Animal welfare evaluation of Liquid Nitrogen breech application to minimise the risk of flystrike in sheep.

Final report

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Summary

This study compared the responses of lambs to breech modification, using Sheep Freeze Branding or surgical mulesing, for the prevention of flystrike in sheep. The study concentrated on the animal behavioural responses indicative of pain, wound inflammation/healing and early growth rates.

A total of 120 lambs in 3 groups were subjected to one of four treatments (30/treatment): i) Ring castration and/or Tail-docking (hot iron) with Meloxicam injection (T/C), ii) as i) with the addition of Freeze Branding (FB), iii) as i) with the addition of surgical mulesing (MM), iv) as i) with the addition of surgical mulesing but using Tri-Solfen spray instead of Meloxicam injection for pain relief (MT).

Behaviour was observed every 15 minutes using scan sampling for 6 hours on Day 1 and for 2 hours on Day 2 and 3. Lambs were weighed prior to the application of the procedures and both body weight and assessment of the wounds were taken at 3-4 (Assessment 1), 10-12 (Assessment 2), 22-24 (Assessment 3) and 36-38 days (Assessment 4) post-procedure.

Behaviour on days 1 and 2 and weight at Day 3-4 were similar for all breech modification treatments (FB, MM and MT) and different from the T/C treatment. Behaviour was similar for all treatments on Day 3, however final body weight of mulesed lambs (MM and MT), but not FB, were significantly lower than T/C lambs.

Wound assessment (both visually and using Infrared Thermography) revealed that both mulesing treatments resulted in open wounds covered by scabs, while the FB treatment resulted in an internal ‘ridge’ where the skin was clamped together without displaying an open wound and appeared similar to animals not subjected to breech modification (T/C).

We conclude that we were unable to detect major behavioural differences between Freeze Branding and the Mulesing treatments when both were accompanied by pain relief. However, some differences were observed between all breech modification treatments (FB, MM and MS) and lamb marking (T/C) only, with pain relief provided for all treatments, in the first 2 days post procedures. Over the longer term, the slightly better weight gain and the described differences in wound healing, suggest that the Freeze Branding application may provide these animal welfare benefits over surgical mulesing.

Introduction

Mulesing in wool sheep is a painful procedure, performed to reduce the risk of flystrike in the breech area of susceptible sheep. While there is increasing pressure to phase out mulesing and use alternative methods to control flystrike, the wool industry still requires breech modification to prevent flystrike and economic loss, particularly in the higher rainfall regions.

The use of pain relief (primarily Tri-Solfen) is common in commercial industry; however, this does not completely abolish the pain associated with this procedure. An alternative method to modify the breech area to minimise the risk of flystrike is the application of liquid nitrogen (freeze branding). Sheep freeze branding is performed through stapling the excess skin on the breech of the sheep using an applicator and performing a cryogenic treatment in the affected area using liquid nitrogen during several seconds. Cryotherapy is a commonly used treatment for warts in humans and results in necrosis of the affected tissue (Mercer and Tyson, 2008).

Preliminary investigations in this technology 5 years ago indicated little to no benefit compared to traditional mulesing (AWI report produced by Small and Lee, 2018), however there were several problems during the trial and modifications were made in the course of that trial that are not accounted for in the results. The liquid nitrogen method has since been refined by the developers with the aim of improving animal welfare outcomes and has been used on more than 25,000 lambs under field conditions. While not a controlled research trial, it was observed by an independent veterinarian during field trials that lambs showed reduced behavioural responses suggestive of pain and grew faster than traditionally mulesed lambs. While animal welfare benefits of the revised methodology are assumed and this technology is currently being used on commercial farms, this assumption needs to be assessed in a controlled scientific study.

Mulesing commonly takes place at the same time as tail docking and castration (of male lambs). This study aimed to compare the animal responses after the application of liquid nitrogen to the breech (freeze branding) to surgical mulesing in lambs that were simultaneously castrated and/or tail docked, including the use of pain relief registered for use under commercial conditions for these procedures. The study concentrated on the animal behavioural responses indicative of pain, wound inflammation/healing and early growth rates.

Methodology

The protocol and conduct of the experiment were approved by The University of Melbourne Animal Ethics Committee (Project ID 10462).

This study was performed on a commercial Merino farm near Heathcote, Victoria during September to November 2020. This farm made available 3 groups of ewes with lambs of approximately 6 weeks old at foot. Group size ranged from 98 (group 1), 64 (group 2) and 52 (group 3) ewes with both single and twin lambs.

On the day of the trial (on 3 consecutive days), lambs and ewes were moved to small paddocks (about 50 x 50m) in their group, and they stayed there until behaviour observations were completed on the day of the procedures. From these ewes and lambs, 40 lambs from each group were selected for the study, excluding lambs that were small or not completely healthy (there was some lameness in the groups).

Lambs (n = 10 per procedure/day; 5 male & 5 female, a total of 40 each day x 3 days) were weighed and allocated at random to a procedure, which immediately followed. Weighing was done in a weighing crate next to the area were the procedures were applied. At weighing, lambs were also marked with an individual number using stock mark spray.

