# final report

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## Assessment of pain responses following castration of lambs using the Elastrator ring with and without midline injections of lignocaine

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# Executive summary

There is growing concern for the welfare of animals subjected to animal husbandry procedures that are painful. Several practical methods to reduce pain associated with husbandry procedures on commercial farms are being developed. The 'Numnuts' device is developed to administer a local anaesthetic injection with simultaneous ring application so it can be used without the involvement of a veterinarian. This study is one of several in the development of such a device.

This study concentrated on the question if and when a single midline injection of lignocaine at the time of castration is effective and whether 1.5 mL or a higher dose of 4.0 mL is required for efficacy. Thirty-six lambs were subjected to 3 treatments (12 lambs per treatment), ring castration with either no pain relief or a single midline injection of 1.5 or 4.0 mL of lignocaine at the time of castration. Lambs were observed continuously for 2 h after castration, when most pain is expected, and instantaneous point sampling every 10 minutes was used until 5 h after castration. In addition blood samples were taken prior to castration as well as 30, 60 and 210 min after castration.

Lambs injected with 4.0 ml of lignocaine at the time of ring castration compared to no pain relief or an injection of 1.5 ml showed minor but significant reduction in pain related behaviour. However, other behaviours and the results of the cortisol analysis do not confirm a reduction in pain. The behavioural changes observed do not appear to be biological significant and do not warrant the time and cost involved in administration of this anaesthetic in order to obtain minimal pain relief. It would also not satisfy the expectation of the general public of providing effective pain relief.

More research is required in the most appropriate injection sites and the tissue and nerves affected by ring castration to be able to design an effective castration tool that can be used in the industry and provide effective pain relief from ring castration.

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# 1 Background

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 (Kent et al., 1993; Robertson et al., 1994; Melches et al., 2007). However, castration by elastrator rings leads to chronic ischaemia and tissue necrosis and results in 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 growing concern for the welfare of animals subjected to animal husbandry procedures that involve pain, and internationally pain relief is required by some countries and some retailers in association with several husbandry procedures. Several developments are underway to develop practical methods to reduce pain associated with husbandry procedures on commercial farms, for example application of Tri-Solfen, a topical spray containing lignocaine, after surgical procedures (Lomax et al., 2010; Paull et al., 2009). While ring castration is considered less painful than surgical castration (Lester et al., 1996; Paull et al., 2009) the impetus is to develop suitable practical application of a local anaesthetic for ring castration to provide marked pain relief and for public assurance.

A common definition of pain in humans is ‘an unpleasant sensory and emotional experience, associated with actual or potential tissue damage’ (International Association for the Study of Pain, 1979). In order to assess pain in animals, there is a range of behavioural and physiological indicators of pain that can be used. Some indicators are very responsive to pain, while others may indicate a more general stress response. Several postural and 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 (Prunier et al., 2013).

Physiological responses are mainly due to two related mechanisms (Prunier et al., 2013). Pain is known to be stressful and stimulates the hypothalamic-pituitary-adrenal (HPA) axis, which can be measured by a rise in cortisol. Tissue damage also results in activation of the HPA axis and the immune system and the release of numerous inflammatory mediators.

While behaviour alone may provide a useful indication of the level of pain experienced by the animal, a combination of both behavioural and physical indicators provides a more comprehensive assessment of pain. The rise in cortisol after castration and/or tail docking in lambs has been found to correlate with the presumed severity of noxious stimuli (Mellor and

Murray, 1989) and the rise in cortisol was eliminated by prior injection of a local anaesthetic into the scrotum and tail (Wood et al., 1991). However, Diniss et al (1999) assessed pain in lambs after castration with different castration methods and found that the behavioural pattern of the response depended on the method used. Different methods resulted in different displays of behaviour, indicating that additional measures should be considered highlighting the need for a broad range of behavioural indices. 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).

Injection of local anaesthetics (LA) into the scrotum and scrotal cords which is a specialised and usually veterinary procedure has been shown to reduce the pain of ring castration (Mellor and Stafford, 2000; Dinniss et al, 1999) but the effectiveness of a single mid-line injection into the scrotal neck in reducing pain associated with ring castration has not been assessed.

