Pain Relief During Painful Husbandry Procedures in Livestock

(2008 – 10754)

MAF Biosecurity New Zealand Technical Paper No: 2010/07

Prepared for MAFBNZ Operational Research
By J R Webster[1], M Stewart[1], N. Beausoleil[2], C. Johnson[2], K. Stafford[2].

1. Animal Behaviour & Welfare, AgResearch, Private Bag 3123, Hamilton
2. Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11 222. Palmerston North

ISBN 978-0-478-36345-6 (print)
ISBN 978-0-478-36346-3 (online)

ISSN 1179-5832 (print)
ISSN 1179-5840 (online)

February 2010

Disclaimer

Requests for further copies should be directed to:

Strategic Science Team
Policy and Risk Directorate
MAF Biosecurity New Zealand
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at www.biosecurity.govt.nz

Contents

Page

1. Abstract

A number of prototype methods of coating elastrator rings with local anaesthetic were developed. Three of these were tested using a custom perfusion rig *in vitro* and the release and diffusion of local anaesthetic through sheep skin was determined. Based on the kinetics of this release a single design was chosen for a field study to examine the cortisol and behavioural responses to castration. Sixty lambs were allocated to six treatments (n=10 per treatment); castration with a normal elastrator ring (R), a ring coated with local anaesthetic (RLA), local anaesthetic given 4 min before a normal elastrator ring (LADR), local anaesthetic given immediately prior to a normal elastrator ring (LAIR), injection of local anaesthetic without castration (LA) handling without castration (C). Behaviours were recorded for a 30-min baseline period and for 3 hours after treatment. Behaviour was grouped into 1 hour blocks for analysis. For the first hour, continuous behaviour sampling was used, then for the following 2 hours, periodic scans (for 1 min per lamb once every 5 min) were used. One day later, twenty four additional lambs were allocated to three treatments (n=8 per treatment); C, R and RLA as above. Blood samples were taken via venipuncture at 0, 30, 60, 90, 120 min from treatment and assayed for cortisol. There were no differences ($P>0.05$) between groups during the baseline period. Lying increased and standing decreased in R and RLA ($P<0.01$) for hours 1 and 2 after treatment. By hour 3, these behaviours were no different from C. There was no difference in lying or standing between R and RLA ($P>0.05$), between C and LA, C and LAIR, C and LADR and LADR and LAIR at any time point. LADR and LAIR responded less ($P<0.05$) than R and RLA. All forms of lying (with and without back limbs extended, lateral lying) were higher in R and RLA than C ($P<0.001$) during hour 1, but by hour 2, only lying with back limbs extended was higher ($P<0.001$). Lateral lying was higher in R than RLA in hour 1 ($P<0.001$). Lying with back legs extended was higher in LADR and LAIR ($P<0.01$) than C; and LADR and LAIR were both lower ($P<0.05$) than R and RLA during hour 1. Stomping, stretching, kneeling, kicking ($P<0.001$) and backward walking ($P<0.05$) were higher in R and RLA than C during hour 1, but were no different than C after the first hour. Stomping, backward walking and kicking were higher than C in LADR and LAIR during the first hour ($P>0.05$). There was no difference between LADR and LAIR; however, stretching, kneeling and kicking were lower in both of these treatments ($P<0.05$) than R and RLA. Cortisol increased after ring application in both R and RLA. Cortisol was higher in R than C at all times after treatment ($P<0.01$) and higher in RLA than C at 30 and 60 minutes after treatment ($P<0.01$). Mean cortisol at 120 min after treatment ($P<0.05$), area under the curve and maximum cortisol ($P<0.001$) were also lower in RLA than R.

In summary, elastrator rings were coated with local anaesthetic which successfully diffused through sheep skin *in vitro*. Application of these lignocaine rings did not significantly reduce the pain experienced by lambs during the first hour after placement. Reduced lateral lying and cortisol responses from the lignocaine ring and the success of local anesthetic injected at the time of ring placement together indicate that a lignocaine ring has potential to reduce castration pain if the release kinetics can be modified.

2. Introduction

There is little doubt that pain is an unpleasant sensation and most animals try to avoid it where possible. Animal production systems have routinely involved practices that have potential to cause pain and suffering in animals. In these situations, there is a clear obligation to determine the extent of the pain and distress caused and potential for the pain to be alleviated, by reducing or eliminating the procedure or providing appropriate analgesia. During the past 10 to 15 years, pain induced by routine husbandry procedures used on farm and its alleviation have been extensively investigated (e.g. Mellor & Stafford, 2000; Stafford et al., 2002; Stafford and Mellor, 2005a and b). This research has shown that a combination of local anaesthetic and a non-steroidal anti-inflammatory agent (NSAID) is required to alleviate both the acute pain and pain in the hours after procedures such as surgical castration and dehorning. However, despite evidence which demonstrates the welfare benefits of using such analgesics, it is still common practice to perform such procedures without pain relief in NZ. In the UK, it is accepted that all current forms of castration are painful and recommendations are to work toward a situation where all male lambs are either not castrated or, when this is necessary, castrated using pain relief (FAWC, 2008). The impetus for changing farming practices arises from consumer concerns for the animal welfare standards of the products they are purchasing. It is important that NZ industries keep pace with overseas consumer expectations in order to maintain our market access, given that 27 percent of sheep meat consumed in the UK is produced in NZ. The lack of analgesic use during painful husbandry procedures on-farm is mainly due to practical and economical factors; difficulty in administering the drugs, low cost of the animal versus the cost of the treatment and therefore increased cost of production; however, tradition also plays a role (Mellor *et al.,* 2008). These are ongoing issues for farmers and veterinarians that are difficult to resolve and therefore research is required to develop techniques that are effective for pain alleviation that are also practical and cost-effective for on-farm use**.** In lambs, tail-docking and castration are two common procedures which have been clearly demonstrated to cause acute pain and distress. Castration with tight rubber rings results in large increases in cortisol and changes in behaviour in young lambs (Mellor and Murray, 1989). Paul et al., (2009) investigated the use of a topical analgesia as a 'farmer friendly' alternative for administrating pain relief during castration and tailing. In that study, the topical analgesia was only applied to the tail stump after hot-iron docking, making it hard to judge the benefit of this technique for castration alone.

