Analgesic effects of intraneural injection of ethyl alcohol or formaldehyde in the palmar digital nerves of horses

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Objective—To determine analgesic effects of intraneural injection of ethyl alcohol or formaldehyde in the palmar digital nerves of horses.

Animals—6 horses.

Procedures—Ethyl alcohol was injected in the medial palmar digital nerve of 1 forelimb, and formaldehyde was injected in the contralateral nerve. The lateral palmar digital nerve in 1 forelimb was surgically exposed, but not injected, and the contralateral lateral palmar digital nerve was not treated. For each heel, severity of lameness in response to experimentally induced heel pain (lameness score and peak vertical force), thermal reaction time, and heel skin sensitivity scores were recorded. Heel pain was experimentally induced by advancing a threaded bolt through a custom-made horseshoe to apply pressure to the palmar horned aspect of the hoof. Horses were followed up for 112 days, when a subset of nerves was sampled for histologic analysis.

Results—Alcohol and formaldehyde significantly reduced all measures of heel pain, and analgesia was evident over the 112 days of the study. Pastern circumference was significantly greater for formaldehyde-treated than for alcohol-treated limbs. Histologic evaluation showed preservation of nerve fiber alignment with an intact epineurium, loss of axons, axon degeneration, fibrosis, and inflammation in alcohol-treated and formaldehyde-treated nerves.

Conclusions and Clinical Relevance—Results suggested that intraneural injection of either ethyl alcohol or formaldehyde in the palmar digital nerves of horses resulted in substantial, but partial, heel analgesia that persisted for at least 112 days. No advantage of formaldehyde over alcohol was found, and formaldehyde resulted in greater soft tissue inflammation. (Am J Vet Res 2014;75:784–791)

Many horses with chronic lameness secondary to heel pain become unwanted because of the high costs of long-term management. Palmar digital neurectomy has been used since the late 1700s to treat heel pain in horses, but this procedure is generally reserved for chronic cases refractory to other treatments because of the risks associated with it. Adverse outcomes reportedly associated with palmar digital neurectomy include painful neuroma formation, sepsis of the third phalanx, deep digital flexor tendinosus rupture, luxation of the distal interphalangeal joint, hoof capsule slough, and navicular bone fracture as well as superficial injury resulting from an inability to feel the heel and sole of the foot. A recent retrospective study of the pull-through technique for palmar digital neurectomy reported a total complication rate of 17% and a painful neuroma incidence of approximately 5%.

Alternative methods of neural analgesia that do not include nerve transection might avoid neuroma formation and other potential complications. Also, although navicular syndrome may be the most common cause of chronic lameness in horses, other injuries, such as distal phalanx wing fractures, sidebone, fracture of the navicular bone, and collateral ligament injuries can be a cause of chronic heel pain. For these conditions, partial or transient (weeks to months) pain relief during the healing process followed by a return of sensation once the condition has healed may be beneficial, as this may allow horses to return to normal activities and could potentially decrease the incidence of complications associated with a lack of heel or sole sensation.
Medical-grade ethyl alcohol is approved for perineural injection in human medicine and has been used for relief of multiple painful conditions, including trigeminal neuralgia, ankle-foot spasticity, and intracranial pain secondary to end-stage malignancy. In a second study, a reduction in ankle-foot spasticity was achieved immediately after injection of ethyl alcohol in 99% of patients, and mean pain-free duration was 46 months. In a second study, a reduction in ankle-foot spasticity was achieved in 90% of patients for the full 6-month follow-up period, and in a third study, relief from visceral pain caused by malignancy was achieved in 100% of patients for the first 3 months (or until death) after local infiltration of alcohol around multiple plexuses. In contrast, neither perineural nor intraneural injection of ethyl alcohol for pain relief has, to our knowledge, been studied in horses.

Formaldehyde, like alcohol, functions as a tissue fixative and offers the potential to block nerve transduction while also preserving nerve structure, such as fiber alignment and structure of the perineurium and endoneurium. Preserving nerve structure may lead to reinnervation by nerve regeneration, which could potentially result in a lower likelihood of neuroma formation. To our knowledge, however, the effects of intraneural injection of formaldehyde have not been studied.

