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Production Animals

Evaluating the efficacy of a reduced mulesing wound size on breech strike risk factors and breech parameters.

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Abstract

Objective: The aim of the experiment is to determine the effect of mulesing technique on wound size, wound healing, and breech strike risk parameters in Merino weaner sheep. To provide a scientific basis to inform standards around the mulesing procedure and provide evidence that demonstrates selective mulesing can improve the welfare for Merino sheep.

Design Randomised controlled field trial in a single location.

Animals 307 x 8-month-old Merino ewe weaners, mean weight 30.4 kg

Methods Weaner ewes were randomly assigned to two treatment groups to ensure an even representation of breech wrinkle score across the treatments: 1) the 'modified mules' procedure (n=151), which involved an average of 6 cuts; and 2) the 'conservative mules' procedure (n=156), which involved an average of 4 cuts. Sheep were weighed and scored for breech wrinkle and breech cover. Mulesing was performed and excised tissue was weighed. Photographs of the wounds were taken immediately after mulesing (day 0) and day 32 post-mulesing for assessment of wound surface area and wound healing. Breech scoring was conducted on day 0 and day 98 to assess the effect of the different treatment groups on breech wrinkle and breech cover scores.

Results The conservative mules treatment resulted in a smaller wound surface area (86.4 cm^2 ± 1.3 cm^2) compared to the modified mules (120.8 cm^2 ± 1.6 cm^2). There was also a smaller number of cuts and reduced tissue weight removed from sheep in the conservative treatment compared to the modified mules treatment group. The wound to body surface area percentage reduced more in the modified mules treatment over the 32 day period. Regardless of treatment there was a reduction in breach wrinkle score over time. There was a significant treatment and time interaction for breech cover scores.

Conclusions A conservative mules improves sheep welfare as there is a faster healing period and less chance of prolonged pain. The conservative mules resulted in smaller open wound

period reducing the risk of infection. The number of cuts used in the conservative mules were less than the modified mules, which has the potential to reduce labour time and pain experienced by the animal. Animals that experience less pain, heal faster and are less likely to get infection are likely to also be more productive.

Keywords breech strike; mulesing; weaners; wound surface area; welfare; Australian merino ewes

Abbreviations: BRWR, Breech wrinkle, BCOV, Breech cover, CON, Conservative mules, MOD, Modified mules, MLA, Meat and Livestock Australia, AWI, Australian Wool Innovation

Introduction

Flystrike has a significant impact on the Merino sheep industry of Australia, with estimated losses of $280 million per year in production [1]. The sheep blow fly, *Lucilia cuprina*, causes flystrike when it deposits eggs in damp fleeces. The larvae that hatch then feed on skin dermis causing sheep to suffer, and die if not treated. Sheep health, as well as wool and meat productivity, are all reduced due to the stress associated with flystrike[2]. Meat and Livestock Australia (MLA) and the Australian Wool Innovation (AWI) have identified the main indicator traits for the prevalence of flystrike through a Visual Scoring guide[1,3,4]. These traits are breech wrinkle score (BRWR), breech cover score (BCOV) and Breech dag score (DAG). Each trait is given a score out of five with higher scores indicating a greater susceptibility to breech strike[1,3,4]. Wrinkle of the skin, particularly around the breech area, traps moisture from weather and/or faecal contamination of the wool. This moisture retention leads to an increase in bacteria and inflammation of the skin, which is thought to attract flies to the area[2].

Mulesing is the surgical removal of wool-bearing skin in the breech region to increase perineal bare area and reduce breech wrinkle[1]. It has been found that surgical mulesing is the most effective method of reducing flystrike risk in Merinos[1-18] but it is also well documented

to cause pain and distress[1,19-21]. For this reason, mulesing has a negative influence on the welfare of animals and creates conflict within the general public[11,20,21]. The protests of animal activist groups like PETA and their demand for the cessation of mulesing has prompted the government to commit funding to identify alternative solutions that are as affective at preventing flystrike as mulesing[3,4,6,22-26]. Some of the alternative solutions that have been considered include clips, intradermal injections and chemical control, however all of them appear to be less effective than mulesing[10,14,16,17,22-26]. The long-term solution for flystrike prevention is breeding a plainer breech animal through genetic manipulation and selection[23,24,27,28]. This solution is painless, cumulative and permanent, eliminating the need for the mulesing procedure. However the breeding approach is a long term solution and it has been estimated that it would take 10-15 years before genetic gain is effective across the Australian national flock [29].

Regardless of the shift toward a plainer breech Merino, mulesing is still a widespread practice in the industry yet standards around the procedure remain unchanged. There is no scientific basis to validate whether the current standard procedure for mulesing or whether other less intrusive surgical procedures might be just as effective [30]. Evaluating the current modified 'V' mules against a smaller conservative mules could demonstrate reduced healing time and pain associated with prolonged healing from a larger wound. If the size of the wound is decreased, healing time is shorter which reduces the time period in which the wound is open and at risk of infection. It would also reduce the time period in which pain is felt from an open wound[31-33]. However, for a more conservative mules to be viable it needs to be tested to demonstrate that it decreases breech scores and wound size, whilst still providing the level of protection against flystrike using the current standard 'modified' mules. The scope of this project is to compare the effect of the two different procedures on the key parameters affecting flystrike (BRWR and BCOV), but does not extend to the measurement of the incidence of flystrike in the treated animals.