Minimum standards on the conduct of painful husbandry procedures (including tail docking, castration, dehorning and disbudding) are laid out in the Animal Welfare (Painful Husbandry Procedures) Code of Welfare. In recognition of the pain and distress caused by these procedures, the National Animal Welfare Advisory Committee (NAWAC) notes in this Code that it will consider making pain relief mandatory, within defined periods, for a wider range of husbandry procedures and circumstances than that currently prescribed, by 2010. NAWAC held a workshop in 2006 to identify barriers to implementing this recommendation. A number of criteria, including simplicity of use, were recognised as necessary to facilitate wider use of pain relief. It was concluded that further research is needed to identify novel means of providing pain relief during routine husbandry procedures that meet the workshop criteria and will thus be acceptable and widely implemented.

The aim of this project was to design and develop a novel system for delivering local anaesthetic from rubber rings, as a 'farmer friendly' method for providing pain relief during castration of lambs and then establish *proof of concept* for this novel system. The novel system evaluated was to release local anaesthetic from the rubber ring directly into the underlying tissue. Proof of concept involved a determination of the ability for the rubber rings, impregnated with local anaesthetic, to reduce the pain associated with ring castration.

3. Methods

There were 3 consecutive main phases to this study:

1. Design and development of a method of incorporating local anaesthetic onto a rubber ring
2. Evaluation of the release of local anaesthetic from the rubber ring *in vitro*
3. Evaluation of the efficacy of the rubber rings to reduce pain during castration *in vivo*

Elastrator (Heiniger, Industrieweg, Switzerland) rubber rings were used.

3.1. INCORPORATION OF LOCAL ANAESTHETIC INTO A RUBBER RING

Three prototype methods of incorporating lignocaine hydrochloride into a rubber ring were constructed as follows:

Dry ring – The ring was soaked in concentrated lignocaine solution to allow lignocaine to dissolve into the rubber of the ring. Lignocaine was incorporated into the rings from a concentrated alcoholic solution, which was kept saturated by maintaining the solution in the presence of undissolved lignocaine. This was intended to force as much lignocaine as possible into the rings so that they would function as well as possible. The ring was then dried off before storage and use. This prototype had the advantage that it was dry and easy to handle, but the solubility and concentration of lignocaine in the rubber, size of the lignocaine reservoir in the ring and contact area for transfer of lignocaine to the skin were all unknown.

Wet ring – The ring was covered with an ointment containing a high concentration of lignocaine. The advantages of this prototype are that in comparison with the dry ring, the wet rings could form a better interconnection with the skin across which the lignocaine can diffuse and contained a larger reservoir of lignocaine to drive diffusion. However, the ointment coating meant that this ring was slightly more difficult to handle.

Coated ring – This was a wet ring with an additional coating of dry material to encase the ointment. The construction of this ring was more complex and required placement of the ring into a mould with the lignocaine ointment. This was then frozen and removed from the mould. The ring was then coated with PVA adhesive and dried to give a hard shell. This shell was broken by the action of stretching the ring prior to placement. This ring had all the advantages of the wet ring, but was easier to handle. There was however, the possibility that freezing and covering the ring with PVA affected the efficacy of the lignocaine.

3.2. RELEAE OF LOCAL ANAESTHETIC FROM THE RUBBER RING *IN VITRO*

3.2.1 Design and construction of testing apparatus

A skin perfusion apparatus was constructed using Delrin^{TM}, which consisted of a convoluted channel through which saline was slowly perfused immediately above an enclosed chamber through, which warm water was pumped to heat the rig to near-body temperature. Delrin was chosen because it is easy to machine and produces a finished product which is suitable for the task eg, impermeable to water, easy to clean etc. Delrin is a polymer of acetal, but is easier to machine than many other acetal polymers. Warm water was provided at 40°C from a hot water perfusion system and the flow of saline was regulated using a syringe driver. A sample of fresh sheep skin was prepared by removing all subcutaneous tissue and wool, and sutured in place over the perfusion channel such that the inside of the skin was in contact with the perfusing saline (Photo 1).

