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Efficacy of precisely injected single local bolus of lignocaine for alleviation of behavioural responses to pain during tail docking and castration of lambs with rubber rings

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ABSTRACT

Delivery of local anaesthetic at the time of castration and tail docking (marking) could improve welfare outcomes in lambs. This study examined pain responses in lambs marked using rubber rings, with or without local anaesthetic precision injected using the Numnuts® instrument. On each of two commercial farms, 150 prime lambs aged 4 to 10 weeks, balanced for sex, were randomly allocated to 3 treatments: handled in a lambing cradle (Sham), handled and marked with rubber rings (Ring) or handled and marked with rubber rings and treated with 30 mg lignocaine using the Numnuts® instrument (NNLA). Time to mother up (one trial site only), acute pain related behaviours at 5, 20, 35 and 50 min, and postures at 10 min intervals from 60 to 180 min were recorded. NNLA lambs tended to mother up more quickly than Ring lambs ($P = 0.09$), and more slowly ($P = 0.07$) than Sham lambs. Acute pain behaviours were significantly more frequent in Ring and NNLA than Sham ($P < 0.001$) from 5 to 50 min. NNLA was significantly lower than Ring at 5 min ($P < 0.001$) and 20 min ($P = 0.001$). Ring and NNLA did not differ at 35 or 50 min. Abnormal postures were higher in Ring and NNLA than Sham at 60, 70, 80, 90 and 150 min ($P < 0.048$). Ring and NNLA did not differ at any time point between 60 and 180 min. Delivery of lignocaine with the Numnuts® instrument improved welfare outcome of lambs during the acute pain response caused by castration and tail docking with rubber rings.

1. Background

The majority of lambs produced in Australia are tail-docked and (if male) castrated between the ages of 4 and 12 weeks (Howard and Beattie 2018). These procedures, together termed 'marking', are carried out to reduce the risk of fly-strike associated with soiling of the tail, to prevent unwanted matings, to prevent fighting between rams and to improve operator safety when handling the animals (many entire ram lambs attaining puberty prior to slaughter, particularly those utilised for wool production). The use of rubber rings to cause ischaemic constriction of the tail and scrotal neck is a common means of marking lambs: it is bloodless, and considered by many to be less painful than surgical (knife) castration or tail docking (Kent et al. 1993; Lester et al. 1996; Lester et al. 1991; Melches et al. 2007; Paull et al. 2009; Robertson et al. 1994; Thornton and Waterman-Pearson 1999). Nevertheless, marking using rubber rings does cause a degree of pain or discomfort, evidenced by a high degree of active pain avoidance type behaviours such as kicking/stamping; rolling; tail-wagging; licking/biting at the ring; restlessness (repeated lying and standing); jumping; easing quarters and vocalising, and an increase in time spent lying and time spent in abnormal postures (Grant 2004; Mellor et al. 1991; Mellor and Murray 1989; Thornton and Waterman-Pearson 1999). Notwithstanding societal pressure, there is an increasing desire within industry for analgesic therapy to be provided at the time of castration and tail docking, but traditionally, use of local anaesthetic agents has been limited to veterinarians as a result of the potential for operator error leading to self-injection, or inappropriate or inaccurate injection techniques, and the challenges posed by sharps disposal requirements.

The Numnuts® tool provides consistent delivery of local anaesthetic to the site of ring application, with a shielded needle arrangement to reduce the risk of self-injection, while also acting as a ring applicator, reducing the requirement for multiple operations to be carried out by the operator at the time of castration and tail docking.

This study is a field-based evaluation of the welfare effects of

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providing local anaesthesia (LA; Lignocaine 2%) using the Numnuts® tool to the scrotal neck (males) and tail (males and females) of lambs undergoing marking in mixed-sex groups. The specific configuration of the Numnuts® instrument results in a very precise repeatable deposition of the local anaesthetic relative to the ring, which is unachievable using manual injection. As such this study is focused on the efficacy of the local anaesthetic as delivered using the Numnuts® device as opposed to efficacy of the local anaesthetic per se.

The hypotheses tested were as follows:

- Local anaesthesia, deposited midline using the Numnuts® tool, will provide alleviation of pain-related behaviours and postures in female lambs subjected to tail-docking, in a paddock setting (behaviours);
- Local anaesthesia, deposited midline using the Numnuts® tool, will provide alleviation of pain-related behaviours and postures in male lambs subjected to castration and tail-docking, in a paddock setting (behaviours);
- Lambs that have received local anaesthesia at the time of marking (castration and/or tail-docking) are more able to locate their mother than lambs receiving no local anaesthesia (mothering up);
- Lambs that have received local anaesthesia at the time of marking (castration and/or tail-docking) show less negative impact on growth than lambs receiving no local anaesthesia (body weights).

2. Materials and methods

The study was a blinded controlled randomized block design field study, incorporating detailed individual behavioural pain indicators. It was conducted on commercial sheep farms at two trial sites at Uralla near Armidale, NSW (April 2017) and at Goorambat near Benalla, VIC (May 2017).

The NSW study was approved by the CSIRO Armidale Animal Ethics Committee, ref. ARA16/32. The VIC study was approved by the University of Melbourne Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee, ref. 1,714,158.

At each trial site, 150 lambs (75 male and 75 female, aged between 4 and 10 weeks, bodyweight 9.4–31.9 kg at the time of marking) were included in the trial. The lambs were second cross lambs from Dorset sires over Merino X Border Leicester ewes, joined by natural mating, and lambed in a paddock situation. They were of apparent good health and individually identified by both electronic RFID and visual ear tags. The lambs and their mothers were kept in a communal paddock situation prior to the start of the study. During the week prior to treatment, the lambs and ewes were mothered up, and allocated to groupings, such that at least 15 lambs were available for each cohort studied. Each grouping contained a mixture of single and twin lambs, selected randomly from the larger flock. In the NSW trial, each grouping contained 20 lambs, from which the lambs to be included in the cohort could be selected. In the VIC trial, each grouping contained 15 lambs.

At each site, the study was conducted across 10 cohorts of 15 lambs, each cohort containing three lambs in each treatment group (Table 1). Two cohorts were studied per day.

