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The age of surgical castration affects the healing process in beef calves[1]

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ABSTRACT: Castration is painful for calves. Castrating at an earlier age is often recommended, but little is known about how this affects the healing process or the pain experienced. We compared incision closure, swelling and pain sensitivity of beef calves surgically castrated at 3 (range 0 to 8 d; n = 16) or 73 (range 69 to 80 d; n = 15) d of age. Closure of the incision, as measured with a 5-point scale (1 = fresh wound, 5 = no longer visible), weight gain, and inflammation (skin temperature and swelling, as measured by scrotal circumference) were recorded on d 1, 3, 7, 11, 15, 18, 21, 25, 32, 39, 45, 61, and 77 after the procedure, until all incisions were fully healed. On these same days, pain sensitivity was assessed by applying a known and increasing force with von Frey hairs (0.02 to 300 g-force) at the edge of the castration wound and at a control site, approximately 2 to 5 cm anterior to the teats, until animals showed a behavioral response or the highest force was reached. The incisions of younger calves healed more quickly than older ones [fully healed, median (95% confidence interval); 39 (32 to 61) vs. 61 (61 to 77) d; P = 0.002], however, they had relatively more swelling in the days after castration ($P < 0.001$). Younger animals reacted to lighter pressure of von Frey hairs compared to older calves especially in the first stages of healing process ($P < 0.001$), and there were other signs indicative of inflammation processes in this region at this time. However, there was no difference in the control site for either age group. In addition, it took longer for older calves to recover their daily weight gain after the procedure ($P < 0.001$). Taken together, these results paint a mixed picture about the effects of age of surgical castration. Calves castrated soon after birth experience more tissue swelling and show more signs of pain, but their incisions heal sooner and their weight gain is less affected, when compared to animals castrated around 73 d of age.

Key words: age, castration, cattle, pain, von Frey

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INTRODUCTION

A majority of beef calves are castrated and surgical method of castration is the most common (USDA, 2007) and is known to be painful. It results immediately in vocalizations, increases in plasma cortisol and substance P, restlessness, decreases in stride length (e.g., Coetzee et al., 2008; Currah et al., 2009; Molony et al., 1995), and altered standing posture (Ting et al., 2003; Webster et al., 2013). However, it is not clear how long the pain associated with the procedure lasts.

Healing after surgical castration is thought to take between 10 d (Molony et al., 1995) and 9 wk (Fisher et al., 2001; Stafford et al., 2002; Mintline et al., 2014). Variation among studies is due, in part, to how healing is assessed. Some emphasize swelling and other measures of inflammation (e.g., Molony et al., 1995), while others also include wound closure (e.g., Stafford et al., 2002). The shortest healing time reported (10 d) also involved the youngest calves, castrated at the age

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of 5 to 7 d (Molony et al., 1995). We hypothesize that age of procedure may affect the healing rate of these wounds for 2 reasons: 1) younger animals may have faster growth rates (and therefore may have quicker tissue regeneration) and 2) there is less tissue damaged initially, as calves near birth are smaller than those castrated at the industry norm of 70 to 80 d (USDA, 2007).

Assessing pain during the healing process can be evaluated by observing the avoidance behavior during palpation of the area (Prunier et al., 2013). Topical anesthetic reduces responses to palpation of surgical castration wounds, compared to controls, for 24 h after the procedure (Lomax and Windsor, 2013), but no research has evaluated pain sensitivity in the days and weeks after surgical castration, nor the effect age of the procedure has on these responses.

Our objective was to assess the effect of age on healing and pain sensitivity after surgical castration of beef calves.

MATERIALS AND METHODS

This experiment was conducted at the University of California Sierra Foothills Research and Extension Center from November 2013 to January 2014. It was reviewed and approved by the University of California, Davis Institutional Animal Care and Use Committee. Surgical castration without any form of pain control is part of the standard operating procedure at this facility. No animals were castrated solely for the purposes of this experiment.

Calf Husbandry

Thirty-one Angus-Hereford cross-bred bull calves were divided into 2 replicates (n = 11 and 20). Calves were with their dams and all treatments and replicates were grazed together. All animals were on pasture except for the first 24 h after castration of the first replicate, when they were kept in a pen (18 × 21 m; 16 m^2/pair) adjacent to the chute. This facilitated testing initially, but was not needed for the subsequent replicate. All calves were nursing at the time of the experiment. Water was available ad libitum and grazing was supplemented with hay, due to drought conditions.

