Iron and laterality effects on healing of cautery disbudding wounds in dairy calves

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ABSTRACT

Hot-iron disbudding, the process of cauterizing the horn buds of calves or goat kids at an early age to prevent horn growth, is routinely practiced in dairy production. The wounds take weeks to heal and are painful to touch throughout this time. Possible strategies to hasten the healing of disbudding wounds are not well understood, but the type of iron used may be an important factor to consider. When evaluating strategies to hasten healing, a within-subjects design may be preferable, but laterality effects might act as a potential source of variation and confounding in this type of experiment. Our objectives were to compare healing after disbudding with 2 commercially available irons, and to determine whether wounds healed differently on the left versus the right side of the head. Ten Holstein calves 4 to 10 d of age were disbudded using the Rhinehart X50A electric disbudder (Rhinehart Development Corp., Spencerville, IN) on one horn bud and the Portasol gas disbudder (Portasol USA, Elmira, OR) on the other; side (left vs. right) was balanced between treatments. We scored wounds daily for the presence of 6 tissue types: attached necrotic tissue, detached necrotic tissue, exudate, granulation, crust, and epithelium. Surface temperature and size of the wound were measured twice-weekly using thermal and digital photographs, respectively. The type of iron used did not affect latency to re-epithelialize, which took on average (mean $\pm$ standard deviation) 53 ± 3 d and 55 ± 3 d for Portasol and Rhinehart wounds, respectively (range: 40–70 d). However, compared with Portasol wounds, those from the Rhinehart had fewer days of granulation tissue and tended to have more days of detached necrotic tissue. The Portasol tip had a smaller total surface area than the Rhinehart, which may have resulted in a less severe burn, causing the necrotic tissue to fall off sooner. The left side tended

to re-epithelialize faster than the right side (mean $\pm$ standard error: left 51 ± 3 d; right 57 ± 3 d) and have fewer days of crust. Left-sided wounds were also cooler and tended to be smaller than those on the right. To assess the external validity of these laterality effects in our primary experiment (experiment A), we analyzed wound healing data from 2 other disbudding studies, one on calves (experiment B) and one on goat kids (experiment C). We observed laterality effects in the opposite direction in Experiment B, but negligible effects in experiment C, indicating that the differences in laterality had low external validity; the biological meaning of this asymmetry is unclear. Nonetheless, if using a within-subjects design, asymmetries in wound healing should be considered to avoid confounding effects. In conclusion, wounds from both irons took 7 to 8 wk to heal, on average; other strategies to accelerate healing should be explored.

Key words: animal welfare, dehorning, cattle, goat

INTRODUCTION

Hot-iron disbudding, a common practice in dairy production, is the process of preventing horn growth through tissue cauterization at an early age (USDA, 2018). Disbudding is a welfare concern, resulting in not only acute pain, but increased pain sensitivity until the wounds have re-epithelialized, which takes from 6 to 13 wk (Adcock and Tucker, 2018). Others have reported increased sensitivity for at least 14 wk after the procedure (Casoni et al., 2019). In addition to potentially prolonging pain, long re-epithelialization times may increase the risk of infectious complications (Church et al., 2006). Thus, it is of interest to evaluate practical strategies to hasten healing after disbudding.

Several brands of disbudding irons are available. They differ in tip dimensions, heat capacity, and suggested application time, all factors that could influence healing. Although most disbudding studies report the brand of iron used, they are often missing other details, such as the model name (e.g., Kleinhenz et al., 2017), iron temperature (e.g., Stilwell et al., 2010), tip

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size (e.g., Mintline et al., 2013), or application time (e.g., Heinrich et al., 2010). To our knowledge, only one study has compared irons for disbudding. Wohlt et al. (1994) found no differences in cortisol response between 2 types of electric disbudding irons when measured for 12 h post-procedure. It is unknown how different irons affect wound healing.

