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Evidence Project Final Report

Department for Environment Food & Rural Affairs

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Project identification

1. Defra Project code: 36033

2. Project title:
   Quantitative assessment of pain associated behaviours of male lambs (less than 1 week and 4-6 weeks old) in response to castration or tail docking with a new method *(Clampeasy/* also known as *ClipFitter)* compared with a 'best welfare practice' method which includes local anaesthesia and analgesia.

3. Contractor organisation(s): SRUC

4. Total Defra project costs (agreed fixed price): £ 45,156

5. Project: start date 01/02/2022
   end date 30/09/2022

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

1. Tail-docking and castration are legally permitted in the UK without the use of anaesthesia or analgesia in lambs aged less than 7 days through use of tight rubber rings, and at older ages with use of anaesthesia or a Burdizzo. However, extensive evidence exists that use of rubber rings cause significant pain in lambs, including in lambs aged under 1 week. FAWC reported on this in 2008 and recommended that it would be appropriate to reconsider this position if practical methods to provide pain relief and improve these procedures were developed.

2. Since 2008 advances in developing immunocastration procedures for lambs have occurred (e.g. Needham et al., 2016; Maslowska, 2016; although not licensed for use in the UK), and various methods have been trialled including rubber rings impregnated with lidocaine (Stewart et al., 2014), and tighter rubber rings designed to achieve a more effective nerve crush (Molony et al., 2012), neither of which have been effective in mitigating pain. Two newer approaches have since been developed: Numnuts (e.g. Small et al., 2021) and ClampEasy (now known as ClipFitter).

3. ClipFitter (which will be referred to as ClampEasy in the report), is a device designed to apply a disposable plastic clip, in a similar way to application of a rubber ring, with the aim of achieving the same desensitisation as can be achieved with a Burdizzo. The Burdizzo crushes the spermatic cords and the nerves to the scrotum by clamping on each side. This has been shown to cause very short term acute pain responses which are significantly lower than are seen with the rubber ring. However, this device requires considerable skill from the applicator, and is not often used by farmers. The Clipfitter is designed to improve the application of this method, and unpublished pilot work on an earlier prototype suggests that this can significantly reduce pain at castration.

4. This study was designed to test the efficacy of the newest version of Clipfitter in mitigating lamb pain at castration or tail docking at two lamb ages (less than 7 days old; between 4-6 weeks old) aligning with the times that rubber rings can legally be applied to lambs or the age at which farmers, particularly hill farmers, would prefer to gather and treat their lambs. The Clipfitter was compared to methods legally permitted at both ages, and to a positive control (handled only). In

young lambs the treatments were: castration using rubber rings, castration using rubber rings with local anaesthetic and analgesia, castration using ClipFitter, tail docking with rubber rings and local anaesthesia and analgesia, tail docking with ClipFitter and lambs that were only handled and not castrated or tail-docked. In older lambs the treatments were: castration with ClipFitter, castration with Burdizzo using local anaesthetic and analgesia, tail docking with ClipFitter, tail docking with rubber rings and local anaesthetic and analgesia, and handled only.

5. The effect of treatment on lamb pain and welfare were assessed by quantitative behavioural measurements (specifically active pain behaviours and time spent in various postures) measured over the first 60 minutes after treatment, Qualitative Behavioural Assessment (QBA) taken at 16 minutes after the treatment, assessments of lesion scores, response to palpation, growth and time taken to shed the tissues over approximately 4-5 weeks after treatment.

6. In young lambs, rubber ring castration caused a significantly greater active pain response and more time spent in abnormal postures than either handled lambs or those castrated with ClipFitter. This response was not greatly reduced through the use of local anaesthesia or analgesia. Lambs castrated with ClipFitter had behavioural responses that did not differ from the lambs that were only handled, suggesting that the device was able to achieve desensitisation that normalised lamb responses to handled controls. QBA data similarly showed a very significant separation of ClipFitter treated and handled lambs from those castrated with a rubber ring either with or without pain relief.

7. In older lambs, ClipFitter castration caused a significantly greater behavioural response compared to handled or Burdizzo treated lambs given local anaesthesia and analgesia. However, compared to the response of young lambs to the rubber ring (approximately 185 active pain responses in the first hour) the response of older lambs to ClipFitter (approximately 22 active pain behaviours per hour) was considerably reduced. QBA data gave similar results to that seen with quantitative behavioural analysis.

8. At both ages, lambs tail-docked with ClipFitter had similar behavioural responses to lambs tail-docked with rubber rings when given pain relief, which was greater than the response seen in lambs that were handled only. QBA data from tail-docked animals did not show any significant differences between treatments.

9. Lambs of both ages, when either castrated or tail docked with ClipFitter, were significantly quicker to shed the necrotic tissue than lambs that were castrated or tail-docked with rubber rings, with or without pain relief. Lesion scores, which assess the size and severity of the tissue damage around the site of castration or tail-docking, were less in ClipFitter treated lambs for castration and equivalent for tail docking. There was little response of the lambs to palpation of the tissues in any treatment group.

10. In younger lambs, full bilateral castration was not possible with ClipFitter for all lambs (for the size and weight of the lambs used in this study) and 50% of lambs were short scrotum castrated. This did not affect the significance of the pain reducing effects of ClipFitter and short scrotum lambs are known to be infertile so would not effect the efficacy of the device for achieving castration. However, this may be an issue with using ClipFitter on very young lambs.

11. In the older lamb group, the analgesic used (under veterinary advice) unexpectedly caused Acute Respiratory Distress Syndrome in some lambs, which lasted beyond the observation period. Although attempts were made to reduce this impact, and a different analgesic was used subsequently, the data on lambs given this treatment may be unreliable.

12. Overall, the data are supportive of ClipFitter as an alternative method of castration and tail docking that induces a lower pain response to use of rubber ring alone and equivalent to the use of pharmaceutical pain relief. Tissues were shed more quickly than with rubber rings, and the lesions

and responses to palpation were equivalent or better than seen by other methods as applied in this study.

Project Report to Defra

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   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

Castration and tail docking of lambs is permitted in the UK and the EU with some exceptions. Castration is allowed in all EU countries although Denmark, and Sweden only permit this to be done by a veterinarian with the use of local anaesthesia. Tail-docking is forbidden in Finland, Lithuania, and Sweden, permitted only for female sheep in Belgium, and only for 3 specified breeds in the Netherlands. In the UK, lambs can be castrated by stock-keepers up to 3 months of age, thereafter only by a veterinarian using anaesthesia, although rubber rings can only be used under 7 days of age in England and Wales, and only over 7 days with anaesthesia in Scotland. Castration using a clamp (Burdizzo) can be done by stock-keepers up to 3 months of age without anaesthesia, although if combined with a rubber ring then the legislation for rubber ring use applies. FAWC has looked at these issues in 2008 and concluded that these procedures should only be carried out when risks of unplanned pregnancies or flystrike cannot be managed in another way and that it would be desirable to use pain relief for castration and tail docking when these procedures are necessary. FAWC (2008) also suggested that this should be reviewed again in 5 years' time, and that if practical methods of delivering acute and/or long-term pain relief are developed it would be appropriate to re-assess permitted methods of castration. Since 2008 several studies have investigated methods of delivering pain relief more practically when these procedures are carried out, as well as looking at alternative methods of castration, such as immunocastration. Of the methods used to achieve pain relief the two methods that appear to have been most successful include the use of *Numnuts* (a device which delivers local anaesthetic as the rubber ring is applied) and *ClampEasy/*Clipfitter (hereafter this will be called ClampEasy) (Eadie Bros Ltd., Selkirk). The *ClampEasy* device is based on the desensitising effect of crushing the nerves to the scrotum and testes or tail as is achieved with the use of the Burdizzo, especially when combined with a rubber ring. This 'Combined' method had been shown previously (Kent et al 2001, 2004) to provoke much less behavioural (pain) responses than rubber rings, the most used method for castration of lambs. In 2018 a 'proof of principle' study on 6–8-week-old lambs demonstrated that an experimental clamp, upon which the *Clampeasy* system is based, did not provoke more behavioural (pain) responses to castration than the 'Combined' method (considered to be the most humane alternative).
The present study set out to investigate the impact of the *Clampeasy* system on lambs aged less than 7 days old, and at older ages (6-8 weeks), often the age at which hill sheep farmers would find it most convenient to handle lambs for both castration and tail-docking.

Aims and Hypotheses
The aim of the present study was to test the ability of the ClampEasy device to mitigate pain from tail docking and castration when compared to unhandled animals and to the methods currently available to farmers within the legislation. We hypothesised that the ClampEasy device would have a similar ability to mitigate pain as seen with the best available methods of legal castration/tail-docking in young and older lambs and will cause less pain than the current most used method in young lambs (rubber ring). We also hypothesised that the level of pain experienced by lambs will not be greater than that experienced by handled lambs which do not experience a painful treatment.

Animals and Methods

The study took place at two different time periods, with young lambs (Scottish Blackface) treated in March 2022, and older lambs (Suffolk-Mule x Texel or Suffolk-Mule x Suffolk) treated in February 2022, with sample periods and breeds affected by lamb availability. Lambs were born as part of the wider flock at SRUC, managed under commercial conditions, and were transferred to the experimental facilities once ewes and lambs had bonded and lambs were deemed healthy. Single and twin-born lambs were used in the study of older lambs, but only twin-born lambs were used in the study in young lambs. Ewes and lambs were housed in large, straw-bedded pens (approximately 12 ewes per pen), and treatment lambs were moved, with their dam and any siblings, to a treatment pen (1.5 x 2 m) adjacent to the home pen in which the procedure was applied. Treated lambs remained in this pen for 60 minutes after treatment.
A total of 88 lambs were used, divided into treatment groups of 8 lambs per treatment (based on previous results from Molony et al., 2002, 2012). Lambs were balanced across treatments for litter size (single or twin, older lambs only), with twin lambs allocated to different treatments. Treatment groups were mixed in all home pens, as to not confound treatment with pen. Best practice treatments for each age group were decided based on veterinary advice and were carried out by a veterinarian, (older lambs) while all other treatments were carried out by an experienced technician.
Lambs were allocated to treatment as described below (and summarised in Table 1):

1) **Young lambs** (Scottish Blackface, <7 days old), 8 lambs per treatment:

a) Castration (male lambs):

i) Negative control, rubber ring (**RR**) castrated: standard rubber rings applied close to the abdominal wall around the neck of the scrotum using an elastrator, without the use of local anaesthetic or analgesic (established current practice).