Castration and Procedures

To be able to evaluate the effect of age on healing calves were castrated at either younger (3 ± 0.5 d, mean ± SE; range 0 to 8, n = 16) or older (73 ± 0.8 d of age, range 69 to 80, n = 15) age. Both age groups were equally represented in the 2 replicates described above. In total, castration took place on 2 separate days, once for Replicate 1 and once for Replicate 2. Older calves were handled for 2 d before the start of the experiment to familiarize the animals to human handling and the calf table (Paul Calf Table, Adrian J. Paul Co., Duncan, OK) used during data collection.

On the day of castration, calves were separated from their dams and placed together in a pen. The first replicate was then caught from horseback with a rope around their back legs. Once on the ground, the front legs were tied together and 2 people restrained the animal. For practical reasons, the second replicate was restrained on a calf table. After the calf was restrained, the scrotal sac was shaved and cleaned with chlorhexidine solution and alcohol. A ring block of 3 mL of 2% lidocaine anesthetic was applied to the distal third of the scrotal sac, which was then cut an average of 3 min later (SE = 19 s; range 1 to 7 min) using a 20-scalpel blade. Each testicle with semen cord was exposed and the surrounding fat pushed proximally before testicle was detached by pulling.

Unless otherwise specified, all measures were collected 13 times after castration: on d 1, 3, 7, 11, 15 (± 1 d), 18 (± 1 d), 21, 25, 32 (± 5 d), 39 (± 2 d), 45 (± 1 d), 61 (± 4 d), and 77 (± 4 d), at which time all calves were healed. For data collection, calves were restrained on the calf table with a head catch and a side squeeze bar. The calf was weighed (Paul Sure-Weigh Scale, Adrian J. Paul Co.), and the table was then tilted horizontally. The back legs were tied with ropes so that the right (upper) leg was lifted and the left (lower) leg was on the table. The calf could move its legs back and forth, but not bring them together. For animals too small to be properly held by the head gate and side squeeze bar, the neck and shoulder were held by a handler.

Healing: Incision Closure, Skin Temperature, and Tissue Swelling. Incision closure was scored by same observer using a 5-point scale (Fig. 1; a modified version of the system used in Mintline et al., 2014). Intra-observer reliability, as measured by kappa statistic (PROC FREQ in SAS Software, version 9.4; SAS Inst. Inc., Cary, NC) was calculated by scoring digital photographs (D60, Nikon Corp., Tokyo, Japan) of the wound twice (κ = 0.91). The skin temperature of the scrotal sac was measured approximately 1 cm distance from the center of the wound using a digital thermometer with thermocouple (model HH-25TC, Omega Engineering, Inc., Stamford, CT). Tissue swelling in the scrotal area was measured with digital calipers (diameter) except for Replicate 1 on the day of castration, when a scrotal measuring tape (circumference) was used. We switched methods because the calipers were faster and more practical. The relationship between the caliper measures and the tape measures was calculated (R^2 = 0.67) and used to generate approximate measures to replace missing data. Values on the day of castration were taken both before and immediately after the testicles were re-

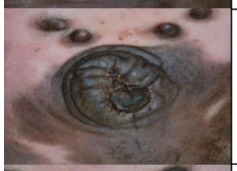
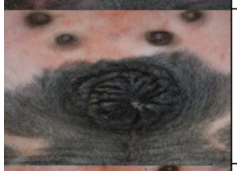
Example	Score	Description
	1	The incision runs the length of the scrotum and tissue is exposed in this area. Scabbing is uncommon but may be present in isolated locations at the edges or across the center of the wound.
	2	The incision is greater than or approximately ¾ the length of the scrotum and scabbing is present.
	3	The incision is scabbed and is less than ¾ of the scrotum
	4	The wound/incision site is less than ¼ of the scrotum, and is smaller than or equal to the size of a teat visible in the photo. A small scab or discoloration is present at the center of the scrotum/wound site.
	5	The incision site is no longer visible. There is no scabbing or discoloration anywhere on the scrotum.

Figure 1. A 5-point system for scoring wound healing following surgical castration of calves, adapted from Mintline et al. (2014).

moved. Scrotal size was not measured before castration for 4 calves (2 older and 2 younger).

Pain Sensitivity. Pain sensitivity was assessed by using 9 von Frey filaments (0.02, 0.4, 1, 2, 6, 10, 26, 100, 300 g-force; Touch-Test 20 Piece Kit, North Coast Medical Inc., Gilroy, CA) applied in sequential order. Two locations were tested: on the scrotal sac at the edge of the wound (wound site) and on the right-side groin area, approximately 2 to 5 cm anterior to the teats (control site). Testing order (wound or control site tested first) was balanced based across age group and body weight. Calves were shaved in both locations before each testing session. A single operator performed all the tests and a second individual rested a hand on the shoulder of the animal. The tip of each filament was pressed against the skin until the filament bent. Movement of the tail or any of the legs was recorded as a response and the test was ceased. The filament was then removed from the skin and, if no response was seen, the next sequential filament was applied. If the test was interrupted (due to a loud noise or other distraction, the animal urinating, defecating, or coughing) testing paused until the animal was still and then resumed. If testing was repeatedly interrupted, or the animal would not lie quietly, the test was abandoned (7% of tests; in total 28 tests of older animals and 25 tests of younger calves).