On day 0, ewes and lambs were separated, and the ewes placed into the observation paddock of approximate area 0.18 ha (42 m × 42 m), while the lambs were held in a holding pen. Lambs were individually captured, sex identified, ear tagged and weighed prior to marking (castration and/or tail docking). Within each cohort, the lambs were randomly allocated to treatment at the time of capture for marking. The time of treatment and the treatment applied to each was recorded, and individual numbers were applied to the lamb's flanks, head and rump, and a colour stripe applied to the rump and shoulders, to aid in identification in the paddock. The lamb was then released into the observation paddock. Lambs were treated at three-min intervals. Three observers, located in a gazebo located in the centre of the observation paddock, counted the number of acute pain behaviours, according to a pre-determined ethogram (Table 2), expressed during a continuous 1-min period at 5 min, 20 min, 35 min and 50 min post treatment, then scan samples were recorded of postures, according to a pre-determined ethogram (Table 3), at 60 min and every 10 min thereafter, until 180 min from treatment had elapsed.

Table 1
Treatment groups.

Description	Lamb sex	Lamb numbers (NSW)	Lamb numbers (VIC)
Sham	M/F	30	30
Tail only with LA (NNLA)	F	30	30
Tail only no LA (Ring)	F	30	30
Castrate + tail with LA (NNLA)	M	30	30
Castrate + tail, no LA (Ring)	M	30	30
Subtotal lambs		150	150
Total lambs		300	

For marking, lambs were captured from the holding pen, weighed on calibrated electronic scales (Tru-test xR3000, Datamars, Banyo, QLD) placed in dorsal decumbency in a marking cradle, one visual identification tag and one electronic ear tag were inserted, then marked. Lambs were marked by application of a rubber ring (Elastrator®, Heiniger Australia, Bibra Lake, WA) to the neck of the scrotum or at the level of the 3rd coccygeal joint of the tail. For lambs not receiving Lignocaine, this was carried out using Elastrator® pliers (Heiniger Australia, Bibra Lake, WA), while for lambs receiving lignocaine, the ring was applied using a proprietary designed instrument (Numnuts®, version 12 prototype, Senesino, Grange, QLD). After extension of the ring over the scrotum or tail, the instrument closes the rubber ring and holds tissue beneath the ring firmly in alignment with an injection mechanism. The operator activates an injection plunger on the instrument to deliver a pre-metered dose of 1.5 mL of 20 mg/mL Lignocaine hydrochloride (Ilium Lignocaine 20, Troy Laboratories, Glendenning, NSW) subcutaneously. For castration, the dose was delivered midline into the posterior aspect of the scrotal neck, adjacent to the ring, immediately after ring application. For tail docking, the dose was delivered midline into the dorsal aspect of the tail, adjacent to the ring, immediately after ring application.

In the VIC trial, mothering up was observed for 180 s following release of lambs into the observation paddock following marking, and the time to mother-up recorded. Lambs were considered to have successfully mothered up when they suckled from the ewe, or were obviously paired and following a particular ewe. Lambs that did not mother-up within 180 s were recorded as 'failed to mother-up'. In NSW, latency to mothering up was not measured, as all the ewes waited at the observation paddock fence for return of their lamb.

Body weights were recorded on the day of marking and at 4 intervals of approximately 1 week spacing, for both trial sites.

3. Statistical analyses

3.1. Mothering up

In the VIC trial, mothering up was assessed for 180 s after release of lambs into the observation paddock. A number of lambs failed to mother up so the results were analysed with Cox's proportional hazards model using survival analysis (Therneau 2015; Therneau and Grambsch 2000) in R (R Core Team 2015). Any lamb that failed to mother up within 180 s was deemed as a censored result, and this was recorded as a 'survival' incidence.

Table 2
Ethogram of active pain avoidance behaviours of lambs for counts over continuous 1 min blocks.

Behaviour	Abbreviation	Description
Restlessness	Rst	Stood up and laid down. Instances of rising as far as its knees included.
Kicking/ft stamping	FSK	Either a front or hind limb (usually hind limb) was lifted and forcefully placed on the ground while standing or was used to kick while standing or lying.
Rolling	rl	Rolled from lying on one side to the other without getting up. Half rolls onto back and then return to lying on the same side included.
Jumping	jmp	All four feet off ground simultaneously.
Pawing	Paw	Front foot scrapes at the ground in a repetitive pattern.
Licking/biting wound site	LBW	Movement of the head beyond the shoulder, including both looking and touching at the source of pain and grooming.
Suckling	SK	Active teat seeking or suckling.
Easing quarters	EQ	Abnormally lowers rear quarters (standing) or attempts to keep quarters off the ground (lying).

Table 3
Ethogram of Postural behaviours for lambs for snapshot scan observations.

Behaviour	Abbreviation	Description
Normal standing	NS	Standing with no apparent abnormalities.
Abnormal standing	AS	Other abnormal standing e.g. Statue standing: immobile standing with an obvious withdrawal from interaction with other pen members and outside stimuli; or stretched standing: legs positioned further back than normal.
Standing other	Su	Standing but unable to clearly categorise the standing posture; e.g. obscured view.
Normal walking	NW	Walking with no apparent abnormalities.
Abnormal walking	AW	Walking unsteadily or stiffly, includes walking backwards, on knees, moving forward with bunny hops, circling, leaning or falling.
Walking other	Wu	Walking but unable to clearly categorise the walking type; e.g. obscured view.
Running	R	Movement across pen at gait faster than walking.
Jumping	J	Forelegs are lifted from the ground, the forepart of the body is elevated in an upward movement.
Grazing	G	Eating pasture.
Suckling	SK	Active teat seeking or suckling.
Playing	P	Agonistic interactions, exuberant skipping.
Normal lying	NL	Ventral recumbency, all legs tucked under body or very close to body.
Abnormal lying	AL	Twisted lying; ventral recumbency with forelimbs tucked under body, one or both hind limbs partially or fully extended; including dog sitting.
Lateral lying	LL	Lateral recumbency with one shoulder on ground, hind limbs and/or forelimbs fully extended.
Lying intention	LI	Attempts to lie down without completing the manoeuvre in a single sequence
Ventral lying other	Vu	Lying ventrally but unable to clearly categorise the lying posture.
Rolling	rl	Rolled from lying on one side to the other without getting up. Half rolls onto back and then return to lying on the same side included.

