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Grace M. Woodward | BVS

Bachelor of Veterinary Science

Honours Research Project

The effect of xylazine on intraoperative pain-related behaviour during castration and tail docking in Merino lambs.

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The effect of xylazine on intraoperative pain-related behaviour during castration and tail docking in Merino lambs.

Abstract

Objective To assess the analgesic effect of xylazine during routine lamb marking.

Procedures Merino lambs (n=150) were allocated to 5 treatment groups: 1) Sham castration and sham tail docking 2) Knife castration and hot iron tail docking 3) Knife castration with hot iron tail docking after 0.1mg/kg intramuscular administration of xylazine (IM) 4) Ring castration with hot iron tail docking 5) Ring castration with hot iron tail docking after 0.1mg/kg IM administration of xylazine. Acute pain-related behavioural response to castration and tail docking was assessed and scored from 0-3 based on severity.

Results There was a significant treatment effect of xylazine on pain responses to castration and tail docking. Xylazine treated lambs showed significantly reduced pain responses than untreated lambs when undergoing knife castration, ring castration and hot iron tail docking.

Conclusion Xylazine is associated with a reduction in pain-related behaviour in lambs during castration by ring or knife and hot iron tail docking. It did not reduce pain-related behaviour to the level of the control group. No adverse side effects were noted. It is a potential analgesic option for use in routine husbandry procedures in sheep. Further research is needed to assess physiological responses, optimal time intervals, side effects and public perceptions before it could be suitably recommended for use during lamb marking.

Introduction

Australian lambs routinely undergo castration and tail docking at 2-12 weeks of age. Castration is necessary for ease of handling and to prevent unwanted matings and aggression. It is commonly performed by knife, rubber ring or Burdizzo clamp. Tail docking is performed for the prevention of cutaneous ovine myiasis by reducing the build up of faecal material at the breech. It is also thought to allow for faster and easier shearing and crutching, although there is limited research to support this idea[1]. Docking can be performed by hot iron, knife, rubber ring, or ring combined with Burdizzo clamp.

All commonly used castration methods and tail docking methods are associated with increased pain-related behaviour and increased blood cortisol concentrations[1-7]. Despite this, these procedures are conventionally performed without any form of pain relief. There is increasing concern from the general public regarding sheep welfare and demand for an improvement in standard practices. The application of effective and practical analgesia during these surgical procedures may help to improve sheep welfare and satisfy these concerns. However in order to be adopted by producers, analgesic options must be practical, financially viable and have minimal impact on labour or time required.

The physiologic pain pathway involves the activation of nociceptors in tissue, transmission of action potentials via sensory neurons to the spinal cord, processing of pain in the dorsal horn of the spinal cord, transmission of action potentials via ascending neurons in the spinal cord to the brain, and processing of the painful stimuli within the brain[8]. Various analgesic agents target different areas of the pain pathways such as initial nociception, sensitisation in peripheral tissues and the spinal cord and/or conscious perception of pain in the cerebrum.

Nociception is the reduced by local anaesthetic agents[8]. It is also believed that the cauterization of tissue (such as in hot iron tail docking) will reduce nociception by damaging nociceptors and sensory nerves[1]. Sensitisation is the target of non-steroidal anti-inflammatory drugs (NSAIDs). Conscious perception of pain is the target of opioids, ketamine and α_2-agonists. In human medicine and companion animal medicine, a balanced multi-modal analgesic approach is recommended for optimal analgesia with minimal side effects. Usually this involves a combination of local anaesthetic agents, NSAIDs and α_2-agonists or opioids. The appropriate use of pre-operative, intraoperative and post-operative analgesia is also important to avoid sensitization (such as dorsal horn wind up)[9]. Currently this “best practice” approach is rarely used in sheep.

