Australian Veterinary Journal

Production Animals

Assessing the effect of a conservative versus modified mules on wound healing and breech parameters in Australian Merino sheep

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Abstract

Objective To assess and compare the effect of the standard modified 'V' mules and smaller conservative mules on breech parameters, wound size and wound healing in Merino hogget sheep.

Design Prospective, randomised and controlled field trial.

Animals 180, 8-month-old Merino ewe hoggets, mean weight 30.08 ± 3.34 kg

Methods Hoggets were randomly allocated to into two treatment groups: (1) Modified mules (n=90); (2) Conservative mules (n=90). At mulesing (Day 0) all excised tissue was weighed. Wounds were photographed for assessment of wound surface area and wound healing on Day 0 and Day 21. Breech scoring was conducted on Day -1 and on Day 42, 6 weeks after mulesing.

Results The conservative mules hoggets had significantly less tissue excised, wound surface area (cm^2) and wound surface area as a percentage of the body surface area than the modified mules group. The conservative mules had a significantly quicker rate of healing over a 3-week period than the modified mules. Both treatments resulted in a reduction in breech wrinkle and breech cover scores. The conservative mules group had significantly higher scores than the modified mules group. Sheep with larger weights tended to have lower breech cover scores.

Conclusion A conservative mules has improved sheep welfare and production outcomes over the modified mules. The reduced initial wound surface means a smaller wound surface area to body surface area ratio and a faster healing time than the modified 'V' mules. A conservative mules generates a reduction in wrinkle and an increase in bare area adequate for the protection of Merino sheep against flystrike.

Keywords breech strike, Merino sheep (*Ovis aries var. merino),* mulesing, hoggets, wound size, welfare

Introduction

Breech strike is a significant welfare and economic threat to the Australian sheep industry. Of the A$280m in lost income and costs associated with flystrike in Australia each year, breech strike represents the largest component costing A$147m, followed by body (103$mill) and then pizzle (30$mill). [1-3]

Surgical mulesing is the most effective method for decreasing the susceptibility to breech strike in Australian Merino sheep. [2,3-10] Mulesing involves the surgical excision of wool bearing skin from the breech and tail, which, upon wound healing, will reduce breech wrinkle and increase the natural perineal bare area. [11,12] From a welfare perspective the mules operation, combined with tail docking, regular crutching and jetting, significantly contributes to the control and prevention of breech strike.[11-13] In addition, crutching sheep with high breech wrinkle is considered more difficult and takes longer than mulesed sheep or plain bodied sheep.[13] Studies have reported that unmulesed sheep with high breech wrinkle have a significantly greater risk of severe breech cuts compared to mulesed sheep (2.0-10.6 x risk). [14,15]

Although mulesing provides animal welfare benefits, the pain of mulesing is well documented and animal welfare groups generally view the practice as unethical.[15-19] Over the past decade, in response to the pressure from welfare groups, the wool industry group Australian Wool Innovation (AWI) has invested over $27 million into research and development of alternatives to assist woolgrowers in managing the risk of breech-strike. [2,10] Current non-surgical alternatives are either still in proof of concept stage, under development and commercialization, or have not proven as effective or practical as the mulesing procedure. [2,3-10]

A genetic approach is the ideal long-term solution to flystrike prevention. [7,21-27] Selective Merino breeding programs to produce breech strike resistant sheep have

identified potential indicator traits that predispose sheep to breech strike. The breech dag score (DAG), wrinkle score (BRWR) and breech cover (BCOV) score are the most important risk factors for breech strike. [21-27] Target indicator scores (DAG:BRWR:BCOV) for a low-strike-susceptible sheep are 2:2:3 respectively. [21,25] Above this range the risk of breech strike increases significantly, while below 2:2:3, the risk of breech strike continues to decrease. [21] Selecting sheep with reduced expression of these traits provides an alternative to mulesing that is painless, cumulative and permanent once traits are fixed within the flock. [21,22] However it is estimated to be 10-15 of years until this genetic gain will take effect across the national Merino flock. [21-27] Consequently, mulesing remains an important procedure in Merino wool production.

In the interim, we must develop methods to minimize the welfare impacts of surgical mulesing to ensure the most humane procedure. The Code of Accepted Farming Practice for the Welfare of Sheep recommends that when performing the mulesing procedure the minimum number of cuts should be used and the size of the wound should be the minimum to achieve sufficient flystrike protection.[28] Currently, the industry standard is to perform the modified 'V' mules. This less invasive approach was modified from the original 'radical mules', developed in the 1970s, as the amount of tissue removed was considered detrimental to the sheep welfare through distortion of the vulva and increased rate of skin cancer.[11,12] With the shift to a plainer bodied Merino flock, there is potential for further refinement of the current practice for reduction in the level of tissue excised. It is important to ensure that and changes must achieve the necessary protection. Training delivered nationwide to contractors and growers through The National Mulesing Accreditation Program (NMAP), is currently being updated by Animal Health Australia (AHA) and Wool Producers

124 Australia (WPA).[2,28] This presents an opportunity for the mulesing technique to be
125 scrutinized and refined to meet industry best practice.[20]
126 The objective of this study was to investigate the effects of a smaller conservative
127 mulesing wound on breech strike indicator scores. This study provides science to
128 support guidelines and allows wool producers to make evidence-based decisions. It
129 was hypothesized that a smaller wound will result in improved rate of wound healing,
130 and reduction in BRWR and BCOV scores for adequate protection from breech strike.

131 **Materials and methods**

132 Experimental protocol was approved by the Animal Ethics Committee of The
133 University of Sydney (Project Title: Promoting welfare solutions for Merino sheep
134 and beef cattle in Australia / Project No: 5832) and was carried out according to the
135 Code of Practice of using animals in experiments.

136 **Animal management**

137 This study was conducted on 180 8-month-old Merino ewe hoggets (mean weight
138 30.08 ± 3.34 kg) on The University of Sydney's commercial sheep property,
139 'Arthursleigh', near Marulan New South Wales. The trial took place in early winter
140 2014.
141 The property has adapted the calendar of operations to mules sheep post-weaning for
142 management purposes. Ewe hoggets were used in the trial, as only females are
143 mulesed on this property due to increased susceptibility for wetting of breech fleece.
144 In addition, wethers are sold before 1yr so remain unmulesed. All sheep had been
145 previously hot-iron tail-docked, ear notched and ear tagged for identification before 2
146 months of age with wounds completely healed at the time of experiment. The hoggets

were crutched one week prior to mulesing to allow scoring of breech parameters, as outlined below.

Study design

On the day prior to mulesing (Day -1), the mob of 180 sheep were randomly allocated to treatment by drafting alternate sheep into two groups: (1) Modified mules (n=90); (2) Conservative mules (n=90).