The 'Numnuts' device aims to combine the use of local anaesthetic injection with simultaneous ring application. However the device relies on a single midline injection of LA into the tissues constricted by the ring but on the proximal side of the ring. Efficacy studies have tended to show the efficacy of midline injection of 1.3 and 1.5 mL of lignocaine up to 1 hour after treatment but good evidence of pain relief after 1 hour has not been shown. In contrast a single dorsal midline injection of LA has been shown to be very effective and quick in reducing pain following the use of the ring for tail docking (unpublished results). The presence of the visceral and parietal layers of peritoneum and other connective tissue structures in the spermatic cords may reduce the diffusion of LA to the sensory nerves supplying the testicles and scrotum. Research is required to precisely characterise if and when the midline injection becomes effective and whether 1.5 mL or a higher dose is required for efficacy.

# 2 Project objectives

The research question to be answered by this project is does using a midline injection of 1.5 mL or a higher volume of lignocaine reduce the pain of castration compared to using the Elastrator ring alone? If so when does this effect commence, when does it reach maximum effect and how long does it lasts?

# 3 Methodology

## 3.1 Animals and housing

Thirty six 8- to 9-week old recently weaned ram lambs (average weight 19.8 kg) with intact tails were transported from the University of Melbourne site at Dookie to the Werribee site. Lambs were familiarized with the indoor facility for 7 days prior to the trial. They were exposed to hay and chaff and were vaccinated with a Clostridial (5 in 1, Ultravac™) vaccination prior to the trial. The lambs were housed in the indoor facility one week prior to castration and in their allocated pen the day before castration so that they would be adapted to the housing facility, feed and handlers prior to treatment

Lambs were allocated to pens and treatment based on weight. Three lambs were allocated to each of six pens, over two treatment days (total of 12 pens). The pens were 1.5 x 2.5 m with wooden slatted floors. The pens were located within the same facility, with the 6 pens on one side and two large pens on the other side. Pens were separated by metal bar divisions. Lambs were fed an ad libitum mixture of lucerne and oaten chaff (Combo chaff) with added oats and they had access to ad libitum grass hay in plastic buckets prior to castration and after observations were completed. The pens were fitted with automatic drinkers. Prior to introduction of the lambs to the pens, video cameras were installed to provide overhead and side views of each pen.

## 3.2 Treatments

The following three treatments were imposed on lambs (12 lambs per treatment) while they were individually held by a handler for 30 s as follows:

1. Ring castration without analgesic. Ring castrations involved the application of a tight rubber ring around the neck of the scrotum proximal to the testes.
2. Ring castration, immediately followed by midline injection of lignocaine on the dorsal side of the ring – 1.5 mL
3. Ring castration, immediately followed by midline injection of lignocaine on the dorsal side of the ring – 4.0 mL

Ring castration was performed using traditional elastrator rings. Lambs were weighed prior to treatment and lambs were ranked by weight and blocked into weight groups of 3 and allocated to a pen. One lamb from each weight group was randomly allocated to one of 3 treatments (using random numbers).

Thus the design is a block design, with blocks corresponding to pens and with individual lambs as the experimental units. Treatments were applied on two separate days three days apart, with lambs on the first day in the six pens treated between 1200-1230 h on and lambs treated 3 days later in the six pens at the same time of day.

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. The age of castration in this study was in accord with the Model Code of Practice for the Welfare of Animals: The Sheep (http://www.publish.csiro.au/Books/download.cfm?ID=5389), the MLA best practice guidelines for sheep (http://www.mla.com.au/News-and-resources/Publication-details?pubid=6106) and standard industry practices.

## 3.3 Measurements

### 3.3.1 Behaviour observations

A total of 24 LED video cameras were used to continuously record the behaviour of lambs in the experiment. For each pen, a camera (Bullet Camera, 1/3" Sony Super HAD II CCD with 2.8-12mm Varifocal lens) 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 the treatment pens on the day of treatment for 5 hours post-treatment was collated from the digital video records. All video records were observed by a single trained observer who was unaware of the treatment applied to each lamb.