The procedures for comparison were:

i) **T/C**: Tail-docking (hot iron) (+ ring castration of male lambs) with meloxicam injection (Metacam® 20 mg/mL administered subcutaneously at registered label dose of 1.0 mL/20 kg bodyweight);

ii) **FB**: Tail-docking (hot iron) (+ ring castration of male lambs) + liquid N2 application with meloxicam injection as above;

iii) **MM**: Tail-docking (hot iron) (+ ring castration of male lambs) + surgical mulesing with meloxicam injection as above

iv) **MT**: Tail-docking (hot iron) (+ ring castration of male lambs) + surgical mulesing with Tri-Solfen application.

Procedures were applied on three consecutive days (n=10 animals per procedure on each day, a total of 40 animals/day). On the relevant day, lambs were separated from the ewes and kept in a separate pen near the treatment area. They were treated as described during an approximate 2-hour period, with procedures balanced over time and the timing between animals based on the duration of the slowest procedure. Mulesing was performed by an accredited mulesing contractor using a standard V-mules for Victorian sheep, while the freeze branding application was applied by Dr John Steinfort, a veterinarian who developed the method of freeze branding to the breech area of sheep (https://steinfortagvet.com.au/). Lambs were placed in a marking cradle where their allocated procedure was performed, and allocated pain relief was administered immediately prior to the procedure.

Following the procedure, lambs were released into a small observation paddock (about 50 x 50 m), where their dams were placed. Five observers, blinded to the procedure as far as practicable, recorded the behaviour of each lamb using scan sampling at fixed intervals relative to the scheduled time when the procedure was applied. Observers were located in a hide in the middle of the paddock area and on the outside perimeter of the observation paddock before application of procedures commenced. The scan sample times were every 15 minutes for 6 hours following the procedures, followed by 8 scan samples every 15 minutes commencing at about 22 hours (day 2) and then again at about 46 hours post-procedure (day 3). An additional observer recorded the time taken for each lamb to mother-up with its dam immediately following release post-procedure for a maximum of 120s. Lamb behaviour was classified as listed in Table 1.

Lambs were re-weighed at 3-4 (Assessment 1), 10-12 (Assessment 2), 22-24 (Assessment 3) and 36-38 days (Assessment 4) post-procedure when their wounds were also assessed. Lambs and their mothers were brought into a yard where they were separated from the ewes. They were weighed individually and identified by their RFID ear tag. The lambs were then lifted and restrained in marking cradles so they could be assessed for wound healing and while images were taken. Wounds on the tail and breech were recorded as open or closed and scored for size and the presence of swelling and exudates on a 5-point scale from 0 (no visible wound or palpable swelling) to 4 (large area of wound or substantial pitting oedema, see Table 2).

Table 1. Ethogram for behavioural observations (adapted from Small et al. 2018)

Behaviour	Abbreviation	Description
Normal ventral lying	NV	Lay on sternum with legs tucked in and head up or down
Abnormal ventral lying	AV	Ventral lying with hind limbs partially or fully extended or keeping hindquarters off the ground (dog sitting).
Ventral lying other	Vu	Lamb was lying ventrally but unable to clearly categorise the lying posture.
Lateral lying	L	Lateral (on side) with one shoulder on ground, extension of hind limbs with head up or down.
Lying intention	Li	Attempts to lie down without completing the manoeuvre in a single sequence
Normal standing	NS	Standing with no apparent abnormalities
Hunched standing - severe	HSS	Lamb stood with mid back arched, with neck and head pointing downward towards the ground.
Hunched standing - mild	HSM	Lamb stood with lower back tilled (tail tucked), with head lower than highest point of back and neck level.
Abnormal standing	AS	Other abnormal standing e.g. Statue standing: immobile standing with an obvious withdrawal from interaction with other pen members and outside stimuli; or stretched standing: legs positioned further back than normal.
Standing other	Su	Lamb was standing but unable to clearly categorise the standing posture; e.g. obscured view
Normal walking	NW	Walking with no apparent abnormalities
Abnormal walking	AW	Walking unsteadily or stiffly, includes walking backwards, on knees, moving forward with bunny hops, circling, leaning or falling.
Walking other	Wu	Lamb was walking but unable to clearly categorise the walking type; e.g. obscured view.
Grazing	G	Nibbling at grass
Suckling	Sk	Drinking from its mother
Running	R	Movement across pen at gait faster than walking
Jumping	J	Forelegs are lifted from the ground, the forepart of the body is elevated in an upward movement
Playing	P	Running and Jumping categories pooled
Easing quarters	EQ	Restless standing with weight shifting from one leg to the other
Restless lying	RL	Laying down and getting up again repeatedly within seconds
Lying rolling	LR	Rolling on the ground (this behaviour is often seen after ring castration)

Table 2. Wound score (appearance and swelling)*:

Descriptor	Wound score
No swelling; dry scab.	0
Slight swelling along wound edges (up to 5 mm either side); Small area (<1 cm) wet and oozing; no visible pus.	1
Large area swelling, but soft; Medium area wet and oozing (1–5 cm); small amount pus. During healing phase: Loose cover; Granulation tissue forming, but still oozing; watery exudate.	2
Large area swelling, moderately hard; Large area wet (>5 cm); small necrotic area; moderate amount of pus.	3
Large area hard swelling; pitting oedema (thumb impression can be made); Large area wet (>5 cm); necrotic; copious pus draining or abscess.	4

* Modified from: Marini et al, 2017

During imaging, lambs were protected from naturally lighting and kept under shade by a temporary marquee set up over the pen. The camera was hand-held, and images were taken at approximately 30-60cm distance from the lamb.

