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Department for Environment Food & Rural Affairs

Evidence Project Final Report

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Project identification

1. Defra Project code: **AW0303**
2. Project title: Evaluation of procaine HCL local anaesthetic for control of pain associated with castration and tail docking in lambs.
3. Contractor organisation(s): SRUC, Peter Wilson Building, West Mains Road, Edinburgh EH9 3JG
4. Total Defra project costs (agreed fixed price): £ £37,241
5. Project: start date 01/02/2022
   end date 31/10/2022

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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. Castration and tail-docking are common procedures applied to young lambs within the sheep industry. Both procedures are commonly conducted by application, around the tail or scrotal neck, of a tight rubber ring, which cuts off blood supply to the distal tissue, eventually causing shedding. These procedures are conducted for various management reasons yet are known to be highly painful for the animals concerned.

2. The welfare concerns relating to these practices have led to substantial interest in developing new approaches to mitigate the pain associated with these procedures.

3. Pain mitigation can be achieved via changes in practice that avoid the painful procedure in the first place, or by alternative methods that are less painful, or by provision of pain mitigation, in the form of local or general anaesthesia or systemic analgesia. In the case of lamb castration and tail-docking all of these approaches have been examined.

4. One potential approach to pain mitigation involves the application of local anaesthetic immediately prior to release of the rubber ring. In recent years, studies have examined the efficacy of such an approach using a novel applicator device, marketed under the trade name "Numnuts®".

5. Application of various local anaesthetic products via Numnuts has been shown to reduce, though not abolish entirely, pain behaviours of lambs either after tail docking, or after combined castration and tail docking.

6. Most prior work with Numnuts® has utilised the local anaesthetic Lignocaine. However, this product is not available for use in the UK. An alternative local anaesthetic product, Procaine, is available for use in sheep in the UK.

7. The objectives of this study were to assess the effectiveness of procaine HCl local anaesthetic for the reduction of pain following castration and/or tail docking in young lambs when administered at the time of application of rubber rings using a Numnuts® applicator.

8. A study was conducted that compared the active pain behaviours, for one hour after procedure, in young lambs that were either handled, or which were tail docked, castrated, or castrated and tail docked either via standard elastrator rubber ring application or with rings applied via the Numnuts® applicator and with a simultaneous injection of 1ml of the local anaesthetic product Procaine (Procamidor Duo). Qualitative Behavioural Assessment (QBA) of the study lambs' emotional expression at ~25minutes post procedure was also conducted.

9. Lambs used in the study were between 3 and 5 days of age and had an average weight of 6Kg. A sample size of 12 animals per treatment was used, based on a power calculation using previous data.

10. In young lambs, delivery of the local anaesthetic product Procaine (Procamidor Duo) via the Numnuts® applicator simultaneous to application of a rubber ring to the tail caused a reduction in active pain behaviour relative to lambs that have a rubber ring applied via an elastrator device.

11. In young lambs, delivery of the local anaesthetic product Procaine (Procamidor Duo) via the Numnuts® applicator simultaneous to application of a rubber ring to the scrotum did not cause a reduction in active pain behaviour relative to lambs that have a rubber ring applied via an elastrator device.

12. In young lambs, delivery of the local anaesthetic product Procaine (Procamidor Duo) via the Numnuts® applicator simultaneous to application of a rubber ring to the scrotum and to application of a rubber ring to the tail caused a reduction in active pain behaviour, for approximately 20mins, relative to lambs that had rubber rings applied via an elastrator device.

13. The most likely explanation for the findings is that local anaesthetic delivery via the Numnuts® applicator provides effective pain mitigation for tail docking but not for castration.

14. This conclusion is in line with existing published findings relating to the use of the Numnuts® applicator with Procaine or other anaesthetic products.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of

the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- 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).

Background

The UK has a large (15.4 million breeding animals in 2020) and diverse sheep industry. Within the sector, castration and tail docking are common management procedures for young lambs. Both procedures are most commonly conducted by application of a tight rubber ring, which cuts off blood flow to either the tail or scrotum. Castration is used to prevent uncontrolled breeding and to minimise behavioural problems with intact male stock. Tail docking is used to prevent fly strike. However, despite being a widespread practice, both procedures are known to cause intense acute pain (Molony et al 2002) and are therefore seen as one of the largest animal welfare issues in sheep production. To date, various practical constraints have limited the development and uptake of valid and economically viable approaches to mitigating pain in young lambs.

A number of possible strategies may apply when there is a desire to minimise or fully abolish animal pain. These have been structured within the '3S' framework as suppress, substitute, and soothe (Guatteo et al 2012). The most basic and effective of these approaches is 'suppress' where the pain is prevented from occurring in the first place by avoidance of the requirement for the procedure. In the case of castration and docking procedures in lambs, this is an option which is available to farmers when, for instance, males are likely to be slaughtered before reproductive maturity or where the risk of fly strike is low. Immunocastration may also be viable as an approach that removes the requirement for physical castration (Zeng et al 2022).

In situations where fully removing the procedure from management is not possible, or human owners are unwilling to take this step, the 'substitute' approach may be applied. In this case pain is minimised, for any given procedure, by selection of approaches that cause the least degree of pain. In the case of castration and docking, a variety of approaches have been investigated. The most effective appears to be the use of a Burdizzo clamp, (Kent et al 1995), with other approaches such use of tighter rubber ring (Molony et al 2012) being less effective. More recently, attempts have been made to replicate the efficacy of the Burdizzo approach by application of a clamp to replace the conventional rubber ring (SRUC Unpublished). The final approach detailed with the '3Ss' is 'soothe' – the provision of pain relief via pharmaceutical or other methods, most commonly via local or general anaesthesia, and/or provision of systemic analgesia. Various castration and docking studies have demonstrated that pain can be wholly or partially (depending on procedures and other factors) mitigated by combinations of local anaesthesia or analgesia (Small et al 2021b). Most pertinent for this study are findings showing that local anaesthetic can be effective in mitigating pain responses in the immediate aftermath of docking or castration procedures (Kent et al 1998). One major issue in relation to either approach is the translation of experimental findings to real-life commercial settings. In the case of administration of local anaesthetic, the requirement for targeted needle insertions on large numbers of animals under field conditions has been a major limitation. One solution to this challenge has been the development of a novel device (Numnuts TM, Sensino, Glasgow), that allows for injection of local anaesthetic to the tail or scrotal neck simultaneous to attachment and release of a standard rubber ring. The Numnuts device is already in use in New Zealand and Australia and has been tested in a small number of research studies under field conditions or in a research setting, and some efficacy in relation to pain mitigation has been demonstrated (Small et al 2020, Small et al 2021ac). The first published Numnuts study demonstrated efficacy in terms of reductions in acute pain behaviours in 4 – 10-week-old lambs following combined castration and tail docking, or just tail docking alone using the local anaesthetic lignocaine (Small et al 2020). Subsequently, this efficacy in relation to tail docking was replicated again with lignocaine (Small et al 2021c). Another study (Small et al 2021a) documented the use of Numnuts with three different local anaesthetic products (lignocaine, bupivacaine and procaine; used in separate experiments) for the combined treatment (lignocaine, bupivacaine), tail docking only (lignocaine, bupivacaine and procaine) or castration alone (procaine). These experiments replicated general finding that local anaesthetic can mitigate pain following the combined treatment or docking alone, with the timing and duration of the benefit varying across local anaesthetic products. However, the use of procaine in relation to castration alone only demonstrated a transient alteration to lamb restlessness.

A further limiting factor in relation to the possible use of Numnuts in the UK is that lignocaine is not licenced for use in sheep in the UK. The current study therefore uses a different veterinary product, the local anaesthetic Procamidor Duo (a procaine formulation licensed for sheep in the UK/EU) to investigate behavioural responses of young lambs to rubber ring tail docking, castration or both together, either with or without local anaesthesia delivered via the Numnuts device.