The present study was designed to evaluate the analgesic effects of intraneural injection of ethyl alcohol or formaldehyde into the palmar digital nerves of horses. Specifically, the purpose of the study reported here was to determine the effects of intraneural injection of medical-grade 98% ethyl alcohol or formaldehyde in the palmar digital nerves on severity of experimentally induced heel pain in horses. Our hypotheses were that both alcohol and formaldehyde would induce measurable analgesia for several weeks after injection of either substance and that formaldehyde would induce a greater decrease in heel pain, with analgesia lasting for a longer duration, than alcohol, but would also result in a greater amount of inflammation.

Materials and Methods

Experimental design—All experimental procedures were approved and monitored by the Institutional Animal Care and Use Committee of The Ohio State University. Six 5-year-old Thoroughbred mares purchased for research purposes were used in the study. All horses were sound (lameness score, 0) and healthy on the basis of results of a physical examination, including thoracic and abdominal auscultation and palpation of the limbs, performed within 1 week prior to the start of the study, with heart rate, respiratory rate, and rectal temperature within reference limits. Horses were housed in box stalls for the duration of the study.

The study consisted of a blocked design controlling for limb (left vs right). Each horse was randomly assigned by means of a random number generator to receive an intraneural injection of ethyl alcohol in the medial palmar digital nerve of the left (n = 3) or right (3) forelimb and an intraneural injection of formaldehyde (40% by volume) in the medial palmar digital nerve of the contralateral forelimb. Additionally, each horse was randomly assigned in the same manner to receive a sham operation consisting of a surgical approach to the lateral palmar digital nerve of the left (n = 3) or right (3) forelimb (surgical control) and no treatment of the lateral palmar digital nerve (negative control) in the contralateral forelimb. Within forelimbs, negative and surgical control treatment assignments were paired with ethyl alcohol and formaldehyde treatment assignments.

Procedures—On day 0, each horse underwent total IV anesthesia, and the left or right medial palmar digital nerve was surgically exposed, isolated with a hemostat, and secured with a soft rubber retractor. Medical-grade 98% ethyl alcohol or formaldehyde was then injected intraneurally with a 27-gauge needle. The volume of chemical injected ranged from 1 to 3 mL, and the total volume was injected into a single location along the exposed portion of the nerve. The end point for injection was a gross change in the appearance of the nerve with at least 1 cm of visible white discoloration and a stiff physical form. Leakage of injectate was recorded but not specifically prevented, and no absorbent material was placed under or around the injected nerve so as to not affect the chemicals’ interaction with the nerve. Once the injection was completed, the retractor was removed (injected nerves did not return to their original position after the retractor was removed) and the incision was closed. The contralateral medial palmar digital nerve was then exposed and treated in a similar manner, except that the other chemical was used for injection. Finally, the left or right lateral palmar digital nerve was exposed, but no injection was performed (surgical control), and the incision was closed routinely. The contralateral lateral palmar digital nerve was not treated (negative control).

Following surgery, light, noncompressive bandages consisting of roll gauze and elastic tape were applied to both limbs. Bandages were maintained for 2 weeks after surgery and changed as necessary.

Assessments—Clinical outcomes recorded for each heel consisted of severity of lameness in response to experimentally induced heel pain (lameness score and PVF), thermal reaction time, and heel skin sensitivity score. Clinical outcome recorded for each limb consisted of circumference of the pastern region. Lameness severity in response to experimentally induced heel pain was evaluated prior to injection (day 0) and 3, 7, 10, 14, 21, 28, 35, 42, 49, 56, and 112 days after injection. Thermal reaction time, heel skin sensitivity, and pastern circumference were measured prior to injection (day 0) and 3, 7, 10, 14, 21, 28, 35, 42, 49, 56, 70, 84, 98, and 112 days after injection. On day 112, horses were anesthetized and palmar digital nerve specimens were obtained for histologic examination.

