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Numnuts® field efficacy studies 2017

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Abstract

4cDesign, Scotland, in collaboration with the Moredun Research Institute, Scotland, and CSIRO, Australia, have developed a prototype tool for delivery of local anaesthetic to the tail and scrotum of lambs at the time of marking (Numnuts®). The tool is a more ergonomic design than the current elastrator tool. This study evaluated the efficacy of Local Anaesthetic (Lignocaine 2%), delivered using the Numnuts® v12 tool in relieving pain related behaviours and postures associated with ring castration and tail docking in unweaned lambs In a trial on 300 lambs aged 4 to 8 weeks and weighing 8.6 to 28.6 kg replicated in 2 geographic locations (VIC and New South Wales). Delivery with the Numnuts® v12 tool of 1.5 mL lignocaine at ring castration and tail docking in males, and tail docking in female lambs reduced acute pain behaviours for 20 to 50 min following marking. Mothering up was improved in female lambs. No effects of Numnuts® treatment were detected on abnormal behaviours between 60 and 180 min following marking, or in growth rate in the 4 weeks following marking. Numnuts® improved welfare in male and female lambs in the period of most intense pain induced by ring marking.

Executive summary

4cDesign, Scotland, in collaboration with the Moredun Research Institute, Scotland, and CSIRO, Australia, have developed a prototype tool for delivery of local anaesthetic to the tail and scrotum of lambs at the time of marking. The tool is a more ergonomic design than the current elastrator tool. Excellent analgesia has been demonstrated in female lambs for tail docking; however the analgesia for castration appears to be limited, as assessed using pen studies. Early proof-of-concept studies did indicate a potential benefit to castrated lambs: these studies were small-scale, and carried out in a paddock environment. It may be that the barren pen environment, providing no distractions, encourages the lambs to focus on the injury, while in a paddock environment, there may be more opportunity for the lambs to perform a greater repertoire of behaviours. Furthermore, a study considering the combined insult of tail-docking and castration in male lambs has not been carried out.

The current study evaluates the efficacy of Local Anaesthetic (Lignocaine 2%), delivered using the Numnuts® v12 tool in relieving pain related behaviours and postures associated with ring castration and tail docking in unweaned lambs, handled in mixed-gender groups, and released onto pasture immediately after marking.

Trials were conducted on commercial properties at "Castlebrook", Uralla near Armidale, NSW (April 2017) and "Fernleigh", Goorambat near Benalla, VIC (May 2017). At each site 150 lambs were allocated to 3 treatments to study the effect of marking by conventional ring applicator (Ring, n=60, males = 30, females = 30), the Numnuts® method with local anaesthetic (approximately 1.5 mL lignocaine) (Numnuts, n=60, males = 30, females = 30), or sham treated by placement in the marking cradle (Sham, n=30, males = 15, females = 15). Actual group sizes differed slightly between sites depending on the availability of male and female lambs. At each site lambs were treated in 10 cohorts of 15, balanced for treatment. Two cohorts were studied per day. Subjects were second cross lambs from Dorset sires over Merino X Border Leicester ewes.

In the VIC trial, mothering up was observed for 180 seconds following release of lambs into the observation paddock following marking. In both trials, acute pain behaviours were examined in 1 min epochs starting 5, 20, 35 and 50 min following marking. Subsequently, abnormal behaviours were scored at 10 min intervals from 60 to 180 min after marking. Body weights were recorded on day of marking and at 4 intervals of approximately 1 week spacing.

Data were combined for analysis of trends across sites. As site by treatment interactions were frequently observed, most data were also analysed within each site.

Mothering up: When data for genders were combined, there was a tendency for Numnuts lambs to mother up more quickly than Ring lambs (P=0.09), and more slowly (P=0.07) than Sham lambs. Ewe Numnuts lambs mothered up significantly faster than Ring lambs (P=0.015) and did not differ from Sham lambs (P=0.320)

Acute pain behaviours: When data for both genders and both sites were combined, Ring caused acute pain behaviours in male and female lambs throughout the 50 min of observation, in

comparison with Sham. In Ring treatment, the count of acute pain behaviours was greatest at 5 and 20 min post marking, and then diminished toward the level seen in Sham treated lambs but still remained significantly elevated at 50 min post marking. The Numnuts treatment significantly reduced acute pain behaviours at 5 and 20 min, in comparison with Ring.

Acute pain behaviours differed between sites and between genders. There were more acute pain behaviours in NSW at 5 and 35 min than in VIC, and more acute pain behaviours in males than females at 20, 35 and 50 min. In data for males combined across both sites, there were significantly fewer acute pain behaviours at 5 and 20 min in Numnuts than Ring lambs. For females, there were significantly fewer acute pain behaviours at 5, 20 and 35 min than for Ring lambs. For each gender, acute pain behaviours in Numnuts lambs remained significantly different from Sham at each time point.

In NSW, the gender by treatment interaction was not significant for acute pain behaviours, although there was a tendency for genders to differ. In accord with the absence of a gender by treatment interaction, when responses were examined within each gender, the trends were very similar. In Numnuts males, there was a significant reduction in acute pain behaviours at 5 min and a tendency for a reduction at 20 min, in comparison with Ring males. For Numnuts females there was a significant reduction at 5 min, and a non-significant reduction at 20 min. The lower level of statistical significance within genders is likely to reflect the reduced number of individuals in the separate analyses. Acute pain behaviours remained significantly higher in Ring marked lambs at 50 min. Thus there was an opportunity to detect a beneficial effect of Numnuts treatment on acute pain behaviours had it been present at 35 and 50 min.

In VIC, there was a significant gender by treatment interaction. In Numnuts males, there was a significant reduction in acute pain behaviours at 5 min and at 20 min in comparison with Ring males. For Numnuts females, there was a significant reduction in acute pain behaviours at 35 min and 50 min in comparison with Ring females. Ring and Numnuts females did not differ at 5 and 20 min.

Abnormal postures and behaviours at 60 – 180 min: When data for both genders and both sites were combined, Ring and Numnuts both caused abnormal behaviours at 60, 70, 80, 90 and 150 min, in comparison with Sham. In the combined data for abnormal behaviours, there were no significant differences between Ring and Numnuts at any time point between 60 and 180 minutes.

There was a tendency for abnormal behaviours to differ between sites. **In NSW**, there was a significant treatment by gender interaction. In Ring males, abnormal behaviours were elevated at 60 and 70 min, then did not differ from male Sham at later time points. Abnormal behaviours in Numnuts males did not differ from Ring males and were significantly elevated in comparison with male Sham at 60 and 70 min. The elevated count of abnormal behaviours at 60 and 70 min in Ring treatment indicates that there was an opportunity to detect a beneficial effect of Numnuts on behavioural response to marking in male lambs had it been present at these time points. Counts of abnormal behaviours in females did not differ between treatments. Thus there was no opportunity to detect a beneficial effect of Numnuts on abnormal behaviours in females from 60-180 min had it occurred.

In VIC, there was also a significant gender by treatment interaction. In Ring males, abnormal behaviours were significantly elevated at 60, 70, 80, 90 and 100 min, and were again elevated at 140 and 150 min in comparison to Sham. Numnuts were elevated at 60, 70, 80, and 90 min. In males, Numnuts was significantly different (lower) than Ring at 140 min but did not differ from Ring at other time points. Counts at 140 min were low, and the magnitude of the difference between Ring and Numnuts males was small. Counts of abnormal behaviours in females were low and did not differ between treatments.

Total lying: Some differences in combined lying behaviours were observed between treatments, with effects more pronounced in males than females. Ring and Numnuts males spent more time lying from 60 – 90 min than Sham lambs. No informative differences were seen between Numnuts and Ring lambs.

Body weight and average daily gain. Bodyweight on the day of marking had little or no influence on behavioural responses to marking. Treatment did not affect subsequent body weight or average daily gain in either trial.

Adverse reactions. Two lambs in the VIC trial exhibited transient hind limb ataxia for several min following the Numnuts treatment. Ataxia probably resulted from anterograde diffusion of lignocaine in the epidural space subsequent to the tail injection.

Conclusions: By the methods of detection used in the study, the Numnuts tool had similar efficacy in reducing pain both in male and in female lambs in the first 20 – 50 min following ring marking. Very similar responses were seen in males at both sites, whereas responses in females were somewhat more variable. The study found that central deposition in the neck of the scrotum of a single injection of approximately 1.5 mL lignocaine together with injection of local anaesthetic into the tail provided significant pain relief when ring marking male lambs. The benefit of marking with the Numnuts tool previously seen in female lambs in a trial at Moredun Research Institute Scotland was confirmed in the current trial. The transience of the pain relief seen in the current trial is in accord with the known pharmacodynamics of lignocaine. 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. The type of 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 benefits from the use of Numnuts 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, a combination of local anaesthetics (e.g. lignocaine + bupivacaine), inclusion of adrenaline with the local anaesthetic or injection of an LA + NSAID combination treatment.

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1 Background

Castration and tail docking are routine husbandry procedures applied to almost all lambs, and are recognised as painful procedures. Ring castration and tail docking are increasingly popular in comparison with incisional methods, due to the reduced risk of haemorrhage, wound infection and fly strike. Notwithstanding external 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.

4cDesign, in collaboration with the Moredun Research Institute and CSIRO, has designed a tool that provides consistent delivery of local anaesthetic to the site of ring application, with a shielded needle arrangement to reduce the risk of self-injection (Smith et al., 2017). The Numnuts® tool also acts 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.

The prototype tool has been evaluated in the field, by farmhands experienced in lamb castration and tail docking, and positive feedback regarding ergonomics and ease of use, as compared with the existing ring applicator tool has been received. Excellent analgesia has been demonstrated in female lambs for tail docking (Smith et al., unpublished data presented at the international Sheep Veterinary Congress 2017, Harrogate, UK); however the analgesia for castration appears to be limited, as assessed using pen studies (Jongman et al., 2016). Early proof-of-concept studies did indicate a potential benefit to castrated lambs: these studies were small-scale, and carried out in a paddock environment. It may be that the barren pen environment, providing no distractions, encourages the lambs to focus on the injury, while in a paddock environment, treated lambs may be more willing to perform normal behaviours than untreated lambs. Furthermore, a study considering the combined insult of tail-docking and castration in male lambs has not yet been carried out.

This study is a field-based evaluation of the welfare effects of providing local anaesthesia (Lignocaine 2%) using the Numnuts® prototype v12 tool to the scrotal neck (males) and tail (males and females) of lambs undergoing marking in mixed-gender groups.

2 Project objectives

To evaluate the efficacy of Local Anaesthetic (Lignocaine 2%), delivered using the Numnuts® v12 tool in relieving pain related behaviours and postures associated with ring castration and tail docking in unweaned lambs.

3 Methodology

3.1 Study Design

The study was a blinded controlled randomized block design field study, incorporating detailed individual behavioural pain indicators. It was conducted at two trial sites:

- “Castlebrook”, Uralla near Armidale, NSW (April 2017); and
- “Fernleigh”, 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 1714158.

At each trial site, 150 lambs (75 male and 75 female, aged between 4 and 10 weeks) 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. 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 replicates of each treatment (Table 1). Two cohorts were studied per day.

Table 1: Treatment groups

Code	Description	Lamb gender	Target replicates (NSW)	Target replicates (VIC)
A	Sham	M/F	30	30
B	Tail only with LA (Numnuts)	F	30	30
C	Tail only no LA (Ring)	F	30	30
D	Castrate + tail with LA (Numnuts)	M	30	30
E	Castrate + tail, no LA (Ring)	M	30	30
	Subtotal lambs		**150**	**150**
	Total lambs		**300**	

On day 0, ewes and lambs were separated, and the ewes placed into the observation paddock, while the lambs were held in the treatment pen. Lambs were individually captured, gender identified and weighed prior to marking. 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 (Figure 1). The lamb was then released into the observation paddock. Lambs were treated at three-min intervals. Lambs were evaluated for behaviour in a paddock situation post marking. Three observers, located in a gazebo located in the centre of the observation paddock (Figure 2), counted the number of acute pain behaviours, according to a pre-determined ethogram (Table 2), expressed during a 1-min period at 5 min, 20 min, 35 min and 50 min post treatment, then took snapshot postural observations, according to a pre-determined ethogram (Table 3), at 60 min and every 10 min thereafter, until 180 min from treatment had elapsed. Examples of observation recording sheets are presented in the appendix, section 9.

Figure 1: Lamb side branded for visual identification from a distance

Figure 2: Recording the behavioural responses of lambs during the study

The Numnuts® tool delivered 1.5 mL of 20 mg/mL Lignocaine hydrochloride (Ilium Lignocaine 20, Troy laboratories Pty Ltd. Batch 161020, expiry Oct 2018) on each use. 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 seconds 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 seconds were recorded as 'failed to mother-up'.

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

Table 2: Ethogram of Active Pain Avoidance behaviours for counts over 1 min blocks

Behaviour	Abbreviation	Description
Active pain avoidance		
Restlessness	Rst	Lamb stood up and laid down. Instances of lamb rising as far as its knees included.
Kicking/foot 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	Lamb rolled from lying on one side to the other without getting up. Half rolls where the lamb rolled on its back and then returned 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 snapshot observations

Behaviour	Abbreviation	Description
Postural behaviours		
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	Lamb was 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	Lamb was 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	Grazing
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	Lamb was lying ventrally but unable to clearly categorise the lying posture.
Rolling	rl	Lamb rolled from lying on one side to the other without getting up. Half rolls where the lamb rolled on its back and then returned to lying on the same side included.

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

3.1.1 Statistical analyses

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).

Mothering up

In the VIC trial, mothering up was assessed for 180 seconds 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 and Grambsch, 2000, Therneau, 2015) 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. The data were analysed in two steps. Firstly, the 'survdiff' function was used to assess differences between the survival curves for each of the treatments. This function generates a log-rank test that compares the curves. A cox proportional hazards model was then conducted on the treatments. This method considers explanatory variables that affect the hazard of an event happening. From the fitted model, hazard ratios can be predicted to investigate the effects of different factors on whether or not an animal was likely to mother up. Hazard ratio values are positive values ranging from zero to infinite. A hazard ratio of >1 indicates a higher likelihood of re-joining with its mother compared with the reference level for each categorical explanatory variable. Values between 0 and 1 indicate a lower likelihood of joining its mother compared with the reference level. Note that the use of the term hazard in survival analysis does not necessarily imply a deleterious outcome and, in this study, the hazard refers to the lambs re-joining their mothers.

Behaviours and weights

Data which were normally distributed or that could be transformed to satisfy normal distribution, was 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, gender 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 that was not able to be normalised by transformation were analysed with a non-parametric test within time points.

4 Results

4.1 Adverse reactions

Two lambs in the VIC trial exhibited transient hind limb ataxia for several min following Numnuts treatment. Ataxia probably resulted from anterograde diffusion of lignocaine in the epidural space.