Since pain can be best understood as a behavioural phenomenon which acts to modify behaviour in order to limit tissue damage, prompt recovery and ensure future avoidance of harm, assessment of behaviour is the best approach to assessing pain in animal studies. Here the various treatment groups (different procedure combinations with and without local anaesthetic) will be compared on: i) quantitative assessment of previously validated lamb pain behaviours (Molony et al 2002) and ii) qualitative behavioural assessment (QBA). QBA is a whole-animal approach, and the premise underlying it is that human observers can integrate perceived behavioural details and signals to judge an animal's behavioural expression, using qualitative descriptors (e.g., relaxed, anxious) that reflect the animals' affective (emotional) state (Wemelsfelder et al 2000). QBA allows for a scientific basis to be applied to the characterisation of behavioural expressions of animals in terms of their affective experience and has previously been shown highly effective in discriminating pain between alternative lamb castration procedures (Maslowska et al 2020). The combination of both quantification of pain behaviour and QBA was intended to maximise the strength of inferences (regarding pain alleviation) drawn from the work.
The study aimed to investigate whether the acute pain associated with castration or tail docking (alone or in combination) in young lambs can be alleviated by use of a novel device (Numnuts TM), which combines application of a rubber ring with a concurrent injection of local anaesthetic. The work will establish whether use of the UK licenced local anaesthetic product provides clear evidence of pain alleviation in lambs that are tail docked or castrated. If proven successful the existence of a practical, quick, easy to use, cost-effective system for provision of pain relief to young lambs would mean a viable solution to a major animal welfare issue in UK agriculture. Widespread uptake of the novel technology would lead to improvements in the lives of many millions of animals each year and to the sustainability of UK sheep farming as a global leader of quality product.

Aims and Hypotheses
This was a partially blinded controlled clinical trial. The aim of the study was to determine whether Procamidor Duo™, a local anaesthetic formulation containing 40mg/ml Procaine and 0.036 mg/ml adrenaline, administered via the Numnuts device, could significantly reduce the pain avoidance behaviours expressed by lambs following castration, tail docking, or both. Procaine HCl used in this study was the commercial product Procamidor Duo, 40 mg/mL procaine HCl plus 0.036 mg/mL adrenaline tartrate (Richter, Austria).

Hypotheses (H): That the pain avoidance behaviours exhibited by lambs after a rubber ring is applied to the tail (H1), or the scrotal neck (H2), or both (H3), will be significantly reduced if 1ml of Procamidor Duo is simultaneously injected through the ring into the tissue by means of the Numnuts applicator.

Animals and Methods

Ethical approval and quality assurance

The study was conducted at SRUC's sheep research facility at Oatridge farm, under Home Office licence (PPL PP3981472) and following ethical approval (AE 05-2022) from SRUC's Animal Welfare Ethical Review Body (AWERB).

The ethical approval and legal authority for the work required clear study endpoints to be in place. Any animal experiencing extreme (beyond that normally expected in response to castration and/or tail docking) reactions would have been immediately removed from study and treated appropriately treatment (including the possibility of euthanasia). If a lamb was observed to have a condition which was unintended, unfavourable, and occurred after drug administration, this would be considered as an Adverse Event.

The study was conducted in compliance with GCP standards (VICH GL9 *Guideline on Good Clinical Practices*) and in accordance with the UK Joint Code of Practice for Research (JCoPR). All SRUC research activity is certified as compliant with ISO9001/2008 (certificate number FS94274) and our quality management system is compliant with ISO 9001:2015. An independent Quality Assurance audit was carried out by SRUC's Quality Manager (in accordance with SRUC SOP SRUC/QA/007) to ensure that the study was performed and reported in compliance with GCP and regulatory requirements, including data collection and documentation.

Animals and treatments

Ewes (Scottish Mule) were kept in groups in straw-bedded pens until they give birth. At, and following, birth ewes and their lambs were managed according to normal farm practice.

Lambs (Mule x Texel) for use in the study (3-6 days of age; N=84) were selected (following a health

check) from the available animals in the SRUC Oatridge flock. The health check included observation for signs of respiratory, gastrointestinal, or musculoskeletal illness or injury. Lambs with any signs of ill-health or exhibiting abnormal behaviour were excluded from selection.

Selected study lambs were weighed and allocated to one of seven treatment groups (n=12 per group; **Table 1**). Based on results from a pilot trial it was calculated that 12 lambs per group would be sufficient to detect a 40% reduction (significant at the 5% level, with more than 80% power) in pain avoidance behaviours in the Procamidor treated groups versus the controls.

Table 1: Overview of treatment groups

Group	Code	Sample size	Procedure	Method
G1	H	n= 12 (6 Male, 6 Female)	Control	Handling only
G2	TD	n= 12 (6 Male, 6 Female)	Tail Docking	Rubber ring only
G3	TDL	n= 12 (6 Male, 6 Female)	Tail Docking	Rubber ring + Procaine (Numnuts)
G4	C	n=12 (Male)	Castration	Rubber ring only
G5	CL	n=12 (Male)	Castration	Rubber ring + Procaine (Numnuts)
G6	CTD	n=12 (Male)	Castration and Tail Docking	Rubber ring only
G7	CTDL	n=12 (Male)	Castration and Tail Docking	Rubber ring + Procaine (Numnuts)

Allocation to treatment was balanced for: i) lamb age and weight, and ii) litter size (twins were used preferentially) but was otherwise conducted according to a pre-determined random schedule. When sibling lambs from the same ewe were used during the study, they were a) allocated to different treatments and b) observed on different days.

Following selection animals were individually identified by a spray marked number on their sides. Study lambs (with their ewe and any sibling) were moved to individual observation pens (~2m x2m, straw bedded, with ad lib access to silage and water) prior to treatment. Each observation pen was equipped with a digital video camera for collection of footage for subsequent behavioural analysis. Ewes and lambs were given a short period to settle into the observation pen before the treatment was conducted.

For treatment, individual lambs were lifted from the observation pen and placed in dorsal recumbency for the application of the appropriate treatment (handling, rubber ring application with elastrator, or rubber ring application via Numnuts and concurrent injection with local anaesthetic). Following treatment, individual lambs were immediately placed back in their observation pen. Ewe and lambs were kept in their observation pen and continually recorded for a period of two hours.

For relevant treatments the local anaesthetic, Procamidor Duo™ (**Table 2**) was administered using the Numnuts® device. The Numnuts® device is an integrated manual system designed to administer a predetermined dose of local anaesthetic at the same time and at the same site as application rubber rings for tail docking and castration in lambs. The local anaesthetic is deposited subcutaneously around the nerves supplying the testes and the tail proximal to the site of the rubber ring.

All treatments were applied by two researchers, who were trained (firstly on an artificial model and then progressing to live animals) in the use of the Numnuts® device, by a highly experienced veterinary representative of the manufacturer. Subsequently, competency was assessed and verified by this veterinarian and by one of SRUC's Named Veterinary Surgeons (NVS). Competency was also assessed and verified, by the NVS, for application of rubber rings via a traditional elastrator.

The study was conducted over 14 observations sessions spread across 13 days. Seven individual observation pens were set up for the study and each treatment was represented once per observation session. In some cases – early in lambing as available numbers were low – partial sessions were necessary, i.e., where all seven observation pens were not occupied. In this case, treatments were still blocked within session by methods, i.e., G2 and G3, G4 and G5, or G6 and G7 always occurred as a pair within the same session. Lambs assigned to particularly treatments (in full or partial sessions) were allocated to observation pens according to a fixed pre-determined schedule.

Table 2: Details of the test article – Procamidor Duo.