FLIR Duo® Pro R (FLIR Systems, Wilsonville, OR. USA) cameras were used for infrared thermography in this study. The HD Dual-Sensor thermal camera combines a high resolution, radiometric thermal imager and 4k colour visible RGB camera.

The thermal imager is an Uncooled V0x Microbolometer with a spectral band of 7.5-13.5µm, sensitivity < 50 mK, resolution 640 x 512, emissivity of 0.985, and is approved for an operating temperature range of -20°C to +50°C. The RGB camera has a visible sensor resolution of 4000 x 3000 and a frame rate of 30 Hz per second. Measurement accuracy of the camera is +/- 5 C or 5% of readings in the -25°C to +135°C range +/- 20 C or 20% of readings in the -40°C to +550°C range. The FLIR camera allows for synchronized RGB and thermal images to be taken together. RGB photos are saved in the 8-bit JPG format, while thermal images are saved in the 16-bit TIFF format on microSD cards.

The thermal TIFF images were processed using a custom developed algorithm in Matlab® R2018b (Mathworks Inc. Natick, MA, USA). The algorithm processed and converted the radiometric information of each image into degrees Celsius and allowed for a box selection of area. An area of best fit (ROI) was selected around the wound site of each image. After selection of the area, the minimum, average, and maximum temperature was extracted. The data was then collated into Microsoft Excel. Only the maximum temperatures are presented.

Statistical analysis

The time to mother up was right censored at 120 s and was therefore analysed by Survival analysis using the Kaplan-Meier Approach. The data were compared with a log rank test (Genstat statistical package). The data on body weight and weight gain were analysed using ANOVA, with treatments blocked on replicate groups and the inclusion of the starting weight as co-variate. Post Hoc analysis was performed using a Bonferroni test. Behaviour data was reported as median and mean and was analysed using the Kruskal-Wallis test due to the non-parametric nature of the data. Post Hoc analysis was performed using a Mann-Whitney U (Wilcoxon rank-sum) test. Wound assessment data is reported as descriptive data, while the data on IR skin temperatures were analysed using ANOVA, with treatments blocked on replicate groups.

Results:

The results of the Survival analysis indicate that the T/C lambs were the quickest to mother up (see Figure 1). Median survival (50%) of lambs in the T/C treatment had found their mother within 20 s (CI 16-28). Sheep in the MT treatment were a little slower, with 50% of lambs reunited with their mother at 28 s (CI 17-75). Both FB and MM lambs were significantly slower (P<0.001) than the T/C lambs, with 50% of the FB lambs reunited at 40 s (CI 31-82) and 50% of the MM lambs reunited at 56 s (CI 32, ..). There were no significant differences between males and females, with 21% of males (32s; (CI 23- 45)) and 27% of females (38 s; (CI 26-50) failing to mother up (P=0.407).

The results of the weights collected prior to commencement of the trial (Weight 0) and during assessments 1-4 after application of the procedures as well as the calculated changes in weight between the observation days are presented in Table 3. Weights of animals that were lame or showed signs of ill health were excluded at the time of observation and thereafter (weight of 1 lamb was removed for Weight 2, 3 lambs were removed from Weight 3 and 4 were removed from Weight 4). Start weights were not significantly different between treatments, however there was a significant difference in start weight between males (22.4 kg) and females (20.39 kg; P=0.001). There was no significant difference (P>0.05) in start weight between the 3 replicate groups.

Table 3. Weights and weight gain (kg) prior to procedures (Weight 0) and at 3-4, 10-12, 22-24 and 36-38 days post procedures during Assessments 1-4. Weight gain was calculated as gain compared to the previous recorded weight.

Treatment-Measures	Tail/Castration (Control)	Freeze Brand	Mulesing	Mulesing TriSolfen	Mean	P-value
Weight 0	22.00	20.95	20.72	21.67	21.33	0.427
Weight 1	21.28^{a}	20.54^{b}	20.27^{b}	20.11^{b}	20.55	<0.001
Weight 2	22.24^{a}	21.61^{ab}	20.90^{bc}	20.31^{c}	21.26	<0.001
Weight 3	26.08^{a}	25.04^{ab}	24.46^{b}	24.41^{b}	25.00	<0.001
Weight 4	29.52^{a}	27.99^{ab}	27.85^{b}	27.19^{b}	28.14	<0.001
Weightgain1	-0.08^{a}	-0.82^{b}	-1.09^{b}	-1.25^{b}	-0.81	<0.001
Weightgain2	0.99^{ab}	1.05^{a}	0.61^{ab}	0.15^{b}	0.70	0.020
Weightgain3	3.69	3.33	3.52	4.08	3.66	0.208
Weightgain4	3.53	2.81	3.40	2.74	3.06	0.088
Total Weight gain	8.17^{a}	6.64^{ab}	6.49^{b}	5.84^{b}	6.78	<0.001

**Values with different superscript are significantly different (P<0.05).*

All treatments resulted in weight loss between the day of application and Assessment 1 (Weight 1). FB, MM and MT lambs lost significantly more weight than T/C lambs, resulting in significant weight differences. All treatments gained weight between Assessment 1 and 2 (Weightgain 2), however MT lambs gained significantly less weight than FB lambs. Weight measured during assessments 2-4 were not significantly different between the FB treatment and T/C while both mulesing treatments weighed significantly less than T/C lambs. Weight gain and actual weight during Assessment 2 was lower in MT lambs than FB and T/C lambs.