Experimental induction of heel pain—At each assessment time, heel pain was experimentally induced by means of custom-made horseshoes fitted to both front feet (Figure 1) and severity of the resulting lameness was assessed by assigning a lameness score and measuring PVF. Custom shoes were maintained for the duration of the study, and shoes were reset and the hooves trimmed every 3 weeks. The custom shoes included medial and lateral curved heel extensions with...
a tapped hole in each extension, allowing a threaded bolt to be advanced through the shoe to apply pressure to the palmar horned aspect of the hoof, distal to the coronary band of the heel. Bolts were in place only during the defined lameness assessment periods. At these times, the bolts were inserted and tightened to apply pressure to each of the heels, one at a time.

On day 0, the bolt length and tightness needed to induce grade 2 lameness was determined for each heel. Lameness was scored on a scale from 0 to 5 by means of a modified American Association of Equine Practitioners grading scale, where 0 = no lameness; 1 = intermittent lameness at the trot; 2 = consistent lameness at the trot but lameness not seen at the walk; 3 = consistent head-bobbing lameness at the trot and lameness evident at the walk; 4 = obvious, consistent lameness at the walk and trot; and 5 = non-weight-bearing lameness. Half scores were assigned when lameness severity was between 2 scores. Once bolt length and tightness needed to induce grade 2 lameness were determined, the thread number was recorded so that the bolt could be inserted to the same position at each subsequent assessment period. When shoes were reset, the bolt length and tightness that, when applied to the negative control heel, resulted in grade 2 lameness were determined, and the other bolts were adjusted to reflect a similar thread number.

Measurement of PVF—At each assessment time, an in-ground, stationary force plate and computer analysis system were used to measure ground reaction forces for each forelimb before and after bolts were tightened to apply pressure to each heel. For each repetition, a curve of vertical force versus time was plotted by the computer analysis system and PVF was determined, as described. Data were obtained by trotting the horse over the force plate at a standard speed (2.5 to 3.5 m/s). A trial was considered valid if the foot in question struck the force plate at or close to the center of the force plate. The mean value for 5 valid trials was calculated for each time point, and PVF was expressed as a percentage of body weight. Relative analgesic potency was determined by calculating the percentage decrease in PVF in the treated heel versus the negative or surgical control heel in the same limb with the bolt applied to induce lameness. The following equation was used:

Relative analgesic potency = 100 \times \frac{(PVF \text{ with bolt applied to treated heel} - PVF \text{ with bolt applied to control heel})}{(PVF \text{ without any bolts applied} - PVF \text{ with bolt applied to control heel})}

Thermal reaction time—Thermal reaction time was assessed as a measure of cutaneous thermal analgesia. To measure thermal reaction time, an incandescent lamp (heat lamp analgesia meter) was focused on the skin of the heel bulb, with the horse standing on both forelimbs, and a digital timer was used to measure the time from application of the lamp to the time when the horse first moved its foot. In a pilot experiment in 1 horse, it was determined that the duration of thermal application with the lamp should be capped at 22 seconds to avoid visible thermal injury to the skin. Therefore, thermal reaction times of 22 seconds were taken to indicate complete cutaneous thermal analgesia. Relative thermal analgesic potency was calculated as the percentage increase in thermal reaction time relative to the control heel in the same limb. The following equation was used:

Relative thermal analgesic potency = 100 \times \frac{(reaction \text{ time for treated heel} - reaction \text{ time for control heel})}{(22 - reaction \text{ time for control heel})}

Heel skin sensitivity—Heel skin sensitivity was assessed by assigning skin sensitivity scores. In brief, pressure was applied to the skin of each heel bulb with a mosquito hemostat to assess the skin’s sensitivity to touch. Skin sensitivity to touch was scored on a scale from 0 to 6, where 0 = no reaction (confirmed as no reaction to needle prick), 1 = notices heavy pressure, 2 = shifts weight with heavy pressure, 3 = picks up the foot with heavy pressure, 4 = notices light pressure, 5 = shifts weight with light pressure, and 6 = picks up the foot with light pressure.