ii) *ClampEasy* (**CE**): This method involves both an applicator and a disposable plastic clip, without the use of local anaesthetic or analgesic. The clip was applied across the full width of the

lamb's scrotum, at its neck, and remained on the lamb until it was shed after healing of the crush wound (Eadie, 2019). This procedure was carried out by a HO licenced technical assistant after advice from the manufacturer of the devices. For 4/8 lambs in this group it was not possible to manipulate the testes to be distal to the clamp and these lambs were therefore castrated by the short scrotum method.

iii) Best Practice (Veterinary-advised; **BP**): 0.1-0.2ml procaine (*Pronestesic*® 40mg/ml Procaine Hydrochloride + Epinephrine 0.2mg/ml: Fatro SpA, Bologna, Italy) was injected into both the spermatic cords and the testes, and 0.3ml meloxicam (***Metacam***® 20mg/ml Boehringer, Ingelheim, Germany) was injected into the neck of the scrotum, five minutes before castration using the rubber ring method, as described for the **RR** group. This procedure was carried out by a licenced technical assistant after advice from a vet (Dr. Fraser Murdoch, University of Edinburgh, Farm Animal Practice).

iv) Positive Control (**H**): lambs were caught and handled in a similar way and for a similar duration as for castration but no castration procedure was carried out.

b) Tail docking (female lambs):

i) ClampEasy **CE**): as described above, with the clip applied across the tail between the 2nd and 3rd caudal vertebrae.

ii) Best Practice (**BP**) (Veterinary-advised) using 0.1-0.2ml procaine (*Pronestesic*® 40mg/ml Procaine Hydrochloride + Epinephrine 0.2mg/ml: Fatro SpA, Bologna, Italy) was injected into both sides of the tail, and 0.3ml meloxicam (***Metacam***® 20mg/ml Boehringer, Ingelheim, Germany) was injected sub-cutaneously five minutes before castration using the rubber ring method, as described for the **RR** group. This procedure was carried out by a licenced technical assistant after advice from a vet (Dr. Fraser Murdoch, University of Edinburgh, Farm Animal Practice).

iii) Positive control (**H**): as described above.

2) **Older lambs** (Mule x Texel, 5-6 weeks of age), 8 lambs per treatment group.

a) *Castration (male lambs)*

i) ClampEasy (**CE**) (as described above)

ii) Best Practice (**BP**): [carried out by vet (Dr F. Murdoch)]: 0.75ml procaine hydrochloride (***Pronestesic***® 40mg/ml Procaine Hydrochloride + Epinephrine 0.2mg/ml: Fatro SpA, Bologna, Italy) was injected into both spermatic cords and testes, and a NSAID (Finadyne™ MSD Animal Health UK Ltd, Milton Keynes, UK) 1.5mg/kg was administered I/V to the first two lambs of this group. These lambs developed Acute Respiratory Distress Syndrome (ARDS) within 20min which lasted for > 6 hrs. The route of injection was changed to S/Cut for the next two lambs, but these lambs also showed signs of ARDS and the dose was reduced from 1.5mg/kg to 1.0mg/kg S/Cut for the last four lambs. Injections were carried out five minutes before castration using the Burdizzo method in which each spermatic cord was crushed for 10s with a Ritchey Nipper (Ritchey Ltd, Ripon, UK).
NB The unwanted and unexpected (side) effects of the NSAID Finadyne™ should be borne in mind in consideration of these results.

iii) Positive control **H**: as described above

b) *Tail-docking (female lambs)*

i) ClampEasy (**CE**): as described above, with the clip applied across the tail between the 2nd and 3rd caudal vertebrae.

ii) Best Practice (**BP**) **[**carried out by vet] using standard rubber rings with the anaesthetic/analgesic regime as described above for castration, injecting the local anaesthetic into each side of the tail and Metacam given sub-cutaneously.

iii) Positive control (**H**): handled as described above.

NB, For older lambs no negative control with rubber rings was used since this is illegal in the UK and was not considered a suitable control. In all cases the new method (CE) was compared to currently available methods and to handled animals (H) without any potentially painful procedures.
Best Practice treatments were as advised by or were carried out by experienced sheep veterinarians (Dr. F. Murdoch BVMS. PhD. MRCVS and Dr. R. Kelly BVSc, MSc, PhD. MRCVS) based on their view of the best methods to mitigate pain at each age. This BP method was considered to represent the situation that

a farmer, wishing to use pain relief, might expect to experience.

Table 1. Summary of experimental design showing allocation of animals to treatment

Group	Age	Code	Sample size	Procedure	Method
G1	<7 days	RR	8 M	Castration	Rubber ring, no anesthesia/ analgesia
G2	<7 days	H	8, 4M, 4F	Control	Handling only
G3	<7 days	CE-cast	8 M	Castration	ClipFitter
G4	<7 days	BP-cast	8 M	Castration	Best Practice (vet advised)
G5	<7 days	CE-TD	8 F	Tail docking	ClipFitter
G6	<7 days	BP-T	8 F	Tail docking	Best Practice
G7	4-5 wks	H	8, 4M, 4F	Control	Handling only
G8	4-5 wks	CE-cast	8 M	Castration	ClipFitter
G9	4-5 wks	BP-cast	8 M	Castration	Best Practice
G10	4-5 wks	CE-TD	8 F	Tail docking	ClipFitter
G11	4-5 wks	BP-TD	8 F	Tail docking	Best Practice

Data collection

1. Live behavioural recording

Live observation of the behaviours of each treatment group of 8 were recorded directly, at the time of treatment, by trained observers using a validated ethogram (Molony et al 2002). These behaviours were recorded in 2 minute blocks for 60 minutes after treatment as previously described (Molony et al., 2002). These live observations were not blind to treatments. However, it was possible for observers to move and so they were better able to see the lambs, which were occasionally obscured from view in the video images. Live recordings were also used to assess the time taken for treatment from picking up the lamb until it was returned to its dam.

Potential pain behaviours of lambs were recorded for up to 30 days; in 3 x 1 hour periods every 3-4 days (three mixed groups/pens were measured each recording day between 13:00 and 16:00hrs) using scan sampling for quantitative assessment of potential chronic pain behaviours in lambs.

2. Video analysis of quantitative behaviours

A digital video camcorder (Canon Legria; Canon Inc., Tokyo, Japan) was placed on a tripod and used to record the full treatment pen, including the focal lamb, ewe and sibling, if present, for one hour directly after treatment. These data were used to assess lamb behaviour using a detailed ethogram outlined in Table 2. Videos were watched in order of spray number, which was random for treatment, with the older lambs first. Although the observers were aware of the treatments they were blinded to the allocations and thus did not know the treatment given to the lamb being observed. Data were collected using continuous focal sampling recorded through the Observer XT program (Version 16; Noldus Information Technology, Wageningen, Netherlands). Intra-observer reliability analysis was conducted in Observer XT by scoring the same 10-minute clip three times throughout the data collection period. Agreement was high between the first and second (agreement = 88.89%, Kappa = 0.85), second and third (agreement = 82.76%, Kappa = 0.77) and first and third (agreement = 92.31%, Kappa = 0.90) observations.

Table 2. Ethogram of lamb behaviours for continuous focal sampling. Adapted from Small et al. (2020) and Molony et al., (2002). Events were recorded as frequencies, states were recorded as total duration, numbers of bouts (frequency) and average bout length.

Behaviour	Definition
Events	
Restlessness	Any postural transition between standing, lying, kneeling and dog-sitting. Postural transitions while teat seeking (e.g. standing to kneeling) were not counted.
Foot stamping and kicking	Either a front or hind limb (usually hind limb) was lifted and forcefully placed on the ground while standing or the leg was forcefully moved backwards or outwards without striking the ground, while standing or lying.
Pawing	Front foot scraped at the ground in a repetitive pattern.
Head turning	Movement of the head beyond the shoulder: included both looking and touching at the source of pain and grooming.
Easing quarters	One action was recorded each time a front or hind limb, including the shoulder and hindquarters was moved in a less forceful manner than stamping or kicking or the whole body was shifted or eased without moving from the place of rest. Tensing of leg muscles was also included.

Tail wagging	A single side-to-side tail movement was recorded as one action. A continuous series of tail movements, without obvious pause with the tail hanging down, was counted as one action. Tail wags while teat seeking were not counted.
REW	Sum of restlessness, foot-stamping/kicking, pawing, head turning, easing quarters and tail wagging (active pain-related behaviours)
States	
Normal standing	Bodyweight was supported equally on all four limbs with no apparent abnormalities.
Abnormal standing	Bodyweight was supported on all four limbs while showing abnormalities (e.g. statue standing: immobile standing with an obvious withdrawal from interaction with other pen members and outside stimuli, stretched standing: legs positioned further back than normal).
Standing other	Bodyweight was supported on all four limbs but unable to clearly categorise the standing posture (e.g. obscured view).
Normal walking	Took more than two steps in any direction with no apparent abnormalities.
Abnormal walking	Took more than two steps in any direction unsteadily or stiffly (e.g. walking backwards, on knees, moving forward with bunny hops, circling, leaning or falling).
Walking other	Took more than two steps in any direction but unable to clearly categorise the walking type (e.g. obscured view).
Normal lying	Body was in contact with the ground, bearing weight on the sternum (ventral recumbency), with all legs tucked under body or very close to body.
Abnormal lying	Body was in contact with the ground, bearing weight on the sternum (ventral recumbency), with forelimbs tucked under body and one or both hind limbs partially or fully extended.
Lateral lying	Body was in contact with the ground, with one shoulder on the ground, and hind limbs and/or forelimbs fully extended.
Lying intention	Attempts to lie down without completing the manoeuvre in a single sequence.
Lying other	Body was in contact with the ground but unable to clearly categorise the lying posture (e.g. obscured view).
Kneeling	Body was supported by front carpal joints and hind legs. Not recorded when teat seeking or transitioning smoothly between lying and standing.
Dog sitting	Bodyweight was supported by two extended front legs and with the hindquarters on the ground.
Rolling	Rolled from lying on one side to the other without getting up. Half rolls onto back and then return to lying on the same side included.
Running	Took more than two steps in any direction at gait faster than walking.
Jumping	Forelegs were lifted from the ground and the forepart of the body was elevated in an upward movement.
Eating	Head was lowered directly into the food trough or bucket and lamb manipulated feed with its mouth.
Teat seeking	Actively moved towards and placed head near the teat of the ewe. Successful suckling involved the lamb holding onto the teat and often tail wagging. No differentiation was made between the lamb seeking and finding the teat with or without suckling taking place.
Playing	Agonistic interactions with other animals or objects (e.g. butting, mounting) or exuberant skipping.
Not visible	Lamb is out of sight.