The overall objective of this study was to determine whether the current mulesing procedure could be modified to be more conservative but equally effective, and to provide a scientific basis for validating mulesing procedures. The hypothesis we tested was that a smaller wound would heal before a larger wound and still reduce indicator traits BRWR and BCOV to a sufficient score.

Materials and methods

Experimental design

Merino ewe wethers (8 months old) were used in this experiment. The animals were weighed, breech scored, and randomly allocated into one of the two treatment groups: 1) conservative mules (CON n=156) and 2) Modified 'V' mules (MOD n=151) based on their wrinkle score, , to ensure an even distribution of breech wrinkle across both treatment groups. A photo was taken of the breech after the mulesing procedure. The number of cuts performed for each treatment was recorded and the excised tissue was weighed (g). Measurements of wound surface area (WSA), body surface area (BSA) and wound healing were taken as well as breech strike risk factors, including breech wrinkle score (BRWR) and breech cover (BCOV) as indicators of the effectiveness of the treatments.

Animal management

The Animal Ethics Committee of the University of Sydney granted approval for the experimental protocol (Protocol number :1126 / Project title "Evaluating the efficacy of a reduced mulesing wound size on breech strike risk factors and breech parameters"). The experiment took place on the University of Sydney farm "Arthursleigh" located near Marulan NSW. A flock of 310 merino ewe weaners approximately 8 months of age born in October of 2016 were used for the purpose of the trial. To reduce the risk of flystrike to the mulesing wound the experiment took place during the winter month of July 2017 when fly activity is minimal. All animals had already undergone the marking procedure (ear-tagging, ear notching, tail-docking and vaccination) at 4-6 weeks of age, and all wounds had fully healed.

One month prior to the experiment all animals were crutched to allow for breech scoring and mulesing.

Assessment of breech strike risk factors/ scores

Breech wrinkle (BRWR) and breech cover (BCOV) were scored on on day 1 and day 98 of the trial using the Visual Sheep Score (VSS) guide developed by Meat and Livestock Australia (MLA) and Australian Wool Innovation (AWI) (Figure 1,2). Scoring using the VSS guide works using a scale of 1 -5 where an animal with a score of 1 has the most extensive expression of that particular trait. Breech wrinkle is the degree of skin folding in the perineal area and breech cover refers to the amount of natural bare skin around the breech area. Scoring took place before mulesing on day 0 when the sheep were in the veterinary examination (VE) machine in lateral recumbency (Figure 3).

Mulesing Procedure

Animals were mustered and penned in the shearing shed with access to Lucerne hay and water the day before the experiment. Sheep were weighed using sheep weigh scales (Ruddweigh weighing system distributed by Gallagher Victoria, Australia). Weights were recorded to the nearest 0.1 of a kg. Sheep were then moved through a race and onto a VE conveyor machine (Robertson sheep handler). On the VE machine sheep were flipped into dorsal recumbency and restrained using standard leg hooks for mulesing. An experienced technician conducted the mulesing using sterilised, sharpened, standard mulesing shears. Shears were disinfected prior to mulesing and in between mulesing procedure using chlorhexidine disinfectant. A photo was taken immediately after mulesing before Tri-solfen® topical anaesthetic was applied to the wound surface as per product instructions as per best practise. The number of cuts performed was recorded and the tissue removed during mulesing was weighed using kitchen scales (SALTER electronic scales, Sydney, Australia) before being disposed of. The sheep were then released into a paddock where they remained until the follow up at 32 days.

146 ***Treatments – Modified 'V' Mules and Conservative mules***

147 Treatment one was the modified 'V' mules (MOD) which was conducted as per the national
148 mulesing accreditation program (NMAP), the industry standard mules, involving 6-8 cuts.
149 This consisted of 4 cuts to the breech region with 2 to 3 cuts on the left and right side of the
150 breech (Figure 4,5). The breech includes four crescent shaped flaps of skin exercised along
151 either side of the perineal bare area beginning next to the bare skin of the vulva. The cuts
152 form a V shape with the widest end starting roughly 2 cm above the tail and ending in a point
153 at the top of the hamstring. Two strips of wool-baring skin are excised along the base and
154 sides of the tail joining up with the breech cut. A 'V' shaped area of wool-bearing skin is left
155 on the ventral side of the tail to protect it from sun damage.

156 Treatment two was the conservative mules (CON), which is a more selective approach that
157 involves a smaller excision (Figure 6, 7). This mules consists of 4 cuts, a single crescent
158 shape from the breech either side of the tail and two tail strips. The tail is mulesed in the same
159 way as the MOD mules, but the breech is mulesed by using only two cuts. The breech and tail
160 cuts join and the same V wool covered area as in the modified mules is left at the base of the
161 tail for sun protection.