Our objective was to compare wound healing after disbudding with 2 common commercial irons: the Rhinehart X50A fitted with a 15-mm tip (Rhinehart Development Corp., Spencerville, IN) and the Portasol Dehorney III with a 17-mm tip (Portasol USA, Elmira, OR). The Portasol has a larger tip circumference than the Rhinehart (17 vs. 15 mm) but a thinner edge (1 vs. 4 mm). We predicted that wounds from the Portasol would re-epithelialize faster, because the tip that touches the skin has a smaller total surface area than the Rhinehart. We used a within-subjects experimental design, because it is generally more powerful than a between-subjects design: it partitions out the variation due to individual differences (Greenwald, 1976). Nevertheless, laterality differences in wound healing can act as a source of variation and potential confounding in within-subjects designs. Thus, our secondary objective was to test whether disbudding wounds healed differently on the right versus the left side of the head.

MATERIALS AND METHODS

Part I: Iron Effects on Wound Healing

This study was carried out at the University of California-Davis Dairy Facility from November 2016 to May 2017. This facility was chosen due to its proximity to the researchers, which made it possible to collect data daily throughout the study. All procedures were approved by the University of California, Davis, Institutional Animal Care and Use Committee (protocol no. 19204).

Animals and Housing. We used all female Holstein calves ($n = 10$) born on the farm between November 14, 2016, and February 19, 2017. Due to time and resource limitations, we could not enroll calves past this period. At birth, calves were housed individually in outdoor plastic hutches (2.0×1.5 m) with an attached wire-fenced pen (2.0 m length $\times$ 1.5 m width $\times$ 0.9 m height). The enclosures were bedded with rice hulls approximately 15 to 20 cm deep and were spaced 0.5 m apart. Calves were offered 3.1 L of colostrum at birth, and then 1.9 L at 930 h and 1630 h for 5 d, after which they were switched to 1.9 L of milk replacer at each of the 2 meals (26% CP, 16% fat, 15% TS; Calva Products Inc., Acampo, CA) mixed at 150 g/L of hot water. Water and starter (8.3% CP, 2.8% fat, 4% crude fat;

Associated Feed and Supply Co., Turlock, CA) were provided ad libitum. Meals were increased by 0.5 L at 14 d of age and again at 24 d of age. At 35 d of age, calves were switched to bucket feeding, and a dry TMR was introduced. Weaning occurred at 60 d of age. At 70 d of age, calves were moved to an outdoor group pen, where they were fed a dry TMR twice daily. Girth circumference measurements were taken twice weekly after disbudding and converted to BW using the equation from Heinrichs et al. (1992) to obtain an estimate of ADG during the experimental period (mean $\pm$ SD: 0.64 ± 0.87 kg/d).

Treatments. Calves were disbudded at 4 to 10 d of age (mean BW $\pm$ SD: 41 ± 2 kg) using a Rhinehart X50A on one horn bud and a Portasol on the other. We chose this age to maximize data collection when calves were housed individually rather than in groups. The side (left vs. right) that each iron was applied to was alternated and balanced between calves. On the day of disbudding, the calf was restrained in a head device in her home pen (Jimenez et al., 2019) and a 5×5 cm patch was clipped on each side of the head to locate the horn bud. The calf received a cornual nerve block of 5 mL 2% lidocaine hydrochloride injected on each side with a 20-gauge $\times$ 25-mm needle. If the horn bud was not numb after 10 min (as assessed by a pin prick), an additional 2 mL was given, which always sufficed. Each iron was placed on the respective horn bud until a copper ring formed (mean duration of application per bud $\pm$ SD: Portasol 10 ± 3 s, Rhinehart 20 ± 5 s; mean iron temperature $\pm$ SD: Rhinehart: $433 \pm 23^\circ\text{C}$, Portasol: $436 \pm 28^\circ\text{C}$). The cauterized horn bud was not removed. The duration of iron contact was similar between sides (mean duration of application per bud $\pm$ SD: left 15 ± 7 s, right 16 ± 7 s), as was iron temperature (mean $\pm$ SD: left $437 \pm 29^\circ\text{C}$, right $432 \pm 22^\circ\text{C}$). Meloxicam tablets were administered orally (1 mg/kg) in a gelatin capsule (3.5 g; Torpac Inc., Fairfield, NJ) immediately after disbudding. One researcher (SJJA) performed all disbudding and, due to the nature of the procedure, was not blinded to the type of iron used.