3. Qualitative Behavioural Assessment

A two-minute video clip was selected 16 minutes after treatment following the protocol of Masłowska et al. (2020) to coincide with the greatest pain expression following castration (Molony et al., 2002). If the lamb was not visible during this period of time, a clip was selected from the closest time after it. The clips were ordered by spray number, which was random for treatment, with the older lambs first. Clips were shared with three observers, who were experienced with sheep behaviour, via an online cloud storage service. All observers were aware of the treatment groups, but were blinded to which treatment was presented in each clip.

The terms generated by participants in Masłowska et al. (2020) through Free Choice Profiling (FCP) were used as a starting point for the development of a Fixed List of terms. Any potentially repetitious descriptors were eliminated and definitions for each term were drafted. The suggested list was then discussed by the three observers and two other individuals with experience in QBA studies. The list of terms and their definitions were then revised by the three observers until consensus was reached; the final list of terms is displayed in Table 3.

Table 3. Fixed list of terms and definitions for Qualitative Behavioural Analysis (QBA). Derived from FCP terms developed in Masłowska et al. (2020).

Term	Definition
Calm	Lamb appears placid and is unhurried when physically active.
Restless	Lamb appears uncomfortable and displays constant activity (e.g. laying down and getting back up again).
Comfortable	Lamb appears to be physically at ease.
Quiet	Lamb appears still and withdrawn.
Irritable	Lamb appears bothered by something/another animal and may respond to interactions in a hostile manner.
Tense	Lamb appears uneasy and its posture may show physical tightness.
Curious	Lamb appears intrigued by the environment/other animals and shows a desire to investigate.
Tired	Lamb appears weary and lacking in energy.
Painful	Lamb appears to be experiencing acute discomfort or injury.
Relaxed	Lamb appears secure and unthreatened.
Stressed	Lamb appears worried or overwhelmed.
Alert	Lamb appears to be vigilant and is observing its surroundings.
Engaged	Lamb appears to be occupied with various tasks (e.g. playing, walking).
Sleepy	Lamb appears dozy and may be nodding off.
Content	Lamb appears peaceful and as if its needs are met.
Sore	Lamb appears to be hurting through its posture and movements (e.g. difficulty walking).
Happy	Lamb appears to be experiencing joy or pleasure.
Playful	Lamb appears to be frisky and lively (e.g. running around with other animals).
Lethargic	Lamb appears sluggish or dull, and it does not appear to be interested in its surroundings.
Agitated	Lamb appears troubled or nervous.

Observers were provided with online surveys (SurveyMonkey; Momentive Inc., San Mateo, United States) to report their scores for each lamb. They were instructed to observe the focal lamb in each clip and then score the 20 agreed terms using the provided visual analogue scales (VAS). The “minimum” score (0) meant that the expressive quality indicated by the term was completely absent, whereas the “maximum” score (100) meant that the expressive quality indicated by the term could not be present more strongly. Terms were randomised for their order for the survey, shown in Table 2, using Microsoft Excel (Version 16.52; Microsoft Corporation, Redmond, United States).

4. Tissue shedding, lesion and palpation scores

The presence or absence of the scrotum or tail were assessed every 3 days for all lambs when lesions were checked and the area palpated. This was continued until approximately day 25 after treatment for young lambs and up to 30 days for older lambs.
Lesions were assessed and scored on 7 occasions over the 25 day period for young lambs, at approximately days 2, 6, 10, 13, 17, 20 and 24, using the procedure of Kent et al., (2000). The affected area was also palpated and scored as described by Mellema et al., (2006). For older lambs similar measures were made on approximately days 2, 5, 9, 12, 16, 19, 23, 26 and 30.

5. Growth rates

Young lambs were weighed at birth, and then at weeks 1, 2, 3 and 4 of age (week 1 was approximately 2

days post castration/tail docking) Older lambs were weighed the week before and after the procedure to assess any effects of treatment on immediate growth rate, and then weighed on weeks 2, 3, 6, 8 and 12 after treatment.

Statistical treatment of data

Data were checked for normality prior to analysis using Anderson-Darling tests (Minitab 17) and by checking residuals. Normally distributed data were analysed by Restricted Maximum Likelihood Procedures (REML) in Genstat, fitting litter size, breed (older lambs only) and treatment in the model with ewe identity as the random factor (to account for within litter effects). Growth data were analysed by repeated measures REML fitting the same model as above. Where data were not normal, and could not be transformed by square root or $\log_e$ transformations, Generalised Linear Mixed Models were used where the distribution of the data approximated to binomial or Poisson distribution. Categorical score data and data that did not fit to the GLMM models were analysed by Kruskal Wallis non-parametric data for the effects of treatment.

Data were analysed separately for older and younger lambs, as they were possibly confounds in time and breed. In addition, tail docking and castration were analysed as separate studies as lamb sex could not be completely included in the model.

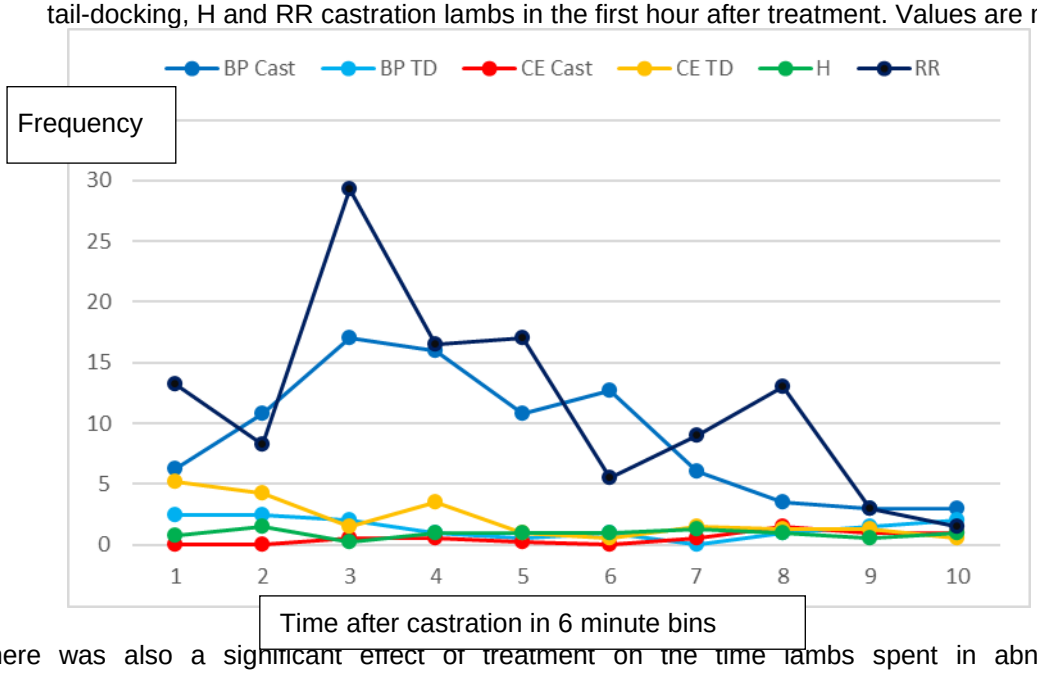
Results

1. Live behavioural recording

i) Young lambs

The effects of different methods of castration and tail docking, and handling alone, on REQ behaviour in 10 x 6 minute bins over the 60 minutes after treatment are shown in Figure 1. There was a significant overall effect of treatment (Generalised Linear Models: Wald=88.16, d.f.=5, P<0.001) and tended to be an effect of time x treatment (Wald=59.92, d.f.=45, P=0.082). Post hoc analysis suggested that RR and BP castrated lambs were significantly different to other groups until the last two observation periods.

Figure 1. Change in REQ behaviour for BP castration, BP Tail docking, CE castration, CE tail-docking, H and RR castration lambs in the first hour after treatment. Values are medians.



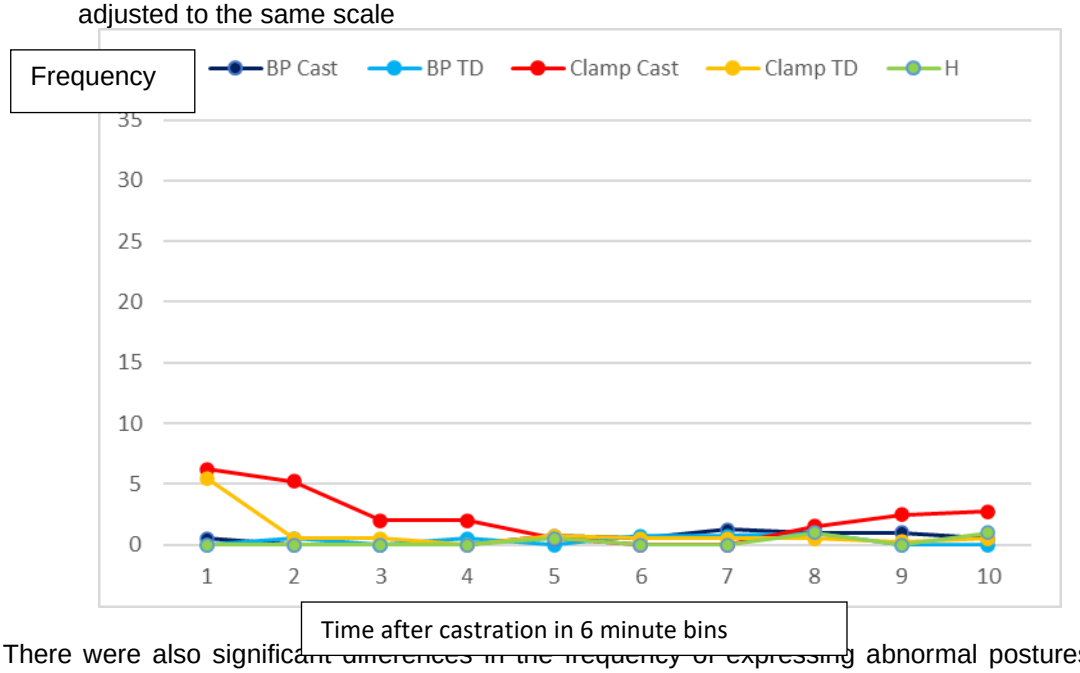
There was also a significant effect of treatment on the time lambs spent in abnormal postures (Wald=23.70, d.f.=5, P=0.002), but no interaction with time. RR and BP Castrated lambs had a higher frequency of expressing these postures compared to other groups throughout the first 60 minutes after treatment.

ii) Older lambs

The effects of castration and tail docking on frequency of expressing REQ in 10 x 6 minute bins over 60 minutes after treatment are shown in Figure 2. There was a significant time x treatment interaction in the expression of REQ over the observation period (Generalized Linear Models: Wald=76.12, d.f.=36, P<0.001). Post hoc analysis revealed a significantly greater REQ frequency in lambs castrated or tail docked with ClampEasy than any other treatment groups in the first 6 minutes after treatment. CE

Castrated lambs also had significantly greater REQ frequency than other groups at 12 minutes after treatment but there were no significant differences from 18 minutes onwards.