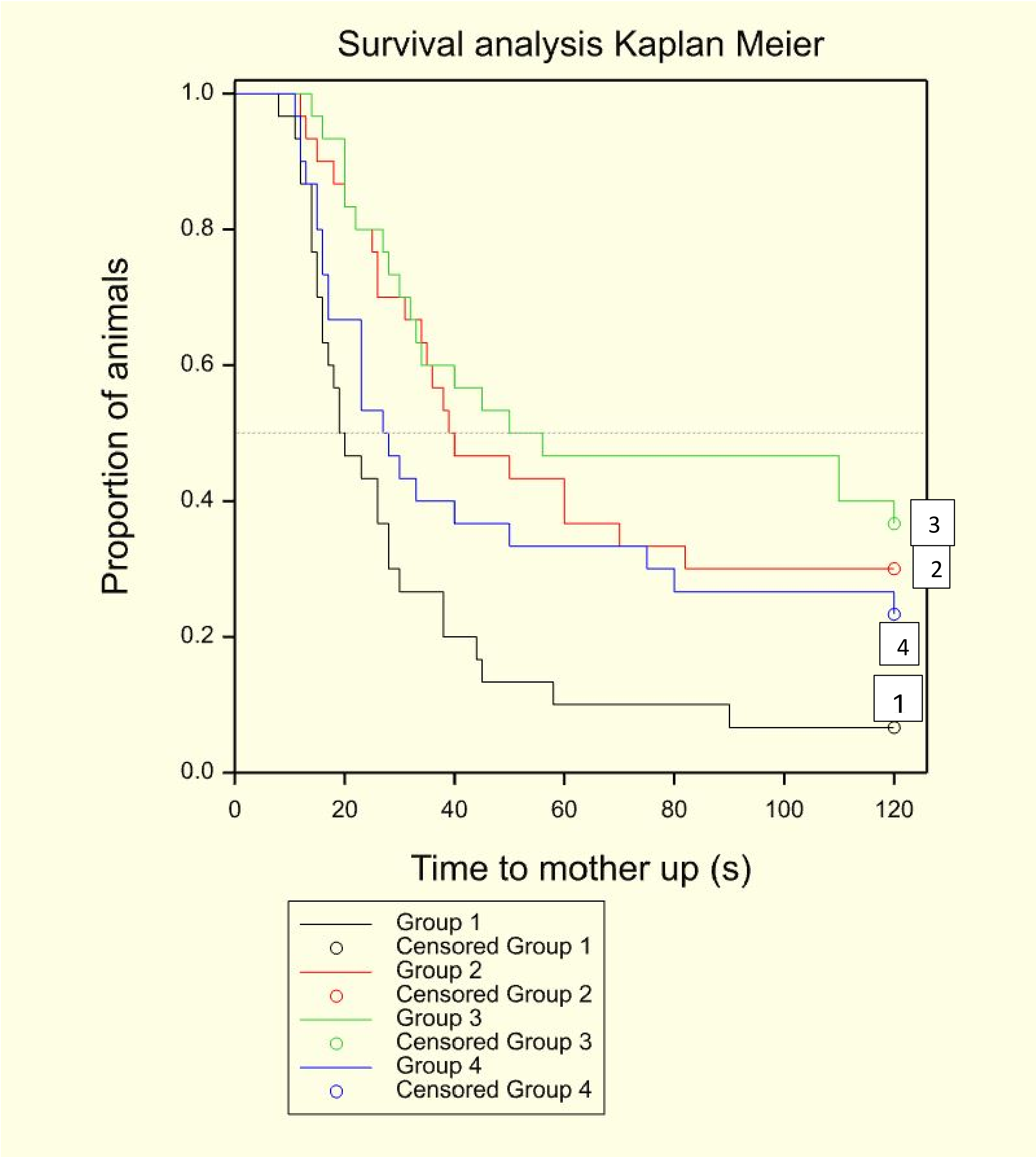

Figure 1. Survival analysis of the time to mother up for the 4 treatment groups (group 1 Control, group 2 Freeze Branding, group 3 Mulesing with Meloxicam, group 4 Mulesing with Tri-Solfen)

The behaviour results on the day of application of procedures (Day 1) are presented in Table 4. The missing data indicates lambs that could not be located within 5 minutes of their allocated observation time. Due to the large number of animals, particularly in Group 1 it was hard to locate every animal without disturbing their behaviour. The data of the individual behaviours contained many zeros and skewed counts and therefore medians and IQR (Interquartile ranges) are presented (as well as means). Individual behaviours were aggregated in categories representing normal and abnormal postures and behaviours, which may be indicative of pain (Marini et al, 2017).

Significant differences were observed in the different behaviours, although care should be taken when interpreting behaviours that were observed at low frequency. Detailed observations with a large number of different behaviours meant that observations of individual behaviours were generally low. Aggregate behaviours may provide a more reliable analysis and the following aggregates are reported: Pain Avoidance (Li, RL, EQ, LR), Abnormal Standing (AS, HSM, HSS), Total lying (NV, AV, VU, L), Abnormal Behaviour (AV, L, AS. AW, HSS, HSM), Normal Behaviour (NV, NS, NW, G, SK, R). They show significant differences of all breech modification treatments compared to T/C, although there was no significant difference in pain avoidance behaviours.

Table 4. Behaviour Day 1, mean and median (IQR, Interquartile ranges) counts of 30 animals/treatment at 15-minute intervals during 6 hrs following procedures (maximum count 24).