Continuous behaviour observation from the digital video records took place for 2 h after treatment, when most intensive pain behaviours are expected (Molony et al., 2002). Behaviour recorded include posture (standing (including head down and head leaning against the wall) and lying postures (lateral or on sternum)); see Melches et al., 2007; Molony et al., 2012) as well as more specific behaviours (see below) for evidence of pain (see Hemsworth et al., 2012). From these data, the following was calculated:

Total time spent standing and lying either lateral or on the sternum, occurrences (bouts) of walking, standing with head down, head leaning against a wall, rubbing quarters against the wall, easing quarters (stepping/walking on the spot or 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 point 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 point sampling, low frequency, short-duration behaviours were rarely seen, so only the main behaviours (standing and lying postures) are reported. Behaviour was not recorded when people were in the pen to collect blood samples. These posture and behaviour data are presented as the percentage of sample points in which the individual posture or behaviour was observed and used as an estimate of time spent in the individual posture or behaviour.

### 3.3.2 Cortisol measurements

The wool on the neck of the lambs were clipped the day prior to castration, to facilitate blood sampling. Blood samples (8 ml) were collected via venipuncture from the jugular vein with a lithium-heparin vacutainer and a 20g needle immediately prior to treatment and at 30, 60 and 210 min after treatment, as cortisol is expected to peak 60 minutes after castration and may stay elevated for more than 120 min (Stewart et al., 2014). Each lamb was sampled within 2 min of two experimenters entering the pen, so as 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).

Plasma concentrations of cortisol were determined using commercial radioimmunoassay kits (Immulite 2000 Cortisol). The sensitivity of the assay was 5.5 nmol/L.

## 3.4 Statistical Analysis

Each behavioural observation and cortisol measurement was analysed using a randomised block analysis of variance, with pens of 3 lambs being the blocks and individual lambs being the experimental units. This analysis provided 22 degrees of freedom in residual error. As well as an overall P value for any difference between the three treatments, for each observation and measurement analysed, P values were also calculated for two non-orthogonal comparisons. These comparisons were (a) no anaesthetic vs high 4 ml dose and (b) no anaesthetic vs low 1.5 ml dose. As the distribution of many observations and measurements had discrete values (e.g. many zeros), all P values were calculated using a non-parametric permutation based on randomly permuting treatment labels to lambs within blocks.

All behaviour observations that were percentages, were angularly transformed. All behaviour observations that were counted as number of bouts were square root transformed. Cortisol measurements were not transformed, as no appreciable skewness was observed in the

residuals of the analyses. One animal, from the 1.5 ml treatment, spent 100% of its time standing in the second hour after treatment, and spent negligible time lying laterally in the third to fifth hours. This animal was deleted as an outlier in the analyses for percent time lying in the second hour, the percent time standing in the second hour and the percent time lying laterally in the 3rd to fifth hours. Thus these analyses were modified to have 21 residual degrees of freedom.

# 4 Results

The results of the continuous behaviour observations of postures in the first 2 hours after castration with and without anaesthetic are presented in Table 1. No effects of the administration of either 1.5 or 4 ml of lignocaine were observed in the first or second hour. The results of bouts of behaviour events, including behaviours indicative of pain, are presented in Table 2 (first hour) and 3 (second hour). The behaviour of the lambs in all treatments is very similar with a couple of minor but significant differences in the first hour only. Lambs provided with 4 ml of lignocaine eased their quarters less ($p<0.05$) and groomed more ($p<0.05$) compared to lambs that did not receive any pain relief. However several behaviours indicative of pain showed a non-significant reduction. When combined as 'All Pain related Behaviours" (*Includes Easing Quarters, Foot Stamping, Head Butting, Standing with Head Down, Head Shaking, Head Leaning on Wall, Rubbing Quarters, Restless Lying, Rolling Lying, Self-Biting and Tail Wagging) lambs provided with 4 ml of lignocaine did show a significant reduction in these behaviours combined ($p<0.05$). No behavioural differences were observed between treatments in the third to fifth hour after castration (Table 4). Cortisol concentration before and after 30, 60 and 210 min after castration also reveal no effect of either amount of lignocaine (Table 5).