Data Collection. Wounds were scored each day by 1 of 5 trained observers until they had completely re-epithelialized (0/1 scoring; Figure 1; inter-observer reliability: Cohen's $\kappa \geq 0.70$). Observers were blind to the type of iron used on each horn bud; however, it was generally possible to differentiate Portasol and Rhinehart wounds while necrotic tissue was present. We took digital photographs of the wounds every Monday and Thursday with a DSLR camera (D5300; Nikon Corp., Tokyo, Japan). Photos were taken 15 cm from the wound. A measuring tape was placed to the right of the wound and included in the photographs to evaluate wound size. During photography, the calf was

restrained and blindfolded in a head device in her home pen (Jimenez et al., 2019).

We used a thermal camera (first 5 calves: RAZ-IR Nano, Sierra Pacific Innovations, Las Vegas, NV; last 5 calves: T430, FLIR Systems Inc., Wilsonville, OR) to measure wound surface temperature every Monday and Thursday until re-epithelialization. Photos were taken out of direct sunlight using an umbrella placed over the calf's pen (2.7 m diameter; Morshade, Williamsport, TN). We placed a 5 × 5 cm cardboard frame around the wound to standardize the image area. The maximum temperature of the framed area was determined using image analysis software (IR Analyzer, Sierra Pacific Innovations; ResearchIR Max, FLIR Systems, Inc.; inter-observer reliability: intraclass correlation coefficient = 0.99).

Statistical Analysis. We used linear mixed models to evaluate the effect of iron (Portasol vs. Rhinehart) on the number of days each tissue type was present and on the latency for the wounds to re-epithelialize. Iron was fitted as a fixed effect and calf as a random effect. Exudate was too rare to warrant statistical comparisons. One calf developed digestive problems (diarrhea, undigested milk, lack of appetite) and was euthanized 46 d after disbudding, at which point the necrotic tissue had fallen off on both sides but neither of the wounds had re-epithelialized. For this calf, we included in the analysis only the number of days that attached and detached necrotic tissues were present.

We used linear mixed models to assess the effect of iron on the size and maximum surface temperature of the wound over 56 d following disbudding, after which time >50% of the wounds had re-epithelialized. For each model, data for each calf were averaged by week for each side, resulting in 6 to 9 data points per side per calf. Iron, days since disbudding, and their interaction were included as fixed effects, and calf as a random effect.

All models were fitted using the REML method implemented in the *nlme* (Pinheiro et al., 2017) package in R, version 3.4.1 (R Core Team, 2017). The unit of analysis was horn bud nested within calf. We controlled for side (left vs. right) by including it as a fixed effect in all of the models. Degrees of freedom were estimated using the containment method. Homogeneity of variance and normality were confirmed using residuals versus fits plots and Q-Q plots, respectively. A first-order autoregressive correlation structure was applied to models that included day relative to disbudding as a fixed effect. This correlation structure was selected based on the lowest Akaike information criterion value. We reported least squares means and standard errors, unless noted otherwise. When interaction effects were present ($P < 0.09$), we performed pairwise compari-

sons using Tukey's method with the *lsmeans* package (Lenth, 2016).

Part II: Laterality Effects on Wound Healing

We used the data collected in the experiment described above (experiment A) to investigate differences in wound healing between the left and right sides of the head. To examine the external validity of our results, we supplemented this analysis with data from 2 other disbudding studies, labeled experiments B and C. These 2 studies were included retrospectively and were designed to describe wound healing following cautery disbudding.

Experiment B. Forty-eight dairy heifers were disbudded or sham-disbudded at 3 or 35 d of age, as described in Adcock and Tucker (2018). Briefly, calves were given a lidocaine cornual nerve block before a heated iron (Rhinehart X50; $467 \pm 22^\circ\text{C}$) was applied to each horn bud for 18 ± 4 s. Calves received 1 mg/kg oral meloxicam immediately after disbudding. Tissue scores and surface temperature were measured twice weekly until the wound had re-epithelialized. Wound size was not recorded.

Experiment C. Eighteen female dairy kids were disbudded at 10 d of age (Alvarez et al., 2019). Briefly, a heated iron (Rhinehart X30; $501 \pm 30^\circ\text{C}$) was applied to each horn bud for 10 ± 2 s. No pain relief was provided, following the farm's standard operating procedure. Tissues in the wound bed were scored daily. Surface temperature and wound size were measured twice weekly.