Treatment-Measures	Tail/Castration	Freeze Brand	Mulesing	Mulesing Tri-Solfen	P-value
Missing	5.13; 4 (3-8)	4.0; 3.5 (2-5)	3.87; 4 (2-6)	3.50; 3 (2-5)	0.300
AS	0.83; 0.5 (0-1)	1.17; 1 (0-2)	0.83; 1 (0-1)	1.87; 1 (1-3)*	**0.017**
AV	0.27; 0 (0-0)	0.17; 0 (0-0)	0.33; 0 (0-0)	0.70; 0 (0-1)	0.220
AW	0.23; 0 (0-0)	0.43; 0 (0-1)	0.53; 0 (0-1)*	0.20; 0 (0-0)	**0.043**
Grazing	4.5; 4.5 (3-5)	3.30; 3 (1-5)	4.87; 5 (3-6)	4.03; 4 (3-5)	0.057
HSM	1.30; 1 (1-2)	2.93; 2 (1-5)*	2.43; 2 (1-3)*	2.23; 2 (1-3)*	**0.016**
HSS	1.10; 1 (0-2)	2.70; 2 (1-4)*	3.57; 3 (1-5)*	2.63; 2 (1-4)*	**<0.001**
L	1.13; 1 (0-2)	0.50; 0 (0-1)	0.63; 0 (0-1)	0.90; 0 (0-2)	0.136
Li	0.13; 0 (0-0)	0.50; 0 (0-1)	0.10; 0 (0-0)	0.03; 0 (0-0)	**0.037**
NS	3.30; 3 (2-5)	3.33; 3 (2-5)	3.13; 2.5 (1-4)	3.23; 3 (2-5)	0.896
NV	2.00; 1 (1-3)	1.20; 1 (0-2)	0.77; 0 (0-1)*	1.20; 1 (0-2)*	**0.005**
NW	1.67; 2 (1-2)	1.13; 1 (0-2)	1.00; 1 (0-2)*	0.93; 0.5 (0-2)*	**0.049**
SK	0.27; 0 (0-1)	0.07; 0 (0-0)	0.07; 0 (0-0)	0.07; 0 (0-0)	**0.034**
SU	0.73; 1 (0-1)	0.93; 1 (0-1)	1.13; 1 (0-2)	1.03; 1 (0-2)	0.781
VU	0.73; 0 (0-1)	0.83; 0 (0-1)	0.33; 0 (0-1)	0.63; 0 (0-1)	0.346
Wu	0.13; 0 (0-0)	0.07; 0 (0-0)	0.13; 0 (0-0)	0.00; 0 (0-0)	0.190
EQ	0.03; 0 (0-0)	0; 0 (0-0)	0.03; 0 (0-0)	0.17; 0 (0-0)	0.257
R	0.03; 0 (0-0)	0; 0 (0-0)	0.00; 0 (0-0)	0.13; 0 (0-0)	**0.031**
RL	0.17; 0 (0-0)	0.17; 0 (0-0)	0.00; 0 (0-0)	0.10; 0 (0-0)	0.125
Aggregates:					
Pain Avoidance	0.33; 0 (0-1)	0.67; 0 (0-1)	0.13; 0 (0-0	0.30; 0 (0-0)	0.235
Abnormal Standing	3.23; 3 (2-4)	6.8; 7 (4-10)*	6.83; 6.5 (5-10)*	6.73; 6.5 (4-9)*	**<0.001**
Total Lying	4.13; 4 (3-5)	2.67; 2 (2-3)*	2.07; 2 (2-3)*	3.43; 2 (1-4)	**0.002**
Normal behaviour	11.73; 12 (10-14)	9.03; 9 (8-11)*	9.83; 9 (8-12)*	9.60; 9 (8-11)*	**0.012**
Abnormal behaviour	4.87; 5 (3-6)	7.90; 8 (5-10)*	8.33; 9 (7-19)*	8.53; 9 (6-11)*	**<0.001**

**Values are significantly different from Control (T/C) (P<0.05).*

There were few significant differences between female and male lambs in behaviour. Female lambs grazed more than male lambs (4.61 vs 3.73 obs; P=0.026) and showed more 'normal behaviours (10.72 vs 9.37 obs; P=0.025), although male lambs were observed to lie more laterally than females (0.990 vs 0.606; P=0.037).

The results of the behaviour observations on Day 2 are presented in Table 5. Incidences of abnormal behaviours were generally low, making statistical analysis less reliable for individual behaviours. The aggregate behaviours are a more reliable measure of these behaviours and show a significant difference for all breech modification treatments compared to T/C, other than for pain avoidance behaviours.

Table 5. Behaviour Day 2, average counts at 15-minute intervals for 2 hrs.