162 ***Assessment of wound area and healing***

163 A spread sheet with the individual animal ID was attached to a clipboard with a 30 cm ruler.
164 The Clipboard was placed horizontally below the wound so that the animal ID and the ruler
165 were visible. Photographs were then taken of the breech area after mulesing. This was done
166 on day 0 and then repeated on day 32 and day 93. Photos were taken using a DSLR canon
167 500D camera with flash (Figure 3).

168 Wound surface area (WSA) was determined using planimetric analysis of the wound
169 photographs in the program Pictzar CDM. The Animal ID was used to enter the animal as a
170 patient in the program to track wound healing. The 30 cm ruler was used to scale the pictures

and work out accurate wound measurements. The body surface area (BSA) was calculated from individual animal weights using the following formula.

$$\text{total body surface area (m2)} = 0.094B0.67$$

where B is sheep weight in kg

Statistical analysis

Weight of tissue removed (g) was analysed using restriction maximum likelihood regression (REML) in Genstat 16th edition (VSN International, UK). The number of cuts used was coded to <4, 5, 6, and ≥7 to avoid bias at the extremes of the scale. All data followed a normal distribution so no transformation was needed. Weight was included as a random effect. Least significant differences (LSD's) were used to compare between wrinkle score and the interaction of the number of cuts and treatment.

Wound Surface Area (WSA), wound surface area to body surface area ratio (WSA:BSA%), and BRWR and BCOV scores were analysed using REML variance component analysis in Genstat 16th edition (VSN International 228 Ltd, Hemel Hempstead, UK).

Treatment, time and their interaction were evaluated in the fixed model. Animal ID was listed as a random effect.

Results

Tissue removed

There was a significant effect of BRWR on the amount of tissue removed (g) ($p < 0.001$). As wrinkle score increased the amount of tissue removed increased (Figure 8).

Number of cuts

There was a significant interaction ($p < 0.001$) between the number of cuts and mulesing treatment. The COD mules used less cuts and removed less tissue than the MOD mules (Figure 9).

Wound surface area

There was a significant treatment x time interaction for wound surface area ($p<0.001$). The mean initial wound surface area was significantly smaller for the CON mules 86.4 cm^2 ± 1.3 cm^2 than the MOD mules treatment 120.8 cm^2 ± 1.6 cm^2 on day 0. There was a significant reduction in WSA for both treatments between days 0 and 98 (Figure 10). The CON mules treatment resulted in a smaller WSA at both day 0 and Day 98 (figure 10).

Wound surface area to body surface area ratio

There was a significant time x treatment interaction for WSA:BSA % ($p<0.001$). Both treatments reduced in WSA:BSA between day 0 and day 32. The MOD mules had a greater reduction in WSA:BSA over the 4 week period.. The CON mules began and ended with an overall smaller WSA:BSA % (Figure 11).

Breech strike risk factors

Mean BRWR score for all sheep was 2.1 on the day of mulesing. There was no significant interaction between treatment x time, and no effect of treatment on BRWR score. There was a significant difference ($p<0.001$) between BRWR scores on day 0 (2.1 ± 0.04) and day 98 (1.6 ± 0.04). Both treatments resulted in a reduction of BRWR over time.

There was a significant treatment x time interaction for BCOV scores. There was no significant difference in BCOV scores between treatment groups on Day 0 pre-mulesing (3.6) (Figure 12). On day 98 there was a significant difference between the BCOV scores for the CON mules (3.46 ± 0.06) and the MOD mules (3.18 ± 0.06) ($p < 0.001$). The CON mules treatment group have a significantly greater BCOV score at day 98 (Figure 12).

Discussion

The results from this study supported the hypothesis that the conservative mules procedure would result in a smaller wound that still reduce BRWR and BCOV. The results from this experiment demonstrate that a more conservative mules (CON) reduced the amount of tissue excised, based on the weight of the tissue, and reduced the number of cuts required. These two factors resulted in a smaller wound area initially and at 32 days post mulesing. This information forms a basis for updating the current regulations and standards around the mulesing procedure, improving the welfare of Merino sheep that are mulesed Australia-wide.

Mulesing remains a widely used practise in Australia despite the pressure from various groups and the community to ban it. Mulesing is done to reduce the risk of flystrike to Merino sheep and involves the removal of skin from around the breech area of the animal by making a number of cuts. The risk of flystrike is influenced by the wrinkles of the skin and amount of wool cover in the breech region, because these factors tend to hold moisture and faecal matter, making it attractive to blowflies to lay their eggs. The two traits that are correlated to the risk of flystrike are breech wrinkle score (BRWR) and breech cover (BCOV) and mulesing is a surgical procedure that can reduce both of these traits. The basis of the mulesing procedure protecting against flystrike is the idea that with wound contraction, BRWR is reduced with the adherence of the wound and the natural perineal bare area is enlarged[34]. However, there are concerns about the welfare of the animals that are mulesed because of the pain they experience during the procedure and the length of time it takes to heal, which is likely to influence how long animals experience pain and the increased risk of infection the longer the healing process takes. Despite this there has been little done to determine whether the current modified 'V' procedure of mulesing, which is the industry standard, could be improved through a more conservative approach without losing effectiveness. There is limited documentation on wound size and wound healing post mules[17,20,21,31,33,35-37], but there is evidence that a slower rate of healing could lead to further complications such as infection as well as prolonged chronic pain associated with the

244 procedure[32-34,38]. The results from this experiment demonstrate that it is possible to take a
245 more conservative approach to mulesing than the current industry standard without losing the
246 effectiveness of reducing BRWR and BCOV.