Trade name:	Procamidor Duo™
Active ingredients:	Procaine HCl 40 mg/mL Adrenaline tartrate 0.036 mg/mL
Batch number:	0621463AA
Expiry date:	31/05/2023
Storage conditions:	Below 25°C

Label:	Commercial label
Dose rate (product):	1mL (single procedure) or 2ml (double procedure) per animal
Dose rate (active ingredient):	40 or 80 mg procaine HCl per lamb i.e. 6.7mg or 13.3mg mg procaine HCl/kg for a 6kg lamb.
Withholding Period:	28 days (for off-licence use under the cascade)
Formulation:	Procaine hydrochloride 40 mg/mL (equivalent to 34.65 mg procaine) Adrenaline tartrate 0.036 mg/mL (equivalent to 0.02 mg adrenaline) Sodium methyl parahydroxybenzoate(E219) 1.14 mg/mL Sodium metabisulphite (E223) 1 mg/mL
Additional excipients:	Disodium edetate Sodium chloride Hydrochloric acid (for pH adjustment) Water for injections

Data collection

Quantitative Behavioural Analysis

Following completion of the animal phase of the study, continuous focal observation of treated lambs for one hour following procedure were conducted (in accordance with SRUC SOP ABW052 and using the Observer software; Noldus: https://www.noldus.com/observer-xt-animal) on recorded footage, according to a pre-determined ethogram focusing on acute pain behaviours (Small et al 2021ac; **Table 3**). In addition to the continuous focal observations, scan samples for postures at five-minute intervals were conducted for two hours following treatment. Inter-observer reliability testing was conducted on whole hour footage from five lambs.

Table 3: Study Ethogram

Category Item	**Description**
Behaviour (events)	
Restlessness	Stood up and laid down. Instances of rising as far as its knees included.
Kicking/foot stamping	Either a front or hind limb (usually hind limb) was lifted and forcefully placed on the ground while standing or was used to kick while standing or lying.
Rolling	Rolled from lying on one side to the other without getting up. Half rolls onto back and then return to lying on the same side included.
Jumping	All four feet off ground simultaneously.
Pawing	Front foot scrapes at the ground in a repetitive pattern.
Licking/biting wound site	Movement of the head beyond the shoulder, including both looking and touching at the source of pain and grooming.
Teat seeking	Active teat seeking
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
Lip curling	Curling of either the upper or both lips away from the teeth, including the flemen response were recorded. It was not counted if the dam urinated or defaecated near the lamb
Lying intention	Attempts to lie down without completing the manoeuvre in a single sequence
Postures (states)	
Normal standing	Standing with no apparent abnormalities.
Abnormal standing	Other abnormal standing e.g., Statue standing: immobile standing with an obvious withdrawal from interaction with other pen members and outside stimuli; or stretched standing: legs positioned further back than normal.
Standing other	Standing but unable to clearly categorise the standing posture, e.g., obscured view.
Normal	Walking with no apparent abnormalities.

walking	
Abnormal walking	Walking unsteadily or stiffly, includes walking backwards, on knees, moving forward with bunny hops, circling, leaning, or falling.
Walking other	Walking but unable to clearly categorise the walking type, e.g., obscured view.
Running	Movement across pen at gait faster than walking.
Suck	Lamb has teat in mouth and appears to be sucking.
Playing	Agonistic interactions, exuberant skipping.
Normal lying	Ventral recumbency, all legs tucked under body or very close to body.
Abnormal lying – partial extension	Twisted lying; ventral recumbency with forelimbs tucked under body, one or both hind limbs partially extended
Abnormal lying – full extension	Twisted lying; ventral recumbency with forelimbs tucked under body, one or both hind limbs fully extended
Sit	Rump in contact with the ground, front of body raised up by extension of front legs.
Kneel	Rump raised off ground by rear legs, front legs flexed, head close to the ground. [Posture must be maintained for at least 3 s – i.e., not recorded during transition from lying to standing].
Lateral lying	Lateral recumbency with one shoulder on ground, with hind limbs partially or fully extended.
Lying other	Lying but unable to clearly categorise the lying posture or leg position.
Out of view	Lamb is not visible on recording.

Qualitative Behavioural Assessment (QBA).

In addition to the quantitative measurement of acute pain behaviours and postures, QBA (using a Free Choice Profiling (FCP) methodology: Wemelsfelder et al 2001) was applied to short clips of lamb behaviour post-treatment. The FCP method consists of two phases. The first phase is known as the term-generation phase, where the observers are free to generate their own terms. The researcher then gives each of these individual terms a visual analogue scale (VAS). In the following second phase, the participants are instructed to use their personal list of terms as a quantitative measurement tool (Wemelsfelder et al*.,* 2001). This study took place remotely due to an insufficient number of observers to participate in person.

The observers (N=12, 11 females and one male) had an understanding of animal behaviour but no specific expertise in lamb behaviour or lamb husbandry procedures. Prior to recruitment an information sheet and a consent form were sent to the participants. The information given to participants informed them that the study related to pain behaviours in lambs but did not inform them about the various procedures and treatments included in the footage they scored.

For each of the 84 lambs, a one-minute video clip was selected by a researcher blinded to treatment group. Windows Movie Maker was used to format the clips. The 25th minute was selected for the clips (69 clips) as behavioural pain sign indicators are expected to peak between the 20th and 30th minute (Kent et al*.,* 1998; Molony et al., 1993). However, when the focal lamb could not be seen at this minute, another minute between 20 and 30 minutes was used (10 clips). First checking the 24th and then the 26th minute, and then the 23rd and then the 27th, and so on until the 10 minutes were checked. If the lamb could still not be seen, minutes 15 to 20 (3 clips) and 30 to 40 (2 clips) were then considered. The lamb which had to be observed was highlighted at the beginning of the clip by circling it. All the clips had audio.

In Phase One, Blackboard Collaborate (Blackboard Inc., Washington, DC, USA) was used to instruct observers on QBA and FCP procedures. For term-generation, observers were asked to watch 16 one-minute clips which had been selected from the two-hour videocam recordings to cover a wide range of different behavioural expressions. The clips were arranged to show varying expressive contrasts to facilitate term generation. After watching each clip, the observers were given two minutes to note down all the terms they thought adequately described the lamb's behavioural expression on a form previously sent to them. At the end of the session, Phase Two was clearly explained to the observers as this phase had to be completed in their own time.

Following the first phase, individual surveys were created on Survey Monkey (SurveyMonkey Inc., San Matteo, California, USA) by collating all the terms the participants had created, thus ensuring that observers only used their own terms for scoring. However, the terms were first reviewed as follows; (1) terms that described more w*hat* the lamb was doing physically rather than its expression (e.g., lying down, walking) were not included in the survey; (2) when terms were given in both their positive and negative forms (e.g., comfortable and uncomfortable) only the positive forms were kept for scoring; (3) when terms

were only given in their negative forms the majority were changed into their positive forms (e.g., uninterested to interested), as they are less confusing to score. However, when the negative forms were clear and common (e.g., uneasy, unsteady), they were left unchanged. Each participant generated between 13 and 30 terms. The order of terms on observers' surveys was pseudorandom, taking care to avoid placing terms with similar meanings adjacent to one another.

The observers were sent three links to three surveys on Survey Monkey. Each survey consisted of the following information 28 times: the clip number; a link to the video on Media Hopper (University of Edinburgh, UK via contract, involving Data Protection Act safeguards, with Kaltura Inc., USA) (this was crucial to ensure the observers watched the correct clip); the list of terms and a 125mm sliding VAS. The observers had to view 28 clips for each survey adding up to a total of 84 clips. The order of the clips was created by dividing all the clips into groups of seven (one of each treatment) and then randomising them. After viewing each clip, the observers were required to quantify the degree of expression shown by the focal lamb for each term by selecting the most appropriate point on the VAS scale that ranged from 'minimum' (0mm) to 'maximum' (125mm). The observers were aware that all the terms had to be scored, and if a term was considered to be insignificant for that particular lamb, it had to be scored as 'minimum' (0mm).

The data from the surveys were exported from Survey Monkey as Excel files. The data consisted of the observer's scoring of their individual terms for all 84 clips. General Procrustes Analysis (GPA), as previously described in detail (Wemelsfelder et al*.,* 2000; 2001), was used to analyse the observer's scoring patterns. In brief, GPA is a multivariate statistical method that does not require common fixed variables. The statistical procedure used to identify the best-fit pattern, known as a consensus profile, occurs regardless of the meaning of the individual terms generated by observers. The Procrustes statistic is determined by quantifying the percentage of variation (between observers in their assessment of individual lambs) that is explained by the consensus. The Procrustes statistic is compared (using a one-sample *t*-test) to the mean of 100 randomised Procrustes statistics to calculate the statistical performance of the consensus profile above chance. Significant values in that test of $P < 0.001$ or better can be interpreted as evidence that the consensus profile represents a common pattern detected by observers and was not merely a methodological artefact. The Procrustes statistic can also determine the degree of agreement between individual observers and the overall consensus profile. To do this, Procrustes statistics are run for all potential pairs of observers. Principal Coordinate Analysis is used to place every observer on a two-dimensional plot (known as the observer plot), along with a 95% confidence region illustrating the 'normal population' of observers. Principal Component Analysis is then used to cut down the numerous amounts of dimensions within the consensus profile to a smaller subset of dimensions, which explain the majority of variation amongst observed animals. The score for individual observer terms can be correlated with the overall dimension score to enable semantic interpretation of these key dimensions. The more strongly correlated a term is with a dimension, the more weight it will carry as a positive or negative descriptor for that dimension. This process is completely post hoc to the calculation of the consensus profile. However, for interpretation purposes, it enables the identification of the individual terms that best represent the anchor points at either end of the primary dimensions.