**Table 1.** Effect of amount of anaesthetic on continuous behaviour observations (% behaviour) in the first 2 hours. All P values are calculated using permutation test.

| Behaviours | Angularly Transformed | | | | Back transformed | | | P Values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| *First hour* | | | | | | | | | | |
| Lying | 43 | 39 | 47 | 2.9 | 47 | 40 | 53 | 0.058 | 0.24 | 0.17 |
| Standing | 47 | 51 | 43 | 2.9 | 53 | 60 | 46 | 0.051 | 0.20 | 0.19 |
| Lying on sternum | 1 | 2 | 2 | 1.3 | 0 | 0 | 0 | 0.62 | 0.43 | 0.38 |
| *Second hour* | | | | | | | | | | |
| Lying[b] | 69 | 68 | 71 | 3.9 | 87 | 86 | 90 | 0.65 | 0.57 | 0.73 |
| Standing[b] | 21 | 22 | 18 | 3.9 | 12 | 14 | 10 | 0.66 | 0.60 | 0.69 |
| Lying on sternum | 1 | 1 | 1 | 1.3 | 0 | 0 | 0 | 1.00 | 0.86 | 0.82 |

[b] Excluded animal 1664/1735 in 1.5 ml treatment as an outlier that spent 100% of time standing.

**Table 2.** Effect of amount of anaesthetic on bouts of behaviour per animal in the first hour during continuous behaviour observations. All P values are calculated using permutation test. P values less than 0.05 are in bold.

| Behaviours | Square Root Transformed | | | | Back transformed | | | P Values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| Drinking | 0.4 | 0.4 | 0.0 | 0.20 | 0.1 | 0.2 | 0.0 | 0.14 | 0.065 | 0.90 |
| Easing Quarters | 6.5 | 6.5 | 5.5 | 0.37 | 41.7 | 42.4 | 30.1 | **0.021** | **0.016** | 0.90 |
| Foot Stamping | 3.7 | 3.3 | 2.7 | 0.65 | 13.8 | 11.0 | 7.4 | 0.32 | 0.14 | 0.53 |
| Grooming | 1.5 | 1.3 | 2.1 | 0.27 | 2.2 | 1.8 | 4.4 | **0.024** | **0.031** | 0.64 |
| Head Butting | 1.7 | 1.6 | 1.1 | 0.45 | 2.8 | 2.5 | 1.2 | 0.43 | 0.24 | 0.89 |
| Standing with Head Down | 5.9 | 5.9 | 5.2 | 0.39 | 34.8 | 34.5 | 27.5 | 0.20 | 0.12 | 0.94 |
| Head Shaking | 0.1 | 0.2 | 0.5 | 0.20 | 0.0 | 0.0 | 0.2 | 0.16 | 0.072 | 0.65 |
| Head Leaning on Wall | 0.2 | 0.2 | 0.2 | 0.15 | 0.0 | 0.1 | 0.1 | 1.00 | 0.90 | 0.82 |
| Lying Lateral on Side | 5.2 | 4.5 | 4.6 | 0.45 | 26.9 | 20.0 | 20.7 | 0.23 | 0.17 | 0.13 |
| Lying on Sternum | 0.3 | 0.5 | 0.4 | 0.28 | 0.1 | 0.2 | 0.2 | 0.81 | 0.68 | 0.54 |
| Rubbing Quarters | 0.2 | 0.2 | 0.2 | 0.16 | 0.0 | 0.0 | 0.0 | 0.92 | 0.82 | 0.88 |
| Restless Lying | 3.0 | 3.1 | 2.7 | 0.37 | 8.9 | 9.5 | 7.5 | 0.61 | 0.49 | 0.78 |
| Rolling Lying | 0.4 | 1.5 | 0.8 | 0.56 | 0.1 | 2.1 | 0.6 | 0.19 | 0.48 | 0.065 |
| Self-Biting | 1.3 | 1.3 | 1.7 | 0.43 | 1.6 | 1.6 | 2.8 | 0.56 | 0.35 | 0.99 |
| Standing | 5.0 | 4.1 | 4.3 | 0.42 | 25.1 | 16.9 | 18.1 | 0.086 | 0.083 | **0.041** |
| Tail Wagging | 3.8 | 3.2 | 2.5 | 0.77 | 14.2 | 10.4 | 6.2 | 0.27 | 0.12 | 0.49 |