Mulesing procedure

On the Day of mulesing (Day 0), sheep were moved in the two groups into the shearing shed for treatment. Mulesing was conducted in a VE machine. Sheep were inverted into dorsal recumbency, with their hind legs secured into leg hooks. An accredited mulesing contractor performed both mulesing operations. Three sets of well-set, sharpened and disinfected shears were used, with those not in use soaking in chlorhexidine disinfectant (Hibitane®, Coopers Animal Health, Baulkham Hills, Australia).

Following mulesing, all excised tissue was weighed using electronic scales calibrated to 0.1g (Salter Spacesaver Kitchen Scale No.1075). To align with industry best practice, 8-10mL of Tri-Solfen® (Bayer Animal Health, Pymble, Australia) topical anaesthetic was applied to the wound surface as per product instructions. All sheep were then released into a 20ha paddock where they remained for 6 weeks until all subsequent data was collected.

Assessment of wound area and healing

Wounds were photographed for assessment of wound size and wound healing on Day 0, immediately following mulesing, and 21 days post-mulesing (Day 21). Wounds were photographed using a digital SLR camera (Canon EOS 50D Digital SLR). A

30cm ruler was used as a scale. This was positioned against the wool immediately above the wound and a tag number for each sheep to allow a correct measurement and identification of the wounds.

Objective wound area calculations were conducted using digital planimetry software (PictZar® CDM, BioVisual Technologies L.L.C. New Jersey, USA) to calculate and record the wound area (cm^2) and percentage change in wound area from day 0 to day 21 post mulesing (Figure 1). Throughout the analysis the same person conducted all wound measurements and was blind to treatment protocol at the time of performing assessments. Body surface area (BSA) was calculated to allow comparison of wound surface area (WSA) as a percentage of the body surface area (WSA:BSA %). BSA was calculated using the formula described by Guyton et al (1973) whereby Total Body Surface Area = $0.084B^{0.67}$ (where B is sheep weight in kg).[31]

Assessment of breech susceptibility scores

Breech scoring was conducted on Day -1 and on Day 42, 6 weeks after mulesing. Sheep were moved through the race, caught and restrained in lateral recumbency using an electronic sheep handler (Hdale Engineering Ltd, Model no: CWC RC) (Figure 2). Scoring for breech wrinkle (BRWR) and perineal bare area (breech cover, BCOV) was conducted. Dag and urine stain scores were not allocated, as the sheep had been crutched only 1-week prior.

BRWR and the BCOV scores were assessed using a 1–5 scale, according to the Visual Sheep Scores (VSS) Guide.[30] BRWR is defined as the degree of wrinkle at the tail set, sides of the tail, adjacent to the anus and vulva and down the hind legs (Figure 3). [30] BCOV refers to the amount of natural bare skin around the perineum and breech area, in particular, the depth and width of bare skin below and immediately surrounding the vulva and anus (Figure 4). [30] The guide provides a 1-5 scoring system

196 (Figure 3 and 4), where a score 1 depicts the least, most optimal expression of the trait
197 and Score 5 depicts the most, least desirable, expression of the trait. [1-3,30]
198 Scoring took place on animals over 4 months of age and within 1-month post-shearing
199 or crutching, as per the VSS guide. [30] It is important to note that if animals were
200 assessed to have an expression of a trait between the single scores, they were awarded
201 a half score. The statistical test used to analyse the scores (ordinal logistic regression)
202 did not allow mid-way scores, so for the purpose of analysis all half values were
203 rounded down.

204 **Treatments**

205 **Modified 'V' mules** -The industry standard for mulesing is to perform the
206 modified 'V' mules, as per the national Mulesing Accreditation Program (NMAP).
207 The modified 'V' mules technique involves an average of 6 cuts, 2 from the tail and 4
208 from the breech area (Figure 5, 6, 7). Two tail strips (cuts 1 and 2 in Figure 5) remove
209 the wool bearing skin at the base and along each side of tail. The breech cuts begin
210 next to the bare skin of the vulva and involve excising four crescents of skin along
211 either side of the bare perianal skin (cuts 3,4,5 and 6 in Figure 6). Cuts starting
212 roughly 2 cm above the tail and finishing in a tapering 'V' on the inside of the leg just
213 above the top of the hamstring. The tail and breech cuts join up, but a V-shaped
214 projection of wool-bearing skin is left approximately 1/3 down the length of the tail
215 from the base to protect from sun damage. [11-15,20-22]

216 The conservative mules - A conservative mules takes a selective approach to
217 dictate the amount of tissue removed based on the individual sheep level of breech
218 wrinkle and breech cover. The tail strip remains the same (cuts 1 and 2 Figure 5),
219 however the breech cuts only involve one strip down either side of the bare area as
220 opposed to two, and not extending as far down the legs. The procedure involves an

221 average of 4 cuts, 2 from the tail and 2 from the breech area (Figure 8, 9). With this
222 approach to mulesing, if an animal presents with high wrinkle and low bare area then
223 more skin can be taken, and the same applies for an animal with a low wrinkle score
224 and high bare area where less tissue can be taken, in some cases only the tail strip.

225 **Statistical analyses**

226 Weight of tissue removed (g) and percentage change in wound area (%) were
227 analysed using general linear regression in Genstat® 16th edition (VSN International
228 Ltd, Hemel Hempstead, UK). The main effect of treatment was fit against the
229 response variable weight of skin removed (g), with the initial weight of the sheep (kg)
230 as a covariate. The main effect of treatment was fit against the response variable
231 change in wound area (%). The data sets were normally distributed for the parameters
232 measured. Means and standard errors are presented in graphs.
233 The WSA analyses was performed using restricted maximum likelihood (REML)
234 variance component analysis in Genstat® 16th edition (VSN International Ltd, Hemel
235 percent area Hempstead, UK). The response variables, WSA (cm^2), WSA:BSA (%),
236 on day 0 to day 21 were analysed in a repeated measures analysis model fitting the
237 main effects of treatment, time, and potential interactions. Initial body weight (BW_0)
238 was found to have no significant effect and so was included in the random effects
239 with sheep. Data for both outcome variables was normalized by log e transformation
240 based on the Anderson-Darling test for normality at the 5% critical value. There was a
241 significant time×treatment interaction, therefore pairwise comparisons were made
242 using LSD's and treatment effects were analysed and presented separately for each
243 time.
244 BRWR and BCOV scores are ordinal data and so were analysed using ordinal logistic
245 regression in ASReml v3.00 software (VSN International Ltd, Hemel Hempstead,

246 UK). The intervals between the scores were not considered necessarily equal. The
247 fixed effects considered for the final model were treatment, time and their interaction,
248 and weight. A spline model for weight was fitted, to allow for any nonlinear effect of
249 initial weight, but this was not significant. Tag was included as the random effect to
250 account for any inter-animal variation. Significant interactions were analysed and
251 presented separately for each time on fitted probability plots.
252 For all analyses *P*-values ≤0.05 were considered significant for statistical
253 associations.