4.2 Mothering up

4.2.1 Both genders combined

Time to mother up was assessed in the VIC trial. By the log rank test on data from both genders combined, there was a statistically significant difference between “survival” curves describing success in mothering up (P=0.006). There was a tendency for Numnuts 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 Numnuts treatment (P=0.09), and for Sham to be 1.5 times more likely to mother up than Numnuts (P=0.07).

Table 4 shows the chance of not mothering up in 180 sec after release into the observation pen for each treatment. The Numnuts treatment group had an 18% chance of not mothering up in the 3 min interval, Ring had a 28% chance and Sham a 6% chance of not mothering up. The Kaplan-Meier plot (Figure 3) shows the time of each mothering up event and the proportion of lambs that failed to mother up.

Table 4: Mothering up data from the VIC trial

	records	Successful mothering up events	0.95 Lower Confidence Limit	0.95 Upper Confidence Limit	Chance of not mothering up (%)	Median time to mother up (sec) (excluding failures)
Numnuts	61	50	20	42	18	26
Ring	57	41	28	97	28	40
Sham	32	30	16	33	6	18

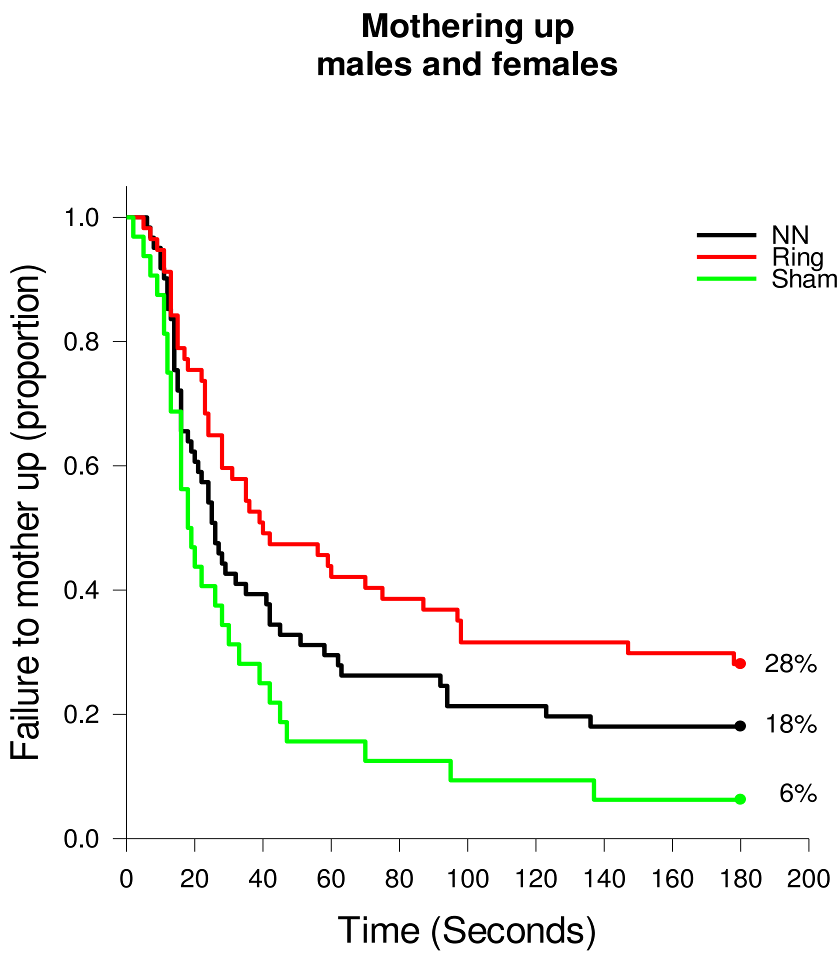


Figure 3: Kaplan-Meier curves for mothering up for all lambs. Every time a lamb joined its mother, the proportion on the Y axis drops.

4.2.2 Female lambs

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

Table 5 shows that for female lambs there was a 38% chance of not mothering up with ring but only 10% for Numnuts and no chance for Sham. The Kaplan-Meier plot (Figure 4) shows the time of each mothering up event and the proportion of lambs that failed to mother up.

Table 5: Mothering up data for female lambs

	records	Successful mothering	0.95 Lower Confidence	0.95 Upper Confidence	Chance of not mothering up	Median time to mother up (sec)

		up events	Limit	Limit	(%)	(excl. failures)
Numnuts	29	26	16	32	10	24
Ring	26	16	24	NA	38	40
Sham	15	15	13	45	0	19

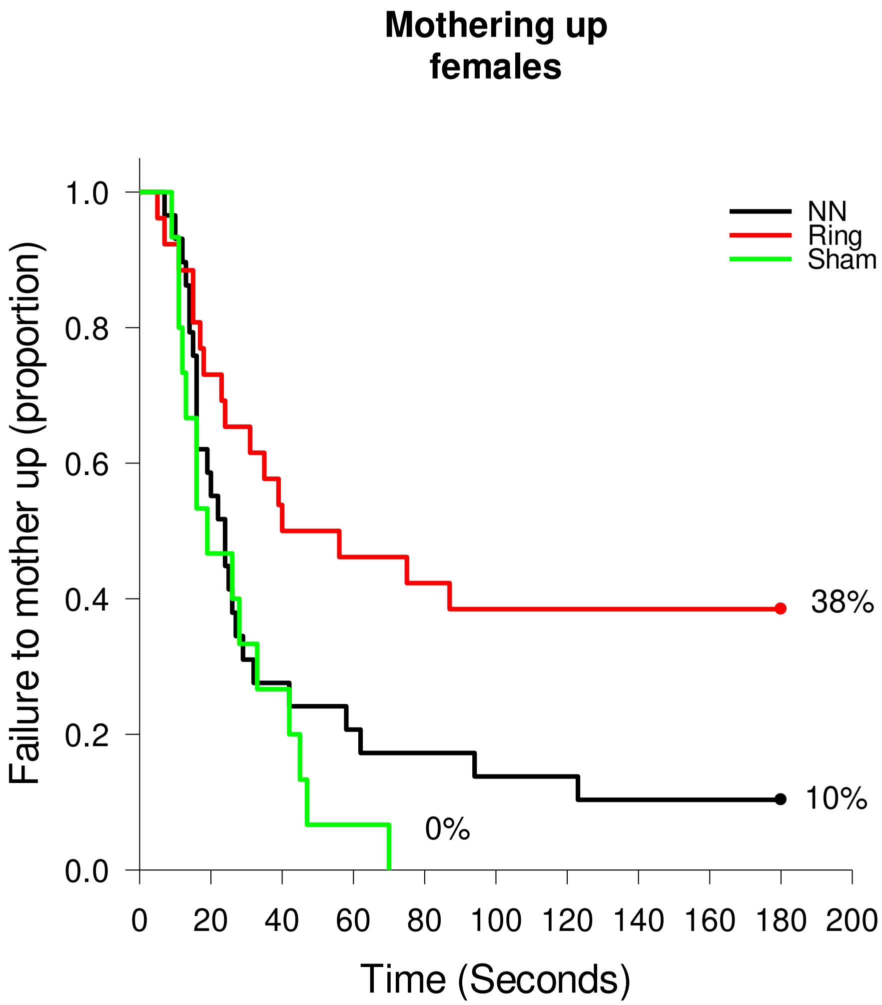


Figure 4: Kaplan-Meier curves for mothering up of female lambs only. Every time a lamb joined its mother, the probability on the Y axis drops.

4.2.3 Male lambs

There were no significant differences between treatments for males. Ring males had 0.995 times less chance of mothering up than Numnuts treatment and this was not significant (P=0.986), while sham lambs were 1.61 times more likely to mother up than Numnuts lambs but this again was not significantly different (P=0.148).

Table 6 shows that there was a 19% chance of not mothering up for Ring, 25% for Numnuts and 12% for sham. These were not significantly different.

The Kaplan-Meier plot (Figure 5) shows the time of each mothering up event and the proportion of lambs that failed to mother up.

Table 6: Mothering up data for male lambs

	records	events	0.95 Lower Confidence Limit	0.95 Upper Confidence Limit	Chance of mothering up (%)	Median time to mother up (sec) (excl. failures)
Numnuts	32	24	21	94	25	35
Ring	31	25	24	98	19	36
Sham	17	15	16	95	12	18

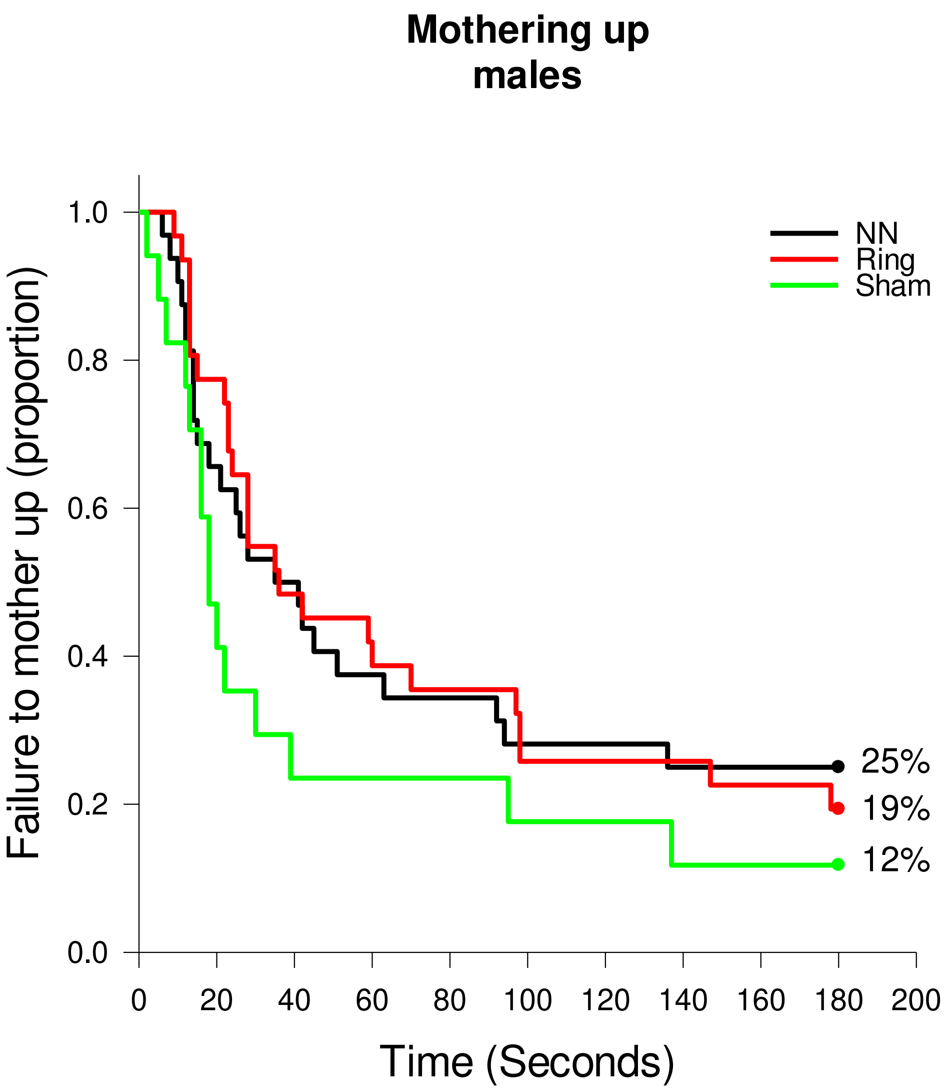


Figure 5: Kaplan-Meier curves for mothering up of male lambs only. Every time a lamb joined its mother, the probability on the Y axis drops.

4.3 Acute pain behaviours

Data were square root transformed for analysis to normalise distributions. Values in graphs are back transformed least squares means, and thus, error bars are not represented.

4.3.1 Combined data for NSW and VIC trials

Data from both trials and genders were combined to examine overall effects of treatments on all lambs. Trial site (NSW or VIC; P=0.027) gender (male or female; P=0.001) and treatment (Numnuts, 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 (P=0.025), time by gender (P=0.008) and time by treatment (P<0.001).

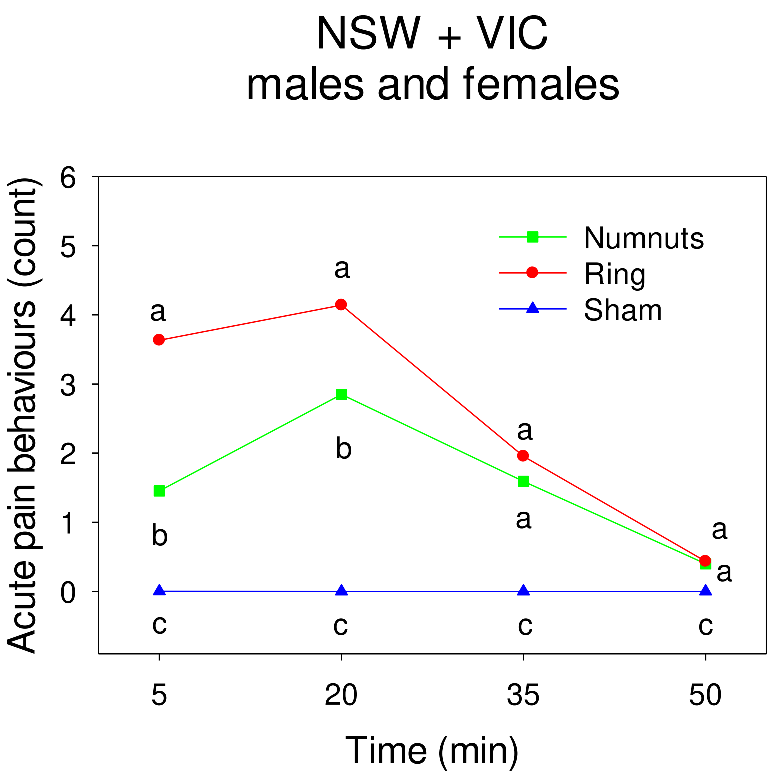
Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.001). Numnuts was significantly different from Ring at 5 min (P<0.001) and 20 min (P=0.001). Ring and Numnuts did not differ at 35 or 50 min (Figure 6).

The significantly greater number of acute pain behaviours in Ring treatment than Sham treatment at 35 and 50 min indicates that the behavioural observation method was sufficiently sensitive to detect a strong effect of Numnuts at these time points should it have occurred.

There were more acute pain behaviours displayed in NSW than VIC at 5 min (P=0.003) and 35 min (P=0.042) (Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.