Treatment-Measures	Tail/Castration	Freeze Brand	Mulesing	Mulesing Tri-Solfen	P-value
Missing	0.63; 0 (0-1)	0.57; 0 (0-1)	0.57; 0 (0-1)	0.37; 0 (0-0)	0.393
Grazing	1.73; 2 (1-2)	1.50; 1 (0-2)	1.40; 1 (0-2)	1.50; 1 (0-2)	0.576
NS	1.47; 1 (0-2)	1.17; 1 (0-2)	1.30; 1(0-2)	1.30; 0.5 (0-2)	0.967
NV	1.27; 1 (0-2)	0.67; 0 (0-1)	0.90; 0 (0-2)	0.60; 0 (0-1)	0.225
HSS	0.10; 0 (0-0)	0.93; 0 (0-1)*	0.80; 0.5 (0-1)*	1.20; 1 (0-2)*	**< 0.001**
HSM	0.53; 0 (0-1)	0.67; 0 (0-1)	0.53; 0 (0-1)	0.80; 1 (0-1)	0.759
AS	0.07; 0 (0-0)	0.23; 0 (0-0)	0.07; 0 (0-0)	0.10; 0 (0-0)	0.282
AW	0.07; 0 (0-0)	0.23; 0 (0-0)	0.20; 0 (0-0)	0.20; 0 (0-0)	0.615
AV	0.17; 0 (0-0)	0; 0 (0-0)	0.10; 0 (0-0)	0.10; 0 (0-0)	0.247
EQ	0; 0 (0-0)	0; 0 (0-0)	0.03; 0 (0-0)	0.07; 0 (0-0)	0.292
L	0.27; 0 (0-0)	0.17; 0 (0-0)	0.17; 0 (0-0)	0.17; 0 (0-0)	0.360
LI	0; 0 (0-0)	0.13; 0 (0-0)	0.07; 0 (0-0)	0.03; 0 (0-0)	0.153
NW	0.63; 0 (0-1)	0.50; 0 (0-1)	0.67; 0 (0-1)	0.30; 0 (0-0)	0.244
R	0; 0 (0-0)	0.03; 0 (0-0)	0; 0 (0-0)	0.03; 0 (0-0)	0.569
SK	0.07; 0 (0-0)	0.07; 0 (0-0)	0.03; 0 (0-0)	0.03; 0 (0-0)	0.874
SU	0.63; 0 (0-1)	0.93; 1 (0-1)	0.83; 1 (0-2)	0.93; 1 (0-1)	0.509
VU	0.37; 0 (0-0)	0.13; 0 (0-0)	0.30; 0 (0-0)	0.20; 0 (0-0)	0.607
WU	0; 0 (0-0)	0.03; 0 (0-0)	0.03; 0 (0-0)	0.07; 0 (0-0)	0.562
Aggregates:					
Normal Behaviour	5.17; 6 (4-6)	3.93; 4 (2-5)*	4.30; 5 (3-5)	3.77; 4 (2-5)*	**0.023**
Abnormal Behaviour	1.20; 1 (1-2)	2.23; 2 (1-3)*	1.87; 1.5 (1-3)	2.27; 2.5 (1-4)*	**0.009**
Abnormal standing	0.70; 0.5 (0-1)	1.83; 2 (0-3)*	1.40; 1 (1-2)*	2.10; 2 (1-3)*	**0.002**
Pain Avoidance	0	0.13; 0 (0-0)	0.10; 0 (0-0)	0.10; 0 (0-0)	0.273
Total Lying	2.07; 2 (1-3)	0.97; 1 (0-1)*	1.47; 1 (0-2)	1.07; 0 (0-2)*	**0.016**

**Values with are significantly different from Control (T/C) (P<0.05).*

The results of the behaviour observations on Day 3 are presented in Table 6. Significant differences between treatments are no longer observed at this time (the difference in “Standing other” can be considered a statistical anomality).

Table 6. Behaviour Day 3, average counts at 15-minute intervals for 2 hrs.

Treatment-Measures	Tail/Castration	Freeze Brand	Mulesing	Mulesing TriSolfen	P-value
Missing	0.93; 0 (0-1)	1.43; 1 (0-3)	1.40; 1 (0-2)	1.37; 1 (0-2)	0.306
Grazing	3.14; 3 (2.8-4)	2.50; 3 (1-4)	2.70; 3 (2-3)	2.60; 2 (1-4)	0.293
NS	1.00; 1 (0-2)	0.80; 0 (0-2)	0.90; 1 (0-1)	0.73; 0 (0-1)	0.710
NV	0.28; 0 (0-0)	0.40; 0 (0-1)	0.23; 0 (0-0)	0.23; 0 (0-0)	0.916
AV	0.07; 0 (0-0)	0.03; 0 (0-0)	0.03; 0 (0-0)	0.07; 0 (0-0)	0.902
AS	0.07; 0 (0-0)	0.07; 0 (0-0)	0.17; 0 (0-0)	0.20; 0 (0-0)	0.254
HSM	0.41; 0 (0-1)	0.23; 0 (0-0)	0.27; 0 (0-0)	0.33; 0 (0-1)	0.407
HSS	0.14; 0 (0-0)	0.17; 0 (0-0)	0.10; 0 (0-0)	0.13; 0 (0-0)	0.963
AW	0.35; 0 (0-1)	0.40; 0 (0-1)	0.23; 0 (0-0)	0.23; 0 (0-0)	0.272
L	0.14; 0 (0-0)	0.13; 0 (0-0)	0.07; 0 (0-0)	0.13; 0 (0-0)	1.000
Li	0.00; 0 (0-0)	0.07; 0 (0-0)	0.03; 0 (0-0)	0.00; 0 (0-0)	0.299
NW	0.90; 0 (0-1)	0.67; 1 (0-1)	0.93; 1 (0-1)	1.03; 1 (0-2)	0.641
R	0.00; 0 (0-0)	0.03; 0 (0-0)	0.07; 0 (0-0)	0.10; 0 (0-0)	0.334
SK	0.07; 0 (0-0)	0.13; 0 (0-0)	0.03; 0 (0-0)	0.00; 0 (0-0)	0.155
SU	0.24; 0 (0-0)	0.87; 1 (0-1)	0.53; 0 (0-1)	0.57; 0 (0-1)	**0.026**
VU	0.28; 0 (0-0.25)	0.07; 0 (0-0)	0.30; 0 (0-0)	0.23; 0 (0-0)	0.219
Aggregates:					
Normal Behaviour	5.38; 5 (4-7)	4.53; 4.5 (3-6)	4.87; 5 (4-6)	4.70; 5 (3-7)	0.320
Abnormal Behaviour	1.17; 1 (0-2)	1.03; 1 (0-2)	0.87; 1 (0-2)	1.10; 1 (0-2)	0.773
Abnormal standing	0.62; 0 (0-1)	0.47; 0 (0-1)	0.53; 0 (0-1)	0.67; 0 (0-1)	0.645
Pain Avoidance	0	0.07; 0 (0-0)	0.03; 0 (0-0)	0.03; 0 (0-0)	0.572
Total Lying	0.76; 0 (0-1)	0.63; 0 (0-1)	0.63; 0 (0-1)	0.67; 0 (0-1)	0.851