247 Currently the best, long-term, practice for controlling flystrike is through selective breeding
248 of a plainer bodied sheep. The objective of selective breeding is heavily focused on lowering
249 BRWR[39-42]. The selective breeding approach can take some years to achieve flystrike
250 resistance, which means mulesing will remain an option for producers at least in the near
251 future. Our results indicate that BRWR scores could be used as a means for decisions around
252 more selective mulesing and improved animal welfare outcomes. The individual wrinkle
253 score of the animal affected the amount of tissue that was removed. Animals with a BRWR
254 score of 1 had significantly less tissue removed (43.2 g) compared to animals with a BRWR
255 of 4 (65.5 g). The amount of tissue removed in grams increased as the BRWR of the
256 individual animal increases. This provides evidence for using BRWR scores for selective
257 mulesing, removing less tissue as the wrinkle score decreases. There are reports that BRWR
258 decreases within the first year of age. An animal has a smaller wrinkle score as a weaner than
259 it does as a lamb [27]. This provides some evidence for mulesing older animals, as less tissue
260 would need to be removed as BRWR score reduces with age[24,27,29]. Being able to be more
261 selective with mulesing means the amount of tissue that is removed can be minimised and the
262 wound area should be decreased.

263 The time taken for a wound to undergo re-epithelialisation and wound contraction is affected
264 by the size and degree of tissue removed[33,34]. By removing less tissue there is less damage
265 being done to the animal, and the wound is likely to recover faster [32-34]. The conservative
266 mules is a smaller less drastic mulesing method and on average takes less grams of tissue and
267 requires a smaller number of cuts. The standard number of cuts for the CON mules is less
268 than for the MOD mules however often the number of cuts vary as the mulesing is done. The
269 standard guidelines require the minimum amount of cuts necessary to be performed. In this
270 study the number of cuts for each mules was recorded and compared against treatment groups

271 and correlated with the grams of tissue removed. It was found that the CON mules was
272 associated with a smaller number of cuts than the MOD mules with a maximum of 6 cuts
273 whereas the modified mules required more cuts with a maximum of 7 cuts. The smaller
274 number of cuts was correlated with less grams of tissue removed. Overall the CON mules
275 required less cuts and removed a smaller amount of tissue.

276 Reducing the number of cuts taken to mules an individual can improve animal welfare
277 because it is likely to reduce pain and the chance of infection[31-34,43]. A-delta nerve fibres under
278 the skin transmit the initial pain response to the brain. These fibres are activated when they
279 are cut in the mulesing process; this initiates 'fast pain'[36,44]. Each time these fibres are
280 activated by a cut the fast pain is felt[36,45]. The more cuts that it takes to mules the animal the
281 greater the number of fibres are being triggered[36]. The CON mulesing procedure involved an
282 average of 4-6 cuts where as the MOD mules involved an average of 6-8 cuts. Since the CON
283 mules involved less cuts than the MOD mules it is likely that less A-delta fibres were
284 triggered and the amount of 'fast' initial pain felt by the animal would have been reduced [36,44].
285 Each time a foreign object enters the body the risk of infection and inflammation
286 increases[34,36,38,45]. An increase in the number of times the mulesing shears are entering the
287 tissues increases the chance of infection from foreign bodies[34,36,38,45]. A CON mules reduced
288 the average number of cuts necessary, which is associated with a reduced risk of infection.

289 The healing time of the wound after mulesing is important because prolonged wound
290 exposure can also increase risks of infection, re-injury and can cause enduring chronic
291 pain[31,33,36] It is important to minimise the time it takes for a wound to heal to reduce
292 complications and encourage effective healing[1,30,34,35,38]. In this study the CON mules was
293 associated with a smaller WSA at day 0 and day 32 whereas the MOD mules had a larger
294 WSA at day 0 and day 35. The conservative mules is associated with a smaller wound size
295 after a 5 week period than the modified 'V' mules. This provides evidence that the CON
296 mules can reduce the time period that there is an open wound. This reduces the risks to the
297 individual against possible complications of a prolonged open wound. If the time period in

298 which a wound is healing is extended due to a larger surface area there is more of a chance
299 that the animal could reinjure the wound by knocking the scab off. When a scab is knocked
300 off this restarts the granulation process and healing is further extended and could become
301 infected [33].