Statistical Analysis

Experiment A. We repeated the analysis described in Part I for the number of days each tissue type was present and on the latency for the wounds to re-epithelialize, but including side as the independent variable, and controlling for iron effects. In the wound size and temperature models, we included side and days since disbudding as fixed effects.

Experiment B. We used linear mixed models to evaluate the effect of side on the number of days each tissue type was present and on latency for the wounds to re-epithelialize. Calf was fitted as a random effect.

We used linear mixed models to assess laterality effects on maximum surface temperature in disbudded and sham calves during the following 21 d, after which time the sham calves were removed from the experiment. We included a 3-way interaction between procedure (disbudded vs. sham), side (left vs. right), and days since disbudding as a fixed effect.

Portasol	Rhinehart X50A	Tissue and definition
Attached necrotic tissue: the edges of the copper ring are not separated from the scalp
Detached necrotic tissue: the edges outside the copper ring have started to separate from the scalp
Exudate: fresh blood; can be moist or freshly dried (discoloration has not yet occurred)
Granulation: light red/dark pink, opaque, bumpy tissue; may be observed underneath necrotic tissue
Crust: dried exudate; may be present underneath detached necrotic tissue; isolated specks are not counted
Epithelium: layer of translucent skin is present; the other tissue types are absent

Figure 1. The system used to evaluate tissue types in wounds after disbudding calves with the Portasol Dehorner III (Portasol USA, Elmira, OR) or Rhinehart X50A (Rhinehart Development Corp., Spencerville, IN) iron. Tissues were scored daily as present or not present until the wound had re-epithelialized. Multiple tissue types could be present in the wound at the same time.

In disbudded animals only, we analyzed the effect of side on surface temperature over a 63-d healing period, after which time >50% of wounds had re-epithelialized. We included the interaction between side and days since disbudding as a fixed effect.

Experiment C. The right side of 1 kid was excluded from the analysis due to early horn regrowth that prevented wound healing. We used linear mixed models to evaluate the effect of side on the number of days each tissue type was present and on the latency for the wounds to re-epithelialize. Kid was fitted as a random effect.

We used a linear mixed model to assess the effect of side on wound size over 50 d following disbudding, after which time >50% of the wounds had re-epithelialized. Side and its interaction with days since disbudding were included as fixed effects, and kid as a random effect.

All Experiments. In all experiments, models were fitted as described in Part I. To estimate how much of the variation in latency to re-epithelialize arose from differences between versus within subjects, variance components were estimated from ANOVA type I sums of squares using the R *VCA* package (Schuetzenmeister and Dufey, 2018) for random models.

RESULTS

Part I: Iron Effects on Wound Healing

Table 1 shows the effect of iron on the tissue types and latency to re-epithelialize. The type of iron used to disbud did not affect latency to re-epithelialize, which took 54 ± 8 d (mean $\pm$ SD; range: 40–70 d). Rhinehart wounds tended to have more days with detached necrotic tissue compared to wounds from the Portasol. Granulation tissue was present more often after disbudding with the Portasol than with the Rhinehart. We found no differences between irons for all other tissue types.

Portasol wounds tended to be larger than Rhinehart wounds 0, 7, and 14 d after disbudding. On d 28 after disbudding, Rhinehart wounds were larger than those from the Portasol (iron $\times$ days since disbudding: $F_{8,118} = 1.86$; $P = 0.072$; Figure 2).

Maximum wound temperature increased from d 42 to 56 ($F_{8,128} = 2.50$; $P = 0.015$; Figure 3), but we found no difference between irons ($F_{1,128} = 0.58$; $P = 0.445$; Portasol: $36.7 \pm 0.2^\circ\text{C}$; Rhinehart: $36.6 \pm 0.2^\circ\text{C}$).

Part II: Laterality Effects on Wound Healing

Experiment A. Table 2 shows the effects of side (left vs. right) on the tissue types and latency to re-epithelialize. Independent of the iron used, the right side tended to take longer to re-epithelialize than the left. The right side also tended to have more days of crust present. We found no differences between sides for other tissue types.