The development of a commercial topical anaesthetic, Tri-Solfen® (Bayer Animal Health, Gordon, NSW, Australia), has revolutionised post-operative wound anaesthesia in sheep. Tri-Solfen® is a spray-on topical anaesthetic and antiseptic gel available by veterinary prescription. It contains lignocaine (40.6g/L) and bupivacaine (4.5g/L) for local anaesthesia, adrenaline (24.8mg/L) for haemostasis and cetrimide (5.0g/L) for antisepsis. The farmer can quickly and easily apply it immediately after knife castration or tail docking. Several studies have shown its efficacy in reducing post-operative pain during marking and mulesing[10-12]. The local anaesthetic agents inhibit the nociception portion of the pain pathway. However, as it is applied after surgery, it affects postoperative pain only, with no effect on the intraoperative pain. Also, local anaesthetic agents are poorly absorbed across unbroken skin and therefore administration via topical spray is ineffective for ring castration.

Xylazine is an α_2-agonist commonly used for dose-dependent sedation and analgesia in livestock. It acts on α_2-receptors found throughout the body, particularly the spinal cord, and causes opening of K+ channels and hyperpolarisation of neurons[13]. As it is centrally acting, it targets the perception part of the pain pathway. Given preoperatively, it theoretically should decrease intraoperative pain perception, as opposed to local anaesthesia and NSAIDs which both act to decrease post-operative pain. Xylazine has been found to be an effective analgesic in sheep against an electrical stimulus[14]. Use of xylazine at mulesing has been associated with reduced intra-operative pain behaviours and reduced peak plasma cortisol concentrations[15]. Xylazine used at dehorning in calves was associated with reduced cortisol

concentrations, and when combined with local anaesthetic associated with elimination of the peak cortisol response[16]. There is minimal literature on the use of xylazine in lambs for marking procedures.

There have been multiple studies that investigate the pain associated with various methods of castration and tail docking[2-7]. A comparison of the techniques is not the focus of this paper. The aim of our research is primarily to assess the efficacy of xylazine as a preoperative analgesic to control intraoperative pain in lambs during the common procedures of knife castration, ring castration and hot iron tail docking.

Materials and methods

A randomized, blinded and controlled experiment was conducted on the commercial University of Sydney property "Arthursleigh" near Marulan, NSW. The trial was conducted in September 2012. Experimental protocols were pre-approved by the institutional Animal Ethics Committee.

Animals and treatments

The trial was performed on Merino lambs (n=150) undergoing routine marking. Lambs were 8-12 weeks old with a bodyweight of 13 +/- 5kg. Each lamb was individually weighed and marked with a spray-on identification number (1-150). Lambs were then randomly allocated to five treatment groups with n=30 (Figure 1). Lambs in xylazine groups (RX and SX) were given 0.1ml/kg bodyweight xylazine intramuscularly (IM) into the rump, a minimum of 15 minutes to a maximum of 60 minutes before marking to ensure optimal pharmacological activity. Castration (by knife or ring) and hot-iron tail docking were performed by an experienced technician blinded to treatment groups. Immediate behavioural response to castration and tail docking was assessed during the procedures, as described below. Topical anaesthesia (4-6mL Tri-Solfen®) was applied immediately after the procedure to all open wounds (knife castration or tail docking) to minimise post-operative pain[10-12]. After the procedure the lambs were immediately released into a holding pen and observed for up to three hours for any adverse effects of xylazine. They were then returned to a small paddock with their mothers and monitored from a distance for a further one hour.

Methods for castration and tail docking

Marking was performed using lamb cradles with blocks of five lambs restrained at once (one lamb from each treatment group). Lambs in group C underwent sham marking. The scrotum, left testis, right testis and then tail were manipulated gently for 30 seconds in total. No castration or tail docking was performed. Knife castration (groups S, SX) was performed using a clean, sharp knife. The surgery involves incision of the distal scrotum and extraction of each testis individually. 1.5ml of Tri-Solfen was applied deep into the scrotal wound to coat the spermatic cord. The cord was incised 10cm proximal to the epididymis. A further 1.5ml of Tri-Solfen was applied to the cut edge of the scrotal sac. Rubber ring castration (groups R, RX) was performed using an elastrator rubber ring applied around the neck of the scrotum, proximal to the testes. Hot iron tail-docking was performed in groups S, SX, R and RX using a preheated gas tail-docking iron. The tail was elevated and the skin of the tail was pushed proximally. The iron was applied between the 2nd and 3rd coccygeal vertebrae to sever the tail, and then held against the stump for two seconds to ensure cautery of blood vessels. Tri-Solfen was then sprayed onto the wound after the procedure.