254 **Results**

255 **Tissue excised (g)**

256 There was a significant effect of treatment of the weight of tissue excised (P<0.001).
257 The mean weight of tissue (± s.e.) removed in the modified 'V' mules was 81.88±
258 1.786g and the conservative mules was 49.24 ± 1.069g (Figure 9). There was a
259 significant interaction between initial sheep weight (kg) and weight of tissue removed
260 (g) (P < 0.001). Regardless of treatment, for every 1kg of sheep, 1.049g of tissue
261 were removed.

262 **Wound surface area (WSA)(cm^2)**

263 There was a significant time x treatment interaction (P=0.003) on WSA (cm^2). From
264 Day 0 to Day 21 the mean WSA (± s.e.) for the modified 'V' mules group were
265 161.42 ± 5.01 cm^2 and 40.9 ± 1.24 cm^2 respectively. Mean WSA–cm^2 for the
266 conservative mules group were 71.64 ± 2.14 cm^2 and 16.36 ± 0.27 cm^2 respectively.
267 Within each time point (Day 0, Day 21) the modified 'V' mules had a significantly
268 larger effect on WSA than the conservative mules (Figure 10). There was a significant
269 reduction in WSA from Day 0 to Day 21 for both treatments.

Wound surface area: Body surface area (BSA) (%)

There was a significant time x treatment interaction (P=0.003) on the WSA:BSA (%). From Day 0 to Day 21 the mean WSA as a percentage of body surface area (± s.e.) for the modified 'V' mules group were 1.76 ± 0.06% and 0.45 ± 0.01% respectively. Mean WSA:BSA for the conservative mules group were 0.78 ± 0.02 % and 0.18 ± 0.01% respectively. Within each time point the modified 'V' mules resulted in a significantly larger WSA:BSA(%) than the conservative mules (Figure 11). There was a significant reduction in WSA:BSA (%) from Day 0 to Day 21 for both treatments.

Percentage change in wound surface area (%)

There was a significant effect of treatment on percentage change in WSA between Day 0 and Day 21 (P=0.004). The mean percentage reduction in WSA (± s.e.) was 74.25 ± 0.73% for the modified 'V' mules group and 77.13 ± 0.66% for the conservative mules group (Figure 12). Sheep-receiving the modified 'V' mules had a slower healing rate than those receiving the conservative mules, with a difference of 2.873% wound contraction in the 3 weeks post (P=0.004).

Breech Wrinkle (BRWR) Score

Mean BRWR scores pre mulesing (Day -1) and 6 weeks post mulesing (Day 42) for both treatments are presented in Table 1. From the table it can be seen that both the treatments have resulted in a reduction in BRWR scores below 2. There was no significant difference between treatment group BRWR scores at Day -1. Mean BRWR scores (± s.e.) were 2.3 ± 0.083 and 2.19 ± 0.076 for the modified and conservative treatment groups respectively [(Table 1). At Day 42 hoggets across both treatments had significantly different BRWR scores, with mean scores of 1.56 ± 0.02 and 1.64 ± 0.028 for the modified and conservative treatment groups respectively (Table 1). Ordinal analysis resulted in a significant treatment × time interaction (P =

295 0.04). There was a significant effect of treatment on BRWR score at Day 42 (P =
296 0.03), with the conservative mules group having marginally higher mean BRWR
297 scores than the modified 'V' mules group (Figure 13).

298 **Breech Cover (BCOV) Score**

299 Mean BCOV scores pre mulesing (Day -1) and 6 weeks post mulesing (Day 42) for
300 both treatments are presented in Table 2. From the table it can be seen that both the
301 treatments have resulted in a reduction in BCOV scores, the modified ' V' mules had
302 a bigger effect as it reduced to BCOV score to less than 3. There was no significant
303 difference between treatment group BCOV scores at Day -1. Mean BCOV scores (±
304 s.e.) were 4.8 ± 0.046 and 4.75 ± 0.058 for the modified and conservative treatment
305 groups respectively (Table 2). At Day 42 hoggets across both treatments had
306 significantly different BCOV scores, with mean scores of 2.67 ± 0.063 and 3.13 ±
307 0.063 for the modified and conservative treatment groups respectively (Table 2).
308 Ordinal analysis resulted in a significant treatment × time interaction ($P < 0.001$).
309 There was a significant effect of treatment on BCOV score at Day 42 ($P < 0.001$),
310 with the conservative mules having higher mean BCOV scores than the modified 'V'
311 mules (Figure 14). Irrespective of treatment there was a significant effect of weight
312 on BCOV score ($P < 0.001$). Sheep with larger weights tended to have lower BCOV
313 scores (Figure 15).

314 **Discussion**

315 Results indicate that significant reductions in WSA and an improved rate of healing
316 can be achieved in hoggets after mulesing with the conservative mules instead of the
317 modified 'V' mules. This presents the potential for updating the current technique in

318 the industry standards, improving the welfare benefit for sheep having this procedure
319 Australia wide.
320 Currently there is insufficient information documenting wound size and wound
321 healing patterns in mulesing. [14-18,20, 31-34] A slow rate of wound healing can lead to
322 post mulesing complications. The degree of subcutaneous tissue excised from a
323 mulesing wound can affect the time taken for re-epithelialisation, granulation tissue
324 formation and wound contraction to occur.[8,9] In lambs wounds with larger surface
325 areas take a significantly greater time to re-epithelialise than smaller wounds. [8-10,32] A
326 wound that remains open longer has an increased likelihood of environmental
327 contamination with foreign bodies, making the sheep more susceptible to infection
328 and wound strike post mulesing. [8-10,32]
329 Wound contraction is the basis for the theory behind mulesing, during wound
330 adhesion, there is a reduction of breech wrinkle and an enlargement of the natural
331 perineal bare area. [6] The concept of a smaller WSA:BSA is linked explicitly with
332 quicker wound healing. [6] The surface area of the wound contributes directly to the
333 time taken for wound healing as the size determines the level of granulation tissue
334 required to close and heal the wound. [8,9]
335 In the present study there was a significant relationship between the conservative
336 mules and wound healing. The conservative mules had less tissue removal, WSA
337 (cm^2) and WSA:BSA when compared with the modified mules. The percentage
338 reductions in wound sizes over the 3-week period were significantly greater than the
339 modified mules. It is also important to note, the conservative mules involves 2 fewer
340 cuts than the modified mules technique (Figure6,7,8,9). These results are concurrent
341 with the Code of Accepted Farming Practice for the Welfare of Sheep, which