Wounds on the right side tended to be larger than those on the left ($F_{1,118} = 3.89$; $P = 0.051$; left 11.4 ± 0.7 mm; right 12.1 ± 0.7 mm). To examine this relationship further, we visually compared left vs. right wound sizes by days since disbudding. We found that the right side was not larger until 14 d, indicating that the size difference was not due to a larger initial wound but to factors during healing. Wounds were also warmer on the right side compared with the left ($F_{1,128} = 5.02$; $P = 0.027$; left $36.5 \pm 0.2^\circ\text{C}$; right $36.8 \pm 0.2^\circ\text{C}$), regardless of days since disbudding.

For latency to re-epithelialize, 27 and 73% of the total variance arose from variation between and within subjects, respectively.

Experiment B. Wounds on the left side tended to have more days with granulation and took longer to re-epithelialize than those on the right (Table 2).

When analyzing the first 3-wk period, side did not affect surface temperature ($F_{1,376} = 0.218$; $P = 0.641$),

Table 1. Number of days each tissue type was present and latency to re-epithelialize (LSM $\pm$ SE) after disbudding 10 calves with a Portasol Dehorner III (Portasol USA, Elmira, OR) or Rhinehart X50A (Rhinehart Development Corp., Spencer, IN) iron¹

Item	Portasol	Rhinehart	P-value	F-value ² _(num df, den df)
Tissue type				
Attached necrotic	19 $\pm$ 1	20 $\pm$ 1	0.300	1.23 _(1,8)
Detached necrotic	5 $\pm$ 1	8 $\pm$ 1	0.072	4.30 _(1,8)
Exudate	0 $\pm$ 0	1 $\pm$ 0	NA ³	NA
Granulation	16 $\pm$ 1	11 $\pm$ 1	0.008	13.60 _(1,7)
Crust	27 $\pm$ 2	24 $\pm$ 2	0.428	0.71 _(1,7)
Latency to re-epithelialize	53 $\pm$ 3	55 $\pm$ 3	0.525	0.45 _(1,7)

¹One calf was euthanized during the study and had to be excluded from analysis for granulation, crust, and latency to re-epithelialize.

²Subscript values show the numerator and denominator degrees of freedom.

³Not applicable.

Figure 2. Wound size (LSM $\pm$ SE) after disbudding 10 calves with the Portasol Dehorner III (Portasol USA, Elmira, OR) or Rhinehart X50A (Rhinehart Development Corp., Spencerville, IN) iron. Results are shown in relation to the days since disbudding. Measurements were taken until the wound had fully re-epithelialized. There were 20 wounds from 0 to 42 d since disbudding, 16 wounds at 49 d, and 14 wounds at 56 d. $^{\wedge}P \leq 0.075$; $*P = 0.018$.

nor did it interact with days since disbudding ($F_{5,376} = 0.27$; $P = 0.932$) or procedure (side $\times$ disbudded: $F_{1,376} = 2.62$; $P = 0.107$; left disbud $40.1 \pm 0.3^{\circ}\text{C}$; right disbud $40.2 \pm 0.3^{\circ}\text{C}$; left sham $40.6 \pm 0.3^{\circ}\text{C}$; right sham $40.0 \pm 0.3^{\circ}\text{C}$). Similarly, we did not observe a side effect or interaction with days since disbudding when considering the 9-wk period in disbudded calves only ($F_{1-17,716} \leq 0.84$; $P \geq 0.644$).

Between- and within-subjects variation contributed 59 and 41% of the total variance in latency to re-epithelialize, respectively.

Experiment C. Granulation was present for longer on the left side than on the right (Table 2). No effect of side on the presence of other tissue types or latency to re-epithelialize was observed. Side did not affect wound size (left: 14.0 ± 0.4 mm; right: 13.8 ± 0.5 mm; $F_{1,416} = 0.39$; $P = 0.535$), nor did it affect wound temperature (Alvarez et al., 2019). For latency to re-epithelialize, we observed more variation between than within subjects (58 vs. 42%).

DISCUSSION

Part I: Iron Effects on Wound Healing

Wounds took an average of 54 d to re-epithelialize (range: 40–70 d), regardless of the iron used. Using the same scoring system, we found that wounds took 62 d to re-epithelialize (range: 42–91 d) in calves disbudded with the Rhinehart iron (Adcock and Tucker, 2018).

A similar progression in healing has been observed for hot-iron brands, which take at least 10 wk to re-epithelialize in beef calves that were 4 to 7 mo old (Tucker et al., 2014a,b).