Figure 2. Change in REQ behaviour for BP castration, BP Tail docking, CE castration, CE tail-docking, and H lambs in the first hour after treatment for lambs aged 5-6 weeks old. Values are medians. Note to facilitate comparison with younger lambs the Y axis has been adjusted to the same scale



There were also significant differences in the frequency of expressing abnormal postures over the first hour after treatment. CE castrated lambs showed the greatest frequency of these postures in the first 6 minutes after treatment, followed by BP castrated and CE tail docked lambs (Figure 2; H=16.10, P=0.003). By 12 minutes after treatment there was still a significant effect of treatment on abnormal postures (H=15.39, P=0.004), but not thereafter.

2. Video behavioural analysis

i) Young lambs

Castration: There were significant effects of castration treatment on the active pain behaviours of foot-stamping and kicking, head turning, easing quarters and tail wagging, restlessness and the combined active pain behaviour score (RE). Overall, RR and BP lambs had a significantly higher frequency of expressing these behaviours in the first 60 minutes after the procedure than H and CE lambs, which did not differ significantly from one another (Table 4a) The behaviours of dog-sitting, kneeling and rolling were only seen in the RR and BP lambs and could not be analysed individually, although they contributed to the REW scores. RR and BP lambs also spent significantly more time in abnormal postures during the 60-minute observation period than H and CE lambs (92% and 88% respectively, compared to 37.8% and 27% in H and CE lambs; Table 4a).

There were no significant differences between the CE lambs that had been short scrotum (SS) castrated compared to those that were subjected to full bilateral castration for any behaviours, except for a lower frequency of easing quarters in the SS lambs (median frequency: CE=5.511, CE_SS=1.521, H=5.33, d.f.=1, P=0.021). Further, removing the SS lambs from the full analysis did not alter the overall conclusions with respect to treatment effects.

Tail docking: There were significant effects of tail docking treatment on the frequency of expression of easing quarters and the combined REW score in the 60 minutes after procedure (Table 4a), and a tendency for an effect on foot stamping/kicking. Overall, BP and CE lambs had a higher frequency of expressing easing quarters and REW than C lambs and did not differ from one another. Rolling was seen in only two lambs (one each in BP and CE) and jumping, kneeling and running were also too infrequent to be analysed.

Lambs spent the majority of the observation period lying (Table 5a), but there were no significant effects of treatment on time spent standing, lying or walking. However, BP and CE lambs spent more time in abnormal postures (standing, lying and walking, in sum approximately 13% of the observation period) than C lambs (Table 5a), but did not differ from one another in time spent in abnormal postures. Teat-seeking was seen in all but one lamb, but the amount of time spent teat seeking did not differ by treatment (median time spent teat-seeking (mins): BP=2.57, CE=1.12, C=1.39, H=1.52, d.f.=2, NS). Play behaviour was seen in four lambs, across all treatment groups, and frequency and duration of play was not affected by treatment.

ii) Older lambs

Castration: Significant effects of castration treatment were observed in older lambs for the active pain behaviour of easing quarters and for restlessness, as well as the composite pain score REW (Table 3b). However, there were no treatment effects on the frequency of foot stamping and kicking, head turning or tail wagging, and kneeling, dog-sitting and rolling were not seen with any treatments. Overall, CE lambs were observed to show a higher number of active pain behaviours than BP or C lambs, which did not differ. Similarly, CE lambs spent a significantly greater proportion of the 60- minutes following treatment in abnormal postures (7% of the period compared to 0.3% for C lambs; Table 4b).
Tail-docking: Tail docking treatment significantly affected the active pain behaviours of head turning, easing quarters, tail-wagging and the composite active pain score, REW (Table 5b), but there were no significant effects on restlessness or foot-stamping and kicking. Rolling was not seen in any lambs and only 2 lambs (both BP) were seen jumping. Overall, both tail docked treatments caused an increase in easing quarters and REW compared to Control lambs. BP lambs also made significantly more head turning and tail wagging movements compared to either CE or C lambs.
Older lambs spent more time standing and walking than young lambs but overall there were no effects of tail-docking treatment on the amount of time they spent standing or lying, although both groups of docked lambs tended to spend more time walking than control lambs (Table 5b). There was, however, a significant effect of treatment on the amount of time lambs spent in abnormal postures, with BP lambs spending more time (8% of the observation period) in these postures than CE or C lambs. BP lambs also spent more time eating than CE lambs, but neither treatment group differed from controls in time spent eating. There were also no significant effects of treatment on teat-seeking responses, and no effects of breed or litter size on any behaviour.

Table 4. The effect of castration treatment on active pain behaviours and time spent in different postures for a) lambs treated at ≤7 days of age or b) lambs treated at 4-5 weeks of age. Lambs were treated with rubber rings (RR), best practice as advised by a vet (BP), clamps (CE) or were handled only (H). Data are frequencies of expression in 60 minutes for foot stamping and kicking, head turning, easing quarters, tail wagging, restlessness and REW, and duration in minutes for postures. Values are model predicted means, with pooled standard errors of the difference or medians[1] (Kruskal Wallis tests). Significant differences were determined by REML[2] or GLMM[3] tests unless otherwise stated, treatment differences were determined by post hoc ANOVA or Kruskal Wallis tests.

a) Young lambs

Behaviour	RR	BP	CE	H	Pooled s.e.d	Statistics	P
Events (frequency)							
Foot stamping/kicking[3]	12.07^{b}	2.48^{b}	0.02^{a}	0.02^{a}	0.52	$F_{3,10.8}$=155.78	P<0.001
Head turning[2]	7.31^{c}	$6.43^{b,c}$	0.54^{a}	$1.31^{a,b}$	0.62	$F_{3,20.0}$=7.36	P=0.002
Easing quarters[2]	87.96^{b}	69.35^{b}	0.70^{a}	9.33^{a}	16.01	$F_{3,12.2}$=15.48	P<0.001
Tail wagging[1]	2.04^{b}	3.08^{b}	0.0^{a}	0.0^{a}	*	H=8.13,d.f.=3	P=0.043
Restlessness[3]	34.16^{b}	20.25^{b}	1.83^{a}	2.96^{a}	0.46	$F_{3,17.0}$=19.90	P<0.001
REW[2]	185.43^{b}	135.91^{b}	6.32^{a}	17.29^{a}	36.92	$F_{3,18.6}$=12.50	P<0.001
States (duration)							
Standing (mins)	4.77	3.51	4.07	5.94	0.43	$F_{3,18.0}$=0.63	NS
Walking (mins)	3.87^{b}	3.43^{b}	1.68^{a}	$1.77^{a,b}$	0.75	$F_{3,11.6}$=4.51	P=0.025
Lying (mins)	52.58	53.35	50.75	47.15	3.83	$F_{3,14.8}$=0.72	NS
Abnormal postures (mins)	55.57^{b}	52.88^{b}	15.7^{a}	22.67^{a}	5.66	$F_{3,18.8}$=29.19	P<0.001

b) Older lambs

Behaviour	BP	CE	H	Pooled s.e.d	Statistics	P
Events (frequency)						
Foot stamping/kicking	0.00	0.00	0.00	*	H=2.01, d.f.=2	NS
Head turning	0.00	0.00	0.00	*	H=2.61, d.f.=2	NS
Easing quarters	0.00^{a}	5.67^{b}	0.00^{a}	*	H=8.20, d.f.=2	P=0.017
Tail wagging	0.00	0.00	0.00	*	H=0.00,	NS

					d.f.=2	
Restlessness	2.26[a]	11.06[b]	3.35[a]	0.48	$F_{2,13.7}$=7.88	P=0.005
REW	3.59[a]	22.40[b]	4.47[a]	0.56	$F_{2,12.9}$=10.63	P=0.002
States (duration)						
Standing (mins)	15.96	6.40	15.07	5.10	$F_{2,7.5}$=2.72	NS
Walking (mins)	0.74	0.83	1.27	0.33	F2,1.8=1.835	NS
Lying (mins)	33.02	47.23	34.52	7.38	$F_{2,6.8}$=2.83	NS
Abnormal postures (mins)	0.00[a]	4.60[b]	0.19[a]	*	H=7.96, d.f.=2	P=0.019

Table 5. The effect of tail docking treatment on active pain behaviours and time spent in different postures for a) lambs treated at ≤7 days of age or b) lambs treated at 4-5 weeks of age. Lambs were treated with best practice as advised by a vet (BP), clamps (CE) or were handled only (H). Data are frequencies of expression in 60 minutes for foot stamping and kicking, head turning, easing quarters, tail wagging, restlessness and REW, and duration in minutes for postures. Values are model predicted means, with pooled standard errors of the difference or medians[1] (Kruskal Wallis tests). Significant differences were determined by REML[2] or GLMM[3] tests unless otherwise stated, treatment differences were determined by post hoc ANOVA or Kruskal Wallis tests.