302 The idea of a smaller WSA:BSA is linked directly to quicker wound healing. The surface are
303 of the wound is directly associated to the time it takes for a wound to heal. The size of the
304 wound determines the amount of granulation tissue required for the wound to close and heal.
305 Our results show that the CON mules starts and ends with a smaller WSA:BSA, which is
306 linked directly to a quicker overall healing time.

307 A protective physiological response is triggered when injury to the skin and tissue occurs
308 which is aimed at reducing the risk of exacerbating the injury[45]. The area immediately
309 surrounding the injury zone develops reddening caused by vasodilation[19,36,45]. Hyperglasia is
310 then initiated as a larger secondary zone that produces an increased sensitisation to pain[36,45]. A
311 response to normally innocuous stimulus such as light touch initiates a pain response this is
312 known as allodynia[36,44,45]. As the size of the injured area increases the area of hyperglasia and
313 allodynia increases. When an animal is treated with the CON mules the area of hyperglasia
314 and allodynia is reduced compared to the modified mules. The CON mules therefore could be
315 considered as having improved welfare outcomes due to the reduced pain sensitization of the
316 area surrounding the mules. After the surgical procedure behaviour such as lying down is
317 often observed, which would cause pain resting on tissue surrounding the wound. If the area
318 of hyperglasia can be reduced the mulesed sheep may experience a greater level of comfort
319 when preforming this behavioural pain responses.

320 Breech wrinkle and breech cover scores are considered important risk factors in identifying
321 animals that are prone to flystrike in Australian merino sheep. Selective breeding programs
322 for flystrike resistance are developed by identifying animals with low BCOV and BRWR to
323 include in the breeding flock. Breech wrinkle and cover scores less than or equal to 2 and 3

are considered candidates for a breeding program. The BRWR and BCOV were measured pre-mules and 98 days post-mules to determine the effect of the two treatment groups on overall scores. Scoring was based on the Visual Scores Guide (VSG) developed by Meat and livestock Australia and the Australian Wool Innovation (Figure 1,2). These scores are subjective, but are used industry wide. BRWR scores decreased from day 0 to day 98 however treatment did not have a significant effect. Both the CON and the MOD mules resulted in a decrease breech wrinkle scores and there was a significant interaction between time and treatment for BCOV scores. Both treatments reduced the BCOV to an average score of 3. Importantly, the CON and the MOD were both sufficient in decreasing the BRWR and BCOV scores, which suggests it should be possible to be more selective in the mulesing procedure to capture the benefits of reducing the number of cuts, the size of the wound, pain and improving welfare without losing the effectiveness of the procedure. It was outside the scope of this experiment to follow these animals through to see if there were differences in the incidence of flystrike, but this would be a valuable follow-up study that should be done.

A smaller number of cuts, although small, affects the labour required to mules the sheep and therefore can benefit the mulesing contractor[46]. When mulesing large flocks the physical demand on the hand and wrist increases as a greater number cuts need to be made[46,47]. The mulesing shears also require sharpening after a finite number of incisions have been performed[46,47]. Reducing the number of cuts required by doing the conservative mules can decrease labour stress as well as decrease the time needed to sharpen the mulesing shears.

This study was performed in an area subject to a particular environment on a flock with particular genetics. Flystrike, is known to be affected by environment and genetics. It will be important validate the results we have obtained from the CON mules in this experiment further by doing experiments in different environmental zones with a variety of different flock genetics across Australia. The flock that the experiment was conducted on had particularly low initial BRWR and BCOV scores. The experiment should be repeated on a flock with high average BRWR and BCOV (score 4 or above). In these future experiments

the BCOV area pre- and post-mules should be closely analysed. The PictZar program used to measure wound surface area could also be used to measure the bare perineal area before and after the wound has healed. This would be a more accurate means of measuring breech score compared to using the VSG, which is highly subjective. It would also be valuable to follow the animals through to measures of flystrike incidence and consider ways to assess pain more directly.

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APPENDIX

Figures

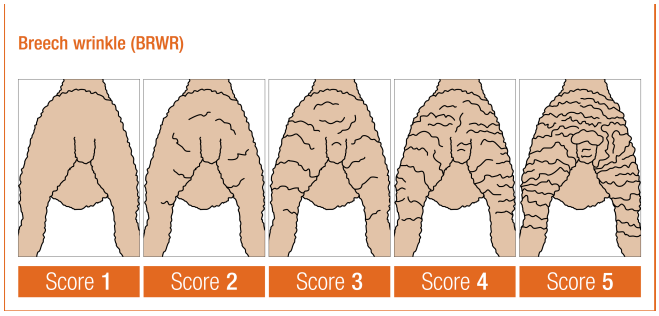


Figure 1: Breech Wrinkle (BRWR) from the Visual Sheep score guide. A sheep with a score 1 has no wrinkle whereas a sheep with a score 5 has significant wrinkling and is at greater risk of flystike.