342 endorses a mulesing procedure that minimizes the number of cuts and the size of the
343 wound. [20,28]
344 The smaller WSA, WSA:BSA and the faster rate of healing is likely attributable to the
345 reduced amount of skin removed in the conservative mules. The conservative mules
346 removed an average of 32.5g less tissue than the modified. The significant difference
347 in the amount of tissue removed between treatments was an anticipated outcome
348 based on the different approach to the number of cuts between each procedure (Figure
349 8,9) The degree of subcutaneous tissue excised from a mulesing wound does affect
350 wound contraction. [6-8]
351 Current industry standards recommend that mulesing is performed at the earliest
352 possible stage, generally at marking, between 2 to 12 weeks of age, to minimize stress
353 and handling. [15-19,28] The reality is the age at mulesing varies from farm to farm and
354 may be determined by fly presence, climate, weather, farming schedule or timing of
355 other husbandry procedures. [6-9]
356 Anecdotal evidence suggests that mulesing sheep older than 6-months of age has
357 benefits for sheep welfare as well as animal management. Since a lice outbreak at
358 Arthursleigh, that prevented mulesing at marking, management has continued to
359 mules sheep at hogget age. This is attributed to the improved ease of management
360 and mothering up at marking, thanks to a reduction in the number of individual
361 procedures a lamb undergoes on one day. Anecdotal reports also state the ability of
362 the older sheep to recover better from the operation than young lambs.
363 In this study, there were significant novel findings associated with variation in wound
364 size. Irrespective of treatment, for every 1kg of sheep, another 1.049g of tissue is
365 removed in the procedure. Larger sheep tended to have lower breech cover scores.
366 Evidence suggests that breech wrinkle and breech cover assessments tend to vary

367 more with increasing age. [25] Since this phenotypic variance is essential for achieving
368 a good response to selection, it is recommended that breech cover scoring be delayed
369 as much as practically possible. [20-27, 35] An age limit at mulesing has important
370 ramifications for producers attempting to reduce mulesing in their flocks through
371 selective breeding. The improved body condition of hoggets, compared to lambs,
372 allows for breech wrinkle and cover scores to reduce with age, potentially making an
373 animal no longer eligible for mulesing. [35]
374 Breech wrinkle and breech cover are identified as important risk factors for breech-
375 strike of fine-wool Merino sheep in Australia. [20-27] Sheep with breech wrinkle and
376 breech cover scores less than or equal to 2 and 3, respectively, are considered
377 candidates for the breech strike resistance breeding plan. [20,25] The breech traits
378 identified with these scores are associated with reduced faecal staining and moisture
379 around the breech, thus reducing the susceptibility to strike. Breech cover is
380 considered a heritable trait at different ages, which supports the results suggesting that
381 allowing the animal to lay down more condition and fill out, could potentially lead to
382 a smaller wound required overall.[23-27, 35]
383 The cohort from the 'Arthursleigh' property were typical fine-wool Merinos and
384 susceptible to breech-strike, having considerable breech wrinkle scores between 2.2
385 and 2.3. The sheep demonstrated little natural variation in the amount of bare breech
386 area, with sheep consistently lacking natural bare area with high scores between 4.75
387 and 4.8. The scores in both treatment groups were reduced as a result of mulesing.
388 The modified mules had significantly lower scores overall. This indicates that the
389 modified mules is more effective at achieving low scores. This is not surprising
390 considering the significantly larger amount of tissue excised and vast initial WSA.

391 Bound up in the codes objective of minimizing the wound size, is the condition that
392 the procedure will reduce breech traits sufficient enough for flystrike protection**.** [28]
393 The breech wrinkle score is under the target goal of 2, meaning the conservative
394 mules was successful in achieving enough breech wrinkle reduction. Whilst the bare
395 cover is not less than 3, both procedures resulted in a reduction of at least 1.5 on the
396 scale and this is important considering a 0.1 score reduction reduces breech strike
397 risk. [20]
398 It is important to note that any half-breech scores for ordinal logistic regression were
399 rounded down. For BRWR there was a total of 67 and 151 half scores recorded on
400 Day- 1 and Day 42 respectively. For BCOV there were no half scores recorded at
401 Day -1, however 66 of the scores at Day 42 were half scores. The half score
402 adjustments that were made could have an impact on the analysis and further
403 investigation in to how to address this is warranted. In future, if this sort of statistical
404 analysis were the most appropriate, it would be advisable to not give half scores
405 during breech assessment.
406 The scoring was assessed based on the VSS guide. [30] These are inherently subjective
407 and can lack sensitivity but are the guides used throughout the industry. In an attempt
408 to limit subjectivity, a single observer assessed the scores. Human error can be easily
409 introduced when manual manipulation of data is carried out. To reduce this error the
410 PictZar program was selected for its highly sensitive and reliable output.[36]
411 In future studies figuring a novel means of being able to analyze the mulesing wound
412 without interrupting the healing process would provide a more detailed account of
413 wound healing. The timing of the visits during this trial were based on reducing the
414 amount of animal handling, so as to not interfere with the true healing rates of each
415 wound. A significant outcome variable that was not considered in this study, due to

the timing of the trial with crutching and shearing, was the ability to dag score the animals. Dag scores have a strong correlation with breech strike and incorporating their assessment in to this procedure would provide important information on the mulesing procedure and its impact on dag formation. [20-27] Follow up investigations in to any cases of post mules strike will provide more information regarding with the true efficacy of each mulesing treatment.

This study is the first of multiple studies that will be conducted on different properties with different environmental factors and different flock genetics, comparing the two-mulesing procedures. Our findings provide new and important information, particularly regarding wound size and WSA. A conservative mules has improved sheep welfare and production outcomes than the modified mules. The reduced initial wound surface means a smaller WSA:BSA and a faster healing time than the modified 'V' mules. A conservative mules generates a reduction in wrinkle and an increase in bare area adequate for the protection of Merino sheep against flystrike.

Acknowledgements

This research program was approved by the Animal Ethics Committee of the University of Sydney. The authors thank the Australian Wool Education Trust for funding this project. The support of Mr Steven Burgun and staff at 'Arthursleigh' and statistical support of Professor Peter Thomson is gratefully acknowledged.