Behavioural analysis

Two veterinarians who were trained in behavioural observation in sheep assessed the behaviour of the lambs during castration and tail docking. They were blinded to the treatment groups, although on observing the procedures the control group could be distinguished and the two knife castration groups (S and SX) could be distinguished from the two ring castration groups (R and RX). One observer focused on central response looking for movement of the face and head such as blinking, eye widening, nasal flare or head rotation. As these are voluntary behaviours, as apposed to local reflexes, they may be reflective of conscious perception of the procedure. The other observer focused on the peripheral response looking at the rump and hind legs such as skin twitching, anal constriction, tail

lifting, struggling or kicking. These behaviours could be due to either voluntary movement or local reflexes. Lambs were allocated a score of 0-3 by each observer in response to castration and subsequently to tail docking. For ring castration and tail docking, which involved only one step, only one score was allocated. For knife castration and sham castration, which involved three steps in the procedure (scrotum, left testis, right testis), one score was allocated for each step and thus three scores in total. Scoring was based on a modified version of a previously developed numerical rating scale[9] (Figure 1). The observers called out the allocated pain score to a third person who recorded the scores.

Statistical analysis

An ordinal generalized linear mixed model was performed using ASREML software. A comparison was made between response to castration for all groups and a separate comparison was made for response to tail docking for all groups. Comparisons were not made between central and peripheral pain scores or between steps in the multi-step procedures of sham castration and knife castration (scrotum, left testis, right testis).

The form of the model fitted is:

$$\log_e\left(\frac{P(Y \le k)}{P(Y > k)}\right) = \theta_k + \text{Treatment} + \text{Observation} + \text{Treatment.Obvservation} + \text{EarTag}$$

where Y is the observed pain score, θ us the threshold or "intercept" for score k, *Treatment* is the effect of C, R, S, RX, SX (fixed), *Observation* is the effect of central vs peripheral (fixed), *Treatment.Observation* is the interaction effect (fixed), and *EarTag* is the effect of sheep (random). As a significant treatment effect was found, the analysis continued on to compare individual treatments on the logit scale.

The cumulative probability of a lamb in each group having a pain score of less than a particular score was calculated. This was converted to an event probability (the probability of a lamb in a particular group being allocated a score of 0, 1, 2 or 3). The standard error of the difference of mean logits (SED) between each group was determined. The difference in Logit value between two groups divided by the SED value was calculated to form a z value and converted to a P value. P values of <0.05 were considered statistically significant.

Results

Data for 11 lambs were missing (four of the RX group and seven of the SX group). Due to human error, these lambs were marked less than the nominated minimum of 15 minutes after IM injection with xylazine. The data points were disregarded for analysis.

Castration

The results are displayed in Figure 3. There was a significant treatment effect ($P<0.01$) on response to castration. The differences between all treatment groups were significant ($P<0.01$), with the exception of R and RX, which did not differ significantly from each other.

Tail docking

The results are displayed in Figure 4. There was a significant treatment effect ($P<0.01$) on response to tail docking. All marked groups (R, S, RX, SX) were significantly more likely to exhibit a higher pain score than the sham group (C). Xylazine treated lambs (RX and SX) were significantly less likely to exhibit a higher pain score than the untreated marked lambs (R or S). There was no significant difference in pain response between R and S groups or between RX and SX groups during tail docking.

Post-operative observations

The behaviour of the lambs post-operatively was not a focus of this study but they were observed for

adverse side effects. An estimated 10% of the xylazine treated lambs showed moderate levels of sedation, becoming recumbent when left undisturbed in the pens. However when approached, the lambs rose and moved about the pen as normal. When returned to the paddock with the ewes, no problems were noted. The farmer reported no associated lamb morbidity, mortality or problems mothering up.

Discussion

Control of intraoperative pain-related behaviour

The results demonstrated a decrease in pain responses associated with xylazine administration for knife castration, ring castration and hot iron tail docking. This is supportive of the hypothesis that xylazine can be used to control intraoperative pain during lamb marking and is consistant with previously published data14,. However, treated groups still showed significantly higher pain scores than the sham group, indicating that while pain appeared to be controlled it was not eliminated.