The postures considered Abnormal were AS, AW, AL, LL, LI and rl.

3.2. Behaviours and weights

Data which were normally distributed or that could be transformed to satisfy normal distribution, were analysed with a repeated measures ANOVA model fitting pre-treatment values as a covariate when significant and the fixed effects treatment, and when significant trial, cohort, sex and first order interactions. $P < 0.05$ was considered significant and $0.1 > P > 0.05$ was considered to indicate a tendency towards statistical significance (Systat version 9). When treatment or interactions between treatment and time were significant, contrasts between least squares means were performed to establish the significant of differences between treatments. Data for abnormal postural behaviours in female lambs were not able to be normalised by transformation and so were analysed with the non-parametric test Kolmogorov-Smirnov test within time points.

4. Results

4.1. Mothering up

For both sexes combined, there was a statistically significant difference between "survival" curves describing success in mothering up ($P = 0.006$; Fig. 1 top). There was a tendency for NNLA to be different to Ring and Sham. There was a tendency for Ring to have 0.7 times less chance of mothering up than the NNLA treatment ($P = 0.09$), and for Sham to be 1.5 times more likely to mother up than NNLA ($P = 0.07$).

For males (Fig. 1 middle), there were no significant differences between treatments in mothering up. Ring males had 0.995 times less chance of mothering up than NNLA treatment and this was not significant ($P = 0.986$), while male sham lambs were 1.61 times more likely to mother up than NNLA lambs but this again was not significantly different ($P = 0.148$).

For females (Fig. 1 bottom), there was a significant difference between NNLA and Ring but not between NNLA and Sham. Ring had 0.46 times less chance of mothering up than NNLA treatment and this was significant ($P = 0.015$), while female sham were 1.39 times more likely to mother up than NNLA but this was not significantly different ($P = 0.32$).

4.2. Acute pain behaviours

Two lambs in the VIC trial exhibited transient hind limb ataxia for several min following NNLA treatment.

Data were square root transformed for analysis to normalise distributions. Values in graphs are back transformed least squares means, and estimated error bars were generated according to JØrgensen and Pedersen (1998).

For both sexes combined, trial site (NSW or VIC; $P = 0.027$) sex (male or female; $P = 0.001$) and treatment (NNLA, Ring or Sham; $P < 0.001$) influenced acute pain behaviours. There was a significant effect of time ($P = 0.001$) and significant interactions between time by trial site ($P = 0.025$), time by sex ($P = 0.008$) and time by treatment ($P < 0.001$). Ring and NNLA treatments were significantly different from Sham at all time points ($P < 0.001$). NNLA was significantly different from Ring at 5 min ($P < 0.001$) and 20 min ($P = 0.001$). Ring and NNLA did not differ at 35 or 50 min (Fig. 2 top). There were more acute pain behaviours displayed in NSW than VIC at 5 min ($P = 0.003$) and 35 min ($P = 0.042$). There were more acute pain behaviours displayed in males than females at 20 min ($P = 0.002$), 35 min ($P < 0.001$) and 50 min ($P = 0.004$).