| | Square Root Transformed | | | | Back transformed | | | P Values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Behaviours | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| Walking | 8.5 | 7.8 | 7.4 | 0.67 | 71.4 | 60.2 | 54.2 | 0.27 | 0.11 | 0.31 |
| All Pain related behaviour* | 11.3 | 11.4 | 9.7 | 0.69 | 128 | 130 | 95 | **0.042** | **0.028** | 0.94 |

*Includes Easing Quarters, Foot Stamping, Head Butting, Standing with Head Down, Head Shaking, Head Leaning on Wall, Rubbing Quarters, Restless Lying, Rolling Lying, Self-Biting and Tail Wagging.

**Table 3.** Effect of amount of anaesthetic on bouts of behaviour per animal in the second hour during continuous behaviour observations. All P values are calculated using permutation test.

| Behaviours | Square Root Transformed | | | | Back transformed | | | | P Values | |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| Drinking | 0.0 | 0.2 | 0.0 | 0.20 | 0.0 | 0.0 | 0.0 | 1.00 | 0.68 | 1.00 |
| Easing Quarters | 2.3 | 2.2 | 1.9 | 0.47 | 5.1 | 4.1 | 3.7 | 0.77 | 0.48 | 0.86 |
| Foot Stamping | 0.4 | 1.0 | 0.7 | 0.44 | 0.1 | 1.0 | 0.5 | 0.39 | 0.45 | 0.17 |
| Grooming | 0.1 | 0.0 | 0.0 | 0.10 | 0.0 | 0.0 | 0.0 | 1.00 | 0.66 | 0.66 |
| Head Butting | 0.1 | 0.2 | 0.2 | 0.45 | 0.0 | 0.0 | 0.0 | 1.00 | 0.66 | 0.75 |
| Standing with Head Down | 1.8 | 1.8 | 1.6 | 0.32 | 3.3 | 3.3 | 2.7 | 0.87 | 0.52 | 0.94 |
| Head Shaking | 0.0 | 0.0 | 0.2 | 0.09 | 0.0 | 0.0 | 0.0 | 0.33 | 0.22 | 1.00 |
| Head Leaning on Wall | 0.0 | 0.0 | 0.2 | 0.15 | 0.0 | 0.0 | 0.0 | 0.34 | 0.23 | 1.00 |
| Lying Lateral on Side | 2.1 | 1.8 | 2.0 | 0.39 | 4.5 | 3.3 | 3.9 | 0.78 | 0.73 | 0.48 |
| Lying on Sternum | 0.2 | 0.1 | 0.1 | 0.14 | 0.0 | 0.0 | 0.0 | 1.00 | 0.76 | 0.77 |
| Rubbing Quarters | 0.0 | 0.0 | 0.0 | - | 0.0 | 0.0 | 0.0 | 1.00 | 1.00 | 1.00 |
| Restless Lying | 0.1 | 0.3 | 0.3 | 0.19 | 0.0 | 0.1 | 0.1 | 0.48 | 0.20 | 0.34 |
| Rolling Lying | 0.1 | 0.4 | 0.3 | 0.36 | 0.0 | 0.2 | 0.1 | 0.83 | 0.58 | 0.45 |
| Self-Biting | 0.1 | 0.2 | 0.2 | 0.07 | 0.0 | 0.1 | 0.1 | 0.67 | 0.22 | 0.44 |
| Standing | 2.0 | 1.5 | 1.8 | 0.40 | 3.9 | 2.1 | 3.3 | 0.40 | 0.68 | 0.19 |
| Tail Wagging | 0.8 | 0.9 | 0.5 | 0.33 | 0.6 | 0.8 | 0.2 | 0.43 | 0.38 | 0.69 |

| Behaviours | Square Root Transformed | | | | Back transformed | | | | P Values | |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| Walking | 2.0 | 1.5 | 1.7 | 0.49 | 4.0 | 2.3 | 2.8 | 0.63 | 0.55 | 0.34 |
| All Pain related Behaviour | 3.4 | 3.6 | 3.3 | 0.65 | 11.8 | 13.0 | 10.8 | 0.89 | 0.82 | 0.81 |