a) Young lambs

Behaviour	**BP**	**CE**	**H**	**s.e.d**	**Statistic**	**P**
Events (frequency)						
Foot stamping/ kicking[1]	2.50 (1.25-7.75)	3.50 (0.0-10.25)	0.00 (0.0-0.0)		H=5.55, d.f.=2	P=0.062
Head turning[1]	5.50 (0.25-21.25)	5.00 (2.0-10.5)	0.50 (0.0-1.75)		H=4.21, d.f.=2	P=0.122
Easing quarters[1]	12.5[b] (7.25-24.75)	11.5[b] (5.0-19.5)	0.0[a] (0.0-3.0)		H=9.60, d.f.=2	**P=0.008**
Tail wagging[1]	4.50 (1.25-10.0)	5.00 (0.25-14.5)	3.00 (0.5-6.25)		H=0.52, d.f.=2	NS
Restlessness[1]	7.00 (3.0-8.75)	10.00 2.25-23.0)	4.00 (2.25-7.25)		H=0.95, d.f.=2	NS
REW[1]	32.50[b] (18.8-71.3)	37.50[b] (13.3-80.3)	13.00[a] (6.75-14.75)		H=6.30, d.f.=2	**P=0.043**
States (duration)						
Standing (mins)[2]	9.10	9.68	11.28	2.86	$F_{2,15}$=0.26	NS
Walking (mins)[1]	1.21 (0.60-1.68)	2.08 (1.62-4.03)	1.37 (0.73-3.46)		H=2.58, d.f.=2	NS
Lying (mins)[2]	43.57	40.65	41.17	4.13	$F_{2,15}$=0.35	NS
Abnormal postures (mins)[1]	8.08[b] (1.22-13.87)	7.59[b] (3.57-17.95)	0.15[a] (0.03-0.40)		H=8.63, d.f.=2	**P=0.013**

b. Older lambs

Behaviour	**BP**	**CE**	**H**	**s.e.d**	**Statistic**	**P**
Events (frequency)						
Foot stamping/ kicking[1]	0.0 (0.0-1.0)	0.0 (0.0-2.75)	0.0 (0.0-0.0)		H=1.62, d.f.=2	NS
Head turning[1]	9.0[b] (6.0-13.0)	4.0[a] (3.0-7.5)	0.0[a] (0.0-5.0)		H=7.37 d.f.=2	**P=0.025**
Easing quarters[1]	15.50[b] (11.0-26.0)	10.50[b] (9.25-20.0)	5.00[a] (0.0-8.0)		H=7.89, d.f.=2	**P=0.019**
Tail wagging[1]	1.0[b] (0.0-2.50)	0.0[a] (0.0-0.0)	0.0[a] (0.0-0.0)		H=6.32, d.f.=2	**P=0.042**
Restlessness[1]	1.0 (0.25-4.0)	0.5 (0.0-3.75)	0.0 (0.0-3.0)		H1.27, d.f.=2	NS
REW[1]	33.50[b] (23.25-41.0)	23.00[b] (18.25-40.50)	8.00[a] (4.0-13.0)		H=8.92, d.f.=2	**P=0.012**
Behavioural states (duration)						
Standing (mins)[2]	25.83	29.95	24.35	6.98	$F_{2,12}$=0.46	NS
Walking (mins)[3*]	3.18 (2.23-4.31)	3.32 (2.34-4.47)	1.49 (0.86-2.29)		$F_{2,12}$=3.18	P=0.082
Lying (mins)[2]	20.68	22.22	15.33	8.98	$F_{2,12}$=1.21	NS

Abnormal postures (mins)[2]	5.02[b]	3.14[a]	1.51[a]	1.11	$F_{2,12}$=5.15	**P=0.022**
Eating (mins)	8.26[b]	2.31[a]	6.50[ab]		F=3.66	**P=0.049**
Teat-seeking (secs)	52.16	44.62	51.25	16.5	$F_{2,12}$=0.13	NS

*Back-transformed data with 95% confidence intervals

c. QBA

i) Young lambs

Castration: Principal components analysis and inspection of eigenvalues suggested two main factors or dimensions explaining 56.3% and 25.4% of the variance for factors 1 and 2 respectively. Factor 1 ranged from 'restless' and 'agitated' to 'calm', 'relaxed' and 'comfortable', and was considered to describe valence (Figure 3), whereas factor 2 had the terms 'tired' and 'quiet' at one end and 'engaged' and 'playful' at the other and was considered to describe arousal or activity.
The distribution of factors scores for castration treatments of young lambs on the two dimensions is shown in Figure 4. There were no significant effects of treatment on the distribution of scores on Factor 2 (arousal: tired/quiet to engaged/playful; Table 6). However, RR and BP lambs were significantly more likely to be scored as restless and agitated (Factor 2: valence), compared to CE and H lambs which were scored as calm and comfortable (Table 6).
Tail-docking: For tail docking two factors were identified, explaining 54.2 and 25.8% of variation respectively. Factor 1 ranged from sore/stressed/restless and tense on one end to calm, relaxed and comfortable on the other; as before this Factor described 'valence' or 'painfulness'. Factor 2 ranged from lethargic, quiet and tired on one end to alert, playful and engaged on the other; this Factor was therefore labelled 'arousal' or 'activity'. There were, however, no significant effects of treatment on either QBA factor (Table 7).

Figure 3. Example loading plot of QBA terms for young lambs following analysis of different castration treatments. Loading plots for castration of older lambs and for tail docking show equivalent distributions of terms on the two factors and are not presented here.

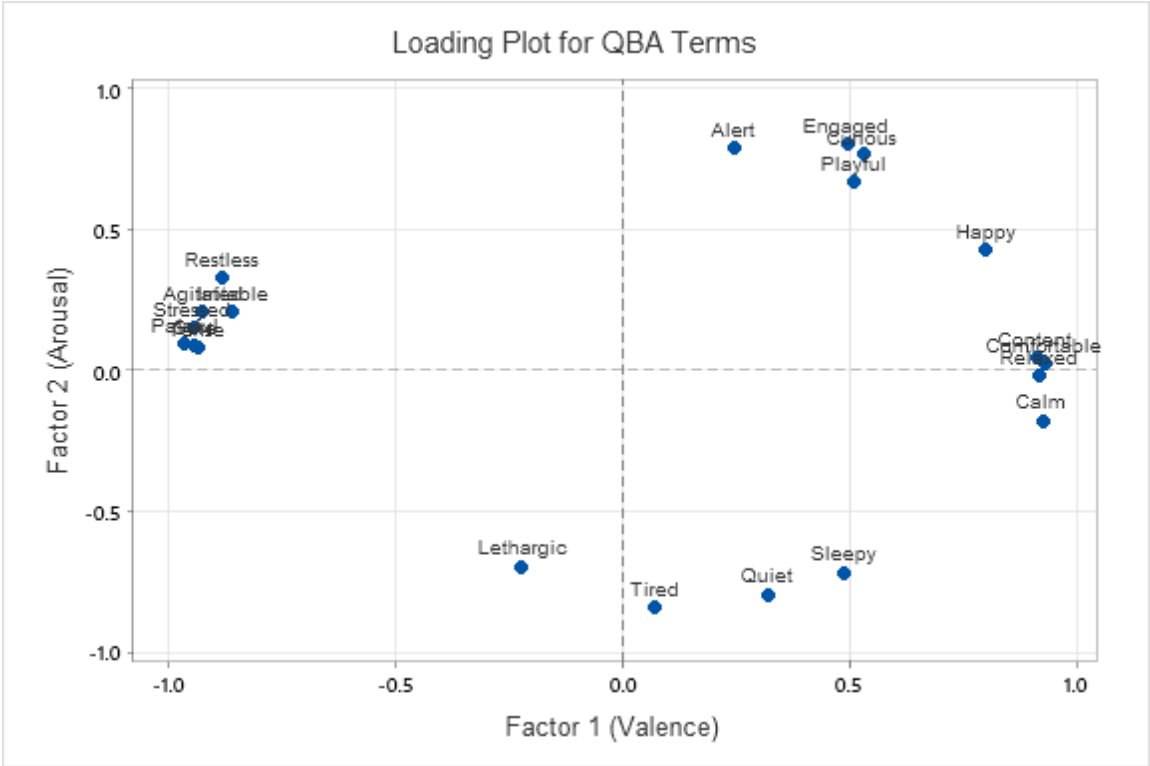


Figure 4. Score plot of young lamb castration data on QBA terms, indicating the different distribution of lambs based on castration treatment. Each marker represents a lamb scored by a single observer.

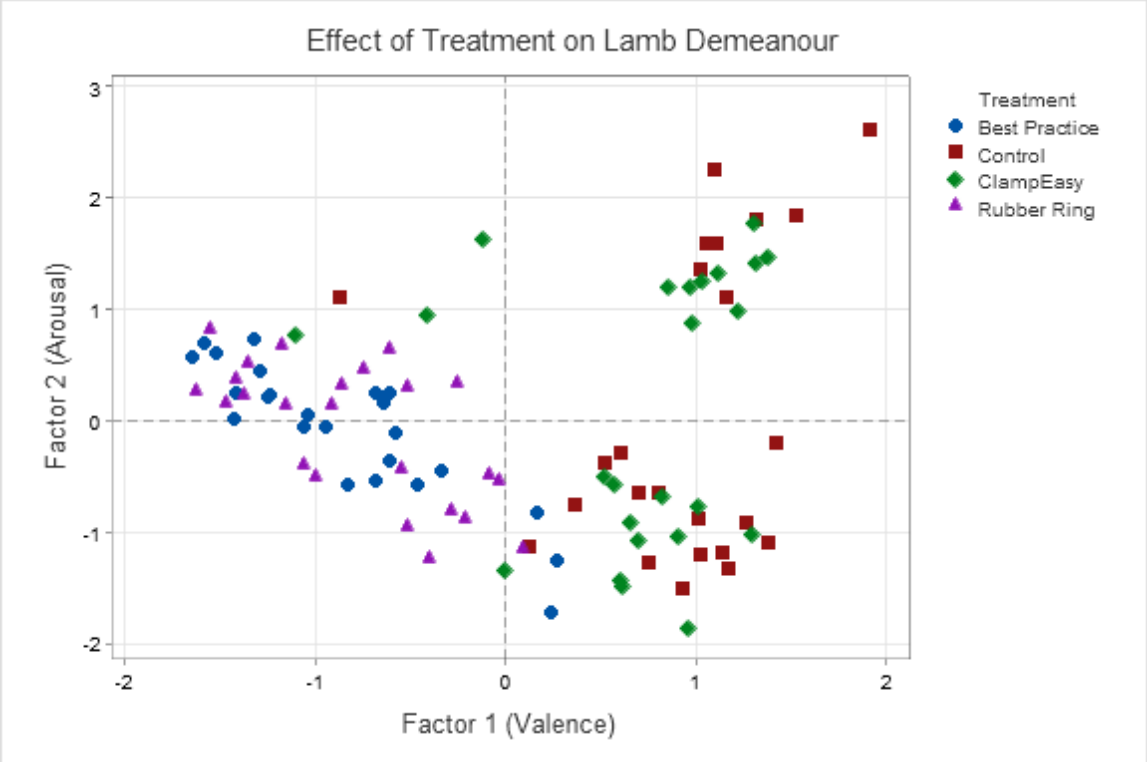


Table 6. Effects of castration treatments on mean QBA scores given to a) lambs and b) older lambs on Factor 1 (valence) and Factor 2 (arousal). Significant differences were determined by REML analysis.