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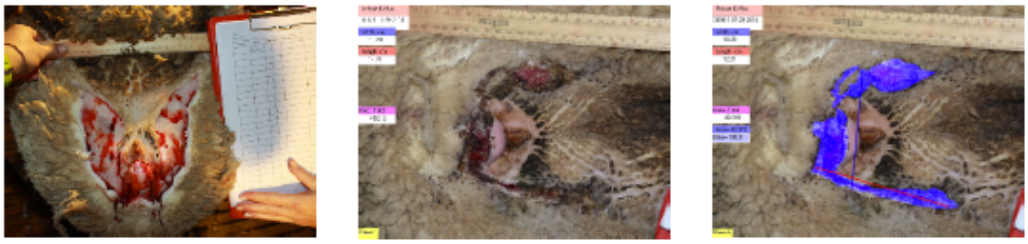
APPENDIX

Figures

University of Sydney Team Pain

PictZar® Wound Measurement Summary Report

Patient Name: Tag 3096 Chart ID: 3096 Wound No: 3096-1 Location: Breech



Original Image	Current Image	PictZar Measured Image
Initial Measurement Date: 07-09-2014	Date Of This Assessment: 07/29/14	Measurement No: 2 of 2

CORE MEASUREMENTS

Initial Area (CmSq)	Current Area (CmSq)	Percent Area Change	Length	Width	Depth	Volume
168.542	40.318	-76.08%	12.21cm	10.28cm	0cm	0cc

DETAIL MEASUREMENT ANALYSIS

Area cmSq.	Color	Percent	Tissue Type by Color	Depth in cm	Color	Volume in cc	Linear Measurements
40.318	Blue	100.0	Not Entered	0	Blue	0	
0.00	Red	0	Not Entered	0	Red	0	Length: 12.21cm
0.00	Green	0	Not Entered	0	Green	0	Width: 10.28cm
0.00	Yellow	0	Not Entered	0	Yellow	0	Circumference: 0cm
40.318	Total Area			0	Aver. Depth	0	Total Volume

Comments:

Figure 1. Wound measurement output from PictZar software (PictZar® CDM, BioVisual Technologies L.L.C. New Jersey, USA).

Figure 2. Sheep restrained in lateral recumbency using an electronic sheep handler (Hdale Engineering Ltd, Auto Weigh Sheep Handler Model no: CWC RC) for breech parameter scoring.

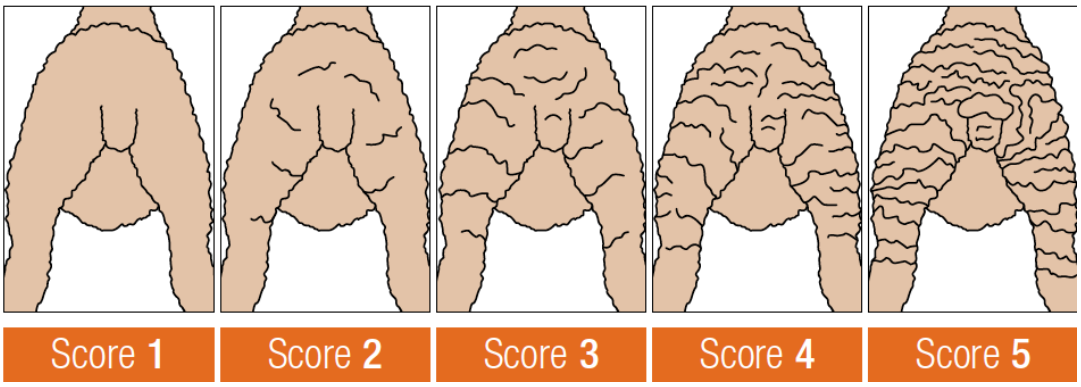


Figure 3. Breech wrinkle (BRWR) standard from Visual Sheep Scores guide. A sheep with Score 1 has no wrinkle. A Score 5 sheep has extensive wrinkle at the tail set, sides of the tail (bat wings), adjacent to the anus/vulva and down the hind legs. [30]

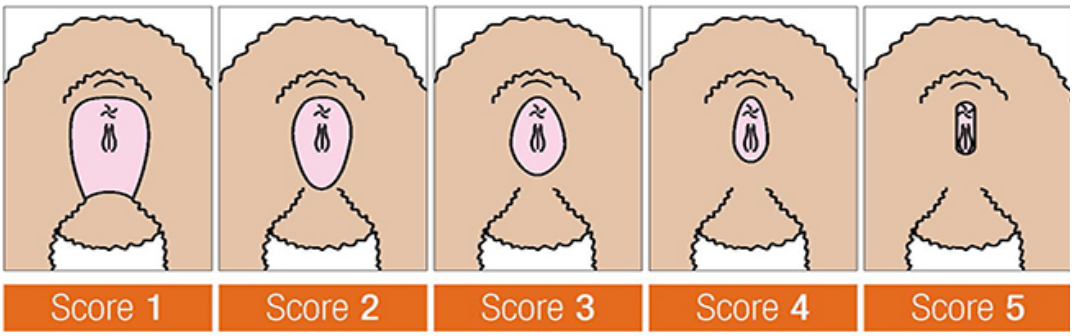


Figure 4. *Breech cover (BCOV) standard from Visual Sheep Scores pocket guide.* A Score 1 sheep has natural bare area that extends outwards around the anus and vulva, and right down to the bottom of the breech area (the channel). A sheep with Score 5 has complete (most) wool cover i.e. no natural bare area at all. [30]

Figure 5. Tailstripping. Cuts 1 and 2 remove all of the wool bearing skin from the sides and end of the tail. A 'V' shaped projection of wool bearing skin remains at the base of the tail (diagram adapted from Gherardi 1996).[37]

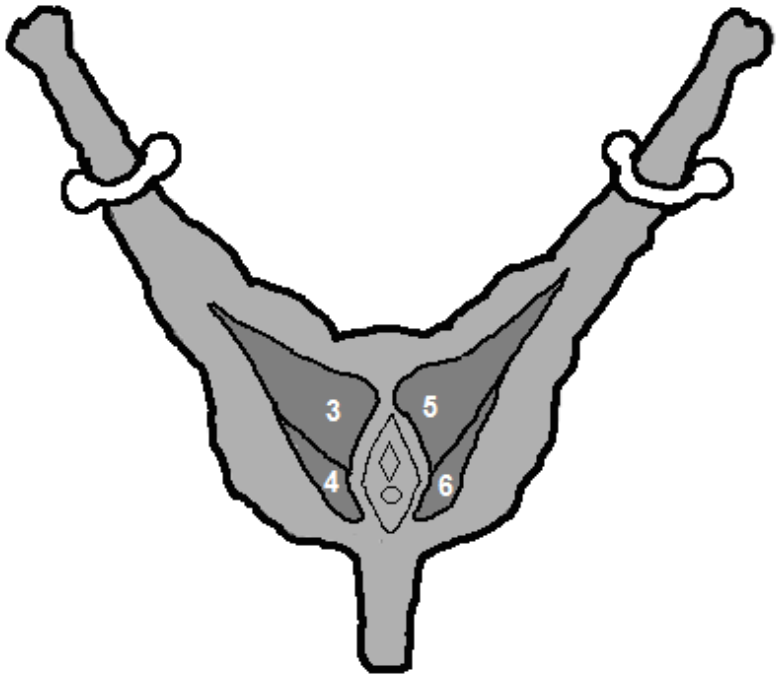


Figure 6. The modified 'V' mules technique involves an average of 6 cuts, 2 from the tail and 4 (cuts 3,4,5,6) from the breech area (diagram adapted from Gherardi 1996).[37]