*Includes Easing Quarters, Foot Stamping, Head Butting, Standing with Head Down, Head Shaking, Head Leaning on Wall, Rubbing Quarters, Restless Lying, Rolling Lying, Self-Biting and Tail Wagging.

**Table 4.** Effect of amount of anaesthetic on prevalence (%) of behaviours in the third to fifth hour during instantaneous point sampling. All P values are calculated using permutation test.

| Behaviours | Angularly Transformed | | | | Back transformed | | | P Values | |
|---|---|---|---|---|---|---|---|---|---|
| | None | 1.5 ml | 4 ml | sed | None | 1.5 ml | 4 ml | Any | None v 4 ml | None v 1.5 ml |
| *Third to Fifth hour* | | | | | | | | | | |
| Drinking | 0 | 0 | 0 | - | 0 | 0 | 0 | 1.00 | 1.00 | 1.00 |
| Feeding Hay | 0 | 0 | 0 | - | 0 | 0 | 0 | 1.00 | 1.00 | 1.00 |
| Standing with Head Down | 14 | 19 | 12 | 4.3 | 6 | 11 | 4 | 0.23 | 0.55 | 0.26 |
| Head Leaning on Wall | 0 | 0 | 1 | 0.9 | 0 | 0 | 0 | 1.00 | 0.67 | 1.00 |
| Lying Lateral[a] | 58 | 55 | 60 | 3.7 | 71 | 66 | 75 | 0.36 | 0.51 | 0.45 |
| Lying on Sternum | 0 | 0 | 0 | - | 0 | 0 | 0 | 1.00 | 1.00 | 1.00 |

[b] Excluded animal 1664/1735 in 1.5 ml treatment as an outlier that was observed to be lying laterally on only one occasion.

**Table 5.** Effect of amount of anaesthetic on cortisol in first day. All P values are calculated using permutation test.

| Cortisol | None | 1.5 ml | 4 ml | sed | Any | P Values None v 4 ml | None v 1.5 ml |
|---|---|---|---|---|---|---|---|
| 30 minutes | 54 | 42 | 45 | 9.5 | 0.47 | 0.38 | 0.23 |
| 60 minutes | 152 | 128 | 138 | 22.9 | 0.56 | 0.54 | 0.29 |
| 210 minutes | 33 | 35 | 36 | 3.7 | 0.68 | 0.40 | 0.57 |

# 5 Discussion

Some minor effects of 4 ml lignocaine administrated immediately after castration were observed in the amount of grooming and easing quarters. Grooming is considered a comfort behaviour and it is generally considered that comfort behaviours are inhibited by stressful or painful events. Therefore the increase in grooming seen in lambs injected with 4 ml lignocaine may indicate a reduction in pain. Easing quarters is thought to be a behaviour indicative of discomfort in the hind quarter and a reduction in this behaviour may indicate a reduction in pain. However, other behaviours such as foot stamping, restless lying, standing with the head down and tail wagging are also thought to be indicative of pain, but were not significantly reduced by administration of lignocaine, although they all appeared somewhat reduced in the lambs provided with 4ml lignocaine. Indeed, when all behaviours considered indicative of pain were combined there was a small but significant reduction in these behaviours. In a previous study on the pain associated with castration of lambs using the WEE Bander all these behaviours were clearly increased in the castrated animals compared to a non-castrated control. Therefore any pain reduction caused by the injection of 4 ml of lignocaine in the first hour in the current study should be considered minor.