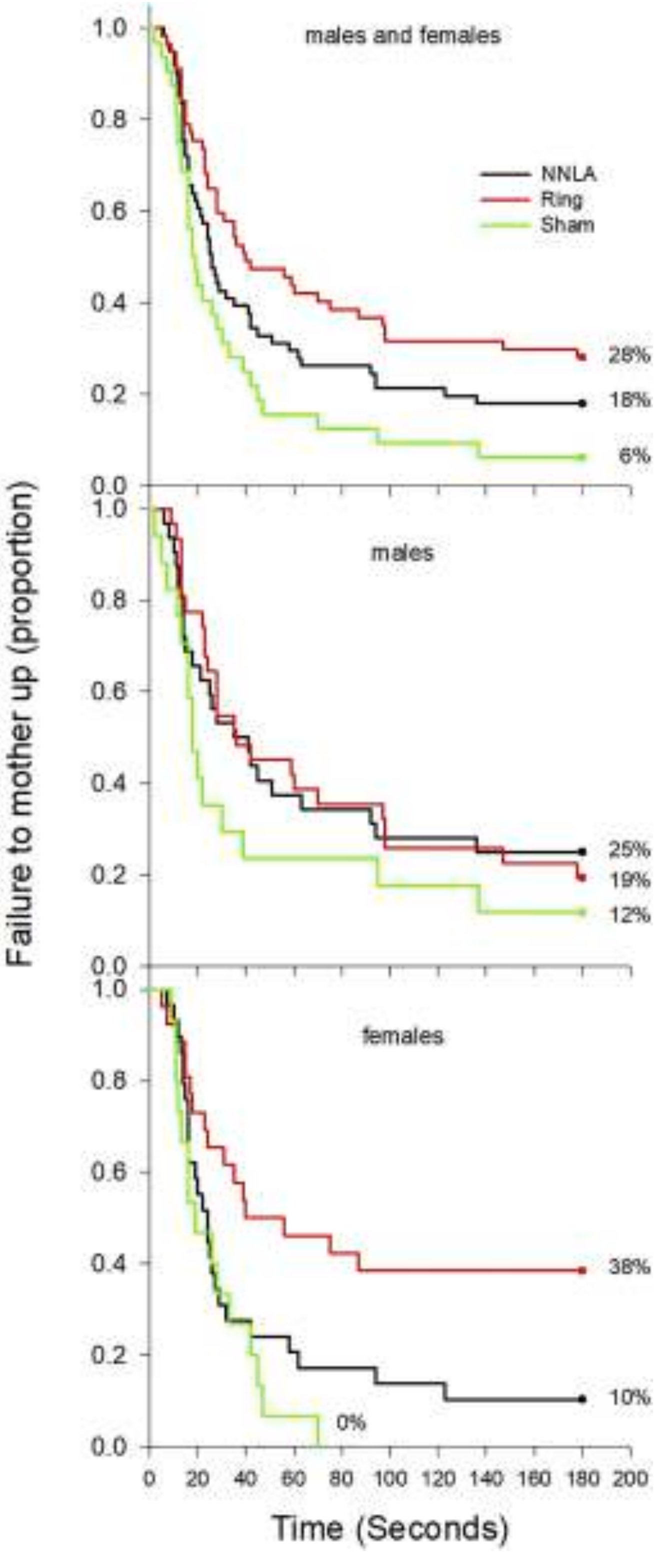

Fig. 1. Kaplan-Meier curves for mothering up for all lambs. Every time a lamb joined its mother, the proportion on the Y axis drops. NNLA: lignocaine delivered at the site of ring application using the Numnuts® tool; Ring: elastration ring applied without local anaesthesia; Sham: lamb handled but no ring applied. Top both sexes combined, $n = 60$ in Ring and NNLA groups; $n = 30$ in Sham group; Middle males (rings applied to both scrotum and tail), n = 30 in Ring and NNLA groups, $n = 15$ in Sham group; Bottom females (rings applied to tail only), n = 30 in Ring and NNLA groups, n = 15 in Sham group.

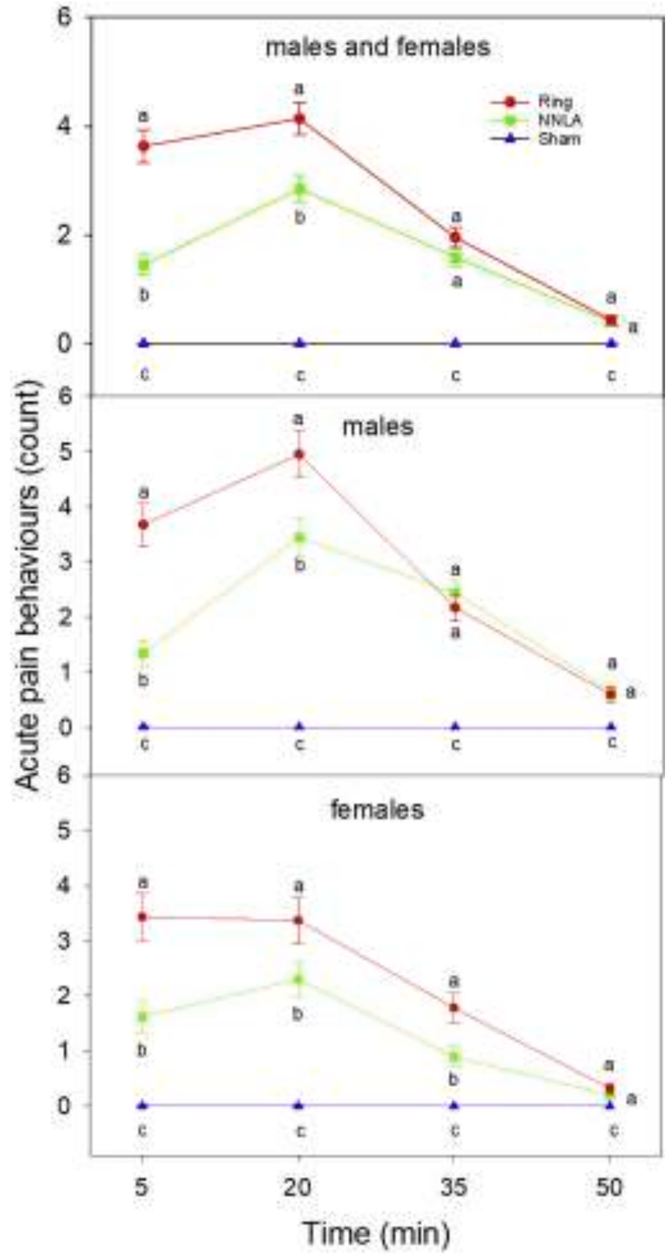

Fig. 2. Acute pain behaviours expressed by lambs, all data pooled across both trial sites and both sexes. NNLA: lignocaine delivered at the site of ring application using the Numnuts® tool; Ring: elastration ring applied without local anaesthesia; Sham: lamb handled but no ring applied. Top both sexes combined, $n = 60$ in Ring and NNLA groups; $n = 30$ in Sham group; Middle males (rings applied to both scrotum and tail), n = 30 in Ring and NNLA groups, $n = 15$ in Sham group; Bottom females (rings applied to tail only), n = 30 in Ring and NNLA groups, n = 15 in Sham group. Data presented are backtransformed means with estimations of standard error according to Jørgensen & Pedersen (1998).

For male lambs, there was a significant effect of treatment ($P < 0.001$) and time ($P < 0.001$). There were significant interactions between time by treatment ($P < 0.001$) and between time and bodyweight ($P = 0.007$). Ring and NNLA treatments were significantly different from Sham at all time points ($P < 0.001$). NNLA was significantly different from Ring at 5 min ($P = 0.001$), 20 min ($P = 0.007$). Ring and NNLA did not differ at 35 or 50 min (Fig. 2 middle).

For female lambs, there was a significant effect of treatment ($P < 0.001$) and time ($P < 0.001$) on acute pain behaviours, and a tendency for responses to differ between trial sites ($P = 0.074$), counts in NSW being greater than in VIC at 5 and 35 min. There were significant interactions between time by treatment ($P < 0.001$), time by trial site by treatment ($P = 0.003$), and a tendency for a time by trial site interaction ($P = 0.051$). Ring and NNLA treatments were significantly different from Sham at all time points ($P < 0.001$). NNLA was significantly different from Ring at 5 min ($P = 0.001$), 20 min ($P = 0.042$) and 35 min ($P = 0.007$). Ring and NNLA did not differ at 50 min (Fig. 2 bottom).

4.3. Abnormal postural behaviours between 60 min and 180 min

For both sexes combined, there was a significant effect of treatment ($P < 0.001$), sex ($P < 0.001$) and time ($P < 0.001$) on abnormal postural behaviours, an interaction between sex and treatment ($P = 0.002$) and a tendency for a difference to occur between trial sites ($P = 0.085$). There was an interaction between time and treatment ($P < 0.001$), time and sex ($P < 0.001$), time by sex by treatment ($P < 0.001$) and between time and cohort nested within trial site ($P = 0.014$). Ring and NNLA treatments differed from Sham at 60, 70, 80, 90 and 150 min ($P < 0.048$). Ring and NNLA did not differ at any time point between 60 and 180 min (Fig. 3 top).