Statistical treatment of data

Methods to minimize bias

Allocation to treatment was balanced for lamb age and weight and, as far as possible, for litter size but was otherwise be conducted according to a pre-determined random schedule. Groups 1, 2, 3 were each balanced for male and female lambs to reduce possible gender bias.

Personnel administering the treatment could not be blinded to treatment. The quantitative observations were conducted by two training observers according to a pre-determined ethogram. The comparison between control and treatment groups could not be fully blinded due to the possible visibility of the rubber ring on the tail or scrotum. But otherwise, observers conducting the behavioural scoring where blinded to treatment (e.g. elastrator versus Numnuts versions of the same procedures). Observers conducting the QBA observations were blinded to treatment group and to the precise purpose of the study.

Outcome variables

The primary outcome variable was a combined index of acute pain behaviours ('REQ' – previously identified as the measure most capable of discriminating between different pain levels in young lambs: Molony et al 2002) consisting of the total frequency of restlessness, kicking/foot stamping, rolling, jumping, and easing quarters. Secondary outcome variables were duration of normal lying (the postural indicator with most discriminatory power: Molony et al 2002) and two dimensions of emotional expression resulting from QBA.

Statistical analysis

All statistical analysis was conducted in Genstat (Genstat Release 16.1, VSN International Ltd., Hemel Hempstead, UK). Treatment groups were compared within procedure and with the handled control group

(i.e. Docking: G1 vs G2 vs G3; Castration: G1 vs G4 vs G5; Castration and Docking: G1 vs G6 vs G7) in terms of frequency of acute pain related behaviours or postures, or on identified dimensions of behavioural expression from QBA. Analysis was also conducted comparing all seven treatment groups. Data were analysed using Residual Estimated Maximum Likelihood (REML) model. Initial models fitted possible confounding variables (session, pen, lamb age, lamb weight, the lamb being a single or twin, lamb sex (where appropriate)) which were discarded from the model if non-significant. The final fixed models included any of these variables that were retained plus treatment, and the random model included Ewe and (where appropriate, i.e., for quantitative data but not for QBA dimensions) observer. The individual animal is the experimental unit. Treatment effects were considered significant at P≤0.05.

Results

General information

In the study no animals reached the defined endpoints requiring removal from the study, and no adverse effects were seen. There was no significant difference in lamb weight (P=0.16) or age (P=0.39) between treatment groups. Mean lamb age was 3.7 days (N=84, SD = 0.78), and all procedures were conducted on lambs between 3 and 5 days of age. Mean lamb weight in the study was 6.02 kg (SD = 1.14 Range: 3.7 – 9.2).

There was a high degree of inter-observer reliability, with highly significant strong correlations seen between the two observers for both REQ (r=0.989, P<0.001) and duration of normal lying (r=0.996, P<0.001).

Preliminary models for all outcome variables found no significant effects of possible confounding variables (session, pen, lamb age, lamb weight, the lamb being a single or twin, lamb sex (where appropriate)), so final models in all cases consist of treatment as the fixed effect and ewe and observer as the random effect (or ewe alone for the QBA dimensions).

Quantitative Behaviour - REQ

There was a significant treatment effect of method on REQ responses to tail docking ($F_{2,32}$=4.19, P=0.024, Fig. 1). The total number of REQ events scored over the first hour after the procedure was significantly (P<0.05) higher in the RR tail docked group (count, mean±SE: 54.7±22.2) compared to the handled (9.7±2.6) and Numnuts docked group (9.5±1.6). There was no difference between handled and Numnuts docking groups (P<0.05).

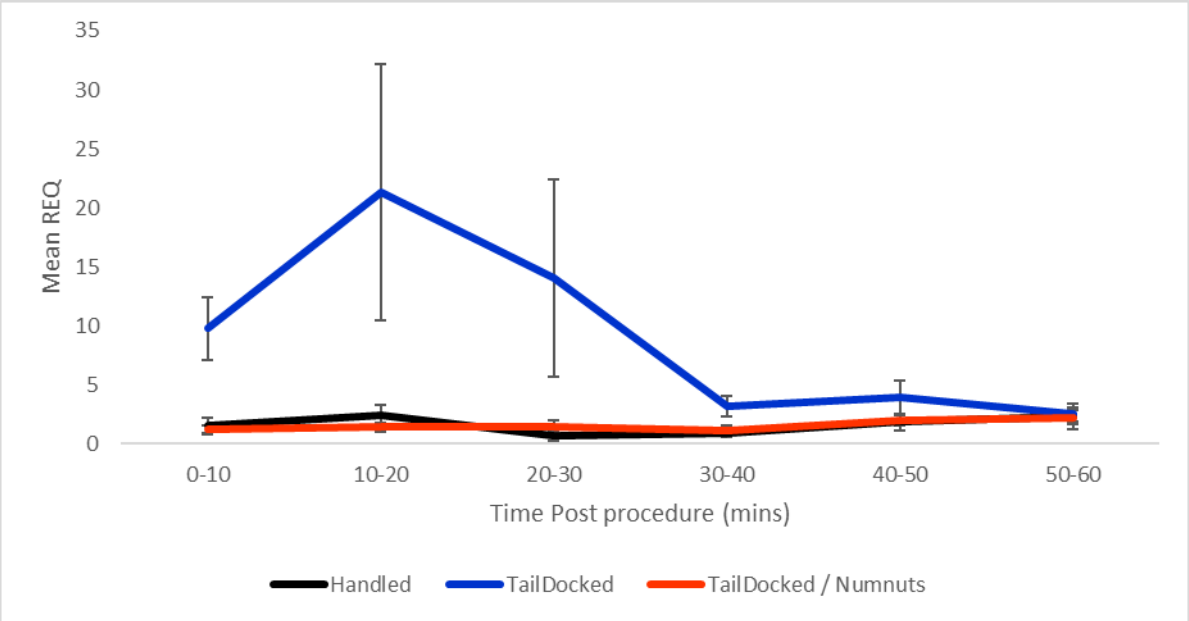


Figure 1: Mean (±SE) REQ total frequency (at different 10-minute periods post treatment) for young lambs either handled (black line), or tail docked with an elastrator application of a rubber ring (blue line), or tail docked with application of a rubber ring and simultaneous injection of Procaine via the Numnuts applicator (red line).

There was a significant treatment effect of method on REQ responses to castration ($F_{2,32}$=18.77, P<0.001, Fig.2). The total number of REQ events scored over the first hour after the procedure was significantly (P<0.05) higher in the elastrator castrated group (count, mean ±SE: 168.0±30.2) and Numnuts castrated group (186.7±26.9) compared to the handled (9.7±2.6). But there was no difference between responses to elastrator castration and Numnuts castration (P>0.05).