No effects of lignocaine administration were seen after the first hour. From 1 h after castration lambs were very inactive and spent most time lying down. This may be an indication of pain, as inactivity avoids irritation of the painful area, or normal behaviour after a period of activity. As this study did not include a control treatment of sham castration, it is not possible to conclude if the lambs are still in pain or if blood supply was cut off sufficiently to numb the area, preventing pain. In a previous study on the pain associated with castration of lambs of similar age using the WEE Bander significant differences were found between castration treatments and control up to 5 h after castration, so it can be assumed that lambs in the current study would also experience some pain for a similar duration. In addition, the lambs were observed to lie laterally with their legs to the side, which is considered an abnormal lying posture in young lambs and may be related to pain (Mellor and Murray, 1989). However the lambs in the current study were older and they were housed on a wooden slatted floor. This floor may have been less comfortable than bedding and may have increased the incidence of lateral lying.

Lambs may have been inactive after the first hour, as they were not supplied with food during the observation period. This may have reduced activity of the lambs, reducing the ability to observe differences as a result of treatment. In a previous study on the pain associated with castration of lambs using the WEE Bander sheep were supplied with ad lib hay and chaff. While they spent little time feeding during the first 2 hours after castration,

feeding behaviour increased during the period of 2 to 5 hours after castration. However feeding behaviour 2 to 5 h after treatment in that study was not affected by castration treatment, including a control treatment.

While lignocaine is an effective local anaesthetic, the restriction in blood supply after ring castration may have reduced the effusion of lignocaine, which may have limited the concentration around the site of inflammation. Indeed, Paull et al (2009) similarly found little effect of carprofen injected subcutaneously 90 min prior to castration, particularly on the cortisol response and considered the lack of effusion as a cause of this result. On the other hand, Wood et al (1991) successfully suppressed all behaviour indicative of pain and increase in cortisol associated with ring castration using lignocaine injections. However, they used multiple injections into each spermatic cord, in a ring around the neck of the scrotum and into each testis 15 to 20 min prior to treatment to block the various nerves affected by ring castration. They suggested that effect on the afferent activity from the testes was responsible for a prolonged effect of lignocaine beyond the expected one hour. In another study 0.3 ml injection of a combination of procaine and adrenaline was injected in the spermatic cords using a needleless injector immediately prior to castration. This was effective in reducing behaviours indicative of pain measured for 60 min after castration (Molony et al., 2012). Steward et al (2014) also successfully reduced behaviour indicative of pain after ring castration with the injection of 2 ml of lignocaine in both testes and scrotal neck 4 min prior to ring castration. They also studied the effect of the use of lignocaine impregnated rubber rings, which had a modest effect on cortisol and behavioural responses after castration.

The amount of lignocaine injected in the present study is close to the level where adverse reactions to the anaesthetic could occur (10 mg/kg bodyweight, White and Taylor, 2000), so increasing the dose injected in an attempt to increase the extend of lignocaine infiltration surrounding the damaged tissue is not advised. In order to increase the effectiveness of lignocaine injections during castration, a different direction of infiltration to more adequately block the afferent innervation of the neck of the scrotum should be considered.

# 6 Conclusions/recommendations

While few minor behaviour changes indicative of reduced pain were seen in lambs injected with 4 ml of lignocaine at the time of ring castration compared to no pain relief and an injection of 1.5 ml, other behaviours and the results of the cortisol analysis do not confirm a reduction in pain. The changes observed do not appear to be biological significant and do not warrant the time and cost involved in administration of this anaesthetic in order to obtain

minimal pain relief. It would also not satisfy the expectation of the general public of providing effective pain relief.

More research is required in the injection sites and tissue and nerves affected by ring castration to be able to design an effective castration tool that can be used in the industry and provide effective pain relief from ring castration.

# 7 Key messages

Lambs provided with a midline injection of 4 ml of lignocaine on the dorsal side of the ring at the time of ring castration showed minor but significant reduction in pain related behaviour compared to lambs castrated with 1.5 ml of lignocaine or no pain relief. However the changes are not of sufficient magnitude to provide evidence of effective pain relief.

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