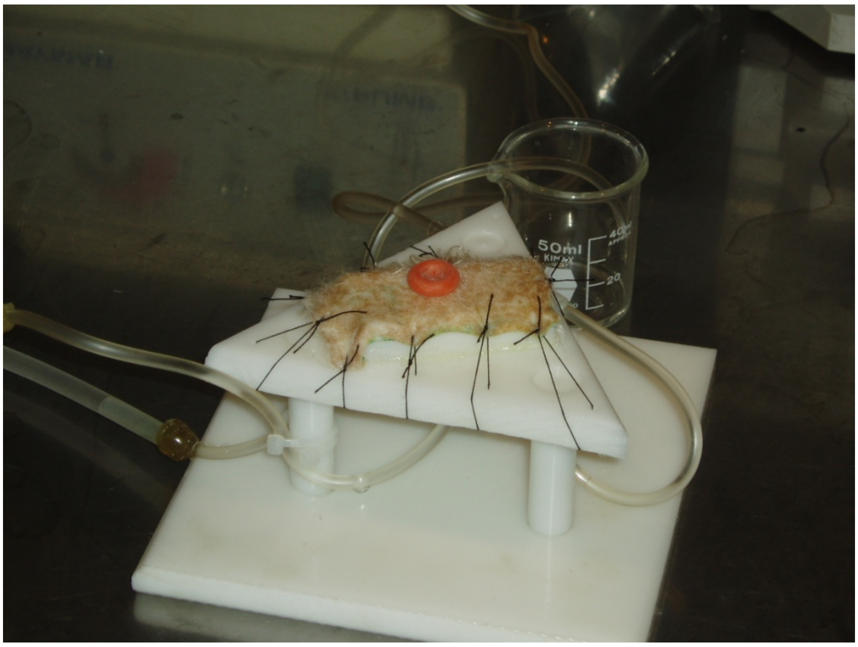
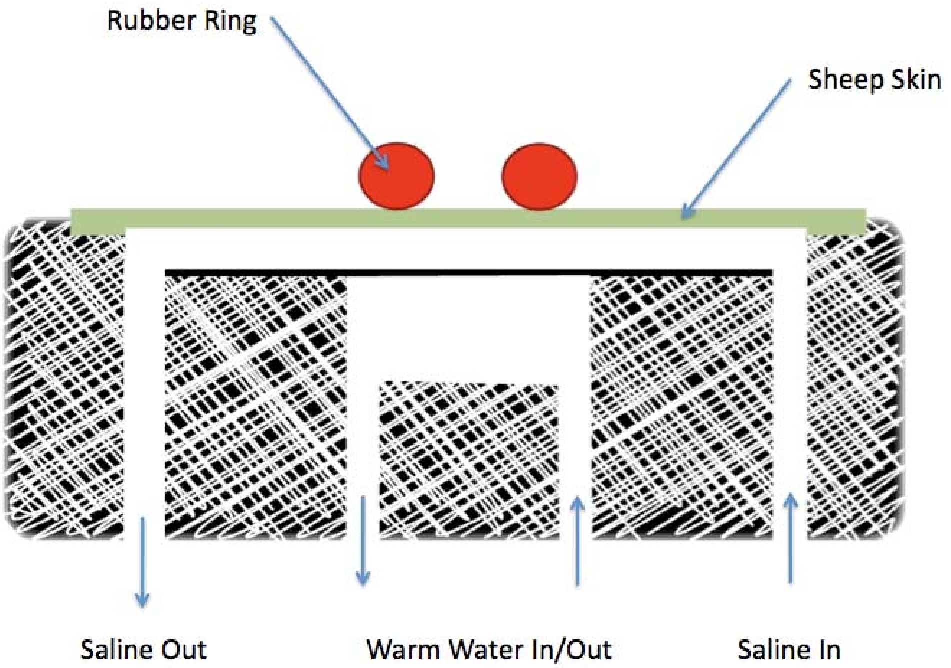


Photo 1. Close up illustrating attachment of sheep skin to top of perfusion apparatus.



Schematic diagram of the perfusion chamber

3.2.2 Testing Protocol

On each occasion, a fresh sample of skin was prepared and mounted on the perfusion apparatus. The ring to be tested was stretched using a commercial ring applicator and then placed onto the apparatus in contact with the outside of the skin and the apparatus perfused with saline at a flow rate of 10 ml/hour for 4 hours. At the end of each hour a sample of 1 ml of saline was collected from the outflow of the perfusion channel for lignocaine analysis.

3.2.3 Lignocaine Analysis

Lignocaine analysis was carried out by high performance liquid chromatography (HPLC) following the methodology described by Bagonluri (2005). All samples were measured in triplicate and the mean of the closest two values for each sample was taken as the lignocaine concentration for that time point. The selection of the ring type to be used in the subsequent animal study was based on the profile of lignocaine concentrations generated. The key characteristics used were the immediate release and the total release of lignocaine over the 4 hour sampling period.

3.3. EVALUATION OF THE EFFICACY OF RUBBER RINGS CONTAINING LIGNOCAINE TO REDUCE PAIN DURING CASTRATION *IN VIVO*

Eighty four singleton male lambs at approximately 4 weeks of age and which had not been previously exposed to any painful husbandry procedures (e.g., ear-tagging, tail docking) were allocated to the study. The trial was carried out over 3 days and comprised two parts utilizing different animals; Day 1 and 2) behavioural responses [n=60 (30 per day)] and Day 3) cortisol responses (n=24). The separation of behaviour and physiological measurements was to eliminate any potential effects of blood sampling on behavioural responses.

Sixty lambs were randomly selected for the behavioural measurements and allocated to six treatments (n=10 per treatment):

1. Control handling (C): The scrotum was handled to simulate the ring castration procedure (approximately 20 sec).
2. Castration with a rubber ring (R): a normal rubber ring was applied to the neck of the scrotum with both testes distal to the ring (in accordance with normal farm practice).
3. Local anaesthetic control (LA): 6 ml of 2 percent lignocaine hydrochloride administered using a 20 gauge x 1 inch needle as 2 ml into each testis and 2 ml into the scrotal neck delivered as the needle is pulled out of the testes into the neck of the scrotum (Mellor and Stafford, 2000).
4. Local anaesthesia plus delayed ring castration (LADR): Lignocaine was administered as above and ring castration carried out as above 4 min later.
5. Local anaesthesia plus immediate ring castration (LAIR): Lignocaine administered as above followed immediately by ring castration as above (the timing of local anaesthetic and ring application was to match the timing of local administration from the lignocaine rings).
6. Castration with a rubber ring coated with local anaesthetic (RLA): castration with a ring as above but using a prototype wet ring which had previously been coated with lignocaine hydrochloride.