Another viable intraoperative analgesic option for lamb marking is injection with local anaesthetic prior to marking. Injections can be made into the testes or spermatic cord before castration by knife or ring. This method differs to the postoperative topical spray (Tri-Solfen) in that it can control intraoperative pain. It has been associated with reduced postoperative pain-related behavior and cortisol concentrations[18, 19] but to our knowledge has not been assessed for true intraoperative analgesia at marking. Successful blockage of the spermatic cord by lignocaine or bupivacaine injection requires some degree of training and skill (more so than an IM injection). To be fully effective, lignocaine requires a time delay of at least 5-10 minutes before the onset of action. This has a practical disadvantage in the field. Farmers would have to inject the local anaesthetic and keep each lamb in the cradle for 10 minutes or alternatively they could cradle each lamb, inject the local anaesthetic, release the lamb and catch it again 10 minutes later. Both of these options would slow down the 'production line' of marking considerably, increase the stress on the lambs, and may be more difficult and time consuming than a simple IM injection of xylazine in the yard 15 minutes prior to marking.

Knife vs ring castration

This study was not designed to compare the different methods of castration (ring vs knife). Comparison of techniques is an area of active debate in the literature and is beyond the scope of this paper[2-7]. Our results showed that the ring groups (R and RX) showed significantly lower pain scores than the knife groups (S and SX). This should not be interpreted as evidence in support for ring castration, The two methods involve different types of tissue damage and nociception that are not directly comparable. Surgery involves an acute traumatic injury with immediate nociception that is soon followed by inflammation and subsequent healing. Ring application may be associated minimal nociception on initial application but is followed by chronic post-operative pain associated with ischemia and subsequent necrosis of the testes. This study assessed only the acute, intraoperative pain and not postoperative pain.

Validity of behavioural analysis

Studies of pain and analgesia in the veterinary field are inherently difficult to interpret as animal patients are unable to communicate their pain levels directly. Instead, pain perception in animals must be inferred from behavioural and physiological responses. As prey animals, adult sheep do not display obvious pain-related behaviour. Lambs are less stoic and their behavioural responses are often used to infer their experience of pain[1-8] .

A major confounding factor in this study is the sedative effect of α_2-agonists. As xylazine is a known sedative in sheep, care must be taken in interpreting behavioural analysis. Sedation is not necessarily an adverse side effect, as it is likely to reduce stress in the lambs, although excessive or prolonged sedation could be detrimental. However, the sedative effect on change in behavior must be distinguished from the analgesic effect. All or part of the reduction in pain response seen in this study may be due to sedation rather than true analgesia. While it is likely that a combination of sedation and analgesia is responsible for the behavioural changes seen, further research is needed to more objectively assess the analgesic effect during marking.

Behavioural observation can also be confounded by observer bias. While the observers were blinded to the treatment groups, they were able to visually distinguish the different methods of castration and tail docking used in this study. They were also not blinded to the pain scores of the other observer.

In future studies, physiological changes such as blood concentrations of cortisol, haptoglobin and acute phase proteins could be used in combination with behavioural analysis and weight gain for a more complete analysis. A good example of such a study in sheep is the research by Paul *et al*. 2007, who investigated topical anaesthesia and NSAIDs in association with mulesing[17]. A similar study involving the use of xylazine is warranted.

Practical considerations

Preoperative xylazine administration requires extra time, labour and mustering. It could be argued that the double handling of lambs required to give preoperative analgesia may be an additional stress that may not be outweighed by the analgesic benefit.

Timing of administration is an important factor. Too little or too much time between injection and marking for any individual lamb will result in reduced or absent efficacy of analgesia. It would be necessary for farmers to keep track of which lambs were injected at what time and ensure they were subsequently marked at appropriate intervals. Even during our study, where diligent records were being kept to track each lamb and time period, human error resulted in premature marking of 11 lambs (these lambs were disregarded for analysis). The interval used in this study was 15-60 minutes, but further research is needed to investigate the optimum time and the effective time range for IM injection in lambs.