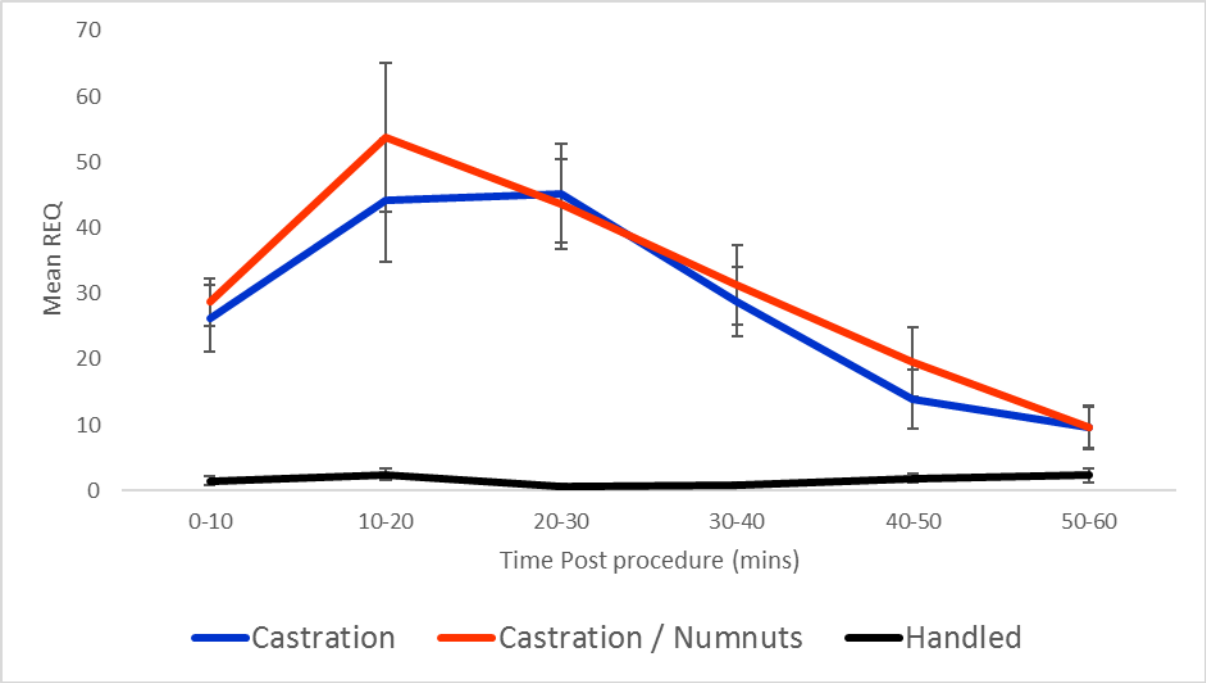


Figure 2: Mean (±SE) REQ total frequency (at different 10-minute periods post treatment) for young lambs either handled (black line), or castrated with an elastrator application of a rubber ring (blue line), or castrated with application of a rubber ring and simultaneous injection of Procaine via the Numnuts applicator (red line).

There was a significant treatment effect of method on REQ responses to combined castration and tail docking ($F_{2,32}$=40.44, P<0.001 Fig. 3). The total number of REQ events scored over the first hour after the procedure was significantly (P<0.05) higher in the elastrator castrated and tail docked group (count, mean ±SE: 282.3±33.5), compared to the Numnuts (202.2±20.5) or handled (9.7±2.6) groups. The number of REQ events was significantly higher in the Numnuts group compared to the handled group.

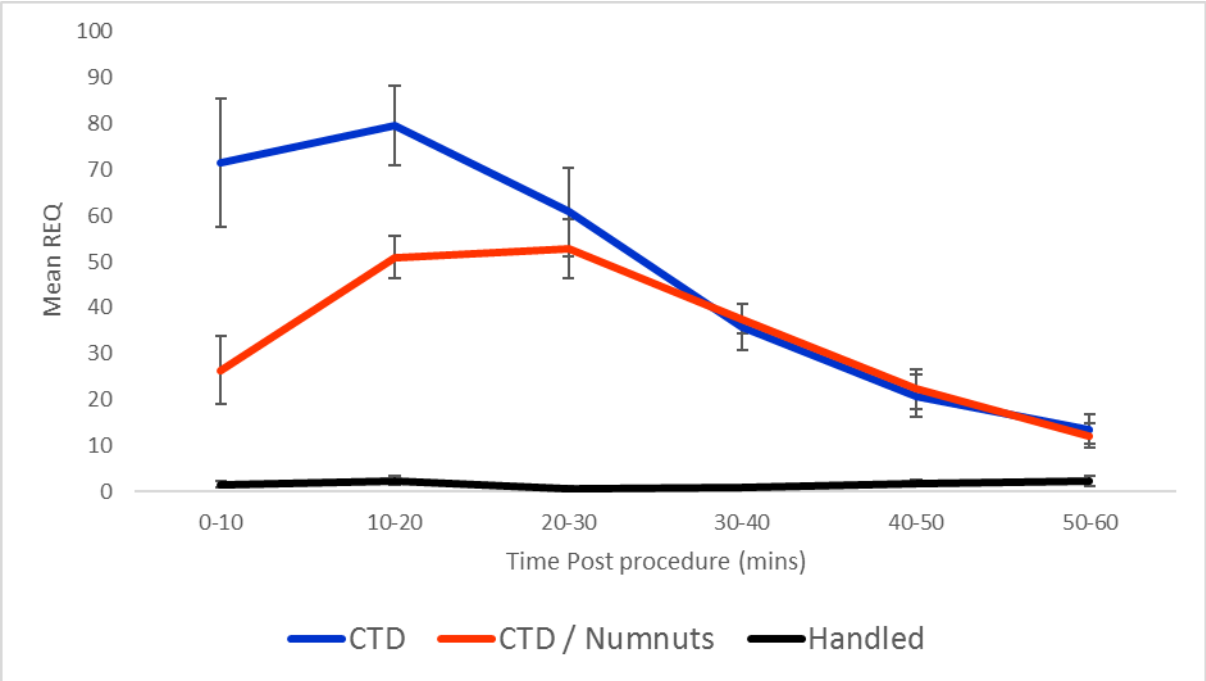


Figure 3: Mean (±SE) REQ total frequency (at different 10-minute periods post treatment) for young lambs either handled (black line), or castrated and tail docked with an elastrator application of a rubber ring (blue line), or castrated and tail docked with application of a rubber ring and simultaneous injection of Procaine via the Numnuts applicator (red line).

Quantitative Behaviour – lying behaviour

Within the tail docking study there was no significant overall effect of treatment method ($F_{2,25.8}$=1.48, P=0.245) on the total duration of lying behaviour seen in the handled (seconds, mean ±SE: 1728±383), tail

docked (900±305) or tail docked with Numnuts (1640±369) groups.

Within the castration study there was a highly significant overall effect of treatment ($F_{2,23}$=14.67, P<0.001), with the duration of normal lying performed by the handled group (seconds, mean ±SE: 1728±383) being significantly higher than both the elastrator castration (20.1±7.3) and Numnuts castration group (122.0±77.3).

Within the castration and docking study there was a highly significant overall effect of treatment ($F_{2,30.1}$=18.32, P<0.001), with the duration of normal lying performed by the handled group (seconds, mean ±SE: 1728±383) being significantly higher than both the elastrator castration and docking (79.7±56.8) and Numnuts castration and docking group (70.8±41.3).

Qualitative Behavioural Assessment

The consensus profile explained a significantly higher percentage of variation between observers (Procrustes statistic: 60.83%) than the mean of 100 randomised analyses (mean ± SE Procrustes statistic: 26.0±0.15, t_{99}=89.9, P<0.001), indicating that the consensus profile represents a fundamental characteristic of the data that was not produced by chance. Two main dimensions were found, accounting for 59.0% and 21.6% of the variation, respectively. Evaluation of the terms positively or negatively associated with each dimension (**Table 4**) demonstrates the semantic agreement generated across observers. Restless and alert (positive) and relaxed and comfortable (negative) were the terms most frequently associated with dimension one, and distressed and agitated (positive) and hungry, inquisitive, and playful (negative) were those most frequently associated with dimension two. Consequently, these terms were used as labels to provide a semantic comprehension of the anchor points of each dimension.

Table 4. Terms (2 for each observer) that demonstrated the highest positive and negative correlation with dimensions 1 and 2 of the consensus profile. Figures in brackets represent the number of observers using that term. Bold terms represent the chosen dimension labels.