Although we found no difference in latency to re-epithelialize between irons, there were differences in tissue types present during the healing process. The necrotic tissue in Portasol wounds fell off after an average of 24 d, compared with 28 d when using the Rhinehart. Due to its thinner edge, the Portasol tip has a smaller total surface area that touches the skin than the Rhinehart. The Rhinehart also required a longer period of application to produce a uniform copper ring than the Portasol. Thus, the Portasol may have produced a less severe burn, explaining why the necrotic tissue fell off sooner. Although not measured in our study, burn depth, a predictor of healing time in humans (Merz et al., 2010), may have differed between irons and influenced latency for the necrotic tissue to detach. In human burn patients, necrotic tissue is excised to accelerate healing (David and Chiu, 2018), but the biological significance of a 4 d difference in latency for necrotic tissue to detach, as observed in our study, is unclear.

In contrast to the results for necrotic tissue, Rhinehart wounds had fewer days of granulation tissue than wounds from the Portasol. Because the Portasol has a larger tip circumference, the resulting wounds tended to be larger in diameter than those from the Rhinehart for the first 2 wk after disbudding. By wk 4, the pattern reversed, such that Rhinehart wounds were larger

Figure 3. Maximum surface temperature (LSM $\pm$ SE) after disbudding 10 calves with the Portasol Dehorner III (Portasol USA, Elmira, OR) or Rhinehart X50A (Rhinehart Development Corp., Spencerville, IN) iron. Results are shown in relation to the days since disbudding. Measurements were taken until the wound had fully re-epithelialized. There were 20 wounds from 0 to 42 d since disbudding, 16 wounds at 49 d, and 14 wounds at 56 d. Temperature increased from d 42 to 56 ($P = 0.035$).

Table 2. Number of days each tissue type was present and latency to re-epithelialize (LSM $\pm$ SE) after disbudding on the left versus the right side¹

Experiment and variable	Left		Right		<i>P</i> -value	<i>F</i> -value ² _(num df, den df)
	No. of days scored	% total scored days	No. of days scored	% total scored days		
Experiment A ³						
Attached necrotic	20 ± 1	38	19 ± 1	33	0.300	1.23 _(1,8)
Detached necrotic	5 ± 1	9	7 ± 1	13	0.249	1.55 _(1,8)
Exudate	1 ± 0	1	0 ± 0	1	NA ⁴	NA
Granulation	13 ± 1	28	13 ± 1	23	0.874	0.03 _(1,7)
Crust	22 ± 2	44	29 ± 2	48	0.059	5.09 _(1,7)
Latency to re-epithelialize	51 ± 3		57 ± 3		0.062	4.90 _(1,7)
Experiment B						
Attached necrotic	4.2 ± 0.3	23	4.0 ± 0.3	25	0.445	0.61 _(1,23)
Detached necrotic	2.6 ± 0.3	15	2.8 ± 0.3	17	0.649	0.21 _(1,23)
Exudate	1.6 ± 0.3	9	1.5 ± 0.3	9	0.802	0.06 _(1,23)
Granulation	5.3 ± 0.4	29	4.4 ± 0.4	27	0.072	3.57 _(1,23)
Crust	9.2 ± 0.6	50	8.9 ± 0.6	52	0.585	0.31 _(1,23)
Latency to re-epithelialize	64 ± 2		60 ± 2		0.007	8.88 _(1,23)
Experiment C ⁵						
Attached necrotic	15 ± 1	30	15 ± 1	31	0.214	1.67 _(1,16)
Detached necrotic	11 ± 1	21	12 ± 1	24	0.201	1.78 _(1,16)
Exudate	2 ± 1	5	3 ± 1	5	0.822	0.05 _(1,16)
Granulation	11 ± 1	22	9 ± 1	19	0.049	4.53 _(1,16)
Crust	29 ± 2	56	29 ± 2	59	0.996	0.00 _(1,16)
Latency to re-epithelialize	52 ± 2		49 ± 2		0.129	2.56 _(1,16)

¹Experiment A (current study), 10 calves; experiment B (Adcock and Tucker, 2018), 24 calves; experiment C (Alvarez et al., 2019), 18 goat kids. Tissues were scored daily in experiments A and C, and twice weekly in experiment B until the wound had re-epithelialized. The percentage of days each tissue type was present (out of the total number of days the wound was scored) is shown for descriptive purposes to allow for comparison of experiment B with the others. The summed percentage is >100 , because multiple tissues could be present in the wound at the same time.