On speaking with farmers and veterinarians, it appears that one major and widespread concern for the use of xylazine at lamb marking is the risk of adverse effects. Known side effects include sedation, bradycardia, hypotension, hypoxaemia, impaired thermoregulation, salivation, transient diarrhoea, and ruminal atony[20, 21]. The frequency and severity of these side effects in lambs is unknown, and none were seen during this trial. This could also be a topic for further research.

There is a 28-day withholding period after administration of xylazine for slaughter for human consumption in Australia[22]. This is not likely to be a preclusion for use at lamb marking as lambs would be unlikely to be sent to slaughter for human consumption at such a young age.

Social climate

Social research would also be useful to determine whether xylazine is actually likely to be adopted and well received. This may include a survey of farmers to assess their receptiveness to the use of xylazine in lambs. An assessment would be useful regarding whether or not veterinarians would feel comfortable recommending and prescribing it for use with or without the presence of a veterinarian at marking. The general public could also be surveyed to assess whether intraoperative pain relief would alleviate public concerns, satisfy animal rights activists, and/or improve wool sales or prices.

Conclusion

Xylazine is a potential analgesic to address intra-operative pain in lambs during castration and tail docking. It is associated with reduced pain-related behaviour during ring castration, knife castration and hot iron tail docking. Pain-related behaviours were not eliminated to control levels. No significant adverse side effects were noted. Further research is needed to more thoroughly assess the effect of xylazine at lamb marking. This could include assessment of optimal time interval between injection and marking, physiological responses, post-operative behavioural observations, proactive observation for side effects, and investigation of the current social climate.

Appendix

Group	N	Systemic Treatment	Castration method	Tail docking method
C	30	None	Sham	Sham
S	30	None	Knife	Hot iron
SX	30	Xylazine	Knife	Hot iron
R	30	None	Ring	Hot iron
RX	30	Xylazine	Ring	Hot iron

Figure 1: TREATMENT GROUPS. Lambs were allocated at random to the five treatment groups above.

Pain score	Peripheral response	Central response
0	No response	No response
1	Minor involuntary motor response, eg. *Local skin twitch* *Anal contraction*	Minor facial response, eg. *Eye widening* *Blinking* *Nostril flare*
2	Partial rump withdrawal reflex, eg. *Lifting tail* *Multiple* *Struggle behaviour*	Partial startle response, eg. *Partial head rotation*
3	Full startle reflex, eg. *Lifting off cradle* *Kicking* *Escape behaviour*	Full startle response, eg. *Major head rotation*

Figure 2. PAIN SCORING SCALE. This scale was used to allocate scores to each lamb during castration and subsequently during tail docking.