	Positive correlation	**Negative correlation**
Dimension One	**restless** (4), **alert** (3), agitated (2), frustrated (2), explorative, attention-seeking, tense, active, bored, uncoordinated, uneasy, energetic, spirited	**relaxed** (5), **comfortable** (4), calm (4), sleepy (3), content, unresponsive, peaceful, settled
Dimension Two	**distressed** (3), **agitated** (3), anxious (2), frustrated (2), unsettled, weak, stressed, groggy, lethargic, uncomfortable, restless, uneasy, tired, irritated	**hungry** (3), **inquisitive** (2), **playful** (2), lively (2), curious (2), explorative, attention-seeking, ambitious, content, happy, eager, active, assertive, interested

QBA – dimension one

There was no significant treatment effect of method on dimension one emotional expression scores ($F_{2,32.3}$=1, P=0.38, Fig. 4) across the tail docking groups.

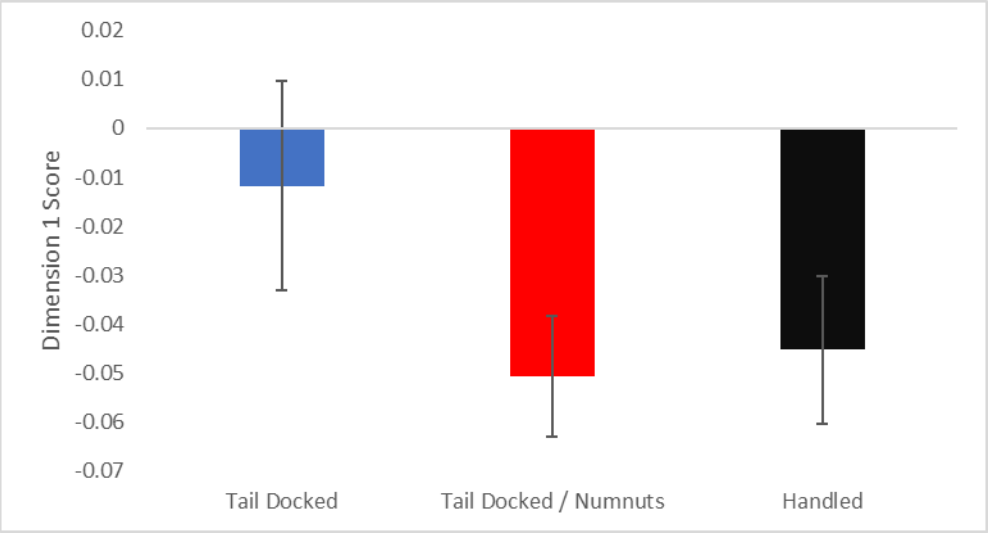


Figure 4: Mean (±SE) scores for each treatment group (tail docking study) on dimension one of the consensus profile produce via QBA

There was a significant overall treatment effect of castration method on dimension one emotional expression scores ($F_{2,27.7}$=6.91, P=0.004, Fig. 5). The score for the handled group (-0.045±0.0150) was significantly (P<0.05) lower (indicating a more relaxed/comfortable behavioural expression) than the scores for either the elastrator castrated group (0.0245±0.014) or the Numnuts castrated group (0.0257±0.0188). These two groups did not differ from each other (P>0.05).

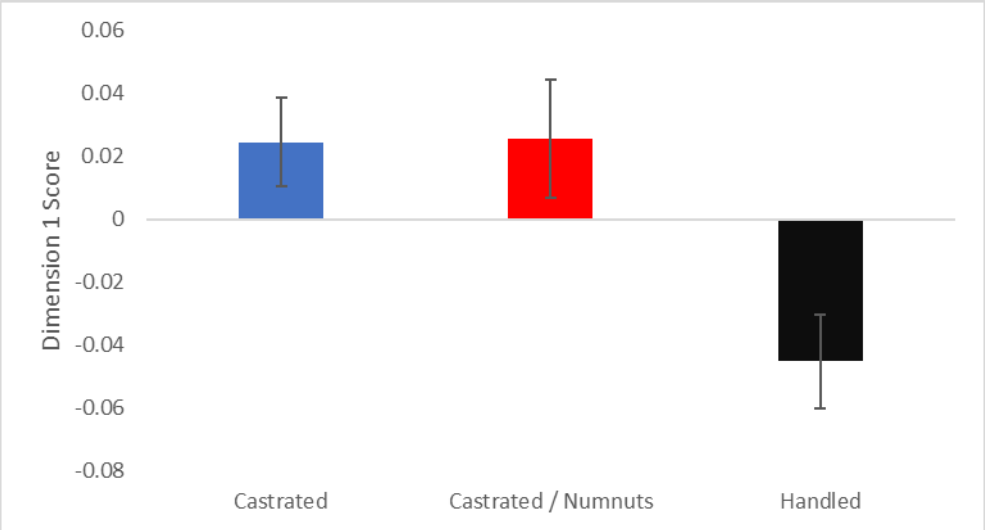


Figure 5: Mean (±SE) scores for each treatment group (castration study) on dimension one of the consensus profile produce via QBA

There was a significant overall treatment effect of castration and tail docking method on dimension one emotional expression scores ($F_{2,32.4}$=7.15, P=0.003, Fig. 6). The score for the handled group (-0.045±0.0150) was significantly (P<0.05) lower (indicating a more relaxed/comfortable behavioural expression) than the scores for either the elastrator castrated and docked group (0.0378±0.0146) or the Numnuts castrated and docked group (0.0199±0.0200). These two groups did not differ from each other (P>0.05).

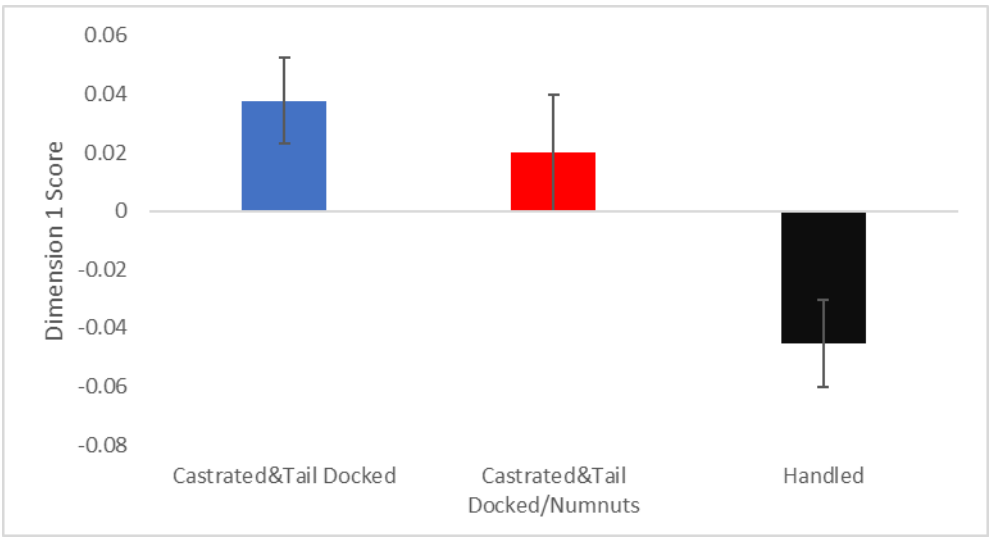


Figure 6: Mean (±SE) scores for each treatment group on dimension one (castration and tail docking study) of the consensus profile produce via QBA

QBA – Dimension two

There was no significant treatment effect of method on dimension two emotional expression scores ($F_{2,32.1}$=0.2, P=0.82, Fig. 7) across the tail docking groups.