Weight Gain. Daily gain was calculated for each calf in 2 ways. The overall ADG was calculated by taking the difference from end weight and the baseline weight and dividing by the number of days between the measures. The ADG during the healing process was calculated by taking the difference between the weight when the calf's incision was fully closed (score of 5) and the average of the weights on d 3 and 7 of measurement (as a baseline, using multiple measures to reduce variability) and then dividing by the number of days between the measures; henceforth called ADG during the healing process.

Statistical Analysis

One older calf contracted a severe illness and 1 younger calf died during the experiment; their data were excluded. In addition, one measurement/animal from 3 animals were missing: 1) a younger calf had no d 1 measures taken 2) another younger calf was not tested on d 11 due to illness and finally 3) one calf was not included in the ADG analysis, due to a missing pre-castration weight. All other data from these 3 animals were included. Threshold for statistical significance was considered $P < 0.05$.

Birth weights of younger and older calves were compared with Wilcoxon 2-sample exact test (PROC NPAR1WAY of SAS). The effect of age of castration on ADG, skin temperature and overall scrotum size as well as the percentage change from baseline (immediately after testicles were removed) were analyzed using lin-

ear mixed models (PROC MIXED of SAS) that included terms for age at the time of procedure, time since castration (as a linear term) and the interaction (time since castration × age of the procedure) with calf (age at the time of castration) as the subject of the random term.

Animals that did not respond to even the highest of von Frey filament were assigned with a maximum value of 850 g to represent of next step in g the force on the logarithmic scale. Data from the control and wound sites were analyzed separately. Responses to the von Frey filaments were analyzed in 2 ways. First, a linear mixed model, as described above, was used to look at time since castration in relation to age of the procedure. Second, the effect of healing score on pain sensitivity was analyzed using a linear mixed model (PROC MIXED) that included terms for age at the time of castration, healing score and the interaction (healing score × age) with calf (age at the time of castration) as the subject of the random term. In this second type of analysis, if a calf was at the same healing score for multiple observations, the average of the von Frey results were used.

For all mixed models, variance components covariance structure provided the best model fit and the Containment method was used to estimate degrees of freedom. To ensure that data met the assumption of normality, residuals were visually inspected and evaluated with the Shapiro-Wilk test statistic. Average daily gain data were Winsorized at the fifth and 95th percentiles to meet the assumption of normality.

The effect of age on the time it took to reach each healing score after castration was analyzed with a Wilcoxon-Mann–Whitney test (PROC NPAR1WAY of SAS). Medians and 95% distribution free confidence limits were calculated with the univariate procedure (PROC UNIVARIATE of SAS). Not all animals were observed at every score, thus the sample size varies from 9 to 15 at each stage of healing. The relationship between ADG during the healing process and the time it took to for the incision to fully close (to reach score 5) was evaluated separately for each treatment using a Spearman correlation (PROC CORR of SAS).

RESULTS

Body Weight and Gain. Calves in the younger group had, on average, higher birth weights than calves in the older group (33 ± 1 vs. 29 ± 1 kg; Wilcoxon 2-sample exact test: S = 157, $P = 0.019$). There was an interaction between age of castration and time after the procedure on ADG ($F_{1,332} = 26.0$, $P < 0.001$, Fig. 2); older animals gained less weight in the days immediately after castration than younger calves. Average daily gain was lower in the days immediately after surgical castration ($F_{1,332} = 21.6$, $P < 0.001$). Overall, younger calves had greater daily weight gain after castration compared to older ones ($F_{1,26} = 27.1$, $P < 0.001$; 0.7 ± 0.03 vs. 0.3 ± 0.03 kg/d).

Skin Temperature, Incision Closure, and Tissue Swelling. Skin temperature at the wound site was lower for younger animals ($F_{1,27} = 5.0$, $P = 0.033$; 29.9 ± 0.2 vs. 30.5 ± 0.2°C). Temperature decreased as healing progressed ($F_{1,27} = 5.0$, $P < 0.001$, Fig. 3). There was no interaction between age and days after castration ($F_{1,334} = 0.3$, $P = 0.603$).