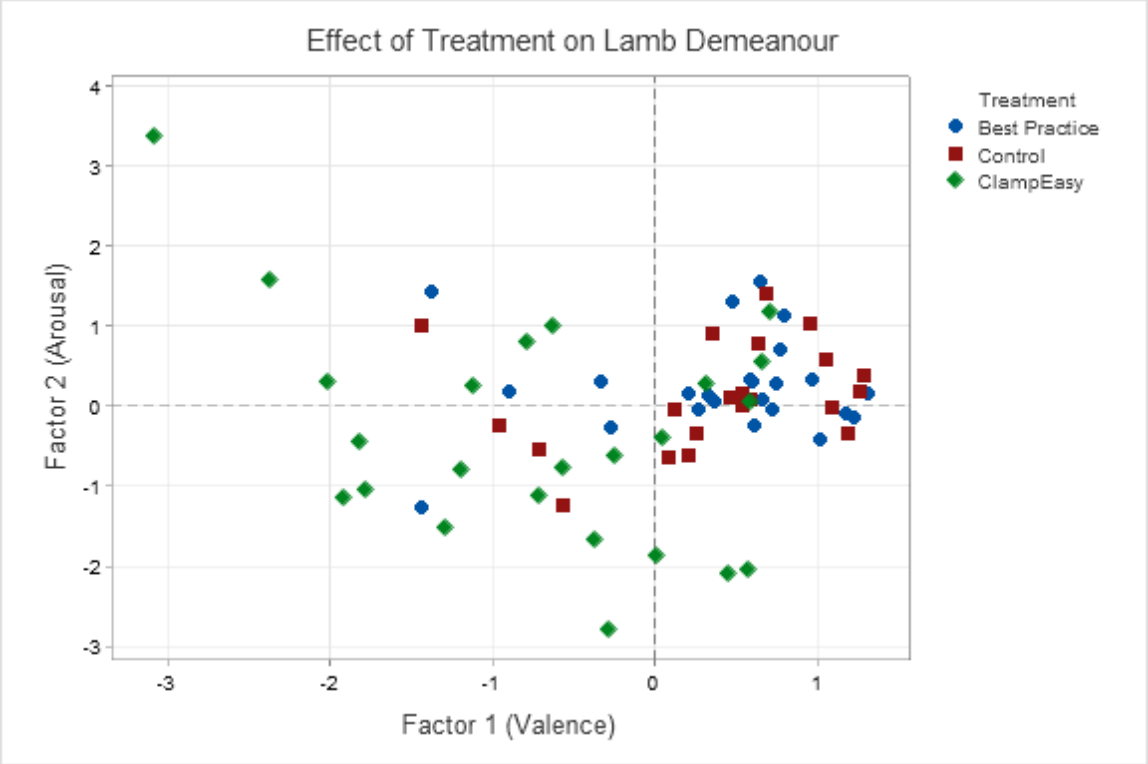
QBA factor	**a) Young lambs**						
	RR	*BP*	*CE*	*H*	*Pooled s.e.d*	*Statistics*	*P value*
Factor 1 (valence)	-0.56[b]	-0.51[b]	1.05[a]	0.88[a]	0.17	$F_{3,8.3}$=52.97	P<0.001
Factor 2 (arousal)	-0.94	-1.20	-0.81	-0.57	0.55	$F_{3,9.6}$=0.42	NS
	b) Older lambs						
	BP	*CE*	*H*		*Pooled s.e.d*	*Statistics*	*P value*
Factor 1 (valence)	0.63[a]	-0.52[b]	0.29[a]		0.46	$F_{2,13.8}$=4.46	0.032
Factor 2	0.41	-0.24	0.00		0.57	$F_{2,12.6}$=0.83	0.457

(arousal)							

ii) Older lambs

Castration: Principal components analysis for castration treatments of older lambs also revealed two main factors of lamb expression (data not shown), accounting for 55.6% and 14.4% of the variation respectively. Factor 1 ranged from 'sore'/'tense' to 'content'/'comfortable' and so was considered to reflect valence, while factor 2 ranged from 'sleepy'/'tired' to 'restless'/'agitated' and so was labelled arousal.
As seen with the younger lambs, there were no significant effects of treatment on the arousal dimension, but CE lambs had significantly more negatively valenced scores compared to H and BP lambs, which did not differ (Figure 5, Table 5).

Figure 5. Score plot of older lamb castration data on QBA terms, indicating the different distribution of lambs based on castration treatment. Each marker represents a lamb scored by a single observer.



Tail-docking: As seen for the castration treatment, PCA analysis of QBA data revealed two main factors of expression, accounting for 50.4 and 17.4% of the variation respectively. Factor 1 ranged from calm, comfortable and happy at one end, to tense, sore and stressed at the other and described valence or painfulness. Factor 2 ranged from restless and engaged at one end to sleepy, quiet and tired at the other was considered to describe arousal. There were no significant effects of treatment, breed or litter size on Factor 1 scores (valence, Table 6). There was a tendency for treatment to have an effect of Factor 2 describing arousal or activity. CE lambs tended to be more sleepy/quiet than either BP or H lambs who tended to be more likely to be described as alert, restless or energetic (Figure 6, Table 6).

Figure 6. Scatterplot of older lamb scores for QBA terms following tail-docking by best practice methods, using ClampEasy or Handled only (Control). Values are individual lamb scores by each observer on the 2 factors.

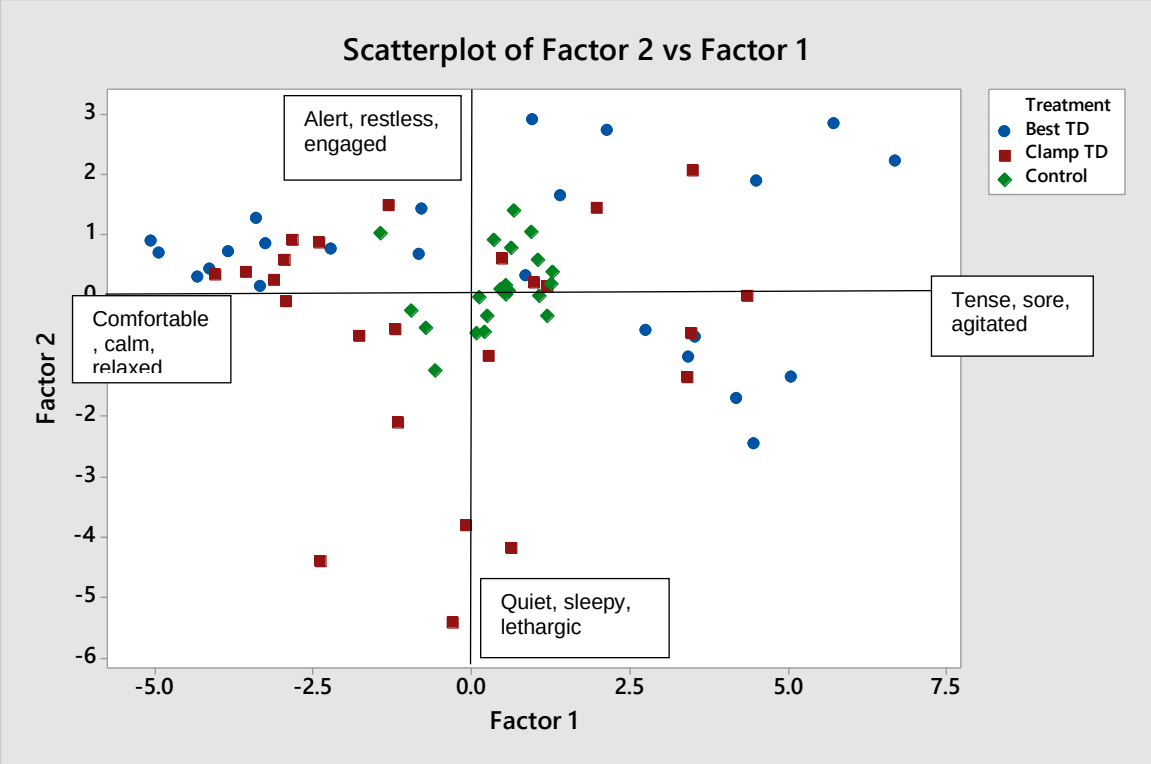


Table 7. Effects of tail-docking treatments on predicted mean QBA scores given to a) young lambs and b) older lambs on Factor 1 (valence) and Factor 2 (arousal). Significant differences were determined by REML analysis.

QBA factor	**a) Young lambs**					
	BP	*CE*	*H*	*Pooled s.e.d*	*Statistics*	*P value*
Factor 1	0.94	-0.26	0.93	1.1	$F_{1,18}$=1.08	NS
Factor 2	0.70	0.50	0.70	0.97	$F_{1,18}$=0..04	NS
	b) Older lambs					
	BP	*CE*	*H*	*Pooled s.e.d*	*Statistics*	*P value*
Factor 1	0.23	-0.56	0.36	1.23	$F_{1,17}$=0.43	NS
Factor 2	0.46	-0.88	0.62	0.74	$F_{1,17}$=3.33	P=0.086

4. **Tissue shedding**

i) Young lambs

Castration: The presence or absence of the scrotum or tail was recorded until approximately day 25 after treatment. By this time all the CE lambs had lost the scrotum (8/8, 100%), but none of the BP lambs (0/8, 0%; Fishers exact: P<0.001) and only 1 RR lamb (1/8, 12.5%; Fishers exact: P=0.0014). CE lambs shed the necrotic tissue at a median time of 18.0 (interquartile range: 16-20.5) days after treatment, the single RR lamb that lost the necrotic tissue did so at d25.
Tail docking: For the tail docked lambs all tails were shed within the observation period for all lambs in the BP and CE groups. Tails were shed significantly more quickly in the CE group than in BP group lambs (median time (d) to loss of tail (with IQR): CE=5.0 (2.25-11.50); BP=20.50 (14.50-22.50); H = 9.02, d.f.=1, P=0.003).

ii) Older lambs

Castration: For older animals the presence or absence of tissues were checked until approximately d30 after treatment. Similarly to younger lambs, all the CE lambs had shed the scrotum by the end of the study period (8/8, 100%). Median time to shed the scrotum in the CE lambs was 23.5 days (IQR: 21.5-27.5), approximately 5 days longer than in the younger lambs. (NB. For older lambs the method used for BP castration, Burdizzo, does not result in loss of the tissue).
Tail docking: all docked lambs shed the tail within the study period but, as with younger lambs, tails were shed significantly more quickly in the CE lambs than in BP lambs (median time (d) to loss of tail (with

IQR): CE=10.0 (9.0 – 11.0); BP=19.50 (18.0-23.75); H = 11.46, d.f.=1, P<0.001).