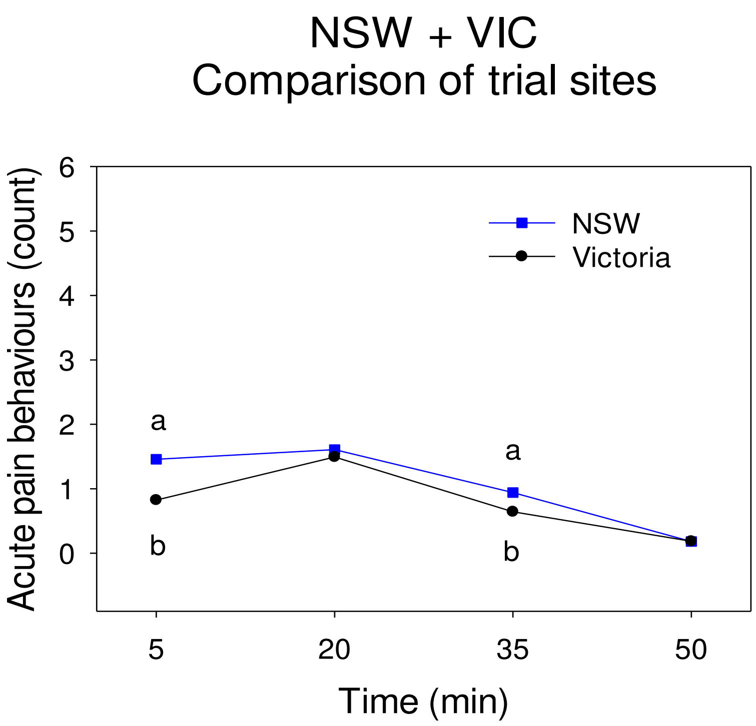
a, b: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 7). 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) (Figure 8).



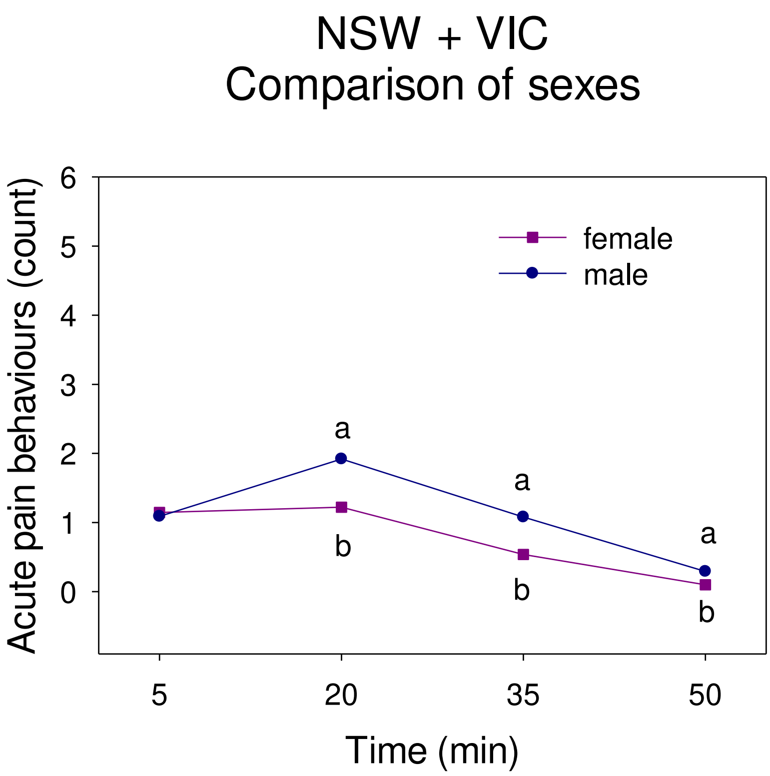
Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 6: Acute pain behaviours expressed by lambs, all data pooled across both trial sites and both genders.



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 7: Acute pain behaviours expressed by lambs at each trial site. Plotted data include Sham lambs



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 8: Acute pain behaviours expressed by lambs of each gender. Plotted data include Sham lambs

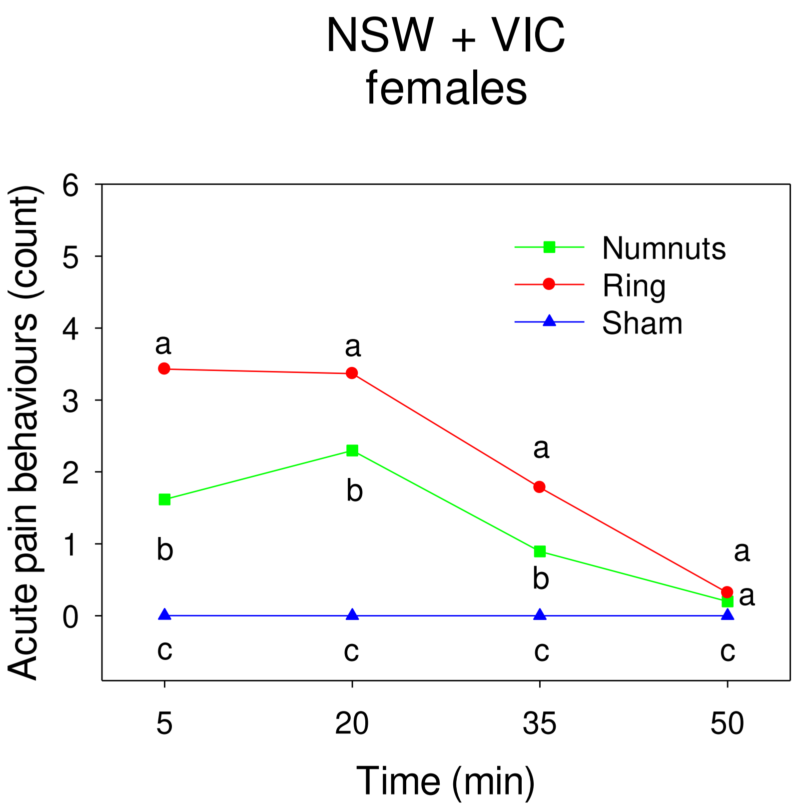
4.3.2 Responses of females for data combined from both trials

There was a significant effect of treatment (P<0.001) and time (P<0.001) on acute pain behaviours expressed by female lambs, and a tendency for responses to differ between sites (P=0.074). 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 Numnuts treatments were significantly different from Sham at all time points (P<0.001). Numnuts was significantly different from Ring at 5 min (P=0.001), 20 min (P=0.042) and 35 min (P=0.007). Ring and Numnuts did not differ at 50 min (Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.

a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 9).



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 9: Acute pain behaviours expressed by female lambs, data from both trial sites pooled

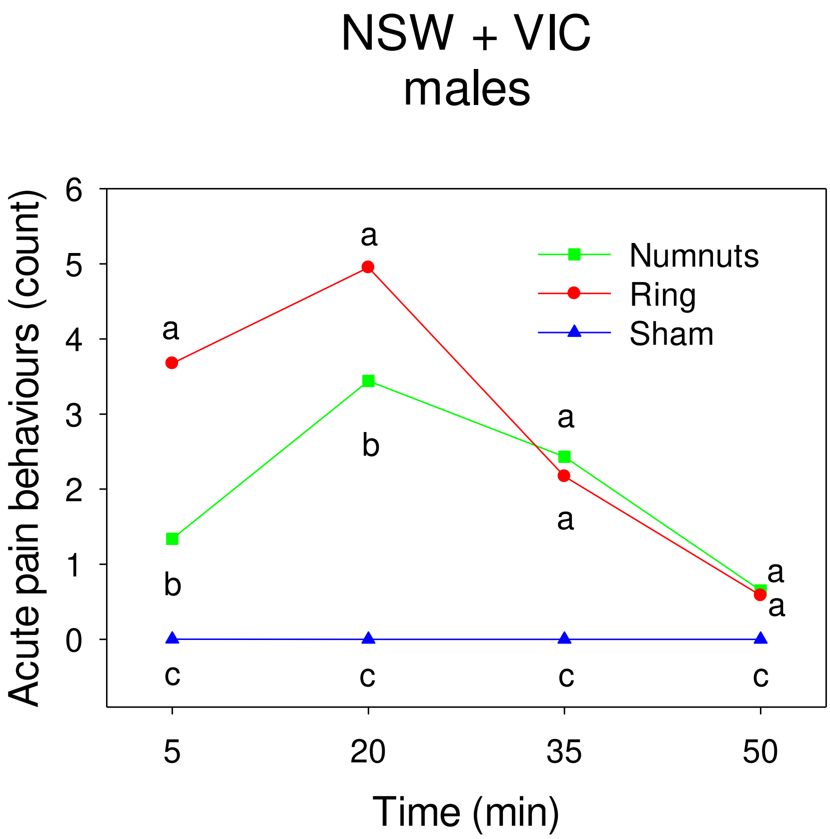
4.3.3 Responses of males for data combined from both trials

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) between time and bodyweight (P=0.007).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.001). Numnuts was significantly different from Ring at 5 min (P=0.001), 20 min (P=0.007). Ring and Numnuts did not differ at 35 or 50 min (Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.

a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 10).



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

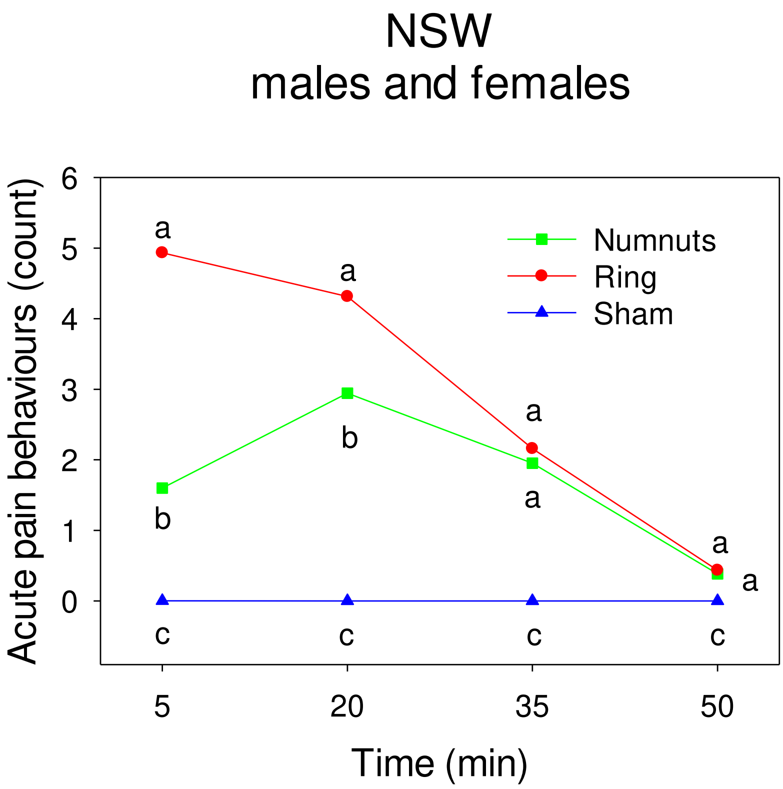
Figure 10: Acute pain behaviours expressed by male lambs, data from both trial sites pooled

4.3.4 NSW Trial, both genders combined

In the NSW trial, there was a tendency for gender to influence acute pain behaviours (P=0.071). There as significant effect of treatment (P<0.001) and time (P=0.001) and significant interactions between time by body weight (P=0.001) and time by treatment (P<0.001).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.001). Numnuts was significantly different from Ring at 5 min (P<0.001) and 20 min (P=0.015). Ring and Numnuts did not differ at 35 or 50 min (Figure 11).

The significantly greater number of acute pain behaviours in Ring treatment than Sham treatment at 35 and 50 min indicates that the behavioural observation method was sufficiently sensitive to detect a strong effect of Numnuts at these time points should it have occurred.



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 11: Acute pain behaviours expressed by lambs at the NSW trial site, data from both genders pooled

4.3.5 NSW Males

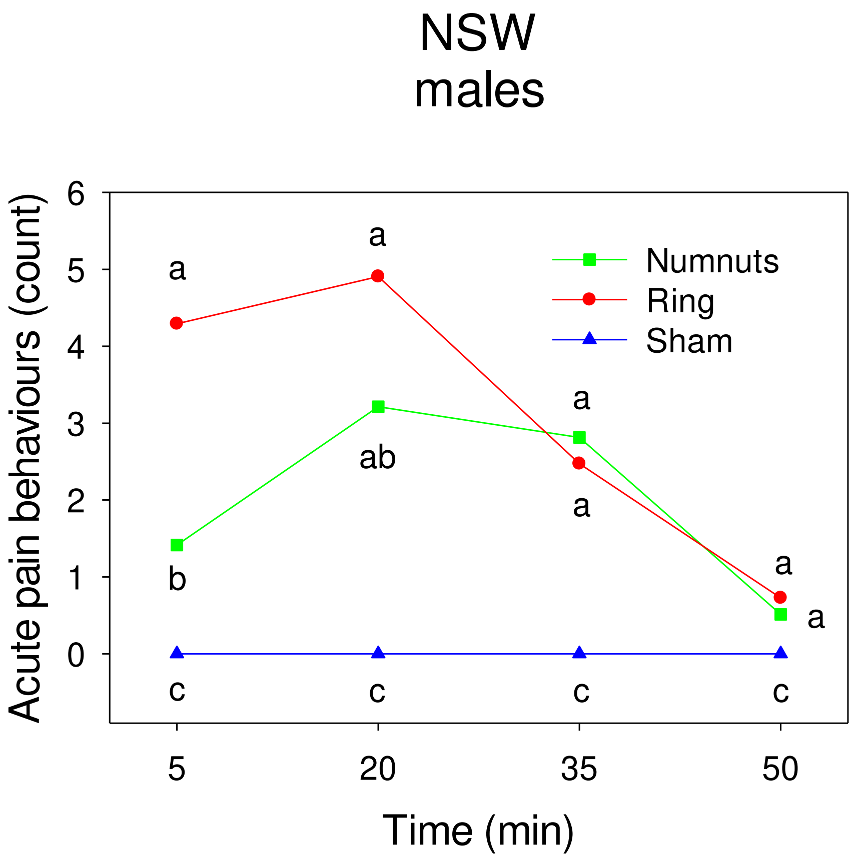
For male lambs at the NSW trial site, there was a significant effect of treatment (P<0.001) and time (P=0.001) and significant interactions between time by body weight (P=0.016) and time by treatment (P<0.001).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.003). Numnuts was significantly different from Ring at 5 min (P<0.001). At 20 min there was a tendency for Numnuts to be different from Ring (P=0.067). Ring and Numnuts did not differ at 35 or 50 min (Figure 12).

The significantly greater number of acute pain behaviours in Ring treatment than Sham treatment at 35 and 50 min indicates that the behavioural observation method was sufficiently sensitive to detect a strong effect of Numnuts at these time points should it have occurred.

There was doubt whether 6 Numnuts males received the full LA injection. Results for these animals did not differ from results for the 24 other Numnuts males in the NSW trial (P=0.779).

There was a slightly negative correlation between body weight and acute pain behaviours at 5 min in Ring (P=0.024) and Numnuts (P=0.033) male lambs.