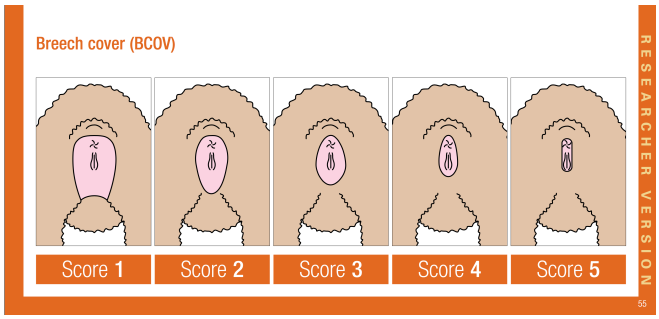


Figure 2: Breech cover (BCOV) from the visual sheep scores guide. Sheep with a score 1 have a large natural bare area that extends outwards and down to the bottom of the breach. A score 5 has no natural bare area

Figure 3: Clipboard with ruler and animal ID visible in photograph for reference.

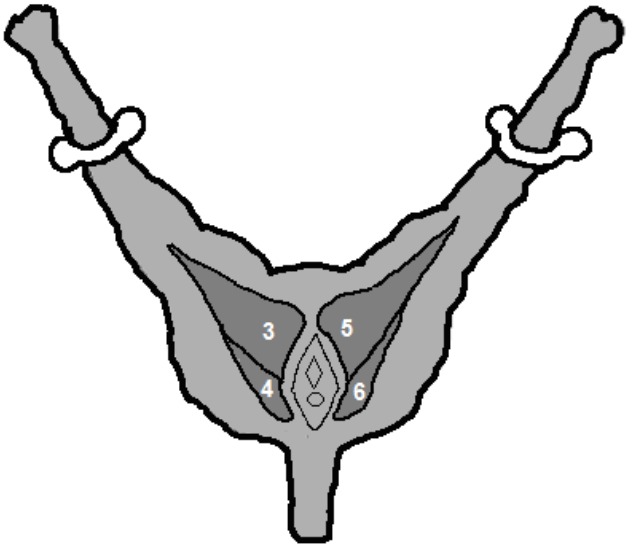


Figure 4: The modified 'V' Mules technique involving an average of 6 cuts (diagram from Costello 2014).[35]

Figure 5: The modified 'V' mules from day 0 of mulesing

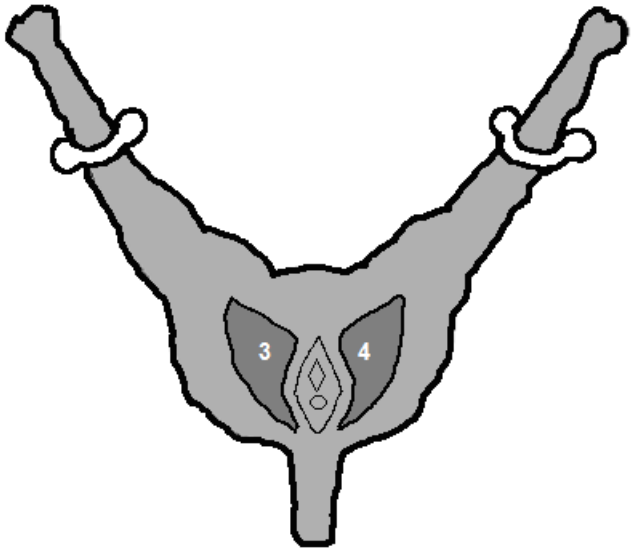


Figure 6: Conservative mules involving an average of 4 cuts (diagra, adapted from Costello 2014)[35]

Figure 7: Photograph of the conservative mules from day 0 of the experiment

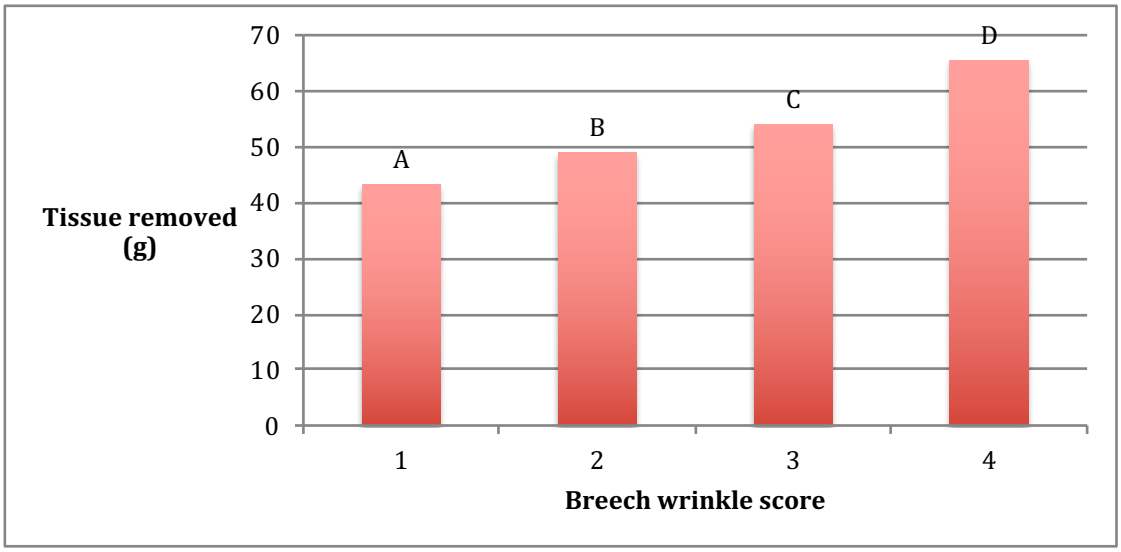


Figure 8: Mean grams of tissue removed from each BRWR score group. BRWR based visual score guide [3]. A, B, C, D denotes a significant difference between tissue removed within BRWR score groups