Circumference of the pastern region—Circumference of the pastern region of each forelimb was measured with a tape measure as an estimate of local soft tissue reaction associated with the skin incisions and intraneural injections. The top border of the tape was placed at the top of the palpable caudal eminence of the second phalanx.

Histologic evaluation—On day 112, horses were anesthetized, and specimens of 2 surgical control later-
al palmar digital nerves, 5 medial palmar digital nerves that had been injected with ethyl alcohol, and 2 medial palmar digital nerves that had been injected with formaldehyde were obtained by means of neurectomy. All specimens included the area of injection or (for the surgical control specimens) the portion of the nerve that had been surgically exposed.

Specimens were fixed in neutral-buffered 10% formalin for 1 week. Fixed nerves were serially sectioned (4 µm) and stained with H&E, Bielschowsky, Masson trichrome, and Luxol fast blue stains. Sections were subjectively evaluated simultaneously by 3 investigators (CPS, MO, and ALB) who were blinded to treatment assignment until a consensus opinion on histologic appearance was reached. Specimens were evaluated for evidence of primary demyelination, axon degeneration, axon dropout, perineural and neural coagulation necrosis, mineralization, inflammation, and fibroplasia.

Statistical analysis—Multivariate repeated-measures ANOVA was performed to evaluate the main effects of heel treatment and time. For PVF, thermal reaction time, and pastern circumference, mixed procedure models for continuous dependent variables were used. A Tukey posttest was used to assess selected relevant comparisons among treatments within day. For ordinal data (lameness score and skin sensitivity score), generalized linear models as well as a 1-way nonparametric Kruskal-Wallis test were used. Patterns of change over time were descriptively reported. For all analyses, values of $P < 0.05$ were considered significant.

Results

Horses—Five horses (mean body weight, 496 kg) completed the study. One horse had a celiotomy to correct a nonstrangulating colon displacement within 8 hours after recovery from the first surgery but still completed the study. One horse required removal from the study at day 19 because of lameness in the formaldehyde-injected limb; data from that horse up until day 19 were included. For all outcomes, horse was not a significant factor in 3-way ANOVA.

Surgical procedure—Mean surgical procedure time (ie, time from anesthetic induction to cessation of anesthetic drug administration) was 47.5 minutes (SD, 17.4 minutes). In all horses, the nerve was readily identified with minimal dissection. For most nerves, some unmeasured amount of leakage of the injected substance could be observed in the surgical field.

Lameness without experimental induction of heel pain—Lameness scores without experimental induction of heel pain (ie, without insertion of the bolts in the custom shoes) did not differ significantly between limbs at baseline (day 0) or after injection of alcohol and formaldehyde. A few horses had mild (< grade 1), intermittent lameness without insertion of the bolt in limbs treated with alcohol or formaldehyde on various days throughout the study. There was no significant difference in PVF between the limbs treated with alcohol versus formaldehyde at any assessment time during the study when bolts were not inserted.

Figure 2—Mean ± SEM lameness scores (A), PVF (% body weight; B), and relative analgesic potency (C) in 6 horses (1 horse was removed from the study on day 19; data from that horse up until day 19 were included) following experimental induction of heel pain. In each horse, medical-grade 98% ethyl alcohol was injected in the medial palmar digital nerve of 1 forelimb and formaldehyde was injected in the medial palmar digital nerve of the contralateral forelimb on day 0. At the same time, the lateral palmar digital nerve in 1 forelimb was surgically exposed, but no injection was performed (surgical control) and the lateral palmar digital nerve in the contralateral forelimb was not treated (negative control). Heel pain was induced by means of custom-made horseshoes that allowed a threaded bolt to apply pressure to the palmar horned aspect of the hoof; bolts were inserted and tightened to apply pressure to each of the heels one at a time. Lameness was scored on a scale from 0 to 5. Peak vertical force was measured with a force plate. Relative analgesic potency was calculated as 100 X (PVF with bolt applied to treated heel – PVF with bolt applied to control heel)/(PVF without any bolts applied – PVF with bolt applied to control heel). *Value is significantly ($P < 0.05$) different from values for negative and surgical control heels. †Value is significantly ($P < 0.05$) different from value for negative control heels. ‡Value is significantly ($P < 0.05$) different from value for surgical control heels.
Lameness with experimental induction of heel pain—Lameness scores assigned following experimental induction of heel pain (ie, with insertion of the bolts in the custom shoes) differed significantly with treatment \((P < 0.001)\) and over time \((P < 0.001)\). Lameness scores were significantly lower following application of the bolts to the heels treated with alcohol or formaldehyde than following application of the bolts to the surgical or negative control heels (Figure 2). Also, lameness scores following application of the bolts to the surgical and negative control heels increased over time. Subjective evaluation of the shape of the lameness score curves following application of the bolts to the heels treated with alcohol or formaldehyde suggested a marked decrease in severity of induced lameness in the first 14 days and a moderate decrease from day 14 through day 28, followed by a plateau for the remainder of the study.