3.3.1 Day 1 and 2. Behavioural responses

A total of sixty lambs were observed over two days; thirty different lambs each day (five per treatment group per day). Lambs and ewes were brought in from pasture and the lambs were allowed 30 min to settle after being separated from the ewes (spatially not visually) before behavioural sampling started. Lambs were held in pens (2 x 2 m) in groups of six (one lamb per treatment per pen). Behaviour was recorded continuously by video (Sony DCR-DVD810 DVD Handycam Camcorder). At the end of the observation period the lambs were released back into the paddock with the ewes. Individual behaviours indicative of castration-related pain (see Table 1) were analysed off the video footage using a combination of continuous and scan sampling. Continuous observations of each lamb were applied for the 30 min while lambs were settling in the pen, an additional 30 mins before treatments were undertaken (baseline), and for 1 hour after treatment. After 1 hour post-treatment, periodic scans (for 1 min per lamb once every 5 min) were carried out for an additional 2 hours from the videos.

3.3.2 Day 3. Cortisol responses

The day after behavioural responses were completed, twenty four additional lambs were randomly allocated to three treatments (n=8 per treatment) all carried out as described above:

1. Control handling (C)
2. Castration with a rubber ring (R)
3. Castration with a rubber ring coated with local anaesthetic (RLA)

Ewes and lambs were brought in from pasture and lambs were separated as described above. Two hours was allowed for the settling period to allow for cortisol to return to baseline after the stress of movement and separation from the ewes. Lambs were held in the same pens described above in groups of six (two lambs per treatment per pen). Blood samples were taken from each lamb via venipuncture (using 20 gauge vacutainer needles) of the jugular vein at 0, 30, 60, 90 and 120 min from the start of treatment into tubes containing lithium heparin. All blood samples were placed on ice and centrifuged (1300 g) at 4°C for 10 min and stored at -20°C until assayed for plasma cortisol concentrations. At the end of sampling, lambs were released back into the paddock with the ewes.

3.3.2.1. Cortisol RIA

Cortisol was measured using a double-antibody RIA (Fisher et al., 2002). The intra assay CV was 12.3 percent. This CV is within the acceptable range for this assay and is the same as that reported for cortisol RIA by Stafford *et al*., (2002).

3.3.3 Statistical analysis

Behaviour data from Day 1 and 2 were combined for statistical analysis. The frequency of behaviours (occurrence per animal per hour) were calculated for the baseline period (30 min prior to treatment) and for hourly periods for 3 hours after treatment. For the main postures (standing and lying) an angular transformation was carried out on the percentage of time each activity was observed, followed by a restricted maximum likelihood (REML) analysis with days, pens and their interaction as random effects and baseline durations as a covariate. There were no effects of day or pen. For activities, a log transformation was carried out with a covariate adjustment for total time observed. Due to the low number of activities in hours 2 and 3 after treatment these were combined. A pooled standard error of the difference between means (SED) was calculated and is presented along with P values, where significant ($P \leq 0.05$), in tables and graphs below. The SED represents the probability that the difference between two means is greater than zero (ie they are different) expressed as the standard error of the difference, and a pooled SED is used for simplicity when comparing multiple means. Cortisol was analysed using a one-way ANOVA for each time point, area under the curve, maximum concentration and time to maximum. Residuals were checked for consistency with normality assumptions. The cortisol area under the curve represents an integrated cortisol response (time and magnitude above baseline) and has been used previously to compare the relative noxiousness of different castation procedures (Mellor & Stafford, 2000).

4. Results

4.1. RELEASE OF LOCAL ANAESTHETIC FROM THE RUBBER RING *IN VITRO*

The three prototype rings were constructed and the release of lignocaine tested in the perfusion rig. Lignocaine concentrations for the three rings are illustrated in Fig 1. The maximum concentrations with the dry ring and the coated ring were 39.9 and 239.21 μg/ml respectively at one hour after the start of the infusion. The maximum concentration with the wet ring was 342.6 μg/ml at 4 hours after the start of the infusion. The profile was based on the single 1 ml sample of perfusate generated each hour and measured in triplicate. This single sample for each method at each time point precluded a formal statistical comparison, but allowed a clear decision on the selection of ring to be used in the *in-vivo* study because of the marked difference in the release profiles of each prototype. Overall, the wet ring gave the highest concentrations over the duration of the study and was therefore chosen as the model to be evaluated *in vivo.*

4.2. EVALUATION OF THE EFFICACY OF RUBBER RINGS CONTAINING LIGNOCAINE TO REDUCE PAIN DURING CASTRATION *IN VIVO*

4.2.1 Behavioural responses

There were no differences (P>0.05) between groups during the baseline period.
Lambs were either lying or standing. Lying behaviour changed markedly after treatment (Fig. 2). The main change was in R and RLA groups in which the percentage of time spent in all forms of lying increased and standing decreased. The time spent in all forms of lying and standing were different than C (P<0.01) for hour 1 and 2 after treatment. By hour 3 after treatment, lying and standing behaviour in these groups had returned to levels no different from C. There was no difference between R and RLA at any time point. There was also no difference in the percentage of time spent lying or standing (P>0.05) between C and LA, C and LAIR and C and LADR at any time point. Interestingly there were no differences between LADR and LAIR, however both these groups were lower (P<0.05) than R and RLA.

When lying and standing were separated into the different behavioural sub-classes, lying with and without back limbs extended and lateral lying increased in R and RLA and were higher than C (P<0.001) during the first hour after treatment (Table 2.) By the second hour post treatment (Table 3.) only lying with back limbs extended remained elevated in these two groups (P<0.001). The only difference between R and RLA was in lateral lying in hour 1, which was higher in R than RLA (P<0.001). Lying with back legs extended was also higher in LADR and LAIR treatments than C and LA during hour 1 (P<0.01), but was not different between LADR and LAIR. Lying with back legs extended in LADR and LAIR were all lower (P<0.05) than R and RLA during hour 1.