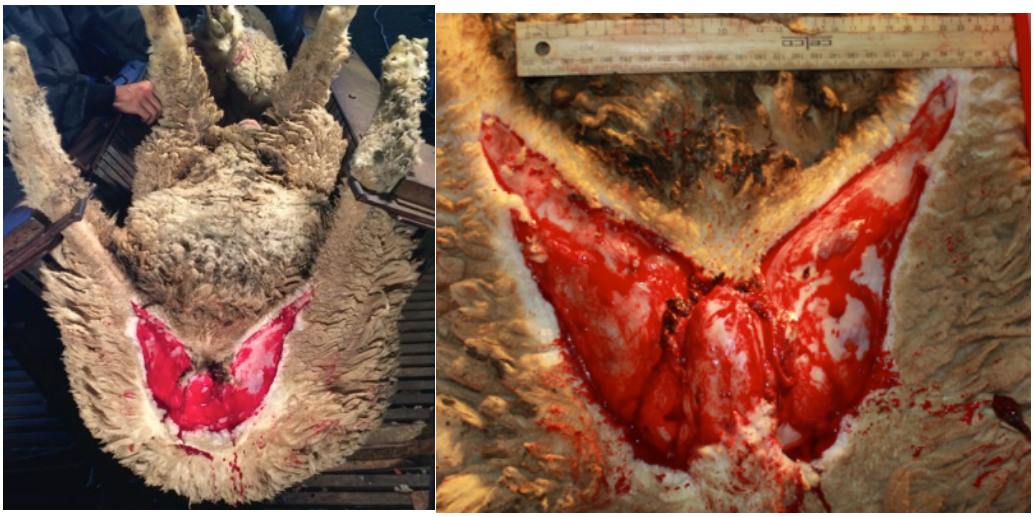


Figure 7. Photographs of the modified 'V' mulesing wound from Day 0 of the trial.

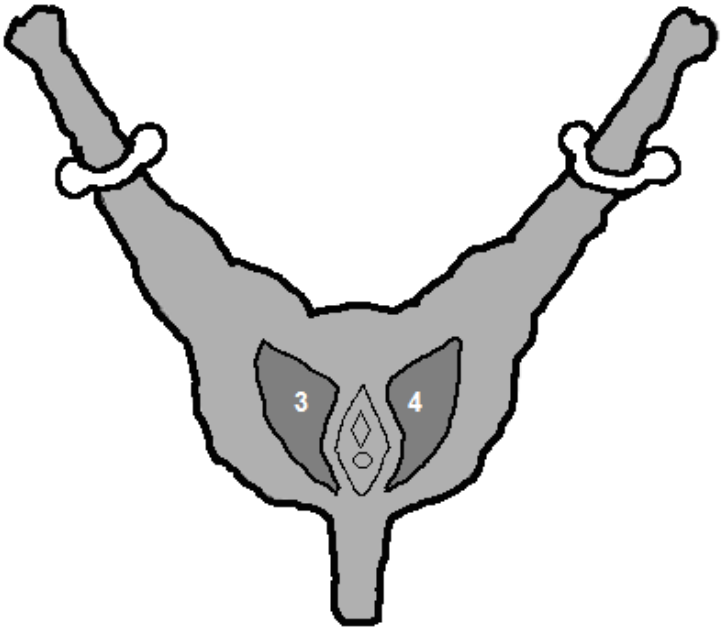


Figure 8. The conservative mules involves an average of 4 cuts, 2 from the tail and 2 (cuts 3 and 4) from the breech area (diagram adapted from Gherardi 1996).[37]

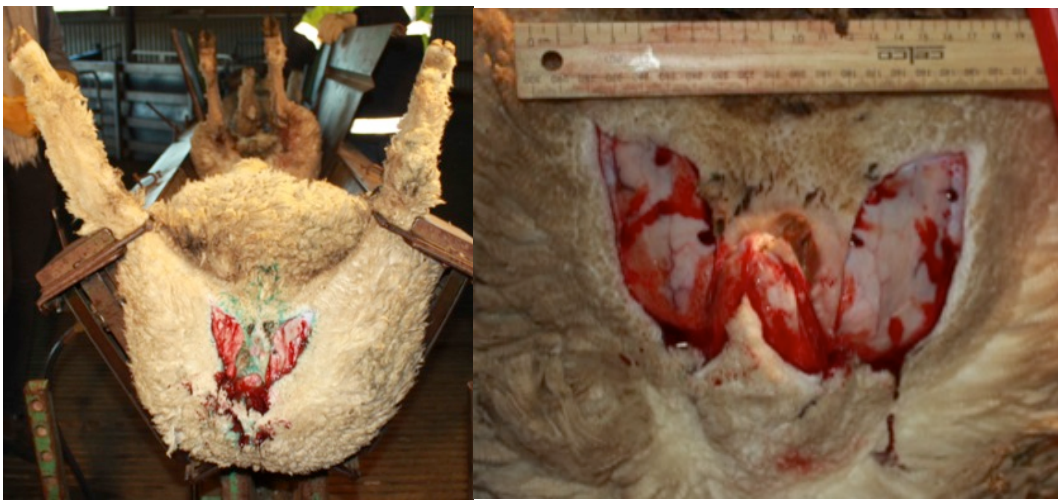


Figure 9. Photographs of the conservative mules wound from Day 0 of the trial.

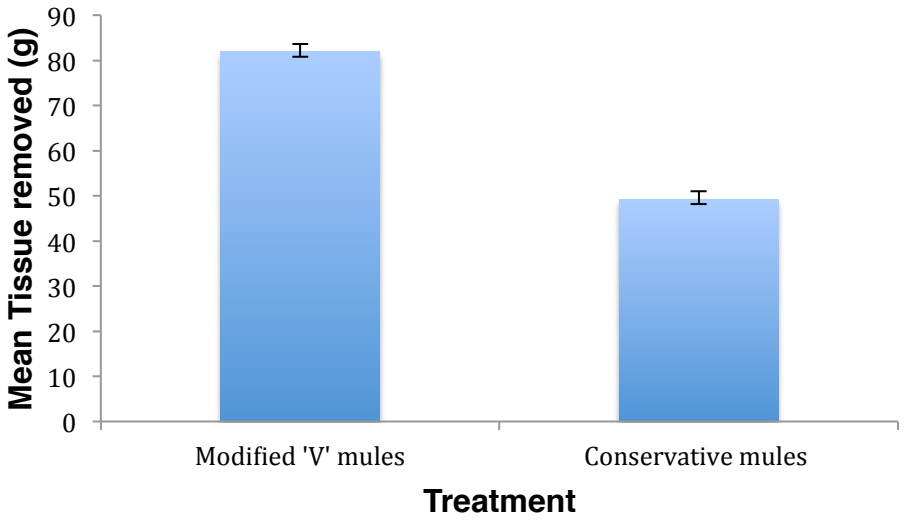


Figure 10. Mean weight of tissue removed in each treatment.