Wound assessment revealed that at 3-4 days after the procedure (Assessment 1) there were no visible wounds (other than those relating to tail docking) in T/C and FB lambs. All lambs in the two mulesing treatments had open wounds with the formation of a closed scab (see Image 1). None of the wounds had exudates and no flystrike was observed. The wound-score for all animals was 0, as the scab formed a closed layer over the mulesing wounds, without visible swelling. The median wound length was 7 cm for both mulesing treatments (IQR 5-9 and 6-9). While FB lambs did not display an open wound, a ridge where the skin was clamped together could be felt, although it was sometimes hard to identify. The median length of this ridge was similar to the wound length (median 7 cm IQR 4-9) of mulesed lambs. The median wound width for mulesed lambs was 9 cm (4-11) for MM and 8 cm (7-10) for MT, while the space between the two ridges (this was measured, rather than the width of the wound) were similarly spaced for FB lambs at 9cm (IQR 4-10).

Image 1. *A typical mulesing wound at 3-4 days after the procedure on the left, compared with a typical breech 3-4 days after Freeze Branding on the right.*

At the second wound assessment all wounds in the FB lambs were assessed as closed, while those from the two mulesing treatments were all open (the scabs had all cracked particularly around the tail, perhaps extenuated by the handling associate with the assessment). The wound-score for FB lambs was an average of 0.3 (30% received a score of 1) while the median was 0 (0-1). The mean score for MM lambs was 1.4 (median 1 (1-2) and for MS lambs 1.2 (median 1 (1-1). Median wound length for FB was 8.7 cm (9 (8-9), for MM lambs 10.2 cm (10 (8-12), and for MT lambs 9.9 cm (10 (9-11). Wound width or area between the two wound edges for FB lambs was 8.5 cm (9 (8-10), for MM lambs 9.6 cm (9.25 (9-11), and for MT lambs 9.8 cm (10 (9-11).

Image 2. *A typical mulesing wound at 11 days after the procedure on the left, compared with a typical breech 11 days after Freeze Branding on the right.*

At the third wound assessment 5 out of 30 FB lambs had open wounds, with one of those lambs receiving a wound score of 2 and the other 4 a wound score of 1. Only 1 lamb in each mulesing treatment had wounds that were fully closed. Mean and median wound scores were: FB 0.23, 0 (0-0); MM 1.1, 1 (1-1); MT 1.1, 1 (1-1). Wound length for FB lambs was 6.6 cm, for MM lambs 7.3 cm, and for MT 7.6 cm. Wound width for FB lambs was 8.4 cm, for MM lambs 9.7 cm, and for MT lambs 9.6 cm. Fly strike was detected in one MT lamb, which was treated immediately. All open wounds had some exudates.

Image 3. *A typical mulesing wound at 23 days after the procedure on the left, compared with a typical breech 23 days after Freeze Branding on the right.*

At the last wound assessment, nearly all wounds were closed and fully healed, with only a few lambs still having a small scab on the tail. The average scar length for FB lambs was 6.5cm, for MM lambs 6.3 cm, and for MT lambs 6.1cm. The average area between the outer scars lines was 9.1cm for FB lambs, 9.0cm for MM lambs and 8.4 cm for MT lambs.

Results of the analysis of the IR images is presented in Table 7. At the 1st assessment after the procedure the temperature of the wounds after both mulesing treatments are significantly higher compared to the TC and FB treatment. At the 2nd assessment only the MT lambs show a significant increase in temperature compared to the TC lambs, with the FB and MM treatment intermediate between those treatments. Significant differences are no longer seen between the treatments in the later assessments, although the high ambient temperature during the 3rd assessment resulted in high skin temperatures, which may have masked any differences between treatments.

Table 7. Maximum skin temperatures (C^o) as measured using IR imaging of skin temperatures during Assessment 1-4.

Time point	Tail/Castration	Freeze Brand	Mulesing	Mulesing TriSolfen	P-value
Assessment 1	33.9^a	34.2^a	36.2^b	36.2^b	**<0.001**
Assessment 2	33.5^a	34.6^{ab}	35.0^{ab}	35.2^b	**0.018**
Assessment 3	37.8	38.1	37.6	38.0	0.565
Assessment 4	30.6	31.2	30.1	30.3	0.370

^{ab}Values with different superscript are significantly different (P<0.05).