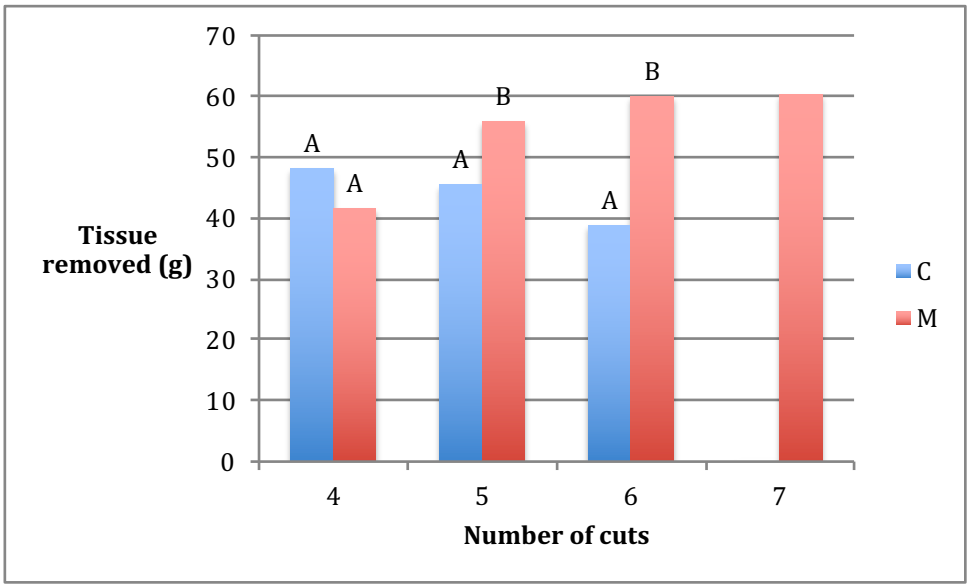


Figure 9: Effect of the treatment (Conservative Mules 'C' and the Modified mules 'M') group on the number of cuts and grams of tissue removed. A,B denotes a significant difference between treatments. Note: there is no significant difference between tissue removed for treatment groups when using 4 cuts and there were no cases where the conservative mules treatment required 7 cuts.

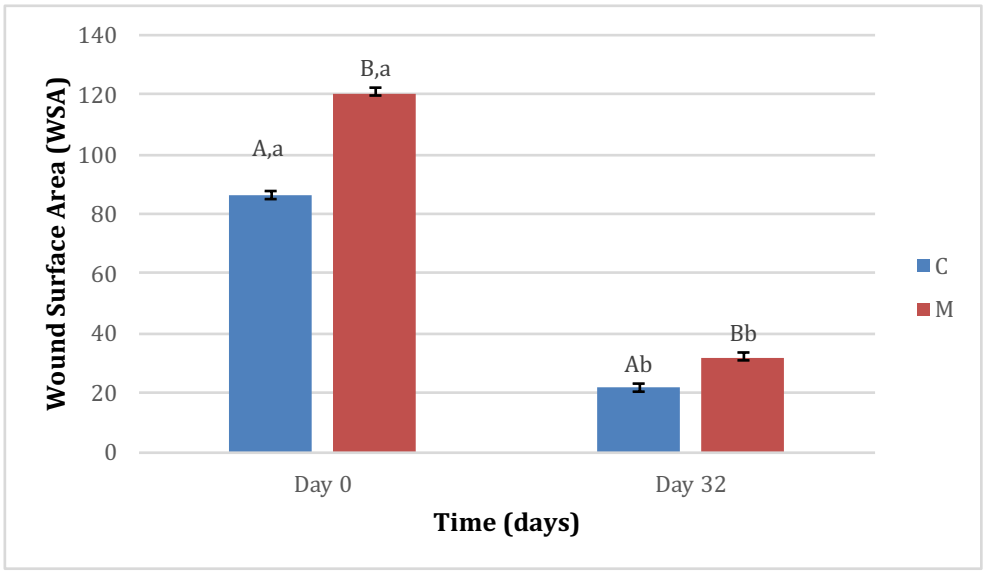


Figure 10: : Effect of treatments (Conservative mules 'C' and Modified mules 'M') on wound surface area of mulesing wounds at day 0 and day 32. A,B denotes a significant difference between treatments. a,b denotes a significant difference between time points. Significance was determined using the LSD 3.33