5. Lesion and palpation scores

i) Young lambs

Castration: In this study the highest lesion score recorded for castrated lambs was 3.5 in a single RR treated lamb at one observation point. There were no significant effects of castration treatment on lesion scores until the third assessment period (d10 post treatment) when BP and RR lambs had significantly higher scores than H and CE lambs (Table 7a, P<0.001). This difference continued until the end of the observation period. CE lambs had larger lesion scores than H lambs at 17 and 20 days after treatment only, and not thereafter.
Few lambs showed any response to palpation of the scrotum and no significant differences were seen. However, only RR castrated lambs showed any response (1 lamb on each of d2, d6, d20 and d24, and 2 lambs on d17).
Tail-docking: For tail docked lambs, a lesion score of 3.5 was also the highest score recorded for a CE lamb on a single occasion. Overall, CE lambs had higher scores than BP lambs from d2 post-treatment (Table 7b) until d20 when there were no significant effects of tail docking method (figure x), thereafter on d24 lesion scores were greater in the BP lambs (Table 7b; H=5.57, d.f.=1, P=0.018) than CE lambs. Responses to tail palpation were only observed on d2 (BP=4/8 lambs, CE=1/8, H=0/8), d6 (BP=2/8, CE=2/8, H=0/8) and d13 (BP=1/8, CE=1/8, H=0/8) only after treatment. On d2 BP lambs tended to have a greater reaction to palpation than other lambs (median palpation scores: BP=0.5, CE=0.0, H=0.0, H=5.84, d.f.=2, P=0.054), no other significant effects were observed.

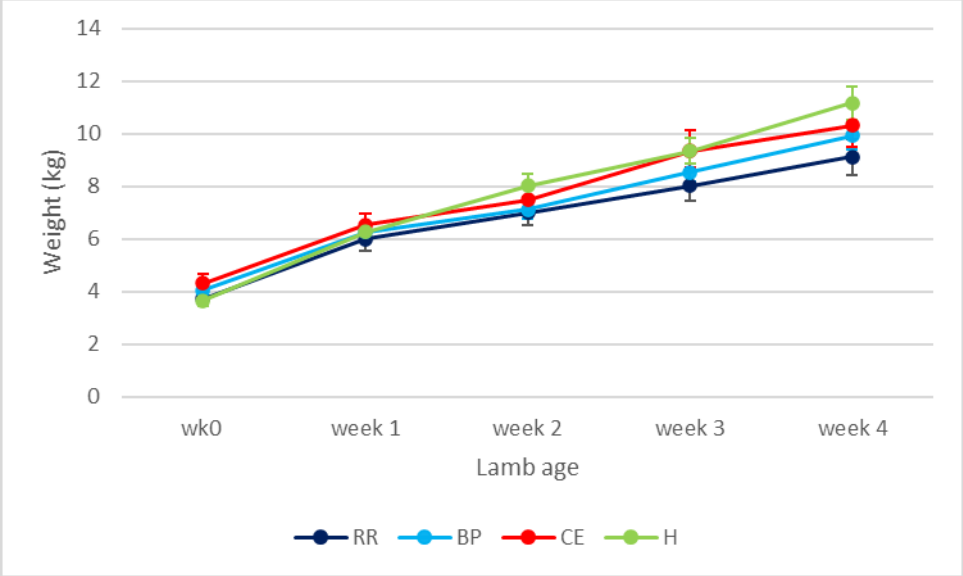
ii) Older lambs

Castration: The highest lesion score recorded in the older lambs was 3.5, which was recorded for the same CE lamb on two consecutive occasions (d19 and d23 post castration). The development of lesions differed by treatment after castration with BP lambs having significantly more severe initial lesions (d2 and 5; Table 8a), which then declined whereas CE lambs had larger lesions than H lambs on d16 and d26 and than both BP and C lambs on d19 and d23 (Table 8a). By d30 after castration there were no significant differences in lesion scores between treatment groups.
Tail-docking: The highest lesion score recorded for tail-docked older lambs was 3.0, recorded for a single CE lamb on d9 after docking. CE and BP lambs had higher lesion scores than H lambs on d2 (Table 8b), and CE lambs continued to have more significant lesions than H lambs throughout. BP lambs had less severe lesions than CE lambs from d5 to d12, but thereafter similar severity of lesions were recorded for BP and CE lambs. BP lamb lesions were not significantly different to H lambs on d5 and d9 (Table 8b). CE and BP lambs tended to have a greater response to palpation of the tail than H lambs at d2 after treatment (median palpation score: BP=0.50, CE=1.0, C=0.0, H=5.74, d.f.=2, P=0.057), but did not differ from each other. Thereafter there were no significant effects of CE treatment on palpation score when compared to H lambs. However, BP lambs tended to be more reactive than either CE or H lambs at d12 and were significantly more reactive at d16 when the tail was palpated (median scores: d12: BP=0.50, CE=0.0, Control=0.0, H=4.65, d.f.=2, P=0.098; d16: BP=0.0 [rank 14.8], CE=0.0 [rank 10.5], Control=0.0 [rank 10.5], H=6.19, d.f.=2, P=0.045).

6. Lamb weights and changes in weight

i) Young lambs

Castration: There were no significant differences in the mean birthweight of lambs allocated to the different castration groups (mean lamb birth weight (kg): RR=3.63, BP=3.95, CE=4.20, H=3.64, s.e.d.=0.31). Lamb treatment tended to affect the change in lamb weight over time (Figure 7, $F_{12,104}$=1.68, P=0.081), with control lambs tending to gain the most, and RR lambs the least, weight over the observation period.
Figure 7. Change in lambs weight over the first 4 weeks of life following different castration treatments applied in the first week of life for lambs castrated by rubber rings (RR), best practice (BP), used of ClampEasy (CE) or handled only. Values are means with standard errors.



Tail-docking: There was also no significant difference in birth weight between lambs allocated to different tail docking treatments, although BP lambs tended to be heavier than CE or H lambs (mean birth weight (kg): BP=4.08, CE=3.54, C=3.67, s.e.d.=0.273, $F_{3,18}$=3.01, P=0.074). There were no significant effects of tail docking treatment on lamb weight gain during the study.

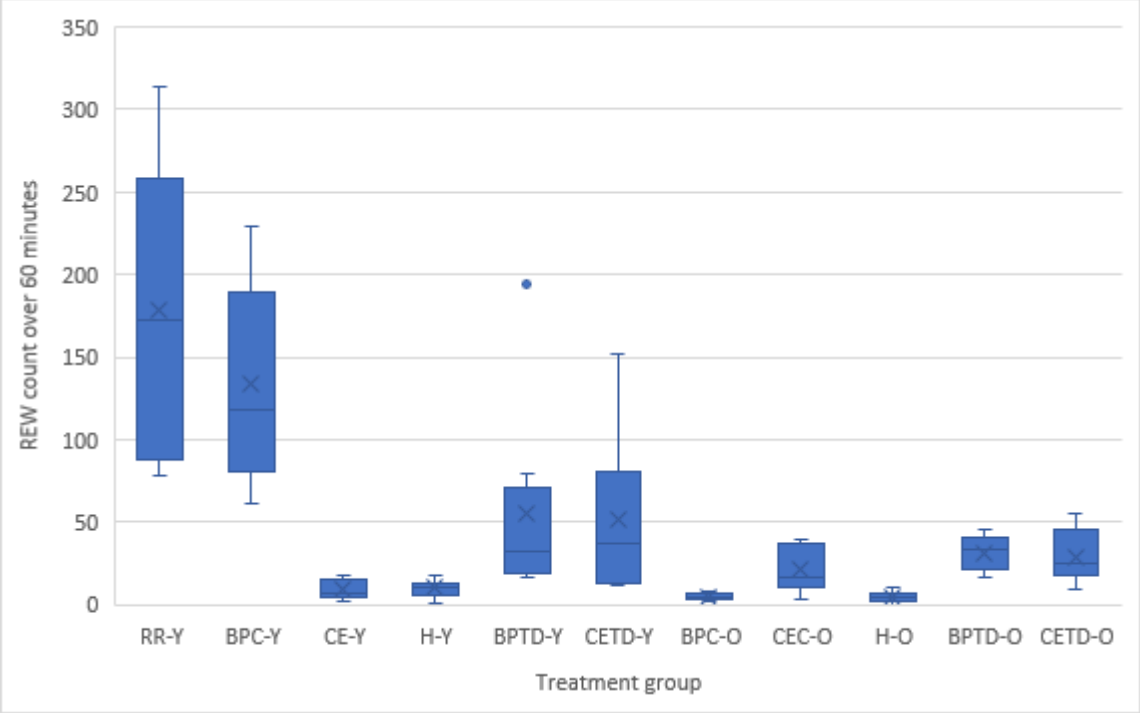
ii) Older lambs

Castration: There were significant effects of litter size (singles heavier than twins, P<0.001) and lamb breed (Suffolk cross lambs heavier than Texel cross, P=0.025) in the weight of lambs prior to the onset of the experiment, but no significant differences between lamb weights within treatment (mean lamb weight (kg): BP=20.92, CE=19.71, H=19.49, s.e.d.=0.82, $F_{2,14}$=1.64, NS). There were also no effects of castration treatment on the change in lamb weight from before treatment on any subsequent weighing days, or over the entire measurement period (mean change in weight from before treatment to week 12 (kg): BP=10.22, CE=6.62, H=4.07, s.e.d.=5.68 , $F_{2,14}$=0.51, NS).
Tail-docking: There was a significant effect of litter size (singles heavier than twins, P<0.001) on the pre-treatment weight of lambs, but no significant differences between the weight of lambs in different treatment groups: (mean lamb weight (kg): BP=16.87, CE=17.91, H=19.25; $F_{2,14}$=1.29, P=0.31). There were also no significant effects of treatment on lamb weights over time for tail docked lamb ($F_{12,92}$=16.89,, P=0.177).

Discussion and implications

The study demonstrates that different castration or tail docking methods evoke different amount of active pain behaviour, duration spent in abnormal postures and QBA outcomes. To facilitate comparison of all 11 treatment groups used in this study, Figure 8 presents the REW data from all groups on the same scale.

Figure 8. Box and whisker plots of REW count data from (left to right) young lambs castrated by RR, BP, and CE methods, young lambs handled only, young lambs tail docked by BP and Ce methods; older lambs castrated by BP and CE or handled only; older lamb tail-docked by BP and CE methods.