Young calves had smaller scrotums before and after castration, compared with older animals ($F_{1,27} = 44.0$, $P < 0.001$, caliper 29 ± 0.4 vs. 33 ± 0.4 mm). In all animals, scrotums were most swollen, as measured by size, in the first days after castration ($F_{1,400} = 357.7$, $P < 0.001$, Fig. 4a). In addition to the absolute size, relative change in scrotum size was dependent on interaction of time and age the procedure was performed ($F_{1,320} = 10.0$, $P = 0.002$, Fig. 4b): younger calves remained swollen longer than the older calves (25 d in younger calves, on average to resolve

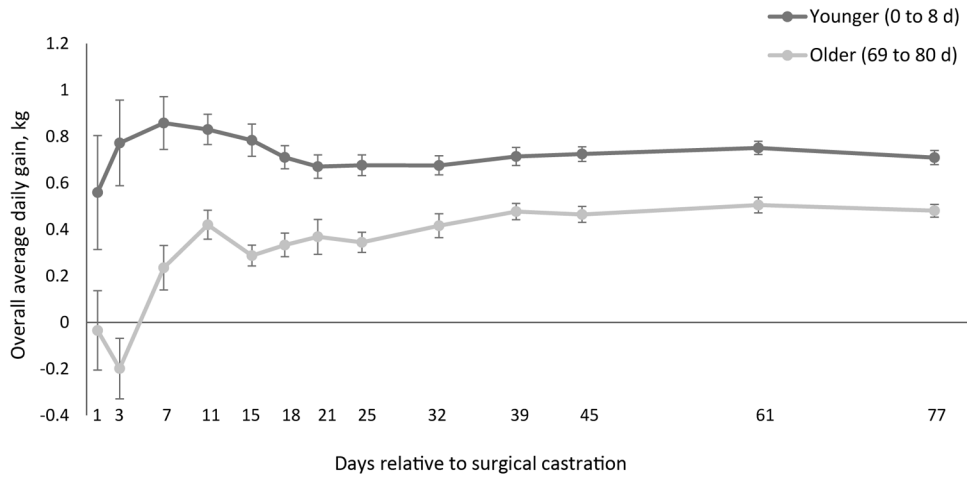


Figure 2. Mean (± SE) overall ADG of calves in the days relative to surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age.

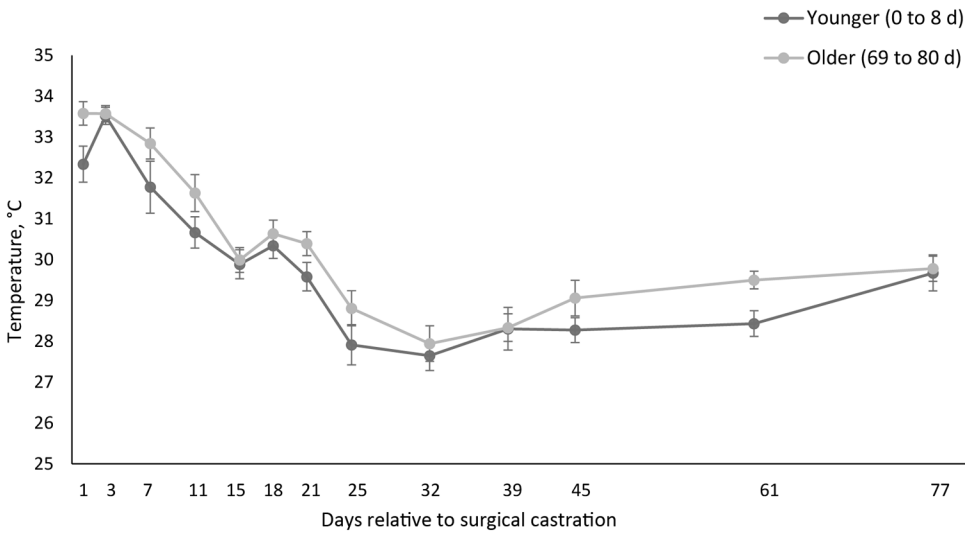


Figure 3. Mean (± SE) skin temperature 1 cm from wound in the days relative to surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age.