The other behaviours that increased markedly and were higher in R and RLA than C during hour 1 were Stomping, Stretching, Kneeling, Kicking (P<0.001) and Backward-walking (P<0.05). These behaviours were no different than C after the first hour. There were no differences in these behaviours between R and RLA (P>0.05). Stomping, Backward-walking and Kicking were also higher in LADR and LAIR than C during the first hour after treatment (P>0.05). There was no difference between LADR and LAIR; however, Stretching, Kneeling and Kicking were lower in both of these treatments (P<0.05) than R and RLA.

4.2.2 Cortisol responses

Mean plasma cortisol concentrations increased after ring application in both R and RLA treatments (Fig. 3). Concentrations peaked 60 minutes after treatment in both RLA and R and were lower in RLA than R ($P<0.05$) at 90 minutes after treatment. The total cortisol response (Area under the curve) was greater in R then RLA ($P<0.05$). Cortisol was higher in R than C at all times after treatment ($P<0.01$). In comparison, cortisol levels were higher in RLA than C at 30 and 60 minutes after treatment ($P<0.01$) but were not different from C ($P>0.05$) by 90 and 120 minutes after treatment. Area under the curve and maximum cortisol were also higher in RLA and R than C ($P<0.001$). There were no differences between RLA and R in maximum cortisol or time to maximum cortisol.

5. Discussion

This study has demonstrated *in vitro* that it is possible to coat an elastrator ring with local anaesthetic in such a way that the local anaesthetic will gradually perfuse into underlying skin. Furthermore, the study has found indications *in vivo* that local anesthetic delivered in this way is capable of providing benefit to the animal. The evidence for this benefit was in cortisol response which was lower in lambs with lignocaine rings than normal-ring lambs. The acute behaviour differences due to ring application (and by inference, the most discomfort), occurred mostly during the first hour after treatment. While lateral lying was lower in lambs treated with lignocaine rings than normal rings at this time, it appears that the release of local anaesthetic was not rapid enough to provide major benefit during the period of most discomfort.

The results of the *in vitro* study did not allow for quantitative analysis of the lignocaine concentrations that these rings would be capable of delivering *in vivo*, but they did allow a comparison to be made between the three prototypes. It was clear that the dry ring was not optimal either because it did not absorb sufficient lignocaine or the lignocaine in the ring was not able to diffuse. The coated ring gave the highest lignocaine concentration in the first hour, but this was reduced at the later time points, possibly due to remnants of the coating interfering with the process of diffusion. Although the wet ring did not give the highest lignocaine concentration in the first hour, its performance subsequently improved and was sustained over the course of the perfusion. Based on these criteria the wet ring was chosen for the field study.

There is clear evidence that castration is a painful procedure for young lambs resulting in nociceptive nerve stimulation, a marked HPA response and behavioural changes, indicating discomfort (Cottrell and Molony, 1995; Dinniss et al., 1997; Dinniss et al., 1999). In the present study, behavioural responses indicate that lambs experience a high degree of discomfort during the first hour after ring application as shown previously (Lester et al., 1996). This effect was unchanged in the lambs given a lignocaine ring with the exception of the increased lateral lying which was attenuated. It appeared from that local anaesthetic administration was successful in reducing this discomfort with no difference between administration at 4 min prior or immediately prior to ring application. These results confirm that local anaesthetic is effective at reducing acute castration pain (Kent et al., 1998) and that delivery of local anesthetic from the lignocaine ring was either too little or too delayed to provide analgesia.

Cortisol levels indicated a clear HPA response in lambs treated with normal and lignocaine rings. This is consistent with elevated cortisol responses as a result of castration with a ring in other studies; see review by Mellor & Stafford (2000). The earlier fall in cortisol with the lignocaine rings may indicate that the lignocaine from these rings was having a delayed effect. The overall effect was in the reduction in totsal cortisol response. The time course of transfer of the lignocaine into the circulation from the lignocaine rings *in vivo* is unknown, and a comparison of cortisol responses in lambs with lignocaine administered at the same time as the lignocaine rings were applied was not carried out.

The effective tissue concentration of lignocaine is of the order of 6μg/ml, so a lignocaine ring should work. A problem is that the *in vitro* preparation probably removed less lignocaine than in the intact animal. A further issue with the lignocaine ring seems to be that whilst some of the ointment is squeezed onto the surface at placement, the coating seems to recover much of the ointment and may result in less being available at the surface for later diffusion. We may be able to improve on this by altering the coating material. It may even be that an alternative local anaesthetic such as mepivicaine or articaine could give better results than lignocaine.

In summary, this study has demonstrated that elastrator rings can be coated with local anaesthetic and that this will diffuse through sheep skin. In its current form, this diffusion appears to be inadequate in speed or quantity to significantly reduce the pain experienced by lambs during the first hour after ring placement. The reduction in the cortisol response indicates that the technique has promise as a easy way to administer lignocaine but this reduction was not transferred into a significant changes in pain-related behaviours, possibly due to a delayed transfer of lignocvaine throught the skin. The effectiveness of local anesthetic at reducing this pain when injected at the time of ring placement suggests that a lignocaine ring does have potential if the release kinetics can be modified. The ease of practically administering local anesthetic in this way warrants further research in order to develop 'farmer friendly' methods for administering pain relief on-farm. The success of lignocaine administration at the time of ring application would indicate that if a ring can be constructed to achieve a similar rapid release of lignocaine into tissue it should be effective at reducing pain.

6. Acknowledgements

The authors would like to thank the staff at the AgResearch Whatawhata Farm, Alison Cullum, Marcie Greenhead, Andrea Rogers and Ondine Habgood for their valuable input into the conduct of this study, Assistant Professor Paul Kenyon (IVABS, Massey University) for the use of video equipment and Neil Cox for conducting the statistical analysis.

7. Figures and tables

Figure 1.
Concentration of lignocaine (µg/ml) during four hours of *in vitro* perfusion with dry rings, wet rings or coated rings. Each time point is based on a single 1 ml sample taken from an hourly 10 ml pool, measured in triplicate and calculated as the mean of the two closest values.