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

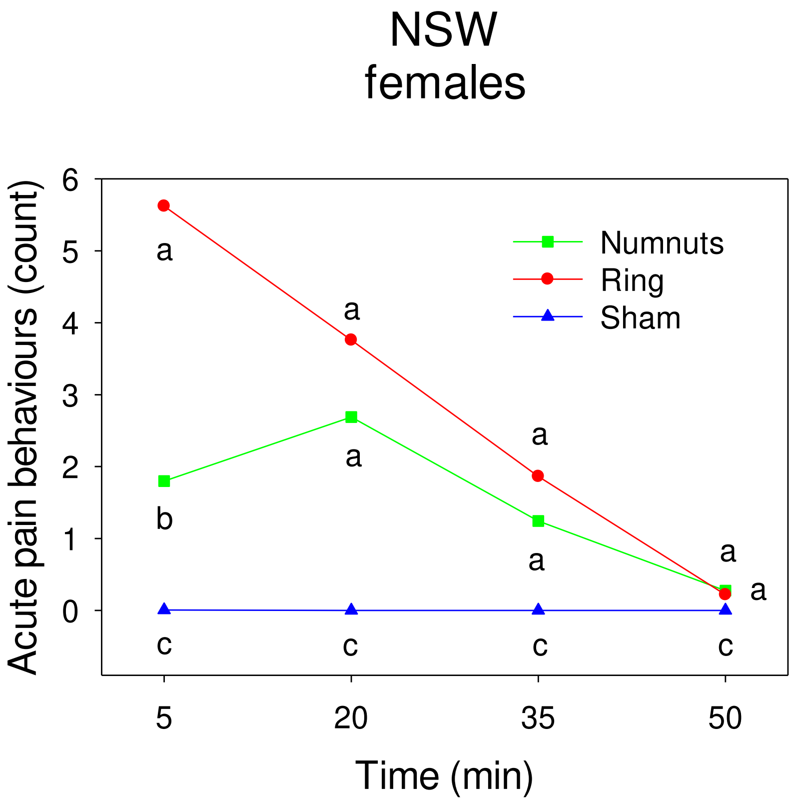
Figure 12: Acute pain behaviours expressed by male lambs at the NSW trial site

4.3.6 NSW Females

There was a significant effect of treatment (P<0.001) and time (P=0.001) and a significant interaction between time by treatment (P<0.001).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.030). Numnuts was significantly different from Ring at 5 min (P<0.001). Ring and Numnuts did not differ at 20, 35 or 50 min (Figure 13).

The significantly greater number of acute pain behaviours in Ring treatment than Sham treatment at 20, 35 and 50 min indicates that the behavioural observation method was sufficiently sensitive to detect a strong effect of Numnuts at these time points should it have occurred.



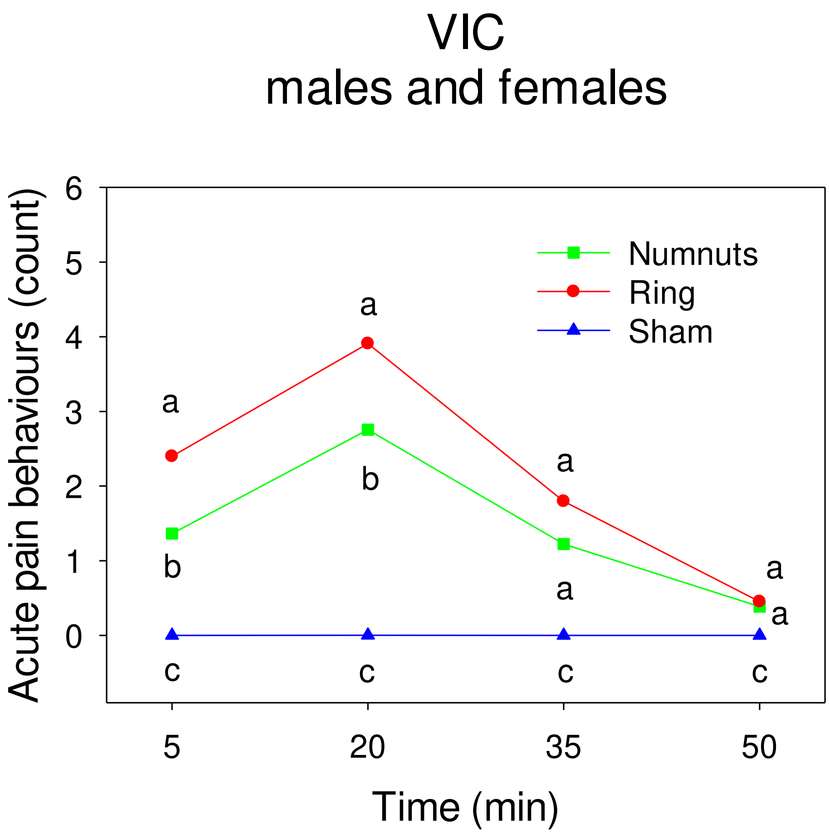
Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 13: Acute pain behaviours expressed by female lambs at the NSW trial site

4.3.7 VIC Trial, both genders combined

At the VIC trial site, there were significant effects of treatment (P<0.001), gender (P=0.008), body weight (P=0.036) and time (P=0.025) and significant interactions between time by treatment (P<0.001) and between time by gender by treatment.

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.001). Numnuts was significantly different from Ring at 5 min (P<0.018) and 20 min (P=0.013), and a tendency for Numnuts to be different from Ring at 35 min (P=0.072). Ring and Numnuts did not differ at 50 min (Figure 14).



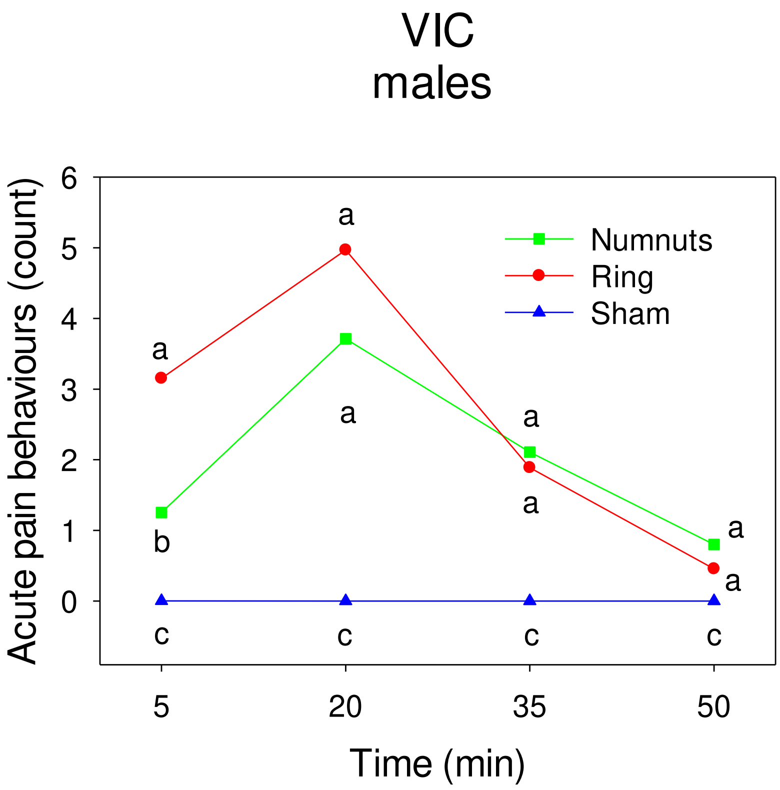
Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 14: Acute pain behaviours expressed by lambs at the VIC trial site, data from both genders pooled

4.3.8 VIC Males

For male lambs at the VIC trial site, there was a significant effect of treatment (P<0.001) and time (P=0.001) and a significant interaction between time and treatment (P<0.001).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.003). Numnuts was significantly different from Ring at 5 min (P<0.002). At 20 min there was a tendency for Numnuts to be different from Ring (P=0.051). Ring and Numnuts did not differ at 35 or 50 min (Figure 15).



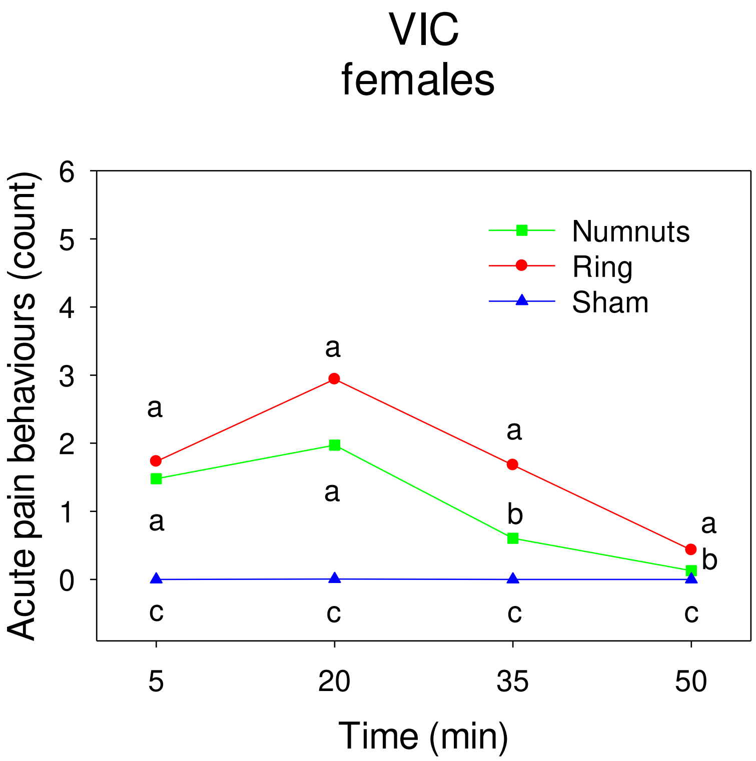
Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

Figure 15: Acute pain behaviours expressed by male lambs at the VIC trial site

4.3.9 VIC Females

In female lambs at the VIC trial site, there was a significant effect of treatment (P<0.001) and body weight (P=0.041). Time was not significant, however there was a significant interaction between time and treatment (P=0.005).

Ring and Numnuts treatments were significantly different from Sham at all time points (P<0.044). Numnuts was significantly different from Ring at 35 min (P=0.016) and at 50 min (P=0.039). At 5min and 20 min Numnuts did not different from Ring (Figure 16).



Back transformed count of acute pain behaviours in 1 min epochs starting 5, 20, 35 and 50 min post-marking.
a, b, c: Means within a time interval with different superscripts are significantly different (P < 0.05)

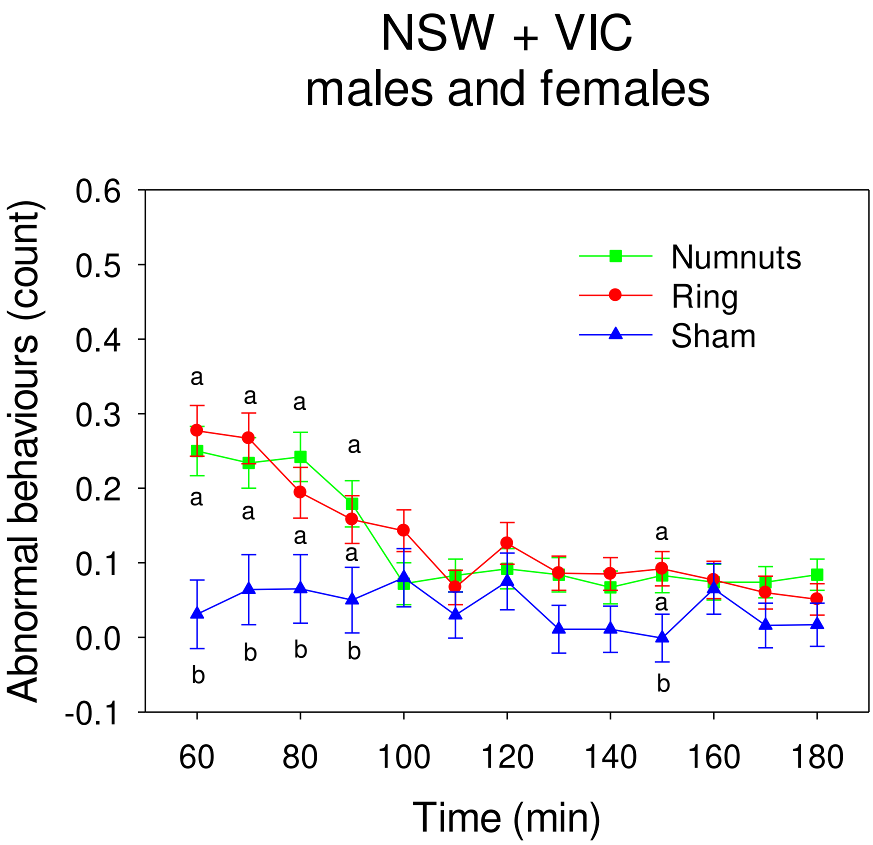
Figure 16: Acute pain behaviours expressed by female lambs at the VIC trial site

4.4 Abnormal postural behaviours between 60 min and 180 min

4.4.1 Data combined for both sites and both genders

For data combining results for both sites from males and females, there was a significant effect of treatment (P<0.001), gender (P<0.001) and time (P < 0.001) on abnormal postural behaviours, an interaction between gender and treatment (P=0.002) and a tendency for a difference to occur between sites (P=0.085). There was an interaction between time and treatment (P<0.001), time and gender (P<0.001), time by gender by treatment (P<0.001) and between time and cohort nested within trial site (P=0.014).

Ring and Numnuts treatments differed from Sham at 60, 70, 80, 90 and 150 min (P<0.048). Ring and Numnuts did not differ at any time point between 60 and 180 min (Figure 17).