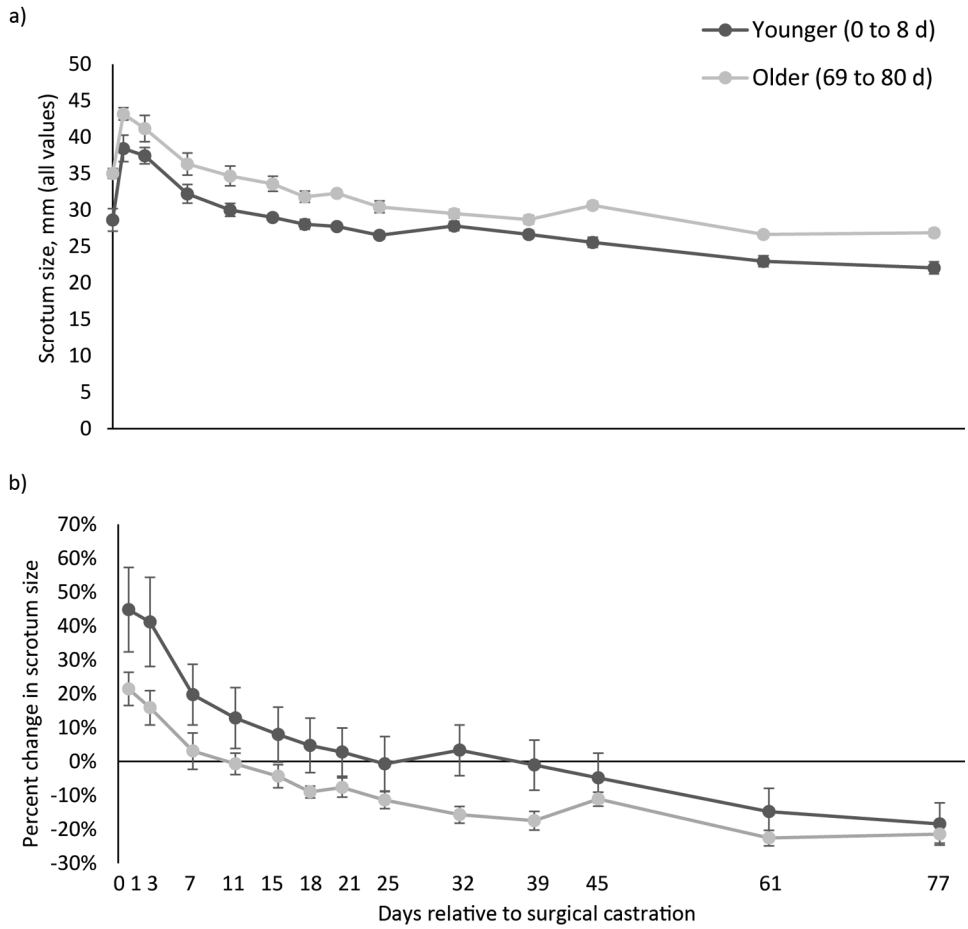


Figure 4. a) Mean (± SE) size of the scrotum and b) relative change of scrotum size of calves in the days relative to surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age.

swelling vs. 10 d in older animals). Relative change in scrotum size was also highest in the days just after castration ($F_{1,320} = 352.7$, $P < 0.001$). There was no main effect of age of castration over the course of the experiment on relative change in scrotum size ($F_{1,25} = 2.0$, $P = 0.165$).

Calves castrated at younger age reached healing scores 2, 4, and 5 sooner than older calves ($Z \geq 2.8$, $P < 0.005$) and there was a trend for reaching score 3 faster ($Z = 1.8$, $P = 0.074$; Fig. 5). There was little relationship between ADG during the healing process and

time to incisions to close for either treatment younger: $r = 0.30$, $P = 0.272$; older: $r = –0.31$, $P = 0.270$).

Sensitivity. Age at the time of the procedure, time since castration and healing score all affected responses to the von Frey filaments. First, there was an interaction between time since castration and calf age in sensitivity of the wound site ($F_{1,319} = 14.6$, $P < 0.001$). Wounds were more sensitive soon after castration, compared to later on ($F_{1,319} = 46.5$, $P < 0.001$, Fig. 6a), but overall, younger calves were more