Although not statistically comparable (across all groups) the data demonstrate that RR castration (which was only carried out on lambs less than 7 days of age induced the greatest pain behaviours, which were significantly greater than for lambs that were handled only or castrated with ClampEasy in this age group (Table 3a). Qualitatively the use of rubber rings to castrate lambs provoked the greatest pain response seen in any of the treatment groups at any age, which is consistent with previous studies that provides substantial evidence for pain caused by this method (e.g. Mellema et al., 2006; Molony et al., 2002). In our study the use of local anaesthetic and NSAIDs in young lambs did not provide significant pain relief for lambs castrated with RR, although previous studies have suggested giving local anaesthetic alone (albeit that these studies gave LA via different routes and timings compared to this study) is effective at mitigating pain behaviour (Mellema et al., 2006; Molony et al., 2002; Thornton & Waterman-Pearson, 1999). However, the timing of the provision of LA is important as Kent et al. (1998) demonstrated that provision of LA at the time of castration was ineffective at reducing pain responses. In this study LA was given 5 minutes before application of the rubber ring and, although Figure 1 suggests some potential reduction of the maximal pain response, this was not significant when considered over the whole 60 minutes after the procedure. Use of LA was more effective at reducing pain in older lambs, however at this age the lambs were castrated with the use of a Burdizzo, which is known to cause significantly less active pain responses compared to RR (Molony et al., 1993), and previous studies have shown some additional marginal benefit of providing LA (Mellema et al., 2006).

Use of the ClampEasy device for castration in young lambs showed a normalisation of behavioural responses to be statistically indistinguishable from H lambs in young lambs. The effect was not as marked in older lambs, and was higher than the level of pain relief that could be achieved by the Burdizzo with local anaesthetic and NSAIDs (although see Limitations below). Previous studies suggest that there is no reduction in behavioural or physiological pain responses in lambs castrated at older ages with RR (e.g. Grant, 2004; Kent et al., 1993; Molony et al., 1993). Our previous (unpublished) data comparing a prototype version of ClampEasy with the Combined method (rubber ring plus Burdizzo) also showed very similar pain responses to ClampEasy application in lambs aged 4-6 weeks old, and significantly lower than was achieved with the Combined method. The data are, therefore, supportive of a reduction in acute pain behaviour through the use of ClampEasy when compared to any methods where a rubber ring is used to achieve castration, regardless of additional pain mitigating approaches combined with the rubber ring.

For tail docked lambs a similar pain response was seen with BP and ClampEasy both within age groups and across ages, although there appeared to be more individual variation in response in the younger lambs compared to older lambs (from inspection of data shown in Figure 8). At both ages, tail-docking did provoke more active pain behaviours than were displayed by lambs that were handled only. However, these pain responses were equivalent whether local anaesthetics were provided, or through the use of the ClampEasy device. Previous studies have suggested that tail docking is less painful compared to castration (e.g. Molony et al., 2002), as it induces a lower frequency of active pain responses and lower plasma cortisol. In both ages of lambs used in the current study the pain responses induced by ClampEasy tail docking appeared to be similar to, or in younger lambs, possible greater than the pain responses of lamb castrated with ClampEasy. In this study care was taken to site the clip in the same

place on the tail in all cases (between the 2nd and 3rd caudal vertebrae), however the time taken for the clamp to crush the nerves projecting along the tail may have taken longer with bony tail tissues compared to the neck of the scrotum, which may account for these effects.

An important consideration in evaluating the pain induced by these different methods is also the potential for chronic pain, that may persist after the acute pain has subsided. In this study we used lesion scores, palpation scores, time to shed the necrotic tissues and weight change as indicators of the pain and discomfort of the procedure in the days following application. Use of ClampEasy resulted in a significantly quicker time to shed the necrotic tissue for both castration and tail docking, and at both ages. This has implications for the amount of discomfort the lambs are likely to experience and suggest that there is likely to be less chronic discomfort through the use of the device compared to rubber rings. Lesion scores tended to be related to the timing of loss of tissue, so a direct comparison, when tissues were shed at different times, is challenging. However, overall lambs castrated or tail docking with the use of a rubber ring (with or without pain relief) tended to have larger lesions than lambs where ClampEasy was used. Lamb responses to palpation were generally infrequent, and restricted to the first few days after the procedures. Although not statistically comparable RR lambs tended to be more responsive than other lambs, but overall there was little evidence for an effect of the different procedures. There were no significant effects of treatment on subsequent growth rates, however the RR lambs in the younger age group tended to gain less weight over the next 4 weeks than the lambs that were not castrated (H).

Implications

The data provide further evidence that use of rubber rings for tail docking and castration are associated with very significant pain responses, which can only be partially mitigated by the use of local anaesthetic and analgesia (at least when applied within a short period before the ring is applied, as is likely to occur in a commercial situation). Rubber rings are also associated with slower healing times than other methods, and may induce a slower growth rate, at least in the first 4 weeks after application. We would therefore, strongly recommend that the use of rubber rings to achieve either castration or tail docking is discouraged.

Use of the ClampEasy (ClipFitter) device was able to mitigate pain associated with castration to a level indistinguishable from uncastrated lambs in younger lambs. In older lambs a better pain reduction could be achieved through the use of local anaesthetic and analgesics, although these are unlikely to be achieved in commercial practice. CE-Cast older lambs exhibited 5 x the number of pain behaviours in the first 60 minutes compared to H lambs. However, this was less than the 10-fold increase in pain response shown by RR young lambs when compared to H lambs, and may represent a possible acceptable alternative method for older lambs. Further in both ages the pain associated with tail docking was equivalent to that seen when local anaesthetic and analgesia was provided. As the necrotic tissues were shed more quickly than by other methods, and the lesion scores were smaller than seen with rubber rings, we conclude that ClampEasy/ClipFitter is a practical and feasible alternative to the use of rubber rings that will cause significantly less pain.

None of the methods used to castrate or tail dock lambs were entirely pain-free, either from the acute pain responses or subsequent lesions (although acute responses to ClampEasy/ClipFitter in young lambs did appear to be similar to lambs that were only handled). We would therefore continue to recommend that, if it is possible to manage lambs without use of castration or tail docking, then this would be the best option for lamb welfare.

Limitations

In using the ClampEasy device to castrate young lambs (Scottish Blackface twin lambs, with a mean weight of 4.2 kg), it was frequently (50% of castrations) impossible to ensure that both testes were distal to the clamp due to their small size and physical constraints. This was not seen to be an issue with older and heavier lambs where the testes could be more easily manipulated into position. A significant proportion of young lambs were therefore short scrotum castrated where the testes were pushed into the abdominal cavity and the scrotum only removed. Although some studies have demonstrated that there is some pain reduction by short-scrotum castration (e.g. Molony et al., 2002), this did not account for the reduction in pain behaviour seen in this study as lambs that were fully castrated also showed a significant decrease in pain behaviour. Our previous work (Maslowska, 2016) has shown that short scrotum lambs are infertile, but nonetheless this could be seen as a disadvantage of the method in very small or low birthweight lambs.

In the older lambs, the use of flunixin as the NSIAD for the Best Practice approach resulted in the development of Acute Respiratory Distress Syndrome (ARDS) in half of the lambs, despite a reduction in the dosage, and some respiratory distress in all lambs, as outlined in the Methods. This was the method

advised by the attending veterinarian, and a change to the use of Metacam was instituted with the younger lambs (where no side-effects were observed). The ARDS response lasted for at least 6 hours and extended beyond the observation of acute behavioural responses reported here. In addition, one of the BP castrated lambs developed a large haematoma at the site of the crush. The lamb was monitored and treated until this had healed, and this had no apparent impact on behavioural measures. However, given these complications and unwanted side effects, the data on BP castration in the older lambs should be treated with caution. It is possible, for example, that the apparent lower levels of active pain behaviour expressed by the BP Cast lambs may be related to being in respiratory distress and not to the pain mitigation supplied by the local anaesthetic and analgesic regime.

In the older lambs, the use of ClampEasy was noted to be close to the limitations for lamb weight (approximately 20 kg) at which it was considered that the clamp could be easily and effectively closed to ensure rapid desensitisation of the tissues. Further research or data would be needed to determine the optimal age, and any upper weight limits, for the use of the clamp to achieve effective castration and pain relief.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Grant, C. (2004) Behavioural responses of lambs to common painful husbandry procedures. *Appl Anim Behav Sci* 87, 255-273.

Kent, J.E., Jackson, R.E., Molony, V., Hosie, B.D. (2000) Effects of acute pain reduction methods on the chronic inflammatory lesions and behaviour of lambs castrated and tail docked with rubber rings at less than two days of age. *Vet J.* 160, 33-41.

Kent, J.E., Molony, V., Robertson, I.S. (1993) Changes in plasma cortisol concentration in lambs of three ages after three methods of castration and tail docking. *Res Vet Sci* 55, 246-251.

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 in week-old lambs. *Vet J.* 155, 39-51.

Maslowska, K. (2016) *Assessment of immunocastration as a means to improve the welfare and productivity of lambs.* PhD thesis, University of Edinburgh.

Mellema, S.C., Doherr, M.G., Wechsler, B., Thueer, S., Steiner, A. (2006) Influence of local anaesthetic on pain and distress induced by two bloodless castration methods in young lambs. *Vet J* 172, 274-283.

Molony, V., Kent, J.E., McKendrick, I.J. (2002) Validation of a method for assessment of acute pain in lambs. *Appl Anim Behav Sci,* 76, 215-238.

Molony, V., Kent, J.E., Robertson, I.S. (1993) Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail-docking. *Res Vet Sci* 55, 236-245.

Molony, V., Kent, J.E., Vinuela-Fernandez, I., Anderson, C., Dwyer, C.M. (2012) Pain in lambs castrated at 2 days using novel smaller and tighter rubber rings with and without local anaesthetic. *Vet J,* 193, 81-86.

Needham, T., Lambrechts, H., Hoffman, L.C. (2016) The influence of vaccination interval on growth, carcase traits and testicular parameters of immunocastrated ram lambs. *Small Rum Res* 145, 53-57.

Small, A., Fetiveau, M., Smith, R., Colditz, I. (2021) Three studies evaluating the potential for Lidocaine, Bupivicaine or Procaine to reduce pain- related behaviours following ring castration and/or tail docking in lambs. *Animals* 11, 3583.

Small, A.H., Jongman, E.C., Niemeyer, D., Lee, C., Colditz, I.G. (2020) Efficacy of a precisely injected single local bolus of lignocaine for alleviation of behavioural responses to pain during tail docking and castration of lambs with rubber rings. *Res Vet Sci* 133, 210-218.

Stewart, M., Beausoleil, N.J., Johnson, C.B., Webster, J.R., Schutz, K.E., Cox, N., Stafford, K.J. (2014) Do rubber rings coated in lignocaine reduce the pain associated with ring castration in lambs? *Appl Anim Behav* Sci 160, 56-63..

Thornton, P.D., Waterman-Pearson, A.E. (2002) Behavioural responses to castration in lambs. *Anim Welf* 11, 203-212.