For males (Fig. 3 middle), there was a significant effect of treatment ($P < 0.001$), trial site ($P = 0.02$), and time ($P < 0.001$) on abnormal postural behaviours. There was an interaction between time and treatment ($P < 0.001$), and between time and cohort nested within trial site ($P = 0.014$). Ring and NNLA treatments differed from Sham at 60, 70, 80, 90, and 100 min ($P < 0.05$). In addition, Ring differed from Sham at 100, 120 and 150 min. NNLA s differed from ring at 120 min.

Data for female lambs were not normally distributed and were not normalised by transformation. Non-parametric analysis by Kolmogorov-Smirnov test detected no differences between treatments in terms of abnormal postural behaviours. Counts of abnormal postural behaviours between 60 and 180 min were very low (Fig. 3 bottom).

4.4. Total lying

The combined measure "Total lying" was analysed because counts of individual lying postures (Normal lying, Abnormal lying, Lateral lying and Ventral lying other) were very low.

For both sexes combined, there was a significant effect of trial site ($P < 0.001$), sex ($P = 0.018$) and cohort within trial site ($P < 0.001$) on total count of lying postures, and interactions between time and treatment ($P < 0.001$), time and sex ($P < 0.001$), time by sex by treatment ($P = 0.001$), time by sex by trial ($P = 0.003$) and time by cohort within trial ($P < 0.001$). Ring and NNLA treatments differed from Sham at 60, 70, 90, 160, 170 and 180 min. Ring and NNLA differed at 130 min ($P = 0.015$) (Fig. 4 top).

For males, there was a significant effect of treatment ($P = 0.027$), trial site ($P = 0.02$), cohort within trial site ($P < 0.001$) and time ($P < 0.001$) on total count of lying postures. There was an interaction between time and treatment ($P < 0.001$), between time and cohort within trial site ($P < 0.001$) and a tendency for time by trial interaction ($P = 0.065$). Ring and NNLA treatments differed from Sham at 60, 70, 90, and 160 min ($P < 0.05$). NNLA was intermediate between ring and sham at 80 min, and Sham was intermediate between Ring and Sham at 130 min. NNLA differed from ring at 90 and 130 min (NNLA > Ring) (Fig. 4 middle).

For females, there was a significant effect of trial site ($P < 0.001$), cohort within trial site ($P < 0.001$) and a tendency for time ($P = 0.062$) to affect total count of lying postures. There was an interaction between time and treatment ($P = 0.023$), between time and trial ($P = 0.001$) and between time and cohort within trial site ($P = 0.001$). Ring differed from Sham at 60 min and 180 min. NNLA differed from Sham at 130 min. Treatments differed from Sham at 60, 70, 90, and 160 min ($P < 0.05$). NNLA differed from Ring at 60 min (Fig. 4 bottom).

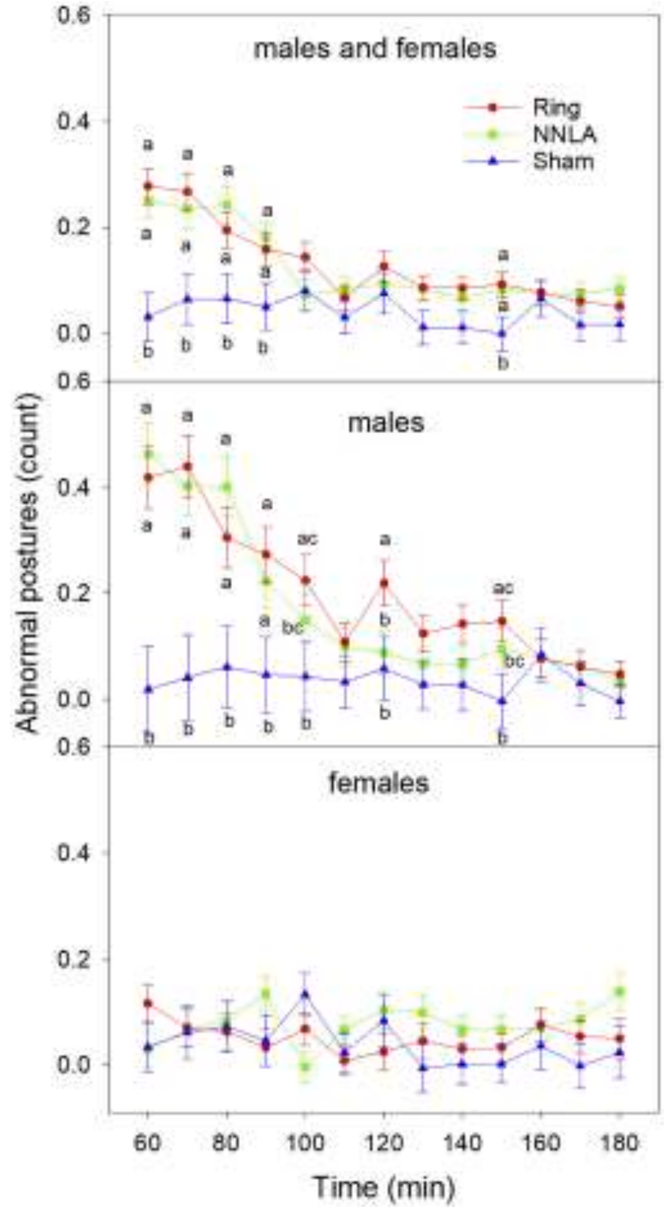

Fig. 3. Total abnormal postures expressed by lambs, data from both trial sites pooled. NNLA: lignocaine delivered at the site of ring application using the Numnuts® tool; Ring: elastration ring applied without local anaesthesia; Sham: lamb handled but no ring applied. Top both sexes combined, $n = 60$ in Ring and NNLA groups; $n = 30$ in Sham group; Middle males (rings applied to both scrotum and tail), $n = 30$ in Ring and NNLA groups, $n = 15$ in Sham group; Bottom females (rings applied to tail only), $n = 30$ in Ring and NNLA groups, $n = 15$ in Sham group.