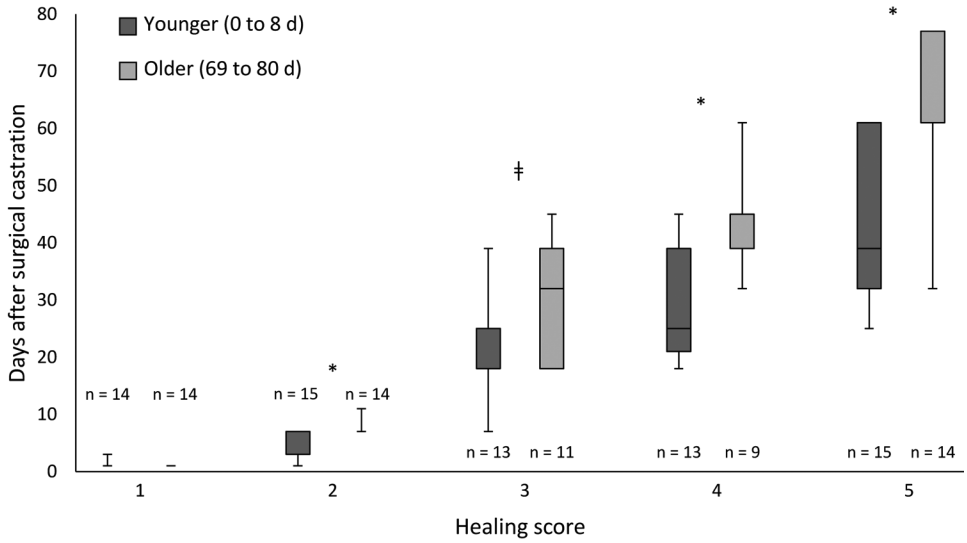


Figure 5. Days to reach each healing score relative to surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age. Not all animals were observed at every score, thus the sample size varies. Differences between treatments are indicated by * ($P \leq 0.005$) and trends by ‡ ($P = 0.074$).

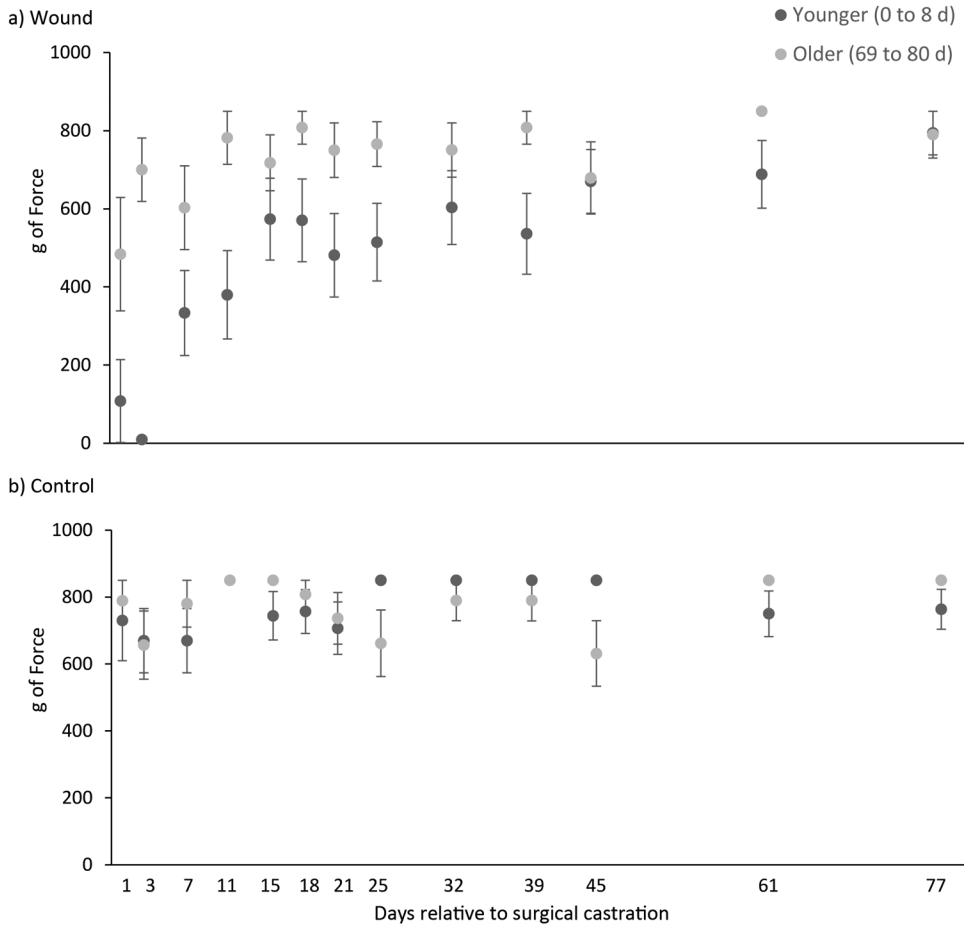


Figure 6. Mean (± SE) sensitivity at the a) wound site and b) control site of calves in the days relative to surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age.

sensitive to palpation of the wound compared with older calves ($F_{1,27}$ = 25.7, P < 0.001, 497 ± 30 vs. 736 ± 21 g force). There were no differences between treatments or time since castration at the control site (treatment: $F_{1,27}$ = 0, day: $F_{1,320}$ = 2.3, $P \geq 0.134$, Fig. 6b). Second, when sensitivity was analyzed in relation to healing scores, there was an interaction between healing scores and age of castration at the wound site ($F_{4,91}$ = 3.4, P = 0.012). The wounds were more sensitive at earlier stages of healing ($F_{4,91}$ = 12.1, P < 0.001, Fig. 7a), and, overall, as above, calves castrated at younger age were more sensitive at the wound site compared with older calves (P < 0.001). Healing score had no effect on sensitivity at the control site ($F_{4,88}$ = 0.9, P = 0.465), nor was there any interaction with age of castration ($F_{4,88}$ = 1.8, P = 0.135, Fig. 7b).