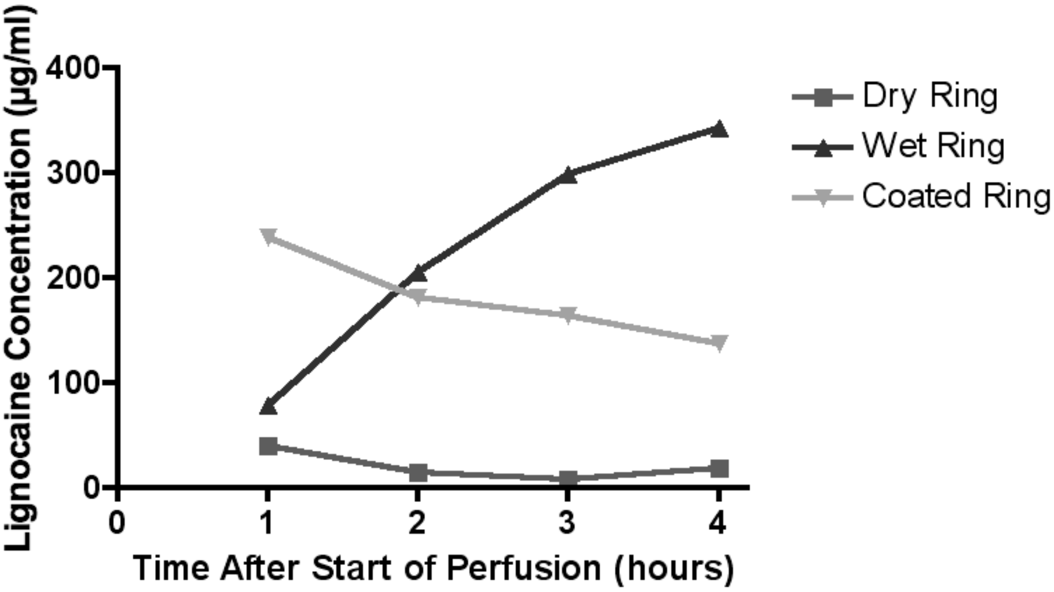


Table 1: Ethogram of behaviours measured.

State		-	Event
Unclassified Lying view of lamb position obstructed for more than 3 sec.	**L Lying** No weight supported by legs and body is in contact with ground	**N**	ventral lying- **N**o extension - No back limbs extended.
		E	Lateral/ventral lying- **E**xtension - 1 or 2 hind limbs are fully extended (completely straight).
		LA	Lateral lying- where **at** least 3 limbs are extended straight and the shoulder plus rump are on the ground.
		R	**R**olling - from lying on one side to the other side of the body. Half rolls where the lamb rolls onto its back and then returns to lying on the same side are also included (Kicking can not be recorded at the same time; it is either one or the other).
			-
	U Upright Not lying	**L**	**L**eaning on exterior walls with whole body in contact with a gate/wall for a minimum of 2 secs.
		St	**St**atue standing - No movement of more than one hoof. The top of the head is below the top of the withers with little or no head movements for longer than 5 seconds. Leg has no contact with head.
		Sp	**S**tom**p** - Rapid up and down movement of 1 leg towards the body and hoof lands in close proximity to starting position.
		B	**B**unny hop -The action of moving forward in a "hop" where both two back hooves leave the ground simultaneously or (within a second of each other) in a leaping action.
		C	**C**ircling - When the lamb walks in a constant circle without distractions, scoring only if the whole circle is completed .
		F	**F**alling - When lamb falls to the ground in one rapid movement in 2 seconds or less.
		S	**S**tretch - a concave arch of the back from a horizontal (neutral) position.
		Bw	**B**ackward **w**alking - 3 or more consecutive steps of either the front or either the back legs.
	(either upright or lying)	**T**	**T**rembling/ Shiver - Any part of the body visibly shivering - forceful voluntary body shake was not included.
		Kn	**Kn**eeling - While upright drops to both front knees and gets back up. While lying gets to knees and back down to a lying position. (The original behaviour must be repeated after kneeling to be classified as a kneel)
		Kc	**K**i**c**king - Rapid movement and extension of a hind leg either outwards or towards the body (if two legs kick, score as two events). A kick is not scored if combined with getting into a standing or lying position, eg: extension
		Na	**N**eck **a**rch backward or upward with a lifted upper lip.

Figure 2.
Mean percentage of time spent lying for lambs prior to (baseline) and at hourly intervals after castration with a normal elastrator ring (R), a ring coated with local anaesthetic (RLA), local anaesthetic given 4 min before a normal elastrator ring (LADR), local anaesthetic given immediately prior to a normal elastrator ring (LAIR), or injection of local anaesthetic without castration (LA) or handling without castration (C). The pooled SED for each time period is denoted by the vertical bars.