4.5. Body weight and average daily gain

Trial sites were analysed separately as the spacing between weighing days differed between sites.

At the NSW trial site, there were significant effects of sex ($P = 0.028$), cohort ($P < 0.001$) and time ($P < 0.001$) on body weight, and an interaction between time and cohort ($P < 0.001$). However, there was no significant effect of treatment ($P = 0.489$), nor were there significant treatment by time, treatment by sex or sex by time interactions. In the absence of an effect of treatment or interactions with treatment no further analysis were performed. Lambs gained

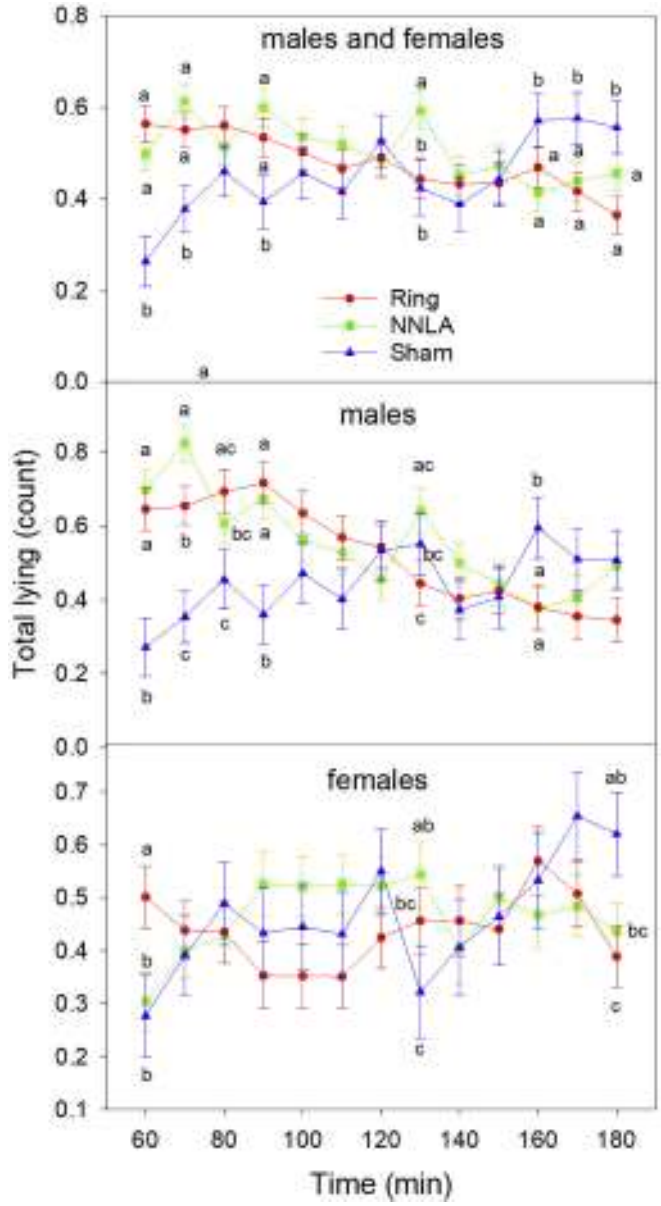

Fig. 4. Total lying postures expressed by lambs, data from both trial sites pooled. NNLA: lignocaine delivered at the site of ring application using the Numnuts® tool; Ring: elastration ring applied without local anaesthesia; Sham: lamb handled but no ring applied. Top both sexes combined, $n = 60$ in Ring and NNLA groups; $n = 30$ in Sham group; Middle males (rings applied to both scrotum and tail), n = 30 in Ring and NNLA groups, $n = 15$ in Sham group; Bottom females (rings applied to tail only), n = 30 in Ring and NNLA groups, n = 15 in Sham group.

approximately 8.2 kg in the 4 weeks after marking. For average daily gain, there was an effect of time ($P < 0.001$) and a time by cohort interaction ($P = 0.04$) but no significant effect of treatment or gender or interactions between these factors and time.

At the VIC trial site, there were significant effects of cohort ($P = 0.017$) and time ($P < 0.001$) and interactions between time and cohort ($P < 0.001$) and between time and treatment ($P = 0.010$) on lamb body weight. Despite the time by treatment interaction, post hoc contrasts within time points detected no significant differences between treatments. Lambs gained approximately 9.5 kg over the 4 weeks following marking. For average daily gain, there was an effect of time ($P < 0.001$) and a tendency for a time by treatment interaction ($P = 0.077$). Sham treatment had the lowest average daily gain at 2 weeks post marking.

5. Discussion

The hypotheses that i) local anaesthesia, deposited midline using the Numnuts® tool, will provide alleviation of pain-related behaviours and postures in female lambs subjected to tail-docking and male lambs subjected to castration and tail-docking in a paddock setting, and ii) that lambs that receive local anaesthesia at the time of marking are more able to locate their mother than lambs receiving no local anaesthesia are supported by the outcomes of the current study.

Marking using rubber rings causes a degree of pain or discomfort, evidenced by expression of active pain avoidance type behaviours such as kicking/stamping; rolling; tail-wagging; licking/biting at the ring; restlessness (repeated lying and standing); jumping; easing quarters and vocalising, and an increase in time spent lying and time spent in abnormal postures (Grant 2004; Mellor et al. 1991; Mellor and Murray 1989; Thornton and Waterman-Pearson 1999). In the current study, these behavioural responses were similarly evident: there was significantly greater expression of acute pain behaviours in Ring lambs than in Sham lambs at 5, 20, 35 and 50 min post procedure (Fig. 1), significantly greater expression of abnormal behaviours/postures in Ring Lambs than Sham lambs at the 60, 70, 80, 90 and 150 min observation time points (Fig. 2), and significantly greater lying in Ring Lambs than Sham lambs at the 60, 70, 90, and 130 min observation time points (Fig. 3).