DISCUSSION

This study paints a mixed picture of how age at the time of surgical castration affects healing and pain experienced. Calves castrated soon after birth have more pronounced scrotal swelling that lasts longer and they show more signs of pain, but their incisions heal sooner and growth is less affected by the procedure, when compared to animals castrated around 73 d of age. All castration wounds were completely healed by 77 d after the procedure and were more sensitive in the early stages of the process for both age groups.

The time needed to heal, 77 d or 2.6 mo, are similar to those reported by Stafford et al. (2002) where calves were surgically castrated at the age of 2 to 4 mo. However, Molony et al. (1995) reported shorter heal-

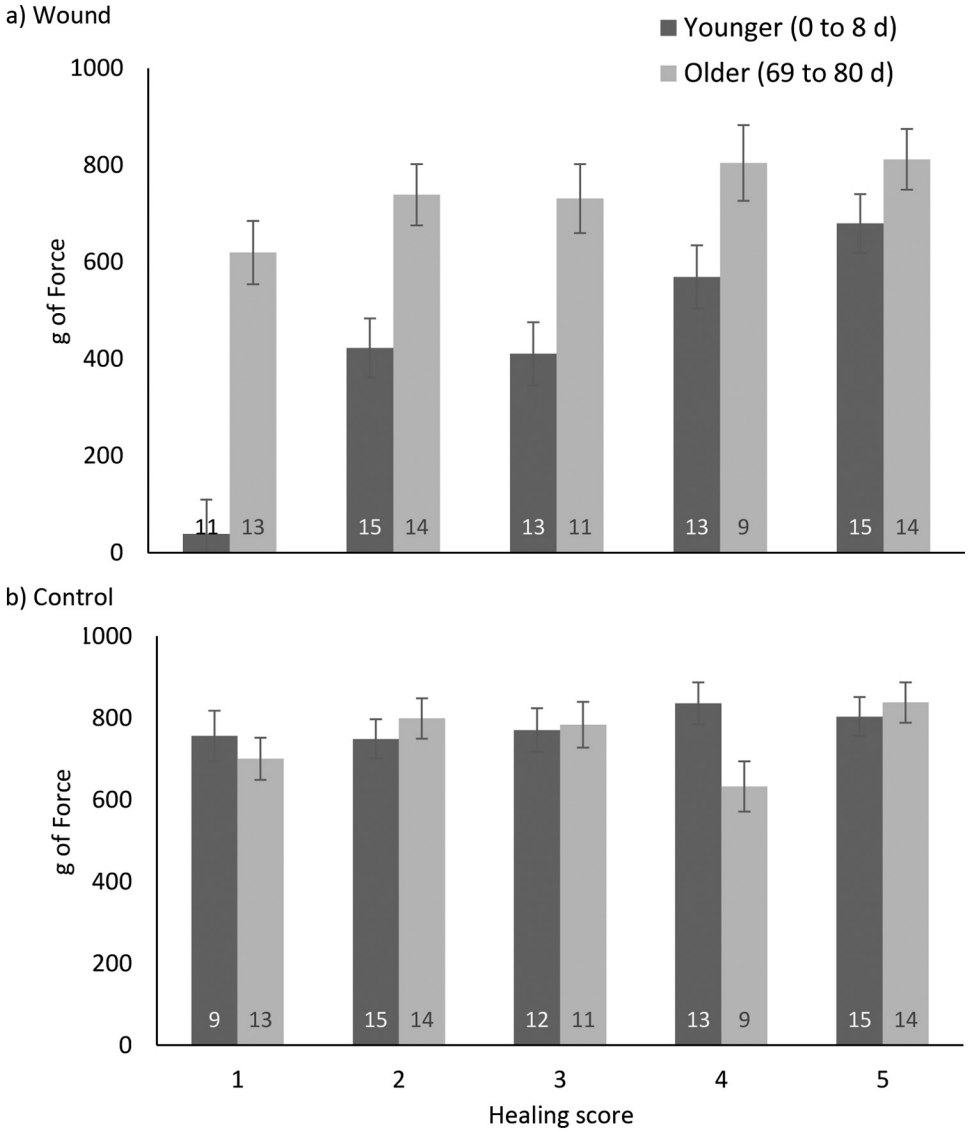


Figure 7. Mean (± SE) sensitivity at each healing score in the a) wound site and b) control site of calves following surgical castration at a younger (0 to 8 d) or older (69 to 80 d) age. Not all calves were observed at each healing score, thus the sample size varies (n reported at the bottom of each bar).