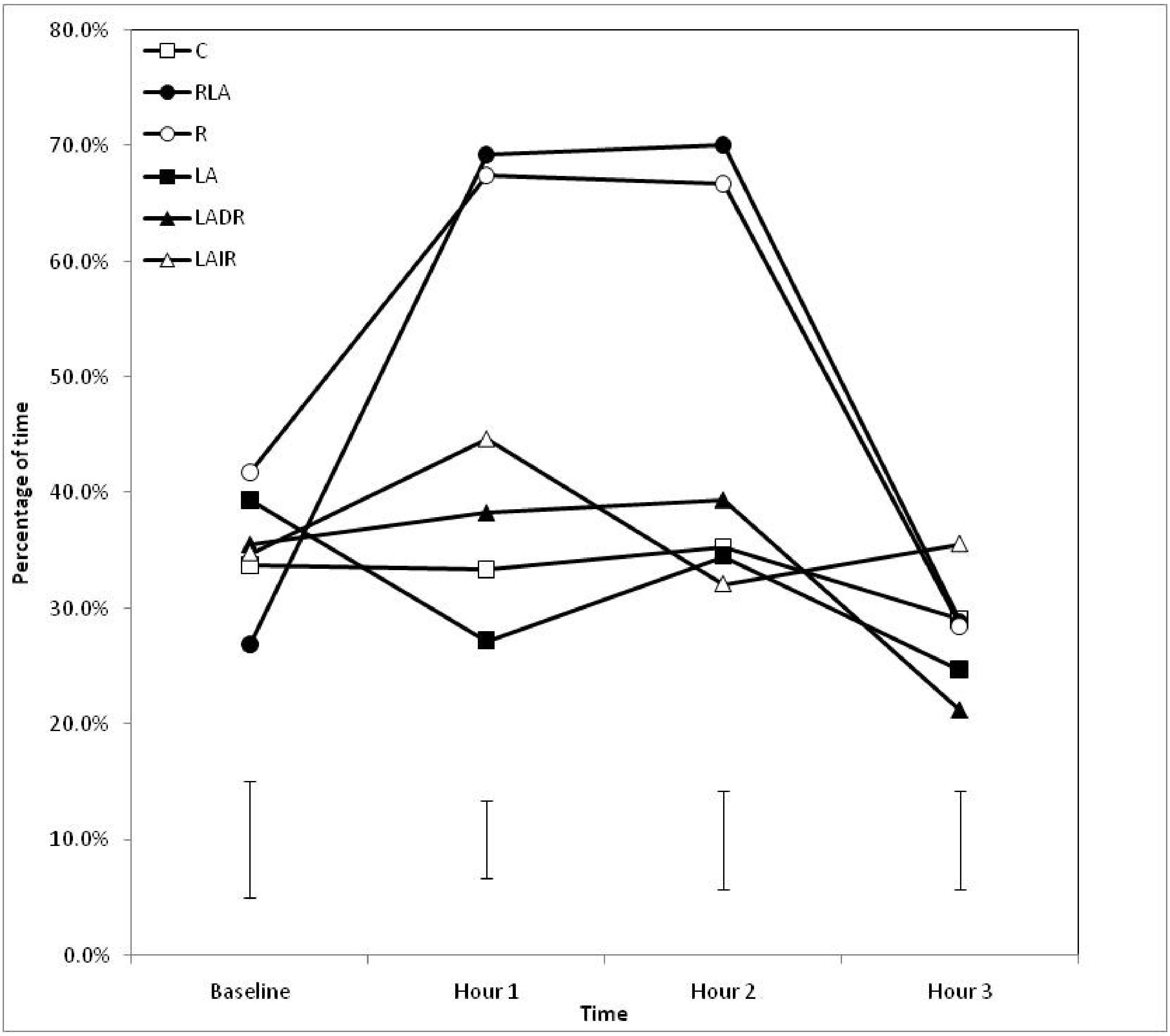


Figure 3.
Mean plasma concentration of cortisol (ng/ml) in lambs treated with a normal elastrator ring (R), a ring coated with local anaesthetic (RLA) or handling without castration (C).

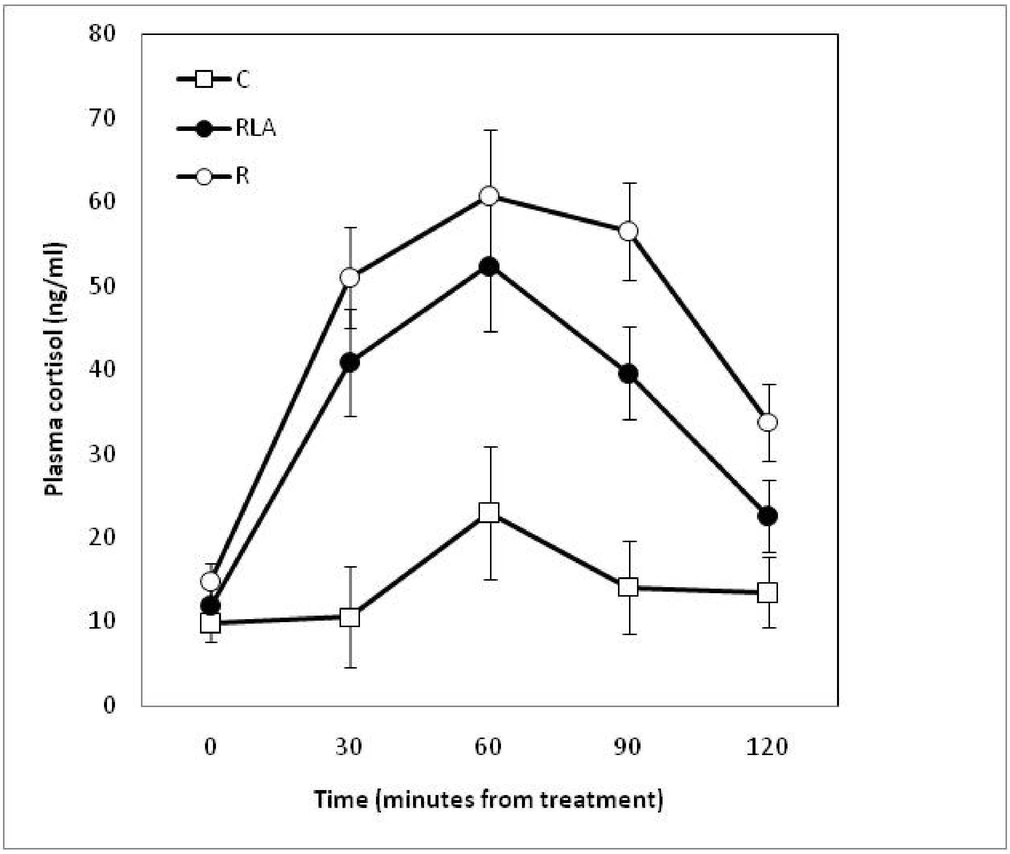


Table 2.