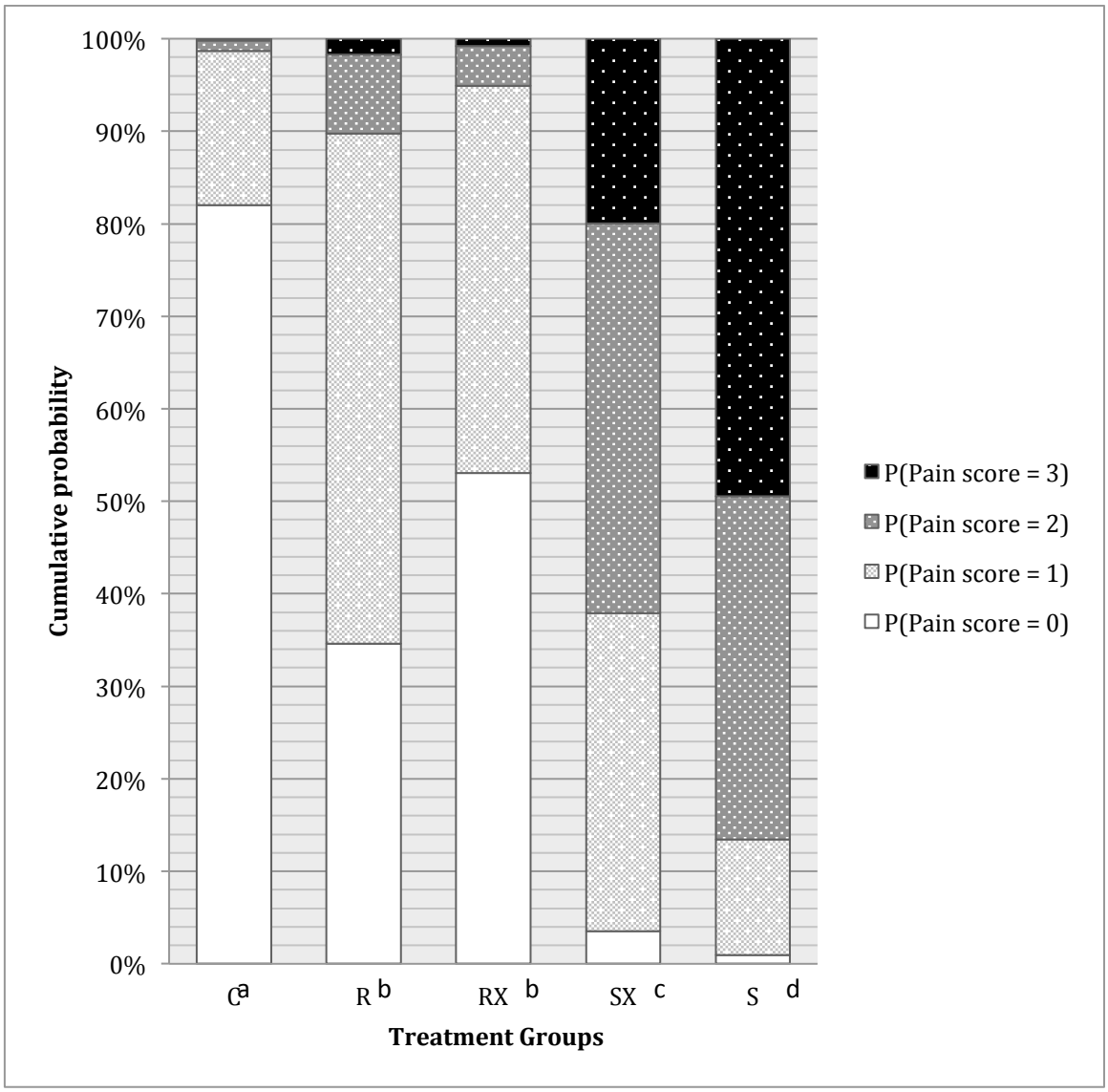

Figure 3. RESPONSE TO CASTRATION. The cumulative probability of a lamb in each treatment group obtaining a particular pain score during castration. Significant difference between groups is indicated by superscripts a, b, c, and d.