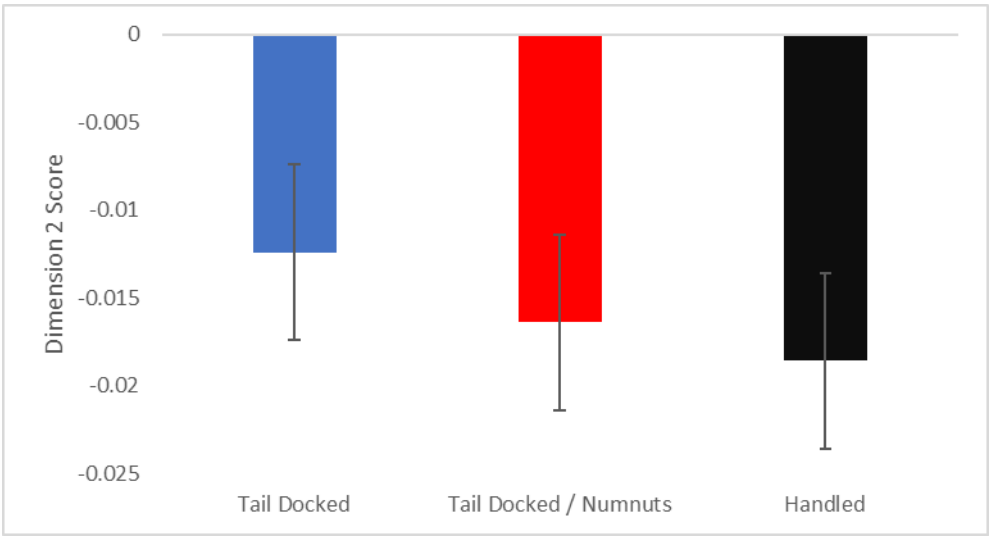
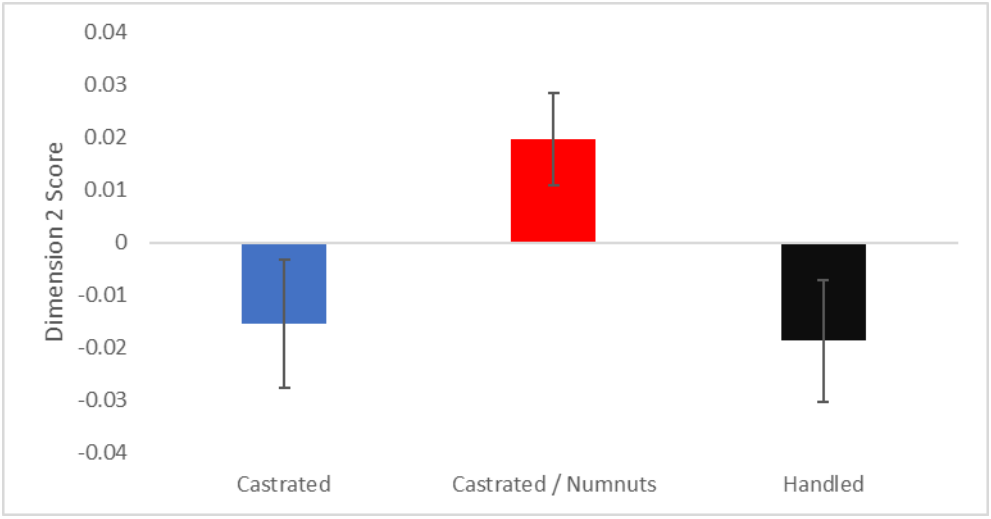


Figure 7: Mean (±SE) scores for each treatment group (tail docking study) on dimension two of the consensus profile produce via QBA

There was a significant overall treatment effect of castration method on dimension two emotional expression scores ($F_{2,32.2}$=3.69, P=0.036, Fig. 8). The score for the handled group (-0.0186±0.0116) and the elastrator castrated group (-0.0153±0.0122) did not differ from each other (P>0.05) but both were significantly (P<0.05) lower (indicating a more inquisitive/playful behavioural expression) than the scores for the Numnuts castrated group (0.01975±0.00884).

Figure 8: Mean (±SE) scores for each treatment group (castration study) on dimension two of the



consensus profile produce via QBA

There was a significant overall treatment effect of castration and tail docking method on dimension two emotional expression scores ($F_{2,31.4}$=5.56, P=0.009, Fig. 9). The score for the handled group (-0.0186±0.0116) was significantly (P<0.05) lower (indicating a more inquisitive/playful behavioural expression) than the scores for either the elastrator castrated and docked group (0.02108±0.00892) or the Numnuts castrated and docked group (0.02192±0.00837). These two groups did not differ from each other (P>0.05).

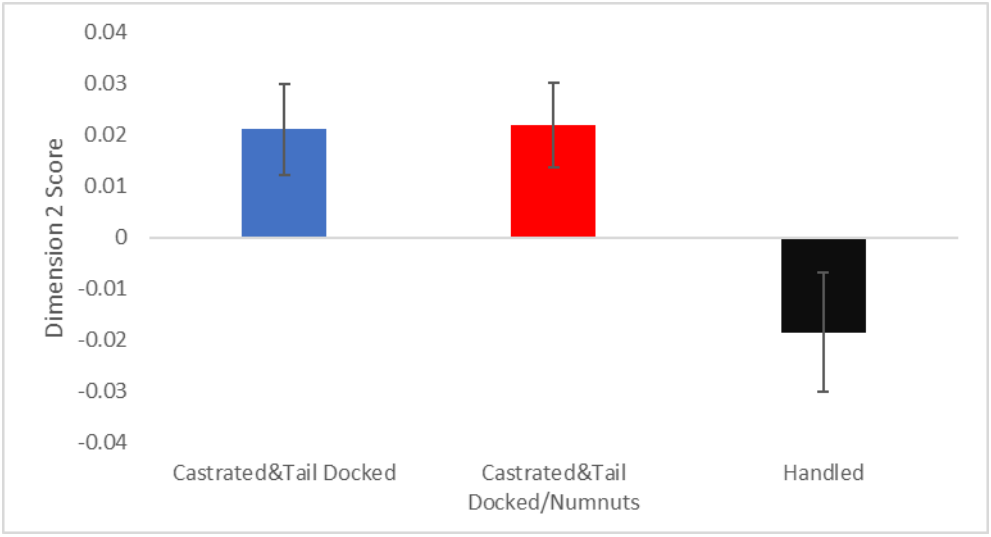


Figure 9: Mean (±SE) scores for each treatment group (castration and tail docking study) on dimension two of the consensus profile produce via QBA

Analysis across all seven treatment

The model fitted across all seven groups found a highly significant effect of treatment on total REQ count (**Table 5**). The elastrator combined castration and docking group was found to show a higher REQ count than all other treatments, with the Numnuts castration and docking treatment not being significantly different from the two castration only treatment groups.

In contrast to the within method analysis there was a significant treatment effect of method on duration of normal lying (Table 4, Fig 10.). The total normal lying time over the first hour after the procedure was significantly ($P<0.05$) lower in the elastrator tail docked group compared to the handled and Numnuts docked groups. There was no difference between handled and Numnuts docking groups. All three groups were significantly different from all four groups involving castration, where the performance of normal lying was very rare, and these groups did not differ from each other.

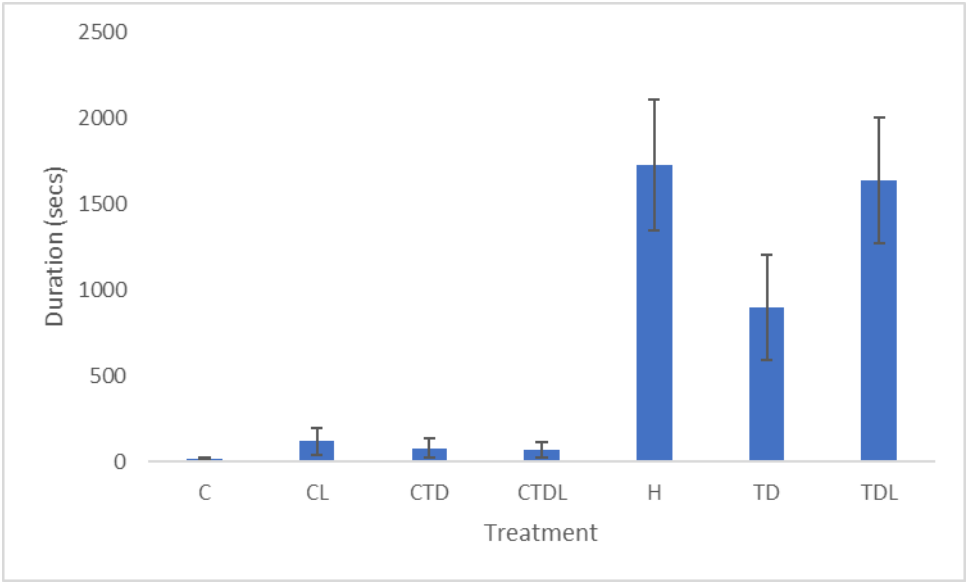


Figure 10: Mean (±SE) duration of normal lying behaviour across all seven treatment groups.