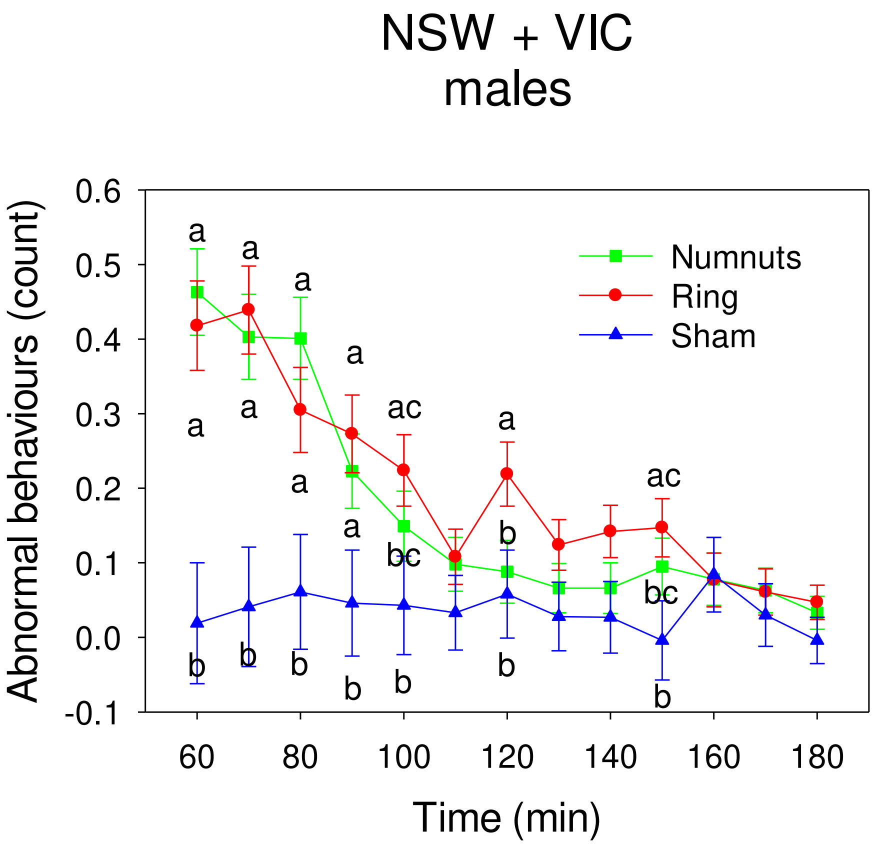
Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 17: Total abnormal postures expressed by lambs, data from both trial sites and both genders pooled

4.4.2 Males lambs, data combined for both sites

For data combining results for both sites from males, 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 Numnuts 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. Numnuts differed from ring at 120 min (Figure 18).

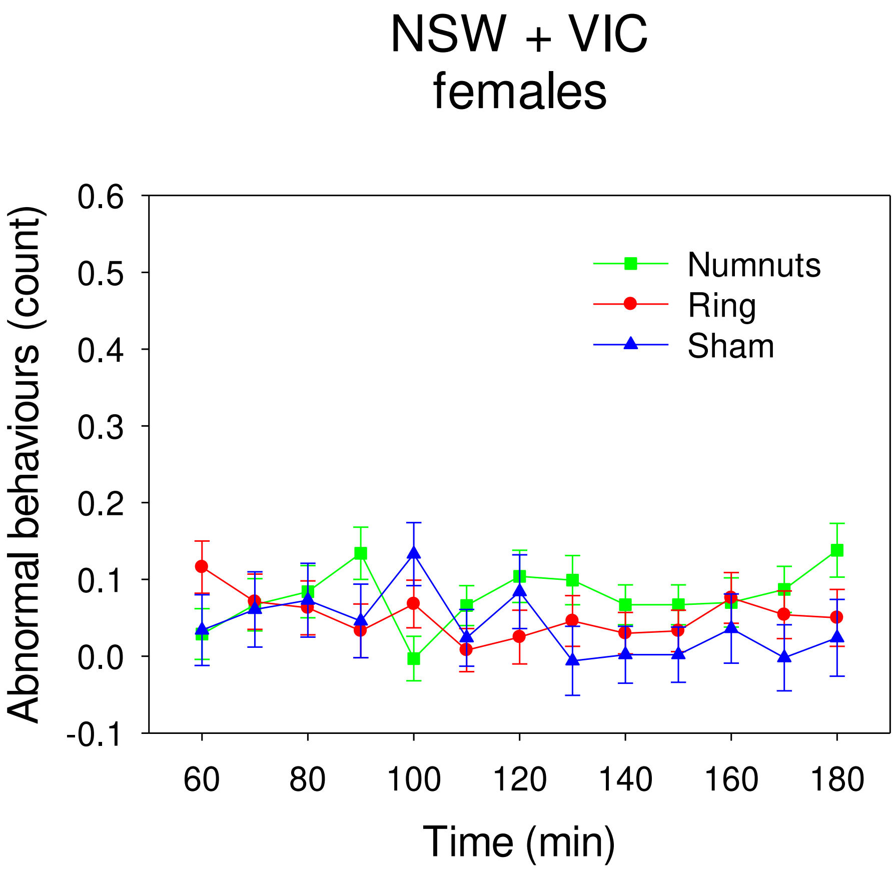


Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 18: Total abnormal postures expressed by male lambs, data from both trial sites pooled

4.4.3 Female lambs, data combined for both sites

The combined 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 (Figure 19).



Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 19: Total abnormal postures expressed by female lambs, data from both trial sites pooled

4.4.4 NSW Trial, both genders combined

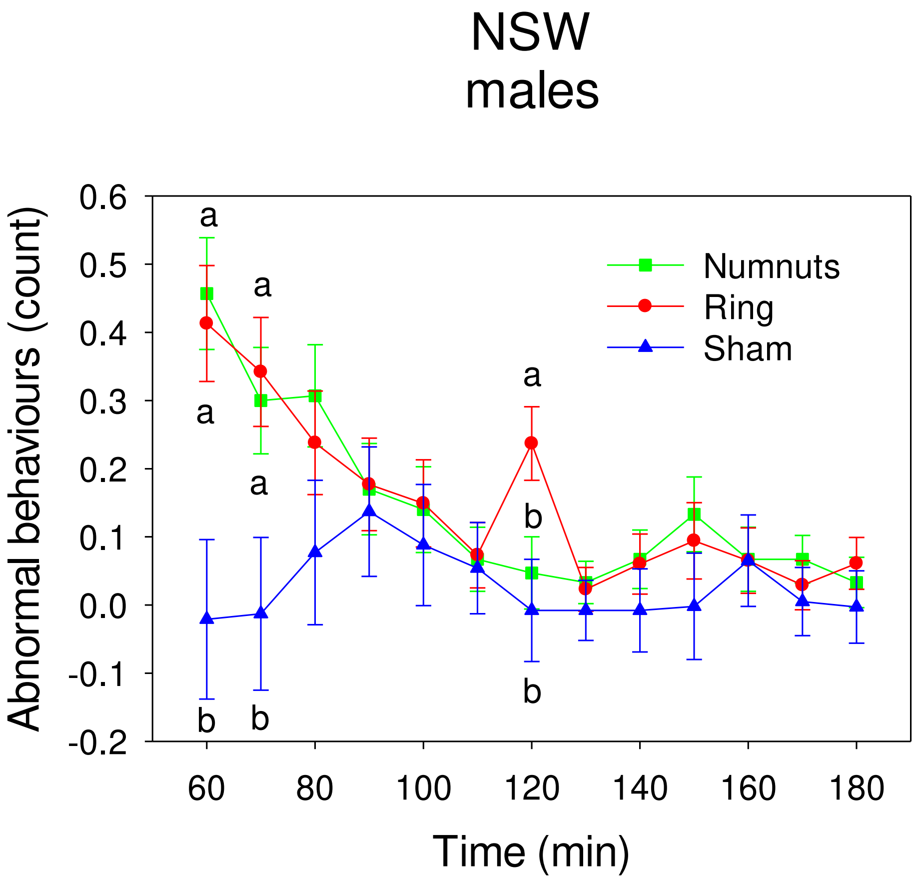
For data combining results from males and females at the NSW trial site, there was a significant effect of treatment (P<0.008), gender (0.024) and time (P < 0.001) on abnormal postural behaviours, and significant interactions between time by gender (P = 0.006) and a time by gender by treatment (P=0.002). In view of the time by treatment by gender interaction, males and females were examined separately.

4.4.5 NSW Males

In male lambs at the NSW trial site, there were significant effects of treatment (P=0.012) and time (P<0.001) and significant interactions between time by treatment (P=0.029) and time by cohort (P=0.050).

Ring lambs expressed significantly more abnormal postural behaviours at 60 (P=0.003), 70 (P=0.11) and 120 (P=0.009) min than the male Sham lambs. At 60 (P=0.001) and 70 (p=0.024) min, the Numnuts males expressed significantly more abnormal postural behaviours than male Sham lambs. Numnuts was not significantly different from Ring at 60 (P=0.711) or 70 (P=0.706) min, and showed significantly fewer abnormal postural behaviours than Ring at 120 min (P=0.014) (Figure 20).

Thus there were 2 time points (60 min and 70 min) in the interval from 60 min to 180 min when the behavioural observation methods was sufficiently sensitive to detect an impact of ring castration and tail docking without analgesia on abnormal postural behaviours. The increase in abnormal postural behaviours at 120 min appears to be an anomaly in the data as it does not appear to be part of a consistent trend, and therefore deserves no further attention. Numnuts treatment provided no observable benefit at 60 and 70 min.



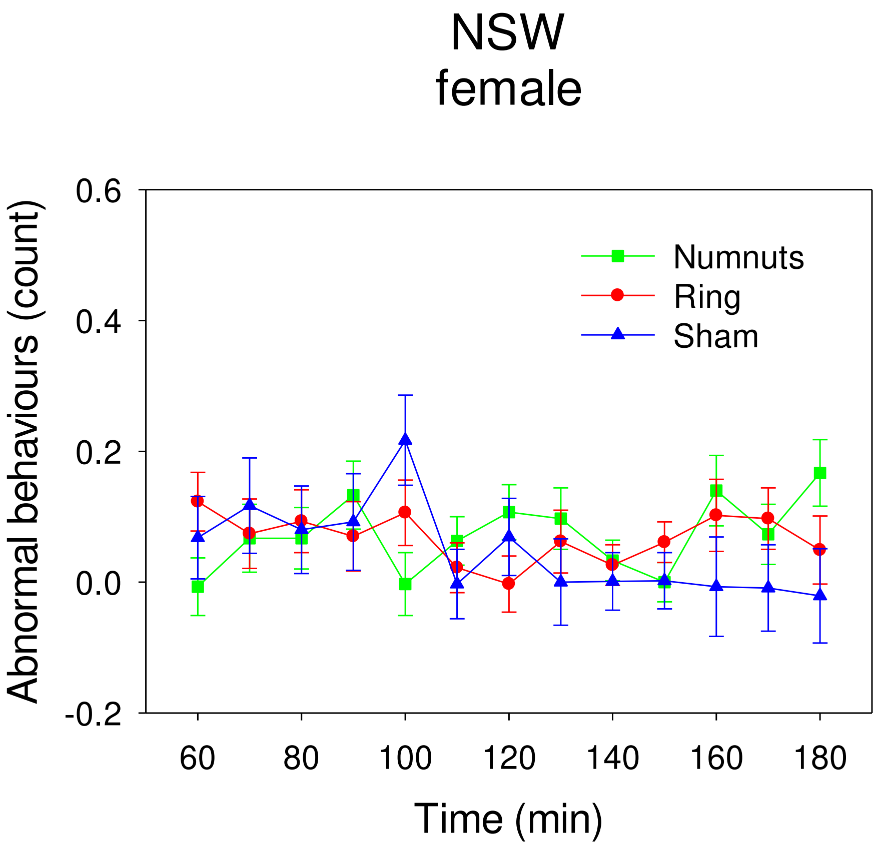
Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 20: Total abnormal postures expressed by male lambs at the NSW trial site

4.4.6 NSW Females

There was a significant effect of cohort (P=0.013), but no effect of time (P=0.600), treatment (P=0.633) or their interaction (P=0.118) on abnormal postural behaviours between 60 min and 180 min in female lambs at the NSW trial site (Figure 21).

The result indicates that in the interval from 60 min to 180 min the behavioural observation methods were not sufficiently sensitive to detect an impact of ring tail docking without analgesia on abnormal postural behaviours. In the absence of an observable effect of ring tail docking on behaviour in this period, there was no opportunity to observe a possible benefit of Numnuts.



Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

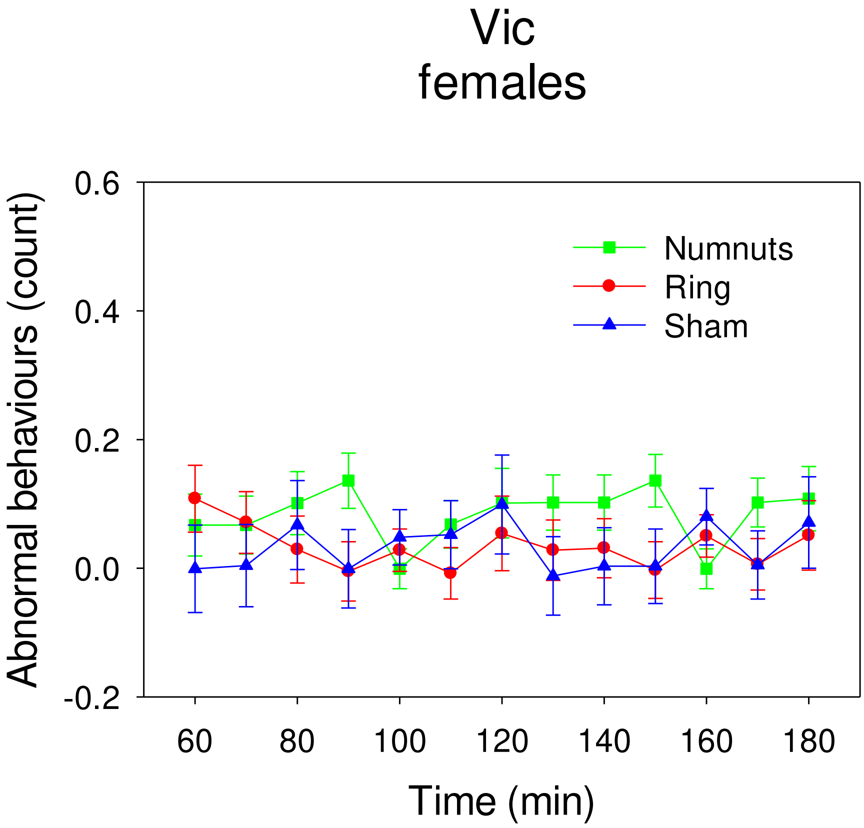
Figure 21: Total abnormal postures expressed by female lambs at the NSW trial site

4.4.7 VIC Trial, both genders combined

For data combining results from males and females for the VIC trial, there was a significant effect of treatment (P<0.003), gender (0P<0.001) and time (P < 0.001) on abnormal postural behaviours, and significant interactions between treatment and gender (P=0.011), time by treatment (P=0.027), time by gender (P < 0.001), time by cohort (P=0.005) and a tendency for time by gender by treatment (P=0.051). In view of the time by gender and treatment by gender interactions, males and females were examined separately.

4.4.8 VIC Females

Data for female lambs at the VIC trial site were not normally distributed and were not normalised by transformation. Non parametric analysis by Kolmogorov-Smirnov test detected no differences between treatments. Counts of abnormal behaviours between 60 and 180 min were very low (Figure 22).