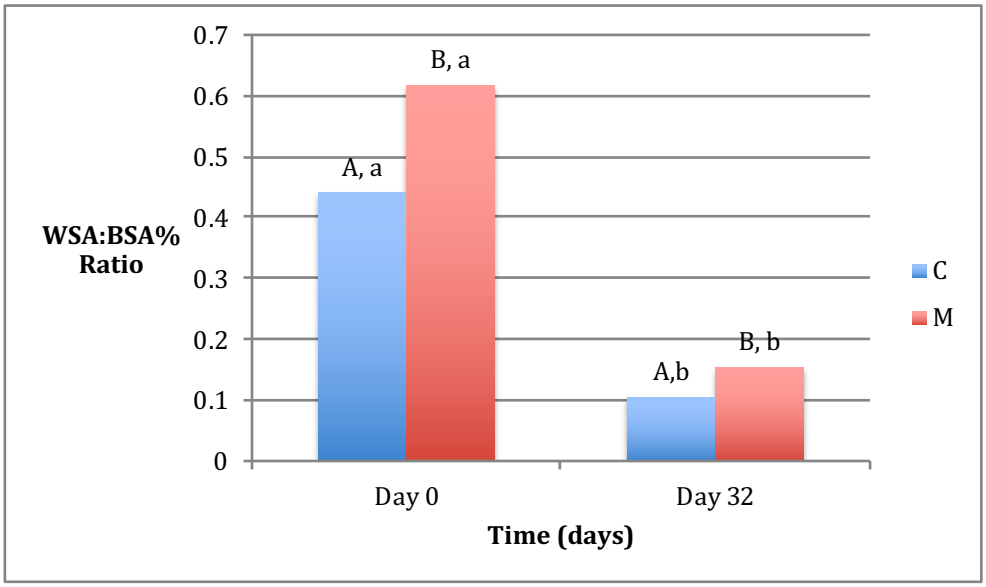
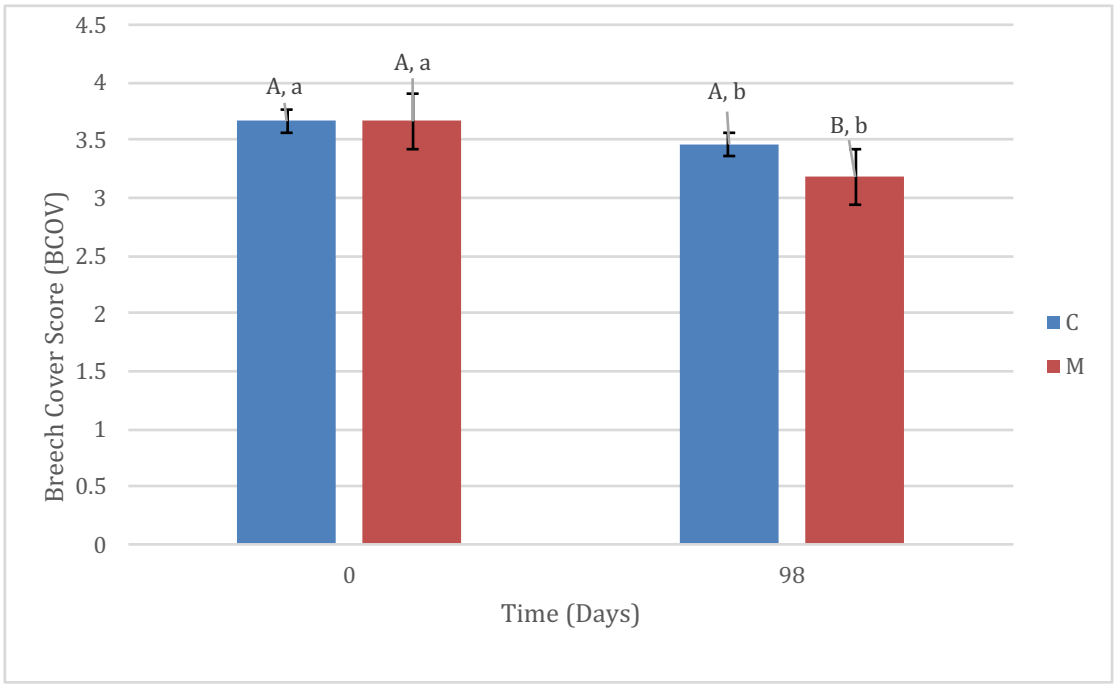


Figure 11: Effect of treatments (Conservative mules 'C' and Modified mules 'M') on WSA:BSA as a % at day 0 and day 32. A,B denotes a significant difference between treatment groups. a, b denotes the significance between time points. Significance was determined using LSD value 0.02



581

582 Figure 12: Effect of treatments (Conservative mules 'C' and the Modified mules 'M') on
583 BCOV scores on day 0 and day 98. A, B denotes a significant difference between treatments
584 (note: no significance between treatments on day 1) a, b denotes significance between time
585 points. Significance determined using LSD 0.11