ing time (10 d) when calves were castrated during their first week of life, possibly because their definition of this process emphasized signs of inflammation over wound closure. Indeed, in our results, swelling resolved in older calves by d 10. Younger calves, however, took 25 d for the wound area to return to the same size it was immediately after castration occurred.

It is unclear why the incisions of younger animals healed more quickly than older animals. There are several possibilities. First, younger animals had smaller scrotums, thus less tissue was damaged and removed, compared the older ones. A smaller amount of tissue damage is often a rationale given for performing painful procedures at a younger age. This may explain why the incisions of the younger calves closed sooner and also why their growth was less affected by the procedure compared the older animals. Similarly, piglets castrated at younger age also healed faster (4 vs. 28 d; Heinritzi et al., 2006). A second explanation for our results is that incision closure might happen more quickly in younger animals because their growth rate is higher than older animals (0.7 vs. 0.3 kg/d in this study), and that growth rate may correspond with degree of tissue regeneration. However, birth weight was confounded in our treatment groups, with the younger animals born heavier than older ones, for reasons that are unclear to us. This confound may have influenced our treatment differences in ADG, or growth rate and thus possibly tissue regeneration. Overall, we found no relationship between growth rate and time to incision closure. Additional work, without a confounding difference in birth weight, is needed. Finally, understanding why there is more swelling in younger calves also remains difficult to explain.

Skin temperature was included as a measure of inflammation, but it does not seem to correspond to a more traditional indicator, namely swelling or scrotal size. Instead, skin temperature declined over the course of the healing process, perhaps because of hair growth. The entire area was shaved initially, but going forward, only the sites of von Frey testing were clipped at each data collection. Indeed, Moya et al. (2014) found no difference in skin temperatures of castrated and non-castrated calves. We also found that younger calves had, on average, 0.6°C lower skin temperature than older ones. The reasons for this are unclear, as are the biological implication of this small difference.

The phases of incision healing and ultimate closure and time since castration corresponded with mechanical nociceptive thresholds. Calves were more sensitive the earlier they were in the healing process. This was a localized effect; there were no differences in responses to the control site associated with the state of the incision. Swelling may also influence sensitivity, at least in young calves. These animals were, on average, more responsive to palpation with von Frey filaments, compared to the older animals.

Younger animals may be more sensitive to palpation than older calves for several reasons. Performing painful procedures near birth has been shown to increase sensitivity to other sources of pain later on in life. For example, lambs castrated at 1 d of age are more responsive to tail docking several weeks later than those castrated at 10 d of age (McCracken et. al., 2010). Similarly, 3- to 4-d old ewes exposed to tail-docking showed more pain-related responses during parturition as adults than control ewes (Clark et al., 2014). Alternatively, or perhaps in addition, older calves may have been less responsive to palpation at the edge of the wound, not because less pain was experienced, but because they perceived the restraint and testing as more aversive than younger calves. Similar to our findings, Guesgen et al. (2011) observed younger male lambs to be more sensitive to thermal stimulus than older ones (1 to 12 d). Fear and stress can reduce the reactivity to painful stimulus (Herskin et al., 2004). The younger calves were habituated to human handling from their first days of life, so perhaps they perceived restraint and contact with us as more benign than their older counterparts. Other measures of pain, without human contact, may help us untangle these possible effects. For example, careful investigation spontaneous facial expression or body postures could also be used to study castration pain, as others have with the sickness response (Gleerup et al., 2015). Similarly, provoked pain during palpation associated with visceral inflammation of an adult cow was detected with aid of heart rate monitoring and observations of posture (Stojkov et al., 2015) thus monitoring heart rate, posture and facial expressions could be used in future experiments.

Conclusions

Overall, surgical castration wounds are inflamed for 1 to 3 wk and take 11 wk for the incisions to heal. There is evidence of pain in response to palpation of the wound during this process, particularly soon after the procedure and at earlier stages of incision closure in calves castrated in the first week of life. Taken together, the effects of age of surgical castration are mixed. Calves castrated soon after birth experience more swelling and show more signs of pain, but their incisions heal sooner and their weight gain is less affected, when compared to animals castrated at 10 to 11 wk of age.

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