of inflammatory mediators in the bloodstream. Indeed, no increase of haptoglobin was found after castration, which would indicate that the blood flow from the scrotum was blocked from the general circulation.

This is similar to a study by Paull et al (2009) where ring castration did not increase haptoglobin over a 2-day period in 5-weeks old lambs. In this previous study, as with many studies on castration, lambs were also tail docked at the time of castration.

Kent et al. (1995) also found that small rubber rings produced more intense pain immediately after application in 5-6 day old lambs than the standard rubber rings, although they found that the increased pain lasted for a shorter time than the standard rubber rings. The additional use of the Burdizzo, whereby nervous tissue is destroyed, did result in less acute pain, although castration with this method was still considered painful. However, in this study too, both castration and tail docking were performed. The results of Molony et al. (2012) were similar to those of Kent et al. (1995) in that no effects on behaviour of 2- to 3-day-old lambs were found in a comparison of traditional ring castration and an alternative method with novel tighter rings. Both of these studies used much younger lambs than the current study. It is unclear if this may have affected the behavioural and physiological responses. As the Wee Bander is specifically designed for use in small and young animals it is possible that the claimed advantage of the Wee Bander, a tighter band compared to the Elastrator, may not be applicable in larger animals and therefore were not found in this study.

In addition, very young lambs may have reduced pain perception compared with older lambs as Johnson et al. (2009) for example found that younger lambs showed less EEG changes to castration in the first 10 days of life. In contrast to most castration experiments in which lambs were castrated in the first week of life, the present experiment studied older animals, lambs aged 10 to 11 weeks of age in which pain responses may be more discernible.

In conclusion, the behavioural and physiological results of the present experiment indicate that the Wee Bander does not reduce pain associated with ring castration in 10- to 11-week-old lambs and may possibly be more painful early after castration than traditional Elastrator rings.

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