²Subscript values show the numerator and denominator degrees of freedom.

³One calf was euthanized during the study and had to be excluded from analysis for granulation, crust, and latency to re-epithelialize.

⁴Not applicable.

⁵The right side of 1 kid was excluded from analysis due to early horn regrowth that prevented wound healing.

than those from the Portasol, likely due to the necrotic tissue remaining attached for longer in the Rhinehart wounds. After the necrotic tissue detaches, granulation tissue allows new epithelial cells to migrate into the wound. The larger the wound bed, the more granulation tissue is needed to complete the epithelialization process (Midwood et al., 2004), which could explain the greater occurrence of granulation tissue in the Portasol wounds.

We found no difference in wound surface temperature between irons. This result was not surprising, given that previous work has found no difference between the surface temperature of disbudded and non-disbudded tissue (Adcock and Tucker, 2018; Mirra et al., 2018). The temperature remained consistent during the first 6 wk after disbudding, and then increased by 2°C from wk 6 to wk 8. The biological significance of this increase is unclear. It is possible that it reflects increasing blood flow as vascular structures are repaired (Kloppenborg et al., 2001), but a sham group would be needed to test this interpretation.

When using healing time as a measure of welfare, neither iron appears to be advantageous. However, differences in operator technique might be important. Hot-iron disbudding can be performed by leaving the ring of tissue containing the horn bud intact, as we did in the current study, or by flicking it out with the iron. These 2 methods result in distinct wounds and, we would predict, unique healing profiles. We have also observed outside of this study that the Portasol can cut to the skull in a few seconds if pressure is applied. To avoid this, we let the Portasol rest on the skin, rather than applying pressure, resulting in a shallower burn. Changes in these techniques may alter the course of healing, as well as the efficacy of preventing horn regrowth (e.g., Sutherland et al., 2019), although the latter was not measured in the current study.

Other strategies to reduce healing time require exploration. A recent study evaluated the efficacy of an aluminum-based aerosol bandage spray on wound healing during a 3-wk period after hot-iron disbudding (Huebner et al., 2017). The authors found no differences between control and treated wounds for the first 2 wk after disbudding, but at wk 3 they observed less scabbing and purulent discharge in treated wounds. To validate this strategy, it would be necessary to follow its effects through formation of a new layer of epithelium. Another possible strategy to hasten healing is performing the procedure at a younger age, but this showed no benefit when comparing calves disbudded at 3 vs. 35 d of age (Adcock and Tucker, 2018). Healing times associated with alternatives to hot-iron disbudding, such as caustic paste and clove oil, have not been investigated.

In goat kids, clove oil causes less tissue damage but may be less effective at preventing horn growth than caustic paste or hot-iron disbudding (Hempstead et al., 2018). A comprehensive evaluation of the welfare outcomes (healing time, acute and chronic pain, pruritus, horn regrowth) associated with different disbudding methods and operator techniques is needed to establish best practices for disbudding.

Part II: Laterality Effects on Wound Healing

We observed laterality effects to varying degrees in all experiments, but the direction of these effects was inconsistent. In experiment A, wounds on the right tended to have more crust and take longer to re-epithelialize, and they were warmer and larger than wounds on the left. Conversely, in experiment B, wounds on the left tended to have more granulation and took longer to re-epithelialize, but showed no difference in surface temperature compared with those on the right side. In experiment C, goat kids had more granulation in the left wound than the right, but no other laterality effects were observed. Species differences in horn anatomy and tissue structure could account for this finding. There were also several methodological differences between the goat study and the calf studies: a different operator performed the procedure; no anesthesia or analgesia was given; the study took place in the spring and early summer; and kids were housed primarily indoors.