Molony et al. (1993) observed that ring marked lambs spent more time lying than control handled lambs, over a 180-min observation period, and this was more marked in 21- or 42-day old lambs than 5-day old lambs. Mellor et al. (1991) also reported increased lying in ring castrated day-old lambs, across a 240-min observation period, and particularly, after the first 60 min, in 63–100% of the observations, that the ring castrated lambs appeared to be asleep. The first 30 min post ring castration in Mellor's study were characterised by restlessness, rolling and kicking, so it is hypothesised that the sleeping behaviour noted was the result of tiredness or exhaustion following this initial phase of vigorous activity. Grant (2004) observed, over a 90-min observation period, that ring marked lambs spent only 22% of their time standing, while control (sham) lambs spent 81% of their time standing. In Grant's study, it appeared that the effect of castration was greater than that of tail docking (ring castrated lambs spent only 30.11% of their time standing, while ring tail docked lambs spent 64% of their time standing). This is corroborated somewhat by the current study, in which Ring females (ring tail docked only) did not differ from Sham in terms of lying after the 60 min time point (excluding lying less than Sham at the 180 min time point), while Ring males (ring castrated and ring tail docked) were more often observed in lying postures than Sham at 60, 70, 80 and 90 min, and lay less than Sham at the 160 min time point.

In the current study, the incidence of acute pain behaviours was greatest in the first half hour post marking, and the expression of acute pain behaviours reduced dramatically over the first hour post ring marking. Molony et al. (1993) recorded high incidences of restlessness at 30 min post procedure in 5-day old lambs, and in 21- and 42-day old lambs, the incidence of restlessness was higher than in control lambs at 60 min, but substantially less than at 30 min post procedure. Mellor et al. (1991) also reported that ring castrated lambs expressed a high level of active pain avoidance activity at 15 min post procedure, but then became progressively less active over the subsequent 45 min, and Barrowman et al. (1954) reported that the behavioural response peaked during the first 15 min post procedure, but resolved over the subsequent 45 min, and at one hour the lambs appeared to be resting normally. This progression is also evident in the current study: active pain avoidance incidence was highest at 20 min post procedure, and progressively reduced to a low level by 50 min post procedure. Abnormal behaviours/postures also resolved to be not significantly different from Sham by 100 min post procedure in the current study.

The theme that marking (castration plus tail docking), as suggested by Grant (2004), may have a greater impact than castration, which in turn has a greater impact than tail docking (this latter contrast also suggested by Mellor and Murray (1989)), continues in the current study's observation that females expressed less acute pain behaviours than males at the 20, 35 and 50 min time points. However, for both sexes, Ring lambs expressed significantly more acute pain behaviours than Sham, and in terms of abnormal behaviours expressed in the 60–180 min observation period, Ring males expressed more abnormal behaviours/postures (significantly greater than Sham at 60 and 70 min in NSW, and at 60, 70, 80, 90 and 100 min in VIC) than Ring females (not significantly greater than Sham at either trial site). Grant (2004), reported that lambs undergoing ring tail docking only performed less abnormal behaviours (> 20% of time spent in abnormal behaviours) than those undergoing ring castration (> 50% of time), and in turn those undergoing both ring castration and ring tail docking (> 70% of time spent in abnormal behaviours).

Local anaesthesia has previously been demonstrated to ameliorate the behavioural consequences of ring castration and tail docking, and indeed the current study demonstrated a beneficial effect of local anaesthesia in the first half hour post marking, at the time when the acute pain behaviour response was greatest. Mellor and Stafford (2000) reviewed the literature and concluded that it was not necessary to 1) delay ring application after delivery of the local anaesthetic, a conclusion subsequently corroborated by Stewart et al. (2014), and 2) neither was it necessary to inject all of scrotal neck, testes and spermatic cords. They therefore recommended the scrotal neck or the testes as the most effective sites for administration of local anaesthetic. It is important to note that many of the previously published studies were performed using lambs of less than one week of age, and larger quantities of the local anaesthetic agent (often containing adrenalin) were administered than was used in the current study. Furthermore, the data on time-course of anaesthesia are often pooled into larger epochs of one hour or more, in contrast with the current study which considered the acute pain avoidance behaviours in one min blocks at 5, 20, 35 and 50 min post procedure. The reduced volume of 2% lignocaine provided in the current study could have limited its efficacy, as compared with previously published studies, particularly with regard to castration. For example, Mellema et al. (2006) delivered 4 mg/kg lignocaine 2% into the scrotal neck and spermatic cords of lambs aged less than 7 days, recording a significant reduction in peak cortisol and active pain related behaviours following ring castration; Stewart et al. (2014) injected 120 mg lignocaine 2% into the testes and scrotal neck of 4-week-old lambs at the time of ring application, and recorded educed lying, activity and postural changes compared to ring application without lignocaine; and using a needleless applicator technique, 8 mg of lignocaine 2% delivered into the testes, scrotal neck and tail prior to ring application in lambs less than 7 days old resulted in a reduced incidence of foot stamping/kicking and tail wagging (Kent et al. 2000), while 24 mg applied using the same method resulted in a significant reduction in visual analogue pain score and active pain-related behaviours (Kent et al. 2001). The only study investigating such low volumes of lignocaine comparable to the current study is that of Jongman et al. (2016), who found little effect of 1.5 mL lignocaine 2%, and only a minor benefit associated with 4 mL lignocaine 2% on lambs undergoing ring castration. In contrast, Dinniss et al. reported reductions in abnormal behaviours over a 4-h period post ring castration (Dinniss et al. 1999) and reductions in cortisol response (Dinniss et al. 1997) after administration of 2.0 mL lignocaine 2% into the scrotal neck (1.0 mL midline, and 0.5 mL either side laterally). They found similar outcomes in association with three other local anaesthesia patterns: 1.0 mL in total (0.5 mL into each spermatic cord); 3.0 mL in total (1.0 mL midline into the scrotal neck, 0.5 mL either side laterally and 0.5 mL into each spermatic cord); and 1.0 mL total (0.5 mL into each testis). Of the four local anaesthesia patterns evaluated, injection into the spermatic cords alone had the least impact on abnormal behaviours, and the integrated cortisol response did not differ significantly from ring castrated lambs.