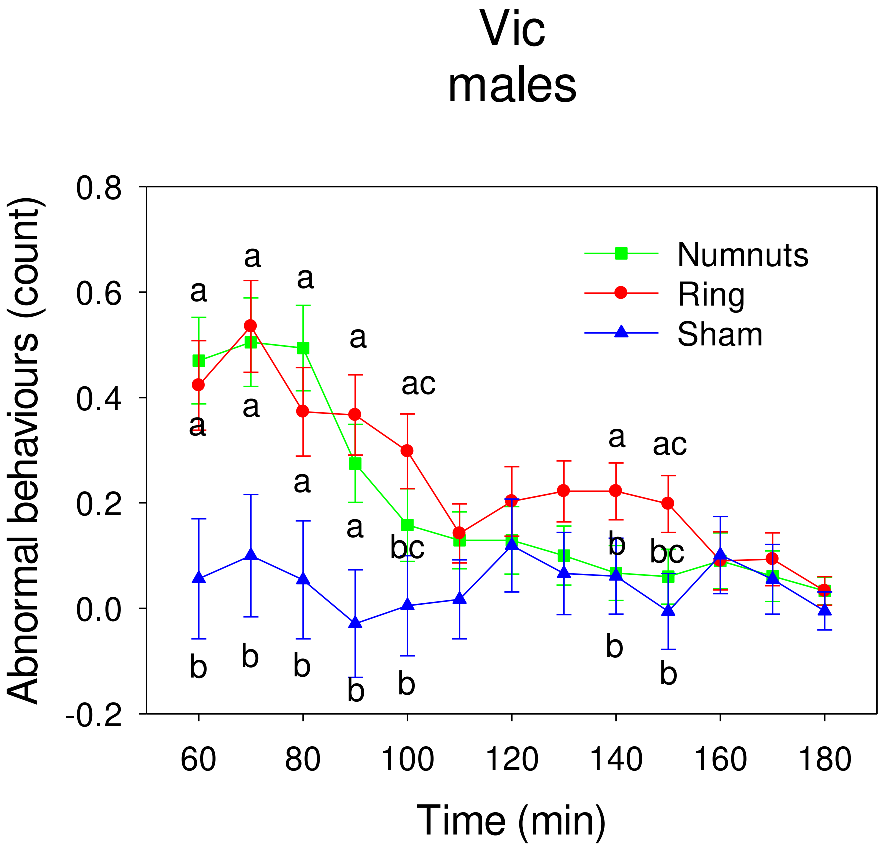
Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 22: Total abnormal postures expressed by female lambs at the VIC trial site

4.4.9 VIC Males

There was a significant effect of treatment (P=0.002) and time (P<0.001) on abnormal postural behaviours, and significant interactions between time by treatment (P=0.002) and time by cohort (P=0.027).

Ring treatment had significantly more abnormal postural behaviours at 60, 70, 80, 90, 100, 140 and 150 min than male Sham treatment. At 60, 70, 80, and 90 min Numnuts treatment had significantly more abnormal postural behaviours than male Sham treatment. Numnuts was significantly different from Ring at 140 min. (P=0.042) (Figure 23).



Back transformed count of abnormal postural behaviours at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 23: Total abnormal postures expressed by male lambs at the VIC trial site

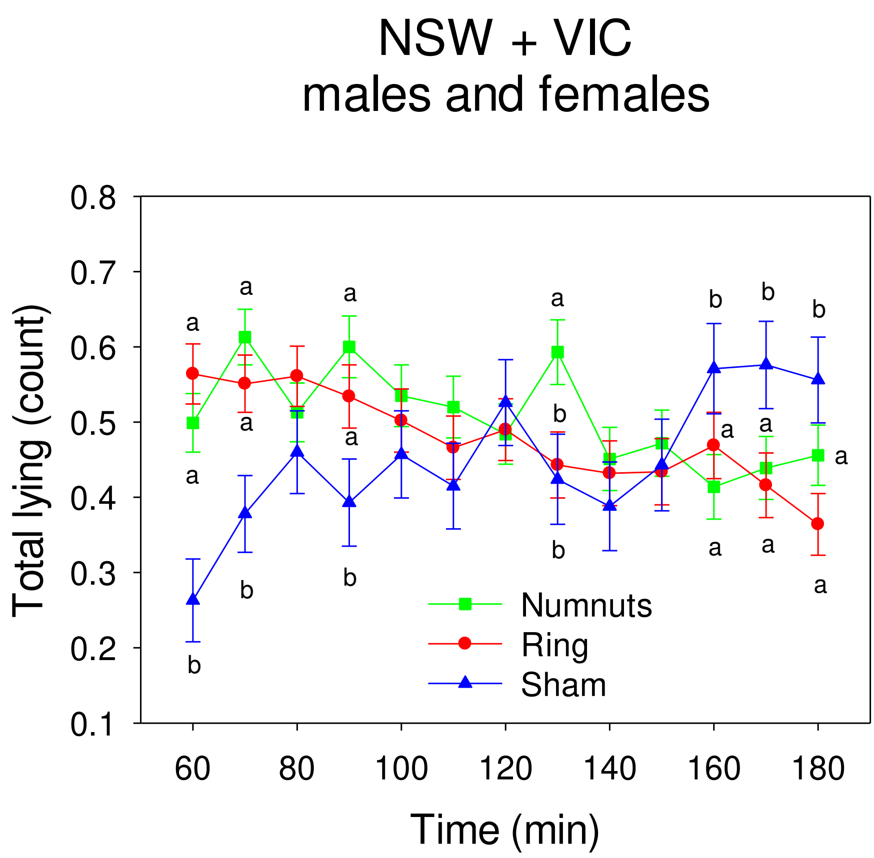
4.5 Total Lying

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.

4.5.1 Combined data for both trial sites and both genders

For data combining results for both trial sites from males and females, there was a significant effect of trial site (P<0.001), gender (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 gender (P<0.001), time by gender by treatment (P=0.001), time by gender by trial (P=0.003) and time by cohort within trial (P<0.001).

Ring and Numnuts treatments differed from Sham at 60, 70, 90, 160, 170 and 180 min. Ring and Numnuts differed at 130 min (P=0.015) (Figure 24).



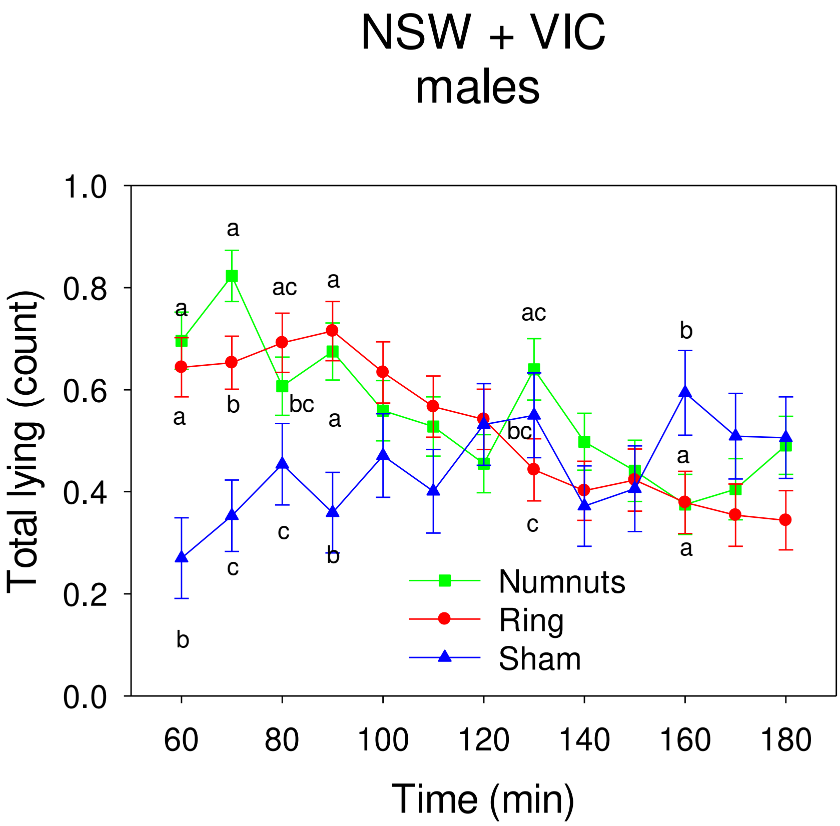
Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b: Data points with different superscripts are significantly different (P < 0.05)

Figure 24: Total count of lying postures expressed by lambs, both trial sites and both genders combined

4.5.2 Combined data for males from both trial sites

For data combining results for both sites from male lambs, 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), and between time and cohort within trial site (P<0.001) and a tendency for time by trial interaction (P=0.065).

Ring and Numnuts treatments differed from Sham at 60, 70, 90, and 160 min (P<0.05). Numnuts was intermediate between ring and sham at 80 min, and Sham was intermediate between Ring and Sham at 130 min. Numnuts differed from ring at 90 and 130 min (Numnuts > Ring) (Figure 25).



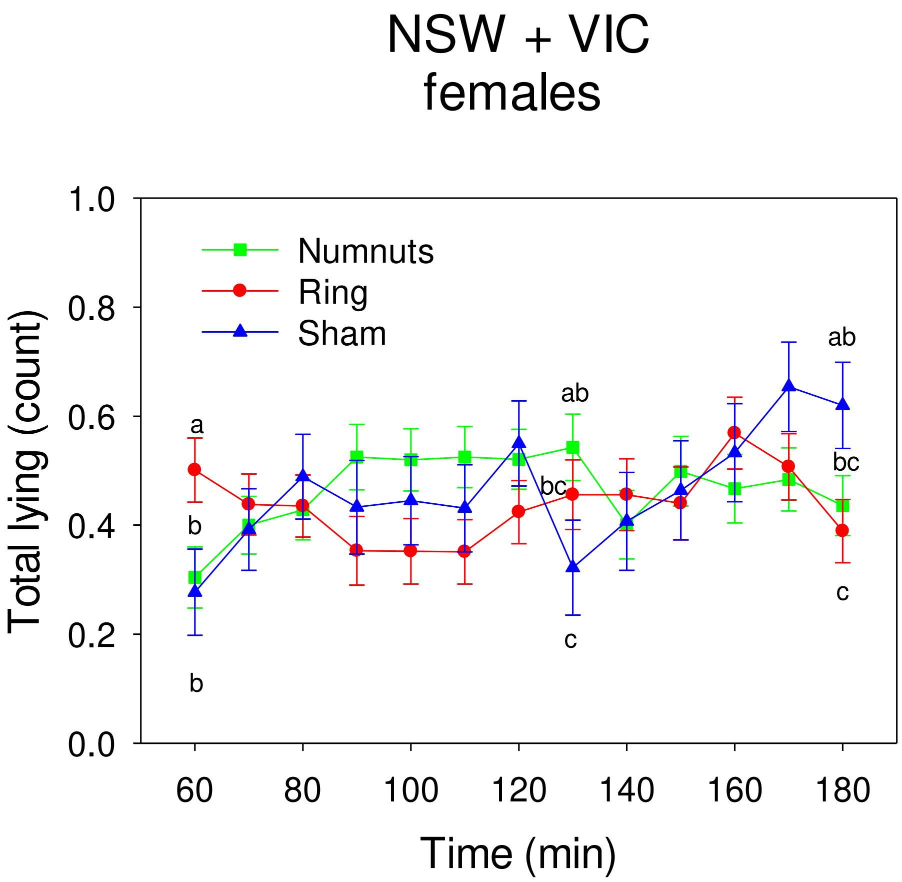
Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 25: Total count of lying postures expressed by male lambs, both trial sites combined

4.5.3 Combined data for females from both trial sites

For data combining results for both sites from 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), and 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. Numnuts differed from Sham at 130 min. treatments differed from Sham at 60, 70, 90, and 160 min (P<0.05). Numnuts differed from Ring at 60 min (Figure 26).



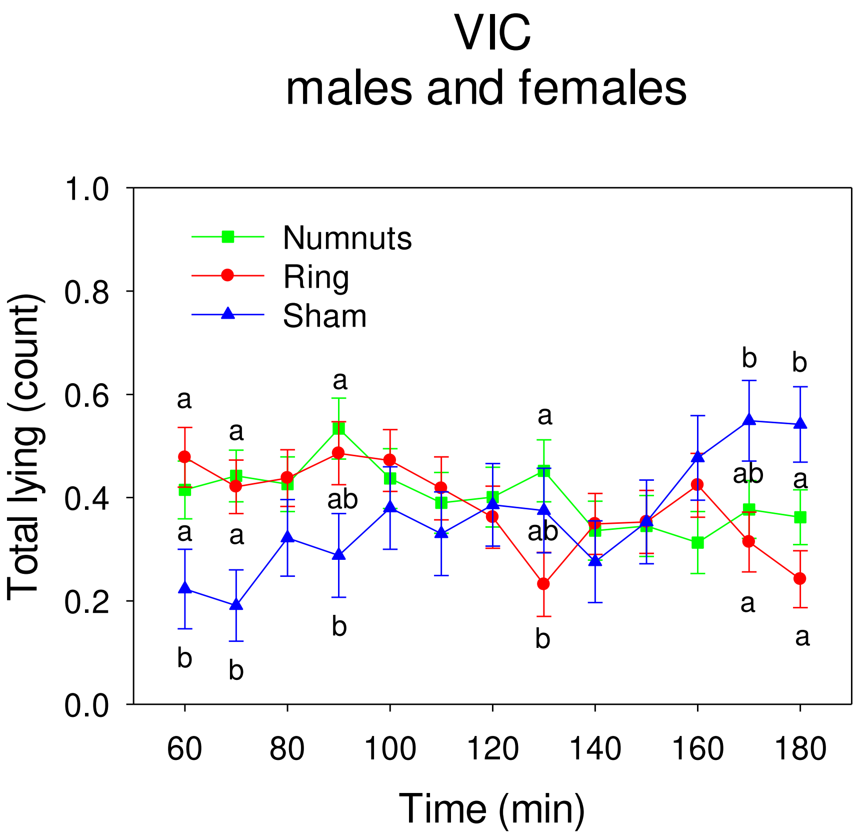
Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 26: Total count of lying postures expressed by female lambs, both trial sites combined

4.5.4 VIC trial, both genders combined

For VIC trial data combining results for males and females (Figure 27), there was a significant effect of gender (P=0.013) and cohort (P<0.001) on total count of lying postures, and an interaction between gender and treatment (P=0.044), time and treatment (P<0.001), time and gender (P<0.001), time by cohort (P=0.001).

In view of the gender by treatment and gender by time interactions, genders were analysed independently.