The mechanisms underlying these laterality differences in wound healing are unclear. The handedness of the operator during disbudding could result in more force being applied on their dominant side, resulting in a more extensive burn. We have no evidence for this, however, because the same right-handed operator performed disbudding in experiments A and B, in which opposite laterality effects were observed. Limited evidence is available in other species that cerebral lateralization may modulate immune responses on the left compared to the right side of the body. Brain lesions in humans and nonhuman species indicate that the left hemisphere potentiates immune function, whereas the right hemisphere suppresses it (Sumner et al., 2011). Some studies have found peripheral asymmetries in immune diseases, such as a greater prevalence of herpes zoster infection on the left side of the body (Dane et al., 2001). Individual differences in cerebral dominance have also been shown to influence wound healing. For example, left-hemisphere-dominant rats healed faster on the right side of their body, and vice versa (Shokouhi et al., 2008). To our knowledge, no other studies have investigated laterality differences in healing time in any species. However, a few studies have looked at laterality

effects on wound sensitivity after disbudding in calves, and have reported mixed results; Heinrich et al. (2010) found no difference in pain responses between left and right wounds, whereas Casoni et al. (2019) observed increased sensitivity on the right side.

In experiment A, the warmer side took longer to re-epithelialize. We did not observe asymmetrical surface temperatures in experiments B or C. The causal relationship between surface temperature and healing time is unclear. On the one hand, higher temperatures may be symptomatic of an increased inflammatory response to injury and, hence, prolonged healing. However, previous work suggests that surface temperature is a poor indicator of inflammation after burn injuries in cattle (Tucker et al., 2014b; Adcock and Tucker, 2018; Mirra et al., 2018). It is also possible that lateralization of skin temperature is independent of injury. Studies in humans report that temperatures are cooler on the left side of the body compared with the right in healthy adults, which the authors suggest may result from cerebral or peripheral immune asymmetry (Rimm-Kaufman and Kagan, 1996; Demirel et al., 2016). However, the inconsistent findings we found for surface temperature across studies in the current paper suggest that this asymmetry may not be biologically meaningful.

We used a within-subjects design to test iron effects on wound healing in experiment A, because this type of experiment is generally more powerful than a between-subjects design (Greenwald, 1976). However, the appropriate choice might depend on the relative magnitude of the between- and within-subject components of variation; a larger contribution of between-subject variation to the total variance would support the use of a within-subjects design. For experiments B and C, slightly more than half of the total variance in latency to re-epithelialize (59 and 58%, respectively) arose from variation between individuals. However, the reverse was true for experiment A. Although experiment A failed to show an effect of the iron on latency to re-epithelialize, it may still have contributed to increased variation within calves, and thus the high magnitude of within-subject variation (73%) may be exaggerated. Overall, however, these results do not provide particularly compelling support for favoring a within-subject design over a between-subject one in studies comparing the influence of various treatments on wound healing after disbudding.

Wounds were highly variable in how long they took to re-epithelialize: some took 5.7 wk, and others up to 10 wk. Adcock and Tucker (2018) and Alvarez et al. (2019) also reported substantial ranges in wound healing times for experiment B (6–13 wk) and C (5–9 wk), respectively. In addition to horn-bud-level factors discussed previously, between-individual differences in

energy status, immune function, chronic stress, and wound-directed behaviors, among other factors, likely contribute to this variation. The nutritional plane may also play a role, because energetic demands can more than double after a severe burn in humans, and nutritional deficits impair healing (Clark et al., 2017). The growth rate of 0.64 kg/d in the current study is below the recommended target of 0.8 kg/d (Sherwin et al., 2016), and healing times may be shorter in calves fed a more biologically appropriate nutritional plane. Seasonally dependent environmental variables, such as duration of daylight, may also contribute to variation in healing (e.g., Nelson and Blom, 1994; Kinsey et al., 2003). Investigating the possible physiological, behavioral, and environmental factors that drive within- and between-individual variation in healing could provide valuable insight into how to hasten this process for all animals.

CONCLUSIONS

Disbudding with the Portasol or Rhinehart X50A resulted in similar healing times: 53 and 55 d, respectively. Wound healing differed between the left and right sides in calves, but this result had low external validity, and the biological meaning of this asymmetry is unclear. A sizable amount of the variation in wound healing was due to differences within individuals, indicating that a within-subjects design does not have a clear advantage over a between-subjects design in this research context. Practical strategies to improve healing after disbudding and an understanding of the factors affecting this process are needed.

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