Lignocaine is known to be a relatively short-acting local anaesthetic, and its plasma half-life in sheep is reported to be between 30 and 60 min (Bloedow et al. 1980; Morishima et al. 1979; Santos et al. 1988), which accounts for the short duration of effect demonstrated in the current study, but also makes it somewhat surprising that observable benefits over a period of up to 4 h have been previously reported. The location of deposition of the local anaesthetic relative to the ring may be of significance, and potentially also the use of local anaesthetic preparations containing adrenalin, a haemostatic agent which may slow local clearance of the agent via the circulation. In the current study, the Numnuts® tool may deliver the lignocaine either proximal (lamb side) or distal (scrotum side) to the ring. In previous studies in which anaesthesia has been given at the scrotal neck alone (Kent et al. 1998; Kent et al. 2004), efforts were made to ensure that the agent was deposited on the proximal side of the ring. However, Thornton and Waterman-Pearson (1999) hypothesised that the reservoir of local anaesthetic in the testis, separated from circulatory flushing, may contribute to prolonging the effect of the agent. When the ring is applied, there is a period of 10–15 s before blood flow to the testis is fully occluded, which may allow sufficient time to deeply anaesthetise the tissues. Indeed a mere 0.1 mL of lignocaine 2% has been shown to abolish, (within 2 min of administration) afferent activity associated with ring application in the superior spermatic nerve (Cottrell and Molony 1995). The transient hind limb ataxia observed in two lambs in the VIC trial may have resulted from anterograde diffusion of lignocaine in the epidural space following injection at the site of ring application on the tail.

Lignocaine was selected as the agent of choice for the current study because it is registered for use in sheep in Australia and is readily available. However, in light of its short half-life in sheep, an alternative agent should be considered. Bupivacaine has been proposed as an alternative, and reductions in acute pain avoidance behaviours and abnormal postures/behaviours associated with ring castration or tail docking have been reported by Graham et al. (1997). The elimination half-life of bupivacaine in sheep is between 60 and 120 min (Edwards 2017; Santos et al. 1997), suggesting that the duration of effect should be prolonged as compared to lignocaine. However, the latency to onset of analgesia is substantially greater in bupivacaine than lignocaine (Ghadirian et al. 2016). Another alternative agent is ropivacaine, reported to have similar properties to bupivacaine, but a wider margin of safety (Santos et al. 1995). The elimination half-life of ropivacaine in sheep is between 70 and 90 min (Santos et al. 1997), suggesting that its duration of action may be intermediate between lignocaine and bupivacaine. Neither ropivacaine nor bupivacaine are registered for use in sheep. Two other local anaesthetic compounds of interest are procaine and benzyl alcohol. Both are currently used in pharmaceuticals to relieve the discomfort resulting from injection of another compound, e.g. penicillin and its analogues, or sodium lauryl sulfate (Colditz et al. 2010), so registration of the agent is likely to be less onerous than for bupivacaine or ropivacaine. However, both are considered to be short-acting. Benzyl alcohol itself is reported to have a duration of 10–15 min in humans (Raposio et al. 1999), and can be used in conjunction with lignocaine to prolong the duration of anaesthesia (Williams and Howe 1994). Procaine is reported to be slower in onset of action than lignocaine, and have a shorter duration of action (Lemke 2014). Formulations of lignocaine that contain adrenaline also provide more sustained analgesia than lignocaine alone (Rastabi et al. 2018; Rostami and Vesal 2012), but such formulations, registered for sheep, were not readily available at the time of the study.

In the current study, administration of local anaesthetic using the Numnuts® tool provided significant benefits in terms of the lambs' ability to mother up post procedure as compared with lambs being ring marked without local anaesthesia, which in turn is expected to decrease the risk of subsequent lamb losses associated with mis-mothering, hypothermia and exposure. To our knowledge, this is the first study to demonstrate benefits of an analgesic treatment on mothering-up of lambs post marking. Small et al. (2014) similarly assessed mothering-up in a field study assessing the benefits of meloxicam for surgically marked lambs, but found no benefit of that agent. Interestingly, the greatest differences in mothering-up between NNLA and Ring were observed in female lambs, while differences between NNLA and Ring were not significant in male lambs. This appears to be counterintuitive: male lambs underwent both castration and tail-docking, while female lambs underwent the single procedure of tail-docking. However, the difference in latency to mother up between Sham and either Ring or NNLA in male lambs was also not significant, so there may be a strong gender effect on mothering up post-procedure. This would concur with findings on mothering up post-separation in the absence of a husbandry procedure: female lambs mothering up more rapidly than male lambs (Freitas-De-Melo et al. 2015; Hernandez et al. 2009).

The hypothesis that lambs that have received local anaesthesia at the time of marking (castration and/or tail-docking) show less negative impact on growth than lambs receiving no local anaesthesia was not supported by the outcomes of this study. In the current study, there was little negative impact of marking on the body weight or growth rates of the lambs, as compared with the Sham treatment group. All lambs gained on average 10 kg bodyweight across the 4 week observation period. Previous studies have tended to demonstrate a negative impact of marking on growth, particularly in the immediate post-treatment period, but this appears to be greater in surgically marked lambs than in ring-marked lambs (Paull et al. 2007).

6. Conclusions

By the methods of detection used in the study, administration of lignocaine using the Numnuts® tool provided similar mitigation of pain in male and female lambs in the first 20–50 min following ring marking. Production benefits of Numnuts® were not seen in the trial; however, improved mothering up in female lambs treated with Numnuts® could result in fewer lamb losses following marking in some commercial situations.

Variability between trials is commonly seen in responses of lambs to husbandry practices. Variability between sites seen in the current trial is likely to occur during commercial use of Numnuts®. The general trends seen across sites in this trial thus indicate the general pattern of responses likely to be seen in use of Numnuts®, however results could vary between properties and could vary within a property from year to year.

The results suggest that injection into the tail and neck of the scrotum via the Numnuts® tool is an effective route for delivery of local anaesthetic for pain relief at ring marking. Greatest opportunity to improve efficacy is likely to lie with use of a more efficacious, longer acting local anaesthetic such as bupivacaine, inclusion of adrenaline with the local anaesthetic or a combination of local anaesthetics providing both rapid onset and sustained analgesia.

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Declaration of Competing Interest

None.

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