For QBA dimension one the handled and tail docked with Numnuts groups were significantly different from all four groups involving castration (Table 5). The elastrator tail docking group was intermediate and was not found to be significantly different from either the those two groups, or from the four groups involving castration. On dimension two, the handled group, both tail docking groups and the elastrator castration group were not found to be significantly different. All four groups were significantly different from the remaining three castration groups.

Table 5: Predicted means for QBA dimensions one and two, total REQ count and duration of normal lying across all seven treatment groups.

Variable	Treatment group							Statistics	
Variable	**H**	**TD**	**TDL**	**C**	**CL**	**CTD**	**CTDL**	**F Stat.**	**P**
QBA									
Dimension 1	-0.045[a]	0.011[a, b]	-0.051[a]	0.027[b]	0.029[b]	0.034[b]	0.021[b]	$F_{6,69.6} = 4.91$	P<0.001
Dimension 2	-0.019[a]	-0.013[a]	-0.016[a]	-0.018[a]	0.021[b]	0.020[b]	0.023[b]	$F_{6,75.9} = 3.74$	P=0.003
Quantitative									
REQ (count, 1hr)	9.1[a]	53.0[a]	11.2[a]	167.4[b]	187.7[b]	280.0[c]	202.0[b]	$F_{6,70.8}= 24.2$	P<0.001
Normal lying (secs, 1hr)	1720.3[a]	901.8[b]	1621.0[a]	105.0[c]	143.0[c]	58.8[c]	26.0[c]	$F_{6,74.7} = 15.5$	P<0.001

Means in the same row with differing superscript letters are significantly different at p<0.05 (Fishers LSD).

Discussion and implications

Summary of findings

The study set out to test three related hypotheses*: That the pain avoidance behaviours exhibited by lambs after a rubber ring is applied to the tail (H1), or the scrotal neck (H2), or both (H3), will be significantly reduced if 1ml of Procamidor Duo is simultaneously injected through the ring into the tissue by means of the Numnuts applicator.* The results of the study suggest that H1 and H3 can be accepted and that H2 should be rejected.

The secondary outcome variables (lying behaviour and QBA dimensions) are broadly in line with this conclusion. Lying behaviour was seen to be normalised in lambs tail docked with use of the Numnuts device relative to those docked using an elastrator. However, performance of normal lying was low in all groups involving castration and this measure did not identify an effect of Numnuts relative to elastrator use in the combined castration and docking group. Similarly, the scores for lamb emotional expression on dimension one suggested a beneficial effect of use of the Numnuts device in relation to the tail docking only treatment (handled lambs and lambs docked with Numnuts showing equivalent emotional expression; animals docked with a standard elastrator method were intermediate between those animals and the four groups involving castration) but this difference was not statistically significant. QBA outcomes may have been more clear-cut if an earlier time point had been chosen as reference to the timeline of acute pain behaviours suggests that the peak behavioural alteration occurred between 10 and 20 minutes after treatment. In the existing literature the inference about when maximum pain might be occurring following whether docking or castration depends on the outcome measure chosen. The active pain behaviour responses (e.g., REQ) appearing to peak slightly earlier than changes in postures (absence of normal lying and positioning the body in various abnormal postures).

Implications

The most plausible interpretation of the findings is that application of Procaine via Numnuts applicator works well to mitigate tail docking pain but is not effective against castration pain. At the current time this is in line with published findings (Small et al 2020, Small et al 2021ac).

Limitations

Use of the Numnuts applicator was successful within the study. The applicator requires an adjustment in technique compared to standard elastrator use but this was readily achievable. In this study the Numnuts applicator was applied by one person with another person holding the lamb in an appropriate position. Commercial implementation might require either some form of restraint / handling device (such as a cradle) or otherwise single person application – both of these might limit practicality.

In these young lambs, ensuring that both testes are present distal to the ring was sometimes difficult. Though this was always achieved in the experimental animals, it did cause a longer period of handling for some animals. This issue might cause problems for farmers in commercial settings, particularly if the procedure was being conducted by a single operator. Though it is also the case that – across differing lamb sizes and breeds – that ensuring testes are captured by the ring can also be difficult with a standard elastrator in some animals.

The injection process was generally easy. In relation to injections in the tail occasional re-positioning of the device is necessary when resistance is felt as the needle is inserted. This involves expanding the rubber ring and slight movements of the device, before releasing the ring again and completion of the injection. Some lambs do demonstrate a momentary reaction to the injection, although this was not quantified formally during the study. Commercial application of Numnuts also requires repeated use of a single needle for multiple injections. This could have some negative implications for the welfare implications of injection or raise the possibility of disease transfer between animals, though such repeated

use of needles is already common in relation to other on-farm treatments such as vaccinations.

Dissemination of findings and Future work

Information regarding the outcomes of the study have been communicated to the UK Animal Welfare Committee, to Defra's Animal Health and Welfare Pathway Sheep Sector Group, and to sheep farmer and other industry groups. The work will be written up and submitted to a peer-reviewed scientific journal.

The findings of this study do not preclude the possibility that the Numnuts® device can work in relation to castration, merely it shows that it does not appear to have efficacy with the specific dose and local anaesthetic product used here. Future studies might investigate whether alternative dosage volumes – of Procaine, or alternative local anaesthetic products – might provide pain relief in relation to castration. Any future additional efficacy studies might also investigate a broader range of ages of animal.

References

Guatteo, R., Levionnois, O., Fournier, D., Guémené, D., Latouche, K., Leterrier, C. & Le Neindre, P. 2012. Minimising pain in farm animals: The 3S approach- Suppress, Substitute, Soothe. Animal 6, 1261–1274.

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. The Veterinary Journal 155, 39-51

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. Research in Veterinary Science 55, 246-251.

Kent, J.E., Molony, V. & Robertson, I.S. 1995. Comparison of the burdizzo and rubber ring methods for castrating and tail docking lambs. Veterinary Record 136, 192-196

Maslowska, K., Mizzoni, F., Dwyer, C. M. & Wemelsfelder, F. 2020. Qualitative behavioural assessment of pain in castrated lambs. Applied Animal Behaviour Science 233, 105143

Molony, V., Kent, J.E. & McKendrick, I.J. 2002. Validation of a method for assessment of acute pain in lambs. Applied Animal Behaviour Science 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. Research in Veterinary Science 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. The Veterinary Journal 193, 81-86.

Small, A. H., Jongman, E. C., Niemeyer, D., Lee, C. & Colditz, I. G. 2020. Efficacy of precisely injected single local bolus of lignocaine for alleviation of behavioural responses to pain during tail docking and castration of lambs with rubber rings. Research in Veterinary Science 133, 210-218.

Small, A., Fetiveau, M., Smith, R. & Colditz, I. 2021a. 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., Fisher, A.D., Lee, C. & Colditz, I. 2021b Analgesia for sheep in commercial production: where to next? Animals 11, 1127.

Small, A., Marini, D. & Colditz, I. 2021c. Local Anesthetic Delivered with a Dual Action Ring and Injection Applicator Reduces the Acute Pain Response of Lambs during Tail Docking. Animals 11, 2242.

Wemelsfelder, F., Hunter, E. A., Mendl, M. T. & Lawrence, A. B. 2000 The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement. Applied Animal Behaviour Science 67, 193-215.

Wemelsfelder, F., Hunter, E.A., Mendl, M.T. & Lawrence, A.B., 2001. Assessing the 'whole animal': a free choice profiling approach. Animal Behaviour 62, 209-220.

Zeng, F., Ding, Y., Wassie, T., Jing, H., Ahmed, S., Liu, G. & Jiang, X. 2022. Recent advances in immunocastration in sheep and goat and its animal welfare benefits: A review. Journal of Integrative Agriculture 22, 299-309

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.