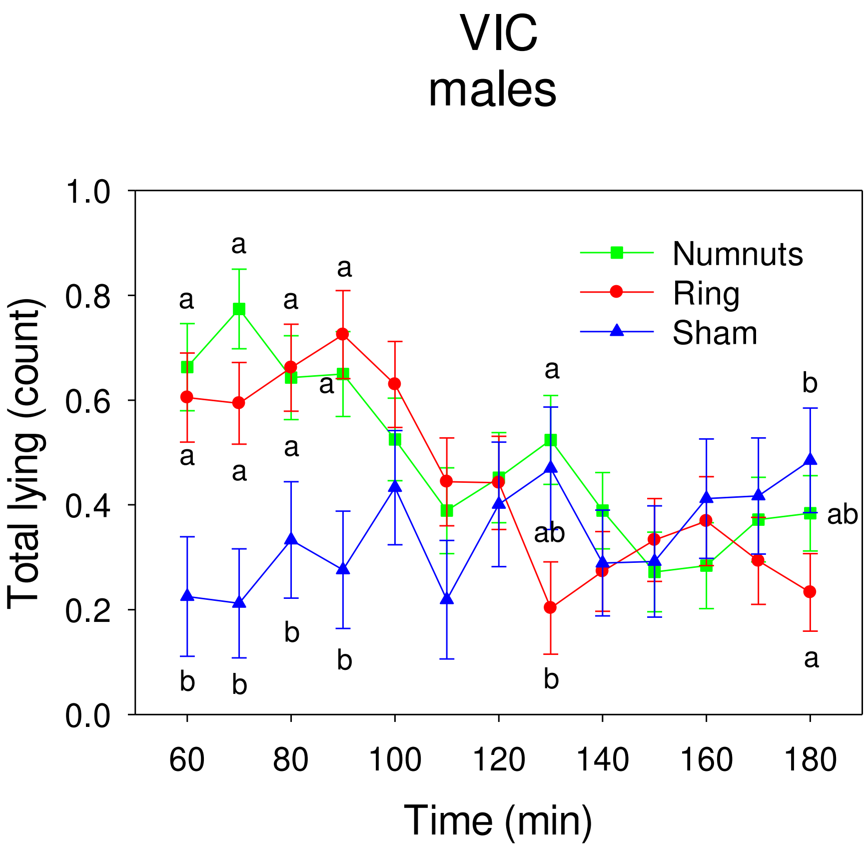
Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 27: Total count of lying postures expressed by lambs at the VIC trial site, both genders combined

4.5.5 VIC Males

There was a significant effect of treatment (P=0.049), cohort (P<0.001), time (P<0.001) and time by cohort (P=0.002) and time by treatment (P<0.001) on total count of lying postures.

Ring and Numnuts were significantly different from Sham at 60, 70, 80 and 90 min. Ring was different from Numnuts at 130 min and Ring was different from Sham at 180 min (Figure 28).

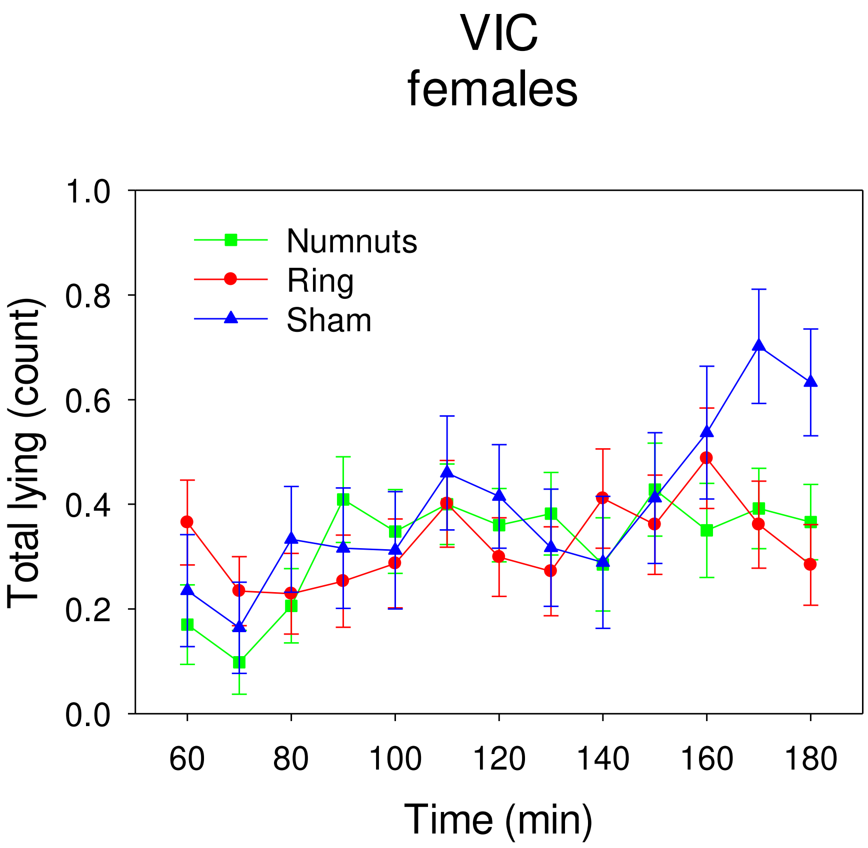


Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 28: Total count of lying postures expressed by male lambs at the VIC trial site

4.5.6 VIC Females

There was a significant effect of cohort (P<0.001), time (P<0.001) and cohort by time, (P=0.002) but no effect of treatment (P=0.358) or time by treatment (P=0.407) on total count of lying postures (Figure 29).



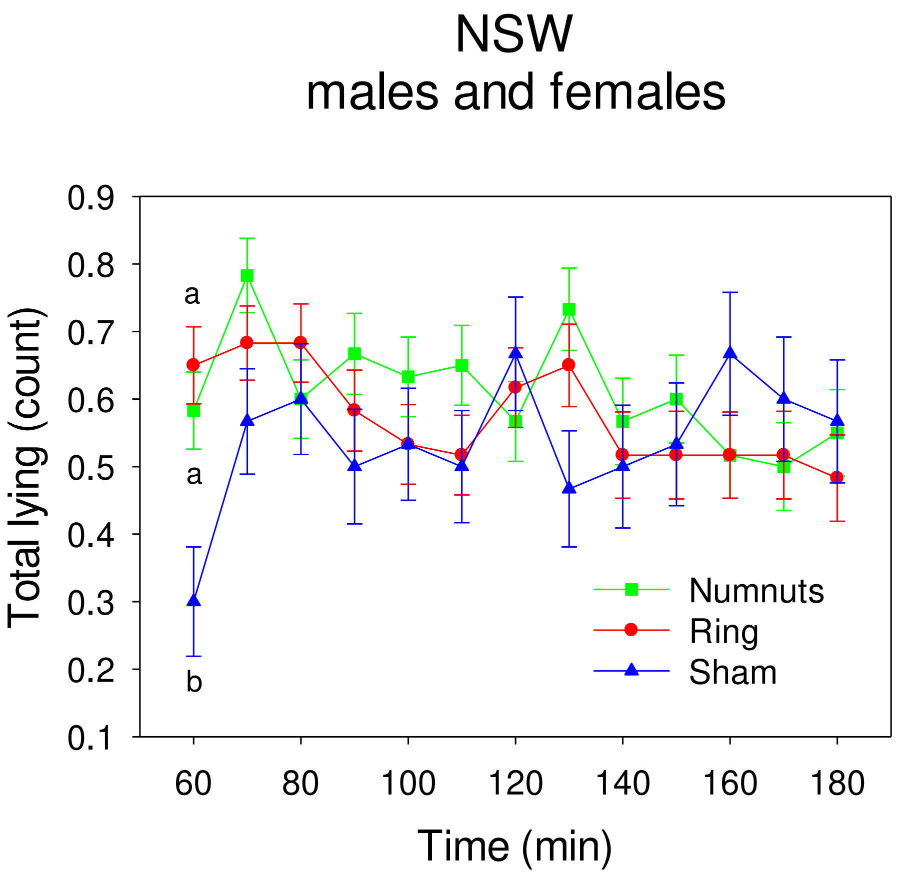
Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 29: Total count of lying postures expressed by female lambs at the VIC trial site

4.5.7 NSW Trial, both genders combined

At the NSW trial site, there was a significant effect of cohort on total count of lying postures (P<0.001). Treatment (P=0.183) and time (P=0.091) were not significant, and the time by treatment interaction approached significance (P=0.068). The interaction was due to less time spent lying in Sham lambs at 60 min than in Ring (P=0.001) and Numnuts (P=0.005) treatments. Ring and Numnuts did not differ at any time point (Figure 30).

In view of the absence of a significant effect of gender, gender by time, gender by treatment or gender by time by treatment interactions, no further analyses were performed.



Back transformed count of lying postures at 10-min intervals, between 60 and 180 min post-marking.
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 30: Total count of lying postures expressed by lambs at the NSW trial site, both genders combined

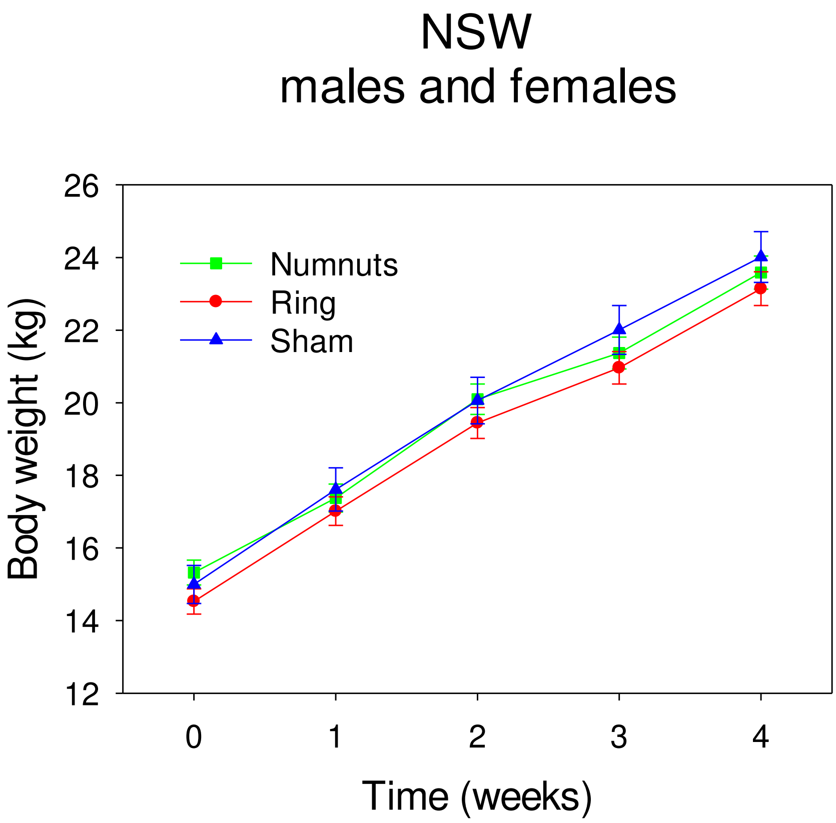
4.6 Body weight and average daily gain

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

4.6.1 NSW Trial

Bodyweight

At the NSW trial site, there were significant effects of gender (P=0.028), cohort (P<0.001) and time (P<0.001) on body weight (Figure 31), 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 gender or gender by time interactions. In the absence of an effect of treatment or interactions with treatment no further analysis were performed.

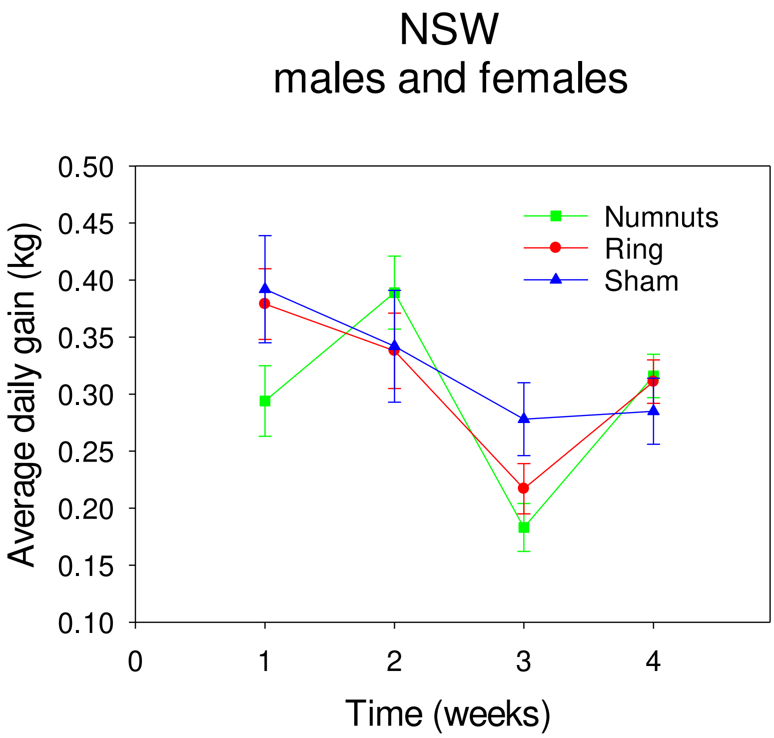


a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 31: Bodyweights of lambs at the NSW trial site, both genders combined

Average daily gain

At the NSW trial site there was an effect of time (P<0.001) and a time by cohort interaction (P=0.04) on average daily gain (Figure 32) but no significant effect of treatment or gender or interactions between these factors and time.



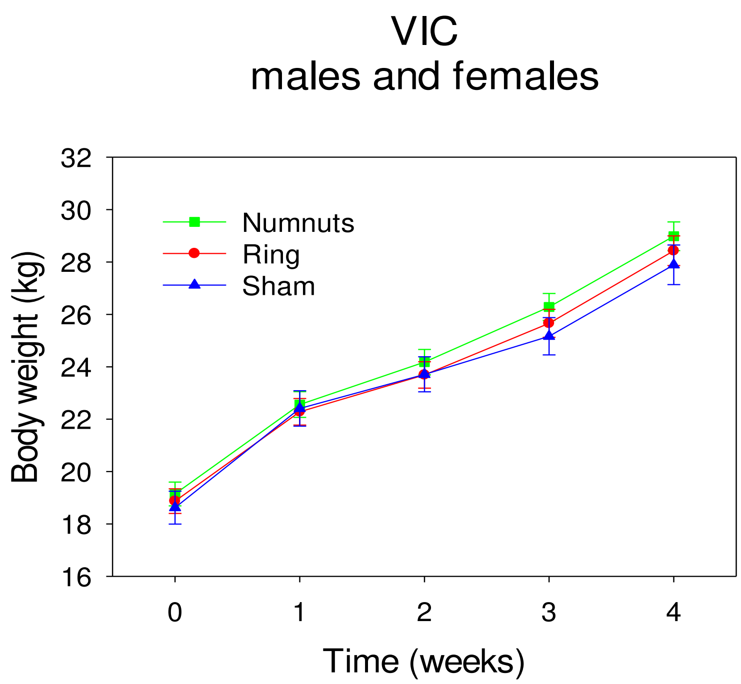
a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 32: Average daily gain of lambs at the NSW trial site, both genders combined

4.6.2 VIC Trial

Bodyweight

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 (Figure 33). Despite the time by treatment interaction, post hoc contrasts within time points detected no significant differences between treatments.