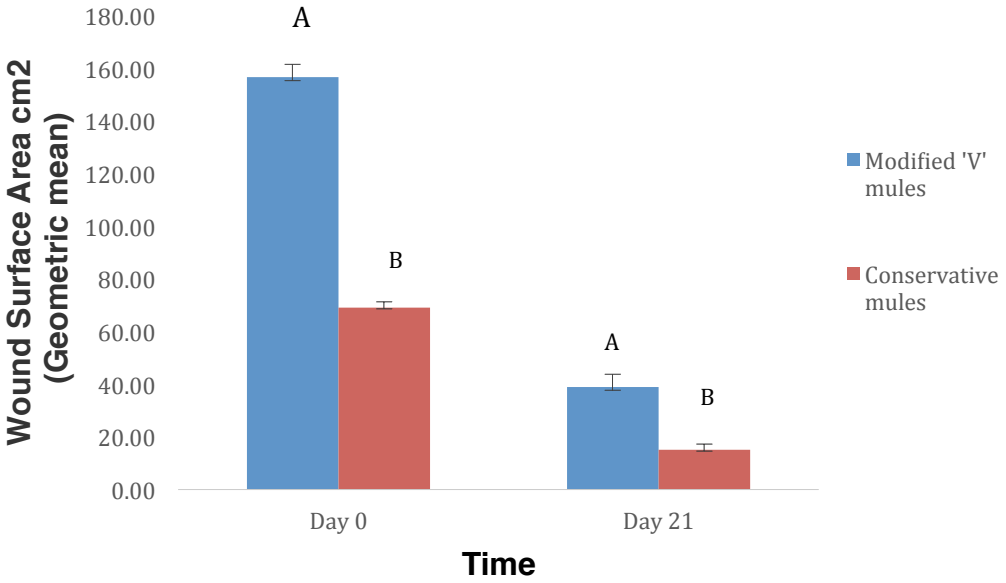


Figure 11. Effect of mulesing treatments on Merino ewe hogget wound surface area (cm^2) at Day 0 and Day 21. ^{AB} Means within a sampling time point without a common superscript are significantly different (P = 0.003).

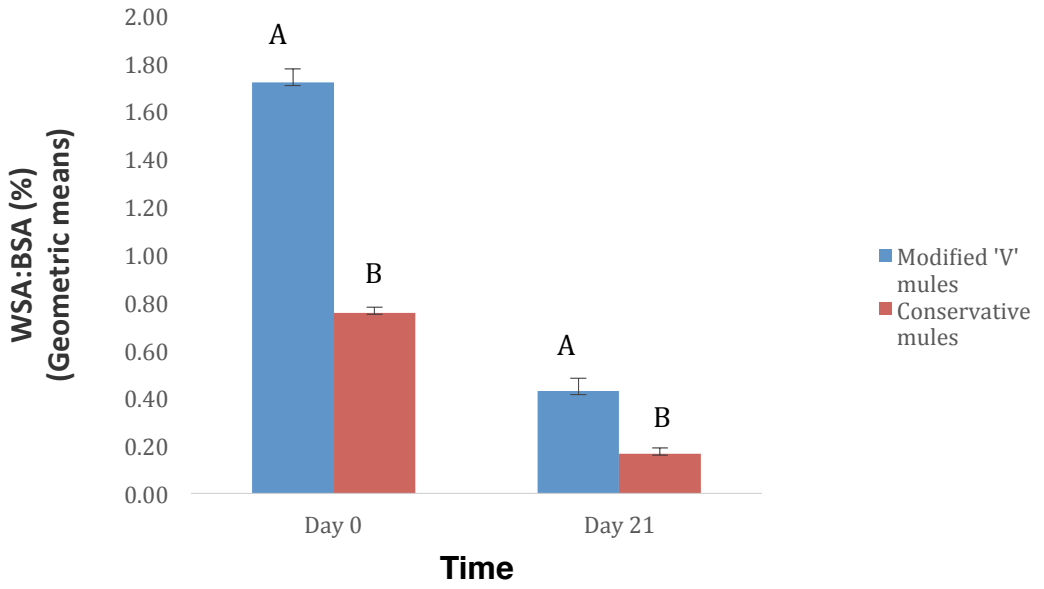
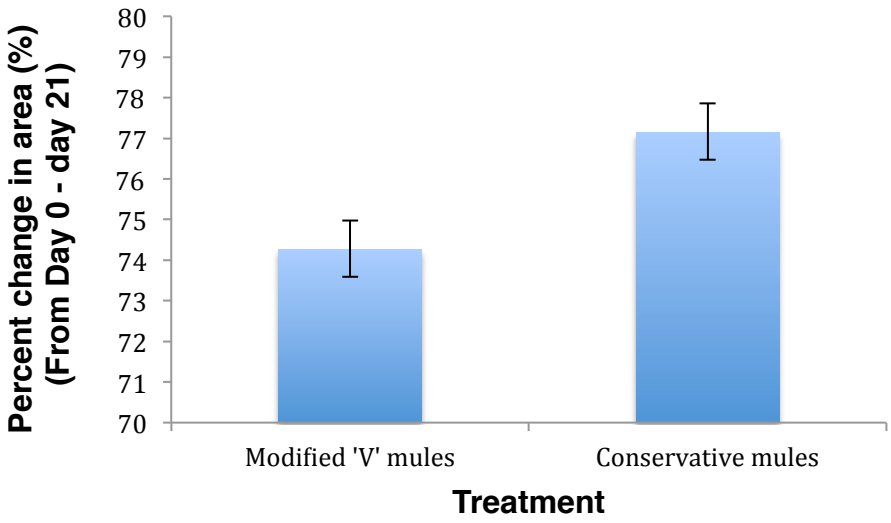
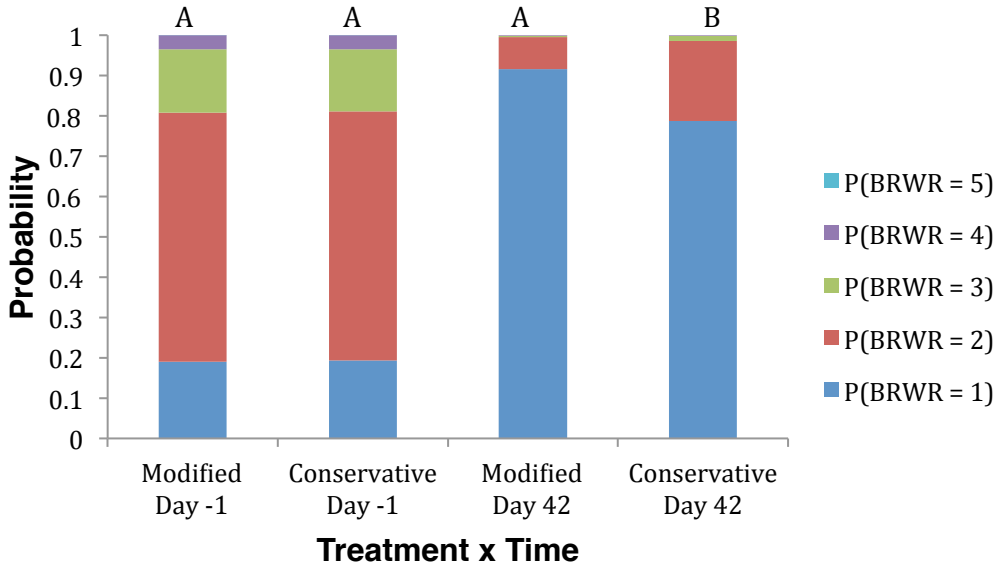