Frequency of behaviours (occurrence per animal per hour) as defined in Table 1, during the first hour after treatment with a normal elastrator ring (R), a ring coated with local anaesthetic (RLA), local anaesthetic given 4 min before a normal elastrator ring (LADR), local anaesthetic given immediately prior to a normal elastrator ring (LAIR), or injection of local anaesthetic without castration (LA) or handling without castration (C). The pooled SED and significance level is presented below the means.

	N	*E*	*LA*	*St*	*Sp*	*B*	*S*	*Bw*	*Kn*	*Kc*
C	7.79	0.36	0.00	0.64	3.01	1.39	0.42	6.04	0.09	-0.01
RLA	49.14	41.56	8.31	0.26	9.81	1.12	2.81	16.36	8.12	24.83
LA	2.78	0.16	0.00	1.05	2.69	0.82	0.43	16.77	-0.05	0.14
LADR	15.09	3.28	0.00	0.73	9.61	1.08	0.35	15.02	0.90	2.52
LAIR	12.57	2.68	0.13	0.92	4.86	1.43	0.32	16.76	1.94	3.75
R	48.34	47.36	19.95	0.10	23.42	1.46	3.37	15.45	10.89	28.96
SED	7.90	4.09	1.45	0.55	4.67	0.64	0.79	3.83	1.63	3.30
P	<0.001	<0.001	<0.001	0.484	<0.001	0.916	<0.001	0.042	<0.001	<0.001

TABLE 3.

Frequency of behaviours (occurrence per animal per hour) as defined in Table 1, during hours 2 and 3 after treatment with a normal elastrator ring (R), a ring coated with local anaesthetic (RLA), local anaesthetic given 4 mins before a normal elastrator ring (LADR), local anaesthetic given immediately prior to a normal elastrator ring (LAIR), or injection of local anaesthetic without castration (LA) or handling without castration (C). The pooled SED and significance level is presented below the means. The grey shaded areas are where there were insufficient occurrences to statistically analyse.

	N	*E*	*LA*	*St*	*Sp*	*B*	*S*	*Bw*	*Kn*	*Kc*
C	16.75	1.37			1.29	3.87		9.11		
RLA	17.53	18.81			0.37	1.71		6.98		
LA	20.15	1.64			1.84	4.80		13.70		
LADR	15.58	3.56			1.54	1.87		15.31		
LAIR	16.67	4.26			0.87	4.22		13.06		
R	17.23	14.53			0.81	4.63		15.53		
SED	6.06	3.48			0.83	2.35		5.01		
P	0.985	<0.001			0.501	0.607		0.426		

8. References

Bagonluri, M., Woodbury, J., Reid, R., Boison, J., 2005. Analysis of lidocaine and its major metabolite, monoethylglycinexylidide, in elk antler by liquid chromatography with UV detection and confirmation by electrospray ionization tandem mass spectrometry. J. Agric. Food Chem. 53, 2386-2391.

Cottrell, D.F., Molony, V., 1995. Afferent activity in the superior spermatic nerve of lambs - The effects of application of rubber castration rings. Vet. Res. Commun. 19, 503-515.

Dinniss, A.S., Mellor, D.J., Stafford, K.J., Bruce, R.A., Ward, R.N., 1997. Acute cortisol responses of lambs to castration using a rubber ring and/or a castration clamp with or without local anaesthetic. N. Z. Vet. J. 45, 114-121.

Dinniss, A.S., Stafford, K.J., Mellor, D.J., Bruce, R.A., Ward, R.N., 1999. The behaviour pattern of lambs after castration using a rubber ring and/ or castrating clamp with or without local anaesthetic. N. Z. Vet. J. 47, 198-203.

Fisher, A.D., Verkerk, G.A., Morrow, C.J., Matthews, L.R., 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. Live. Prod. Sci. 73, 255-263.
FAWC, 2008. Report on the implications of castration and tail docking for the welfare of lambs. http://www.fawc.org.uk/pdf/report-080630. pdf.

Kent, J.E., Molony, V., Graham, M.J., 1998. Comparison of methods for the reduction of acute pain produced by rubber ring castration or tail docking of week-old lambs. Vet. J. 155 39-51.

Lester, S.J., Mellor, D.J., Holmes, R.J., Ward, R.N., Stafford, K.J., 1996. Behavioural and cortisol responses of lambs to castration and tailing using different methods. N. Z. Vet. J. 44 45-54.

Mellor, D.J., Murray, L, 1989. Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. Res. Vet. Sci. 46, 387-391.

Mellor, D.J., Stafford, K.J., 2000. Acute castration and/or tailing distress and its alleviation in lambs. N. Z. Vet. J. 48, 33-43.

Mellor, D.J., Fisher, M.W., Stafford, K.J., 2008. A cost-benefit analysis of pain relief for farm animals. In Scientific assessment and management of animal pain. OIE Technical series Volume 10. 47-55.

Paull, D.R., Lee, C., Colditz, I.G., Fisher, A.D., 2009. Effects of a topical anaesthetic formulation and systemic carprofen, given singly or in combination, on the cortisol and behavioural responses of Merino lambs to castration. Aust. Vet. J. 87, 230-237.

Stafford K.J., Mellor, D.J., 2005a. The welfare significance of the castration of cattle: a review. NZ Vet. J. 53:271-278.

Stafford K.J., Mellor, D.J., 2005b. Dehorning and disbudding distress and its alleviation in calves. Vet J. 169:337-349.

Stafford, K.J., Mellor, D.J., Todd, S.E., Bruce, R.A., Ward, R.N., 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res Vet Sci 73:61-70.