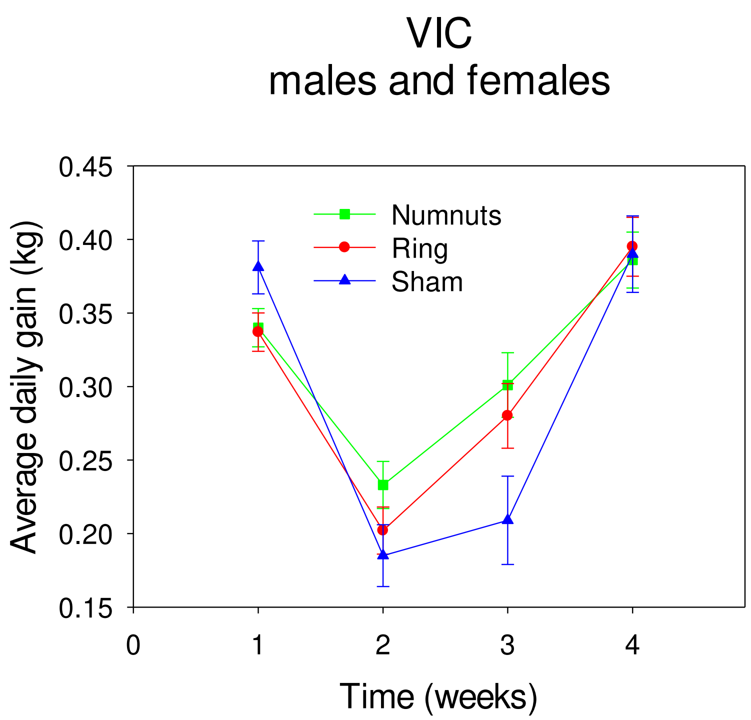


a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 33: Body weights of lambs at the VIC trial site, both genders combined

Average daily gain

At the VIC trial site, there was an effect of time (P<0.001) on average daily gain and a tendency for a time by treatment interaction (P=0.077) (Figure 34).



a, b, c: Data points with different superscripts are significantly different (P < 0.05)

Figure 34: Average daily gain of lambs at the VIC trial site, both genders combined

5 Discussion

The majority of lambs produced in Australia are tail-docked and (if male) castrated between the ages of 4 and 12 weeks. 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 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 (Lester et al., 1991, Kent et al., 1993, Robertson et al., 1994, Lester et al., 1996, Thornton and Waterman-Pearson, 1999, Melches et al., 2007, Paull et al., 2009). 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 (Mellor and Murray, 1989, Mellor et al., 1991, Thornton and Waterman-Pearson, 1999, Grant, 2004). 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, 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, and significantly greater lying in Ring Lambs than Sham lambs at the 60, 70, 90, and 130 min observation time points.

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 lambs, across a 240-min observation period, and particularly that, after the first 60 min, in 63-100% of the observations, 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 22.29% of their time standing, while control (sham) lambs spent 81.33% 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 63.56% 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, although for both genders, 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). This finding also concurs with Grant (2004), who 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 (>709% 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. A list of published data on the use of lignocaine and other local anaesthetic agents is presented in Table 7 and Table 8 respectively. It is important to note that many of these 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. The only study investigating such low volumes of lignocaine 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-hour 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 hours 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 sec 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).

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. (Graham et al., 1997). The elimination half-life of bupivacaine in sheep is between 60 and 120 min (Santos et al., 1997, Edwards, 2017), suggesting that the duration of effect should be prolonged as compared to lignocaine. Another alternative agent is ropivacaine, a newer agent, 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).

In the current study, administration of local anaesthetic using the Numnuts® v12 prototype 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.

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).

Table 7: Summary of findings regarding the behavioural effects of lignocaine for ring marking in lambs.

Reference	Agent	Age of lamb, sample size	Castration	Tail Docking	Dose	Duration of effect	Comment
Current study	Lignocaine 2%	4-10 weeks, n=60	X	X	1.5 mL into midline scrotal neck; 1.5 mL subcutaneously into dorsal surface of tail, using the Numnuts® v12 prototype. Immediate ring application	20-35 min	Measured in 1 min epochs at 5, 20, 35 and 50 min post procedure.
Jongman et al. (2016)	Lignocaine 2%	8-9 weeks, n=12	X		1.5 mL or 4.0 mL into midline scrotal neck. Immediate ring application.	60 min (total abnormal behaviours)	Minor overall benefit of 4 mL in first hour only.
Stewart et al. (2014)	Lignocaine 2%	4 weeks, n=10	X		6 mL total (2 mL into each testis; 2 mL into scrotal neck). Rings applied immediately or after a 4 min delay	80 min	Measured in 20 min epochs, effect greatest in first 40 min. Delay between LA administration and ring application had no benefit over immediate ring application.
Mellema et al. (2006)	Lignocaine 2%	2-7 days, n=15	X		4 mg/kg distributed into each spermatic cord and around the scrotal neck.	120 min	Five observations of 10 min taken over 120 min post procedure.
Kent et al. (2004)	Lignocaine 2% with adrenalin	0.5-3 days, n=200	X	X	0.3 mL into each side of the scrotum under each ring, using a needleless injector, immediately after ring application.	44 min	Data presented in 5 min blocks from 14-44 min post procedure.
Kent et al. (2000)	Lignocaine 2% with adrenalin	0.5-2 days, n=5	X	X	0.3 mL into each side of the scrotal neck, 0.3 mL into each testis (total 1.2 mL) and 0.3 mL into each side of the tail	Up to 6 weeks	Lambs observed for two 3 hr blocks on days 10, 14, 31 and 41 post procedure. Long after pharmacological effect of LA.
Thornton & Waterman-Pearson (1999)	Lignocaine 2%	4-6 days, n=6	X		0.5 mL each spermatic cord, 1.0 mL in a ring around scrotal neck, 0.5 mL each testis (3 mL total) Delay of 15-20 min prior to ring application.	120 min	Lambs observed every 10 min for 60 min, then every 20 min for two hours Active pain response dropped markedly after 80 min.
Dinniss et al.	Lignocaine	4-9 weeks,	X		Scrotal neck only (LASC): total 2	Up to 4 h	Data pooled across 4 h of observations:

Reference	Agent	Age of lamb, sample size	Castration	Tail Docking	Dose	Duration of effect	Comment
(1999)	2%	n=9			mL, 1 anteriomedial, and 0.5 each side lateral		1 min epochs at 15 min intervals till 60 min, then at 30 min intervals till 240 min post procedure. Behaviour/posture recorded every 15 s during the 1 min epoch.
			X		Spermatic cords only (LACD): 1 mL total, 0.5 mL into each spermatic cord	Up to 4 h	Data pooled across 4 h of observations. This treatment had the least effect of the four anaesthesia patterns evaluated.
			X		Scrotal neck and spermatic cords (LASC+CD): total 3 mL	Up to 4 h	Data pooled across 4 h of observations.
			X		Testes only (LAT): 1 mL total, 0.5 mL into each testis	Up to 4 h	Data pooled across 4 h of observations.
Kent et al. (1998)	Lignocaine 2% with adrenalin	5-8 days, n=8	X		0.2 mL into each testis, using a needless injector. Immediate ring application.	96 min (active) 120 min (postures)	Data pooled as 96 min (active), and 120 min (postures) post procedure.
			X		0.2 mL into left and right sides of the scrotal neck, proximal to the ring, immediately after ring application, using a needless injector	36 min (active) 36 min (postures)	Data presented in 6 min blocks to 96 min (active), and 120 min (postures) post procedure.
				X	0.2 mL into left and right dorsolateral aspect of tail, proximal to the ring, immediately after ring application, using a needless injector	24 min (active) 96 min (postures)	Data presented in 6 min blocks to 96 min (active), and 120 min (postures) post procedure.
				X	0.2 mL subcutaneous into left and right dorsolateral aspect of tail, using needle and syringe. Immediate ring application.	96 min (active) 120 min (postures)	Data pooled as 96 min (active), and 120 min (postures) post procedure.
Wood et al. (1991)	Lignocaine 2%	5-6 days, n=6	X	X	Tail – epidural 0.3 mL. Scrotum – 0.5 mL each spermatic	60 min	Data presented in 0-15 min, 15-30 min and 30-60 min blocks.

Reference	Agent	Age of lamb, sample size	Castration	Tail Docking	Dose	Duration of effect	Comment
					cord, 1.0 mL in a ring around scrotal neck, 0.5 mL each testis (3 mL total) Delay of 15-20 min prior to ring application.		LA group spending more time lying than sham control at 120-180 min – and similar to marked group. Cortisol response also suppressed by LA.

Table 8: Summary of findings regarding the behavioural effects of alternative local anaesthetic agents for ring marking in lambs.

Reference	Agent	Age of lamb, sample size	Castration	Tail Docking	Dose	Duration of effect	Comment
Molony et al. (2012)	Procaine 5% with 0.002% adrenalin	2-3 days, n=8	X		0.3 mL into each spermatic cord, using a needleless injector. Immediate ring application.	60 min	Data pooled across 60 min, no shorter epochs reported.
Graham et al. (1997)	Bupivacaine 0.25%	3 weeks, n=7 or 8		X	0.5 mL distributed across each of the dorsal and ventral subcutaneous space at the site of ring application, by slow needle withdrawal. Ring applied 1-2 min later.	Up to 3 hr	Data pooled across 3 hr post procedure. Significant effect on both active pain response (continuous observation in first 96 min, then intermittent), and on postures (recorded every 2 min for first 96 min then every 6 min). Cortisol response also suppressed by LA.
				X	Epidural injection of 0.5 mL. Ring applied 1-2 min later.	Up to 3 hr	Data pooled across 3 hr post procedure. Significant effect on active pain response (continuous observation in first 96 min, then intermittent), but not on postures (recorded every 2 min for first 96 min then every 6 min). Cortisol response also suppressed by LA.

6 Conclusions/recommendations

By the methods of detection used in the study, the Numnuts® v12 prototype tool had similar efficacy in reducing pain in male and female lambs in the first 20 – 50 min following ring marking. Very similar responses were seen in males at both trial sites, whereas responses in females were somewhat more variable. The current study found that central deposition in the neck of the scrotum of a single injection of approximately 1.5 mL lignocaine together with injection of local anaesthetic into the tail provided significant pain relief when ring marking male lambs. The benefit of marking with the Numnuts® v12 prototype tool previously seen in female lambs in a trial at Moredun Research Institute Scotland was confirmed in the current trial. The transience of the pain relief seen in the current trial is in accord with the known pharmacodynamics of lignocaine. 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® v12 prototype 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, a combination of local anaesthetics (e.g. lignocaine + bupivacaine), inclusion of adrenaline with the local anaesthetic or injection of an local anaesthetic + NSAID combination treatment.

Based on feedback from observers, future similar studies should consider including ‘tail wagging’, ‘vocalisation’ and ‘lateral lying’ in the ethogram used for acute pain behaviours in the first hour post procedure.

6.1 Performance against hypotheses tested

- 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);
  - SUPPORTED (active pain avoidance up to 35 min post procedure)
  - NOT SUPPORTED (postures after 60 min)
- 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);

  - SUPPORTED (active pain avoidance up to 20 min post procedure)
  - NOT SUPPORTED (postures after 60 min)
- 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);
  - SUPPORTED (females)
  - NOT SUPPORTED (males)
- 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).
  - NOT SUPPORTED

7 Key messages

- The Numnuts® v12 prototype tool can effectively deliver a local anaesthetic agent to the neck of the scrotum or tail at the time of marking using rubber rings.
- Reductions in active pain avoidance behaviours were evident at 5 min and 20 min post procedure.
- The duration of efficacy is limited by the pharmacokinetics of the agent used (lignocaine 2%).
- Efficacy is likely to be improved if a longer-lasting agent or combination of agents is used.

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9 Appendix

9.1 Example observations record: active pain avoidance

Numnuts Study Location: Date: Observer:

ID	Treat Time	5 min		20 min		35 min		55 min	
		RST		RST		RST		RST	
		FSK		FSK		FSK		FSK	
		LBW		LBW		LBW		LBW	
		RL		RL		RL		RL	
		JMP		JMP		JMP		JMP	
		EQ		EQ		EQ		EQ	
		SK		SK		SK		SK	
		RST		RST		RST		RST	
		FSK		FSK		FSK		FSK	
		LBW		LBW		LBW		LBW	
		RL		RL		RL		RL	
		JMP		JMP		JMP		JMP	
		EQ		EQ		EQ		EQ	
		SK		SK		SK		SK	
		FSK		FSK		FSK		FSK	
		RL		RL		RL		RL	
		RST		RST		RST		RST	
		EQ		EQ		EQ		EQ	
		JMP		JMP		JMP		JMP	
		LBW		LBW		LBW		LBW	
		SK		SK		SK		SK	
		RST		RST		RST		RST	
		FSK		FSK		FSK		FSK	
		LBW		LBW		LBW		LBW	
		RL		RL		RL		RL	
		JMP		JMP		JMP		JMP	
		EQ		EQ		EQ		EQ	
		SK		SK		SK		SK	
		RST		RST		RST		RST	
		FSK		FSK		FSK		FSK	
		LBW		LBW		LBW		LBW	
		RL		RL		RL		RL	
		JMP		JMP		JMP		JMP	
		EQ		EQ		EQ		EQ	
		SK		SK		SK		SK	

9.2 Example observations record sheet: snapshot observations

Numnuts   Cohort: Date: Recorder:

ID	TO	60	Ob	70	Ob	80	Ob	90	Ob	100	Ob	110	Ob	120	Ob	130	Ob

NS-normal standing

AS-abnormal standing (e.g. Statue or stretched)

Su-standing other (unable to characterise)

NW-normal walking

AW- abnormal walking

Wu-walking other

NL- normal ventral lying

AV - Abnormal ventral lying

Vu - Ventral lying other

LL - Lateral lying

LI - Lying intention (attempts to lie down)

rl - rolling

J - jumping

P - playing

Sk-suckling

G-grazing

R- running

9.3 Example treatments record sheet

CSIRO Agriculture and Food

B.AWW.0256 NUMNUTS field trials 2017 SAP reference R-09776-1	Treatment Administration Cohort:.................................. Date..

Gender	Treatment	Time marked	Tag	Side mark	Comments
MALE	NUMNUTS				
MALE	MARK NO LA				
MALE	SHAM				
MALE	NUMNUTS				
MALE	MARK NO LA				
MALE	NUMNUTS				
MALE	MARK NO LA				
MALE	SHAM				
FEMALE	NUMNUTS				
FEMALE	MARK NO LA				
FEMALE	SHAM				
FEMALE	NUMNUTS				
FEMALE	MARK NO LA				
FEMALE	NUMNUTS				
FEMALE	MARK NO LA				
FEMALE	SHAM				

SHAMS – target 2 of one gender, one of the other. Aim to balance across cohorts.