Figure 12. Effect of mulesing treatments on the wound surface area as a percentage of body surface area in Merino ewe hoggets at Day 0 and Day 21. [AB] Means within a sampling time point without a common superscript are significantly different (P = 0.003)



625

626 Figure 13. The percentage change in wound surface area (%) for each
627 treatment between Day 0 and Day 21 (P=0.004)



628
629 Figure 14. Probability of BRWR scores in each treatment over time. On Day
630 42 there was a significant effect of treatment (P = 0.03), with the conservative
631 mules having higher BRWR scores on average than the modified 'V' mules.
632 [AB] Means within a sampling time point without a common superscript are
633 significantly different (P = 0.03).

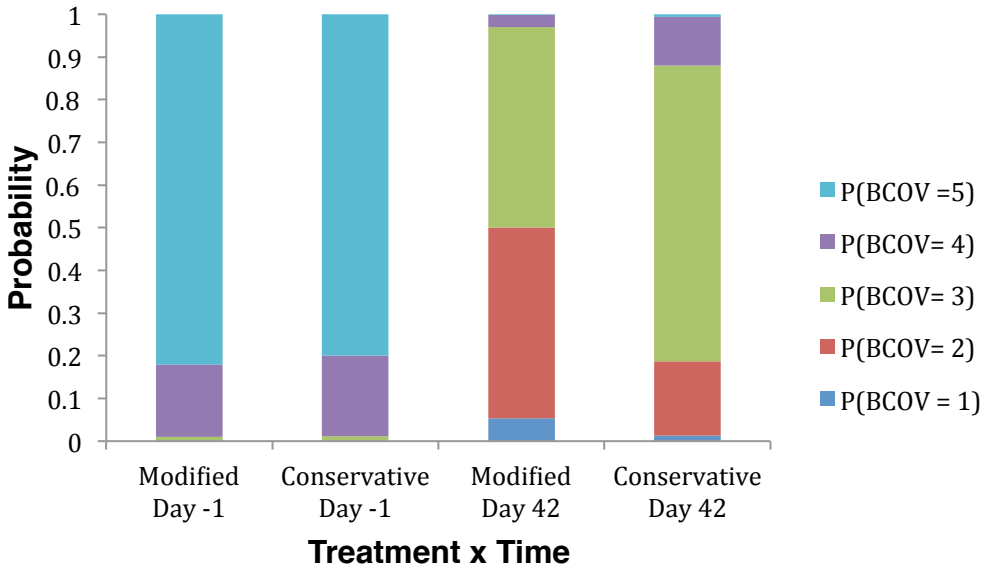


Figure 15. Probability of BCOV scores in each treatment over time. On Day 42 there was a significant effect of treatment ($P < 0.001$), with the conservative mules having higher BCOV scores on average than the modified 'V' mules. [AB] Means within a sampling time point without a common superscript are significantly different ($P < 0.001$).

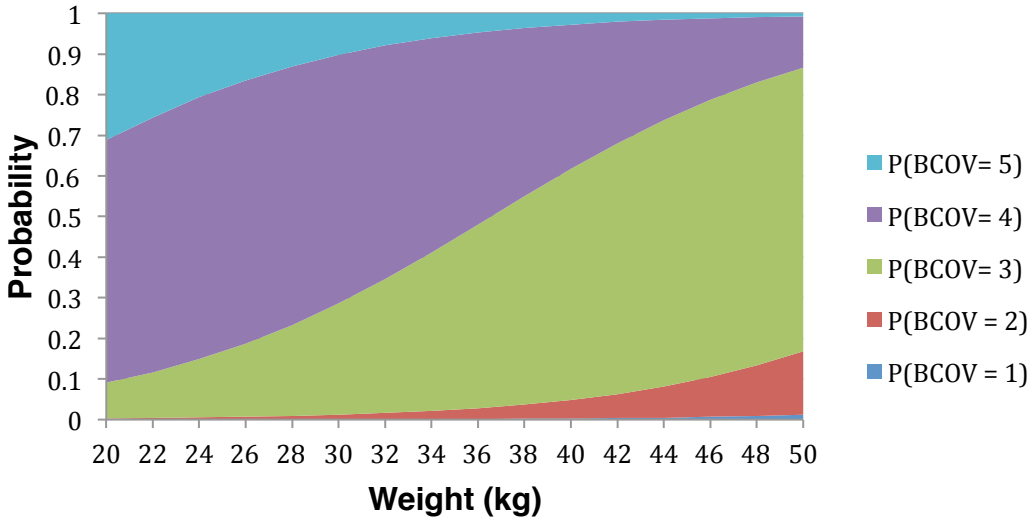


Figure 16. Sheep with larger weights tended to have lower BCOV scores ($P < 0.001$).

644 ***Tables***

645 Table 1. The effect of mulesing on BRWR scores at Day -1 and Day 42.
646 [AB] Means within a sampling time point without a common superscript are
647 significantly different (P = 0.03).

	Mean BRWR Day -1	Mean BRWR Day 42	Change in BRWR
Modified 'V' mules	2.30[A]	1.56[A]	-0.74
Conservative mules	2.19[A]	1.64[B]	-0.55

648

649

650 Table 2. The effect of mulesing on BCOV scores at Day -1 and Day 42. [AB]
651 Means within a sampling time point without a common superscript are
652 significantly different (P < 0.001).

	Mean BCOV (Day 0)	Mean BCOV (Day 75)	Change in BCOV
Modified 'V' mules	4.80[A]	2.67[A]	-2.13
Conservative mules	4.75[A]	3.13[B]	-1.62

653