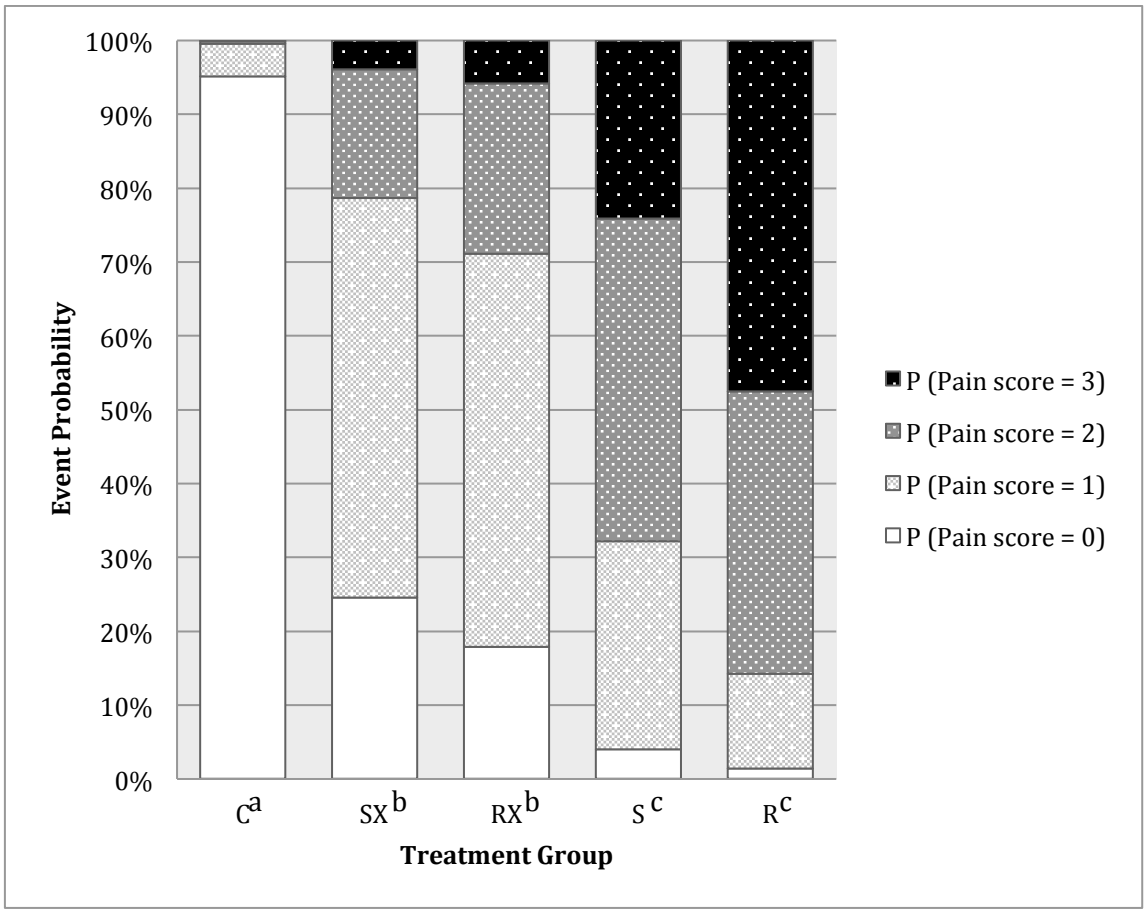

Figure 4. RESPONSE TO TAIL DOCKING. The cumulative probability of a lamb in each treatment group obtaining a particular pain score. Significant difference between groups is indicated by superscripts a, b and c.

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Reflective Statement

This was the first time I have undertaken a university-standard independent research project. This research project was a huge learning curve for me, not only in learning about analgesia and welfare in sheep but in learning about the process of conducting research.

I had planned to finish all the trials by the end of 2012 so that I would have ample time to write the paper thoroughly. Our first trial in September 2012 (the subject of this paper) went smoothly. However I was concerned about the sedative effect of xylazine interfering with our behavioural analysis. I hoped to expand on the project by using a more objective assessment of analgesia, plasma cortisol levels.

Momentum was gained when we were award a research grant by the Australian Wool Education Trust. A second trial was planned for March, between my other commitments for university internships. I had planned to complete all the field work by the end of March but we found it difficult to get access to a flock of lambs or sheep to work with. The trial was then postponed to July.

The second trial was an attempt at a cortisol curve, based on the work by Paull *et al.* 2007. We wanted to assess xylazine, carprofen and Tri-Solfen in various combinations at mulesing. However this trial proved too ambitious for an honours project. Due to our limited time we tried to complete the trial in a 36 hour period. This meant the sheep had no time to acclimatize to the environment and we were unable to block sheep across several days the way that Paul *et al.* had done. We used hoggets and found on the day that it was more difficult than expected to draw blood from the jugular vein. We made the decision early in the day to shear the necks, which was an added stress to the sheep. After collecting blood samples for baseline and at 0.5, 6, 12 and 24 hours after mulesing, we had them analysed for plasma cortisol in the laboratory. Our results were inconclusive due to a huge variation in the baseline cortisol levels. A decision was made to focus the paper on the original trial. I hope to continue the cortisol research at a later date when we would have the time to do a properly designed, more thorough investigation.

Although we had many challenges in completing this trial, I was fortunate to work with an excellent research team. My supervisors were excellent sources of knowledge and advice throughout the year. They were also a fantastic support during both field trials, even when it involved staying up to the early hours of the morning to collect blood samples from sheep and specific time points. Their support and enthusiasm has been non-stop and this project has certainly inspired me to do further research at some point in the future.