Discussion

It is well accepted that lamb marking (tail docking and castration) causes a degree of pain or discomfort, and results in behaviour such as active pain avoidance and increased time spent in abnormal postures as well as reduced total lying time (see Small et al, 2020). In addition, mulesing is considered more painful than castration and tail docking (Jongman et al, 2000; Fisher, 2011).

In this study, all breech modification procedures (freeze branding and both mulesing treatments) resulted in significant differences in behaviour in the first 2 days after the procedures and weight gain until weaning, compared to the T/C treatment. In addition, T/C lambs were fastest in mothering up and MT lambs were intermediate to MM and FB lambs. This faster time of MT lambs compared to MM lambs is likely because of the fast onset of pain relief of Tri-Solfen compared to the slower acting Meloxicam (Small et al, 2018). This agrees with Small et al (2020), who reported an increased ability to mother up in lambs that were ring-marked with the addition of a fast-acting local anaesthetic (using a Numnuts tool). On the 3rd day after the application of the procedures, no differences in behaviour could be detected.

Both the behaviour data and the weight gain measured during the first assessment indicate that all 3 breech modification procedures were more painful than tail docking and castration with Meloxicam, although total weight gain and final weight of FB lambs was intermediate between the two mulesing treatments and T/C lambs. The study was conducted in Spring, when there was an abundance of fresh grass, so nutrition was not limited, and compensatory growth was possible. When nutrition is limited, the impact of husbandry procedures on growth may be greater than was found in the present study (Ryan, 1990). Weight gain in lamb marked (T/C) lambs at 37 days post procedure was more than 8.5 kg, whereas in a previous field trial reported by AgVet Innovations weight gain at 35 days was only 2.5 kg, which may be partly explained by the available nutrition.

There were a number of lame animals (both ewes and lambs) in the flocks and while lame lambs were excluded from the study at the commencement of the study, an additional 2 lambs were removed mid-way through the study due to lameness. Lameness was more prevalent in the adult ewes, which probably resulted in more lying behaviour, which may in turn have affected the behaviour of the lambs.

On commercial farms pain relief (and in particular Meloxicam) is rarely used, so it is not clear how the behaviour response to breech modification treatments compare to castration and/or tail docking if no pain relief is used for the latter. Pain relief is now compulsory in Victoria when sheep are mules and most commercial farms apply Tri-Solfen. However, while Tri-Solfen provides pain relief for surgical castration and tail-docking (Lomax et al, 2010), many farms use ring marking without pain relief, which is usually performed at the same time.

Nonetheless, the use of pain relief in combination with husbandry procedures is increasing. While Tri-Solfen is routinely used with mulesing, Meloxicam is routinely used with the Freeze Branding procedure on commercial farms. It has been shown that Meloxicam or Tri-Solfen alone provide some pain relief for mulesing, however multimodal analgesia (using both) has been shown to be much more effective in reducing pain related behaviour (Inglis et al, 2019).

In the present study we were unable to detect major behavioural differences between Freeze Branding and the Mulesing treatments when both were accompanied by pain relief. However, the slightly better weight gain, and the described differences in wound healing, suggest that the Freeze Branding treatment may provide these animal welfare benefits over mulesing. While there were some lambs observed with skin sloughing after Freeze Branding, this was probably more related to an error in the application, rather than the technique itself. This was seen in some lambs that were treated in the first group, when an older prototype of the handset was used. A newer handset on subsequent days did not result in this effect. On the other hand, all mulesed lambs presented with open wounds that took some time to heal. Particularly the wound on the tail took longest to heal, and interestingly this was longer for the mulesing treatments compared to Freeze Branding. Open mulesing wounds or an attractant to flies, which may result in oviposition and possibly flystrike (Cook and Steiner, 1990) and may cause irritation of the wound (Dr J. Webb-Ware, pers. com).

The size of the wound and the scar lines indicate that FB lambs had a similar impacted area than both mulesing treatments (in terms of length of scar and width between the two scar lines). This may result in a similar bare area in the adult ewe, however assessment of this area in lambs in the current study would provide additional information.

Infrared thermography has been used to assess infection, inflammation, and normal healing wounds, using relative temperature maximums of the wound and skin in humans (Chanmugam et al, 2017). However, wool covers much of the region of interest (ROI) in this study, making a comparison between all the treatments difficult. By comparing maximum temperatures in the ROI, the assumption is made that skin has a higher temperature than wool. Increased blood flow to the area due to inflammation and healing is expected to have an effect on maximum skin temperatures. Maximum skin temperatures were significantly higher for the mulesing treatments compared to both T/C and FB during the first assessment. At the second assessment FB and MM were intermediate to MT and as healing progressed this difference slowly disappeared. Unfortunately, ambient temperatures during the 3rd assessment were high, resulting in much higher skin temperatures, which may have masked any treatment differences.

Conclusions

In the present study we were unable to detect major behavioural differences between Freeze Branding and the Mulesing treatments when both were accompanied by pain relief. However, some differences were observed between all breech modification treatments (FB, MM and MS) and lamb marking (T/C) only, with pain relief provided for all treatments, in the first 2 days post procedures. Over the longer term, the slightly better weight gain and the described differences in wound healing, suggest that the Freeze Branding application may provide these animal welfare benefits over mulesing.

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