Peak vertical force measured following experimental induction of heel pain (ie, with insertion of the bolts in the custom shoes) also differed significantly with treatment \((P < 0.001)\) and over time \((P < 0.001)\). Peak vertical force (expressed as a percentage of body weight) following application of the bolts to the heels treated with alcohol or formaldehyde was significantly greater than PVF following application of the bolts to the surgical or negative control heels (Figure 2). Relative analgesic potency peaked on day 10 for heels treated with alcohol (mean, 90.3%; SD, 6.2%) and on day 7 for heels treated with formaldehyde (mean, 97.3%; SD, 2.7%). Relative analgesic potency was not significantly \((P < 0.08)\) different between heels treated with alcohol and those treated with formaldehyde. On day 112, mean relative analgesic potency was 42.2% (SD, 15.5%) for heels treated with alcohol and 65.3% (SD, 18.7%) for heels treated with formaldehyde.

Thermal reaction time—Thermal reaction time differed significantly with treatment \((P < 0.001)\) and over time \((P < 0.001)\). Thermal reaction time was significantly longer for heels treated with alcohol or formaldehyde than for the control heels on most days after injection (Figure 3). Considering all time points, relative thermal analgesic potency was significantly \((P = 0.03)\) greater for formaldehyde than for alcohol. For heels treated with alcohol, relative thermal analgesic potency peaked on day 3 (mean, 65.3%; SD, 15.1%) and remained between 30% and 50% through the remainder of the study. For heels treated with formaldehyde, relative thermal analgesic potency remained high throughout the study, ranging from 45% to 70%.

Skin sensitivity score—Skin sensitivity scores differed significantly \((P < 0.001)\) with treatment but not over time. Scores did not differ for the surgical control, negative control, and alcohol-injected heels (Figure 4). However, scores for heels treated with formaldehyde were significantly lower than scores for the surgical control and negative control heels.
Pastern circumference—Pastern circumference differed significantly with treatment ($P < 0.001$) and over time ($P < 0.05$). Specifically, pastern circumference was greater and more variable for limbs treated with formaldehyde than for limbs treated with alcohol (Figure 5). Pastern circumference for limbs treated with alcohol did not change over time, whereas circumference for limbs treated with formaldehyde increased over time.

Histopathologic findings—The degree of fibrosis seen with intraneural injection of alcohol permitted a neurectomy to be performed without difficulty. Subjectively, it was more difficult to isolate nerves that had been injected with formaldehyde and to dissect the nerves free from the surrounding fibrosis, but this could be done proximal to the injection site. Nerves injected with alcohol had primary demyelination (loss of myelin with conservation of axons) and preservation of fiber alignment with an intact epineurium and mild fibrosis. Alcohol injection also caused loss of axons (axon dropout), axon degeneration, fibrosis, and inflammation, all of which were mild. Nerves injected with formaldehyde had less preservation of fiber alignment than did nerves injected with alcohol. Nerves injected with formaldehyde also subjectively had greater loss of axons (axon dropout), moderate to severe axon degeneration, and subjectively greater fibrosis and inflammation (Figure 6). Mineralization was noted only in nerves injected with formaldehyde.

Discussion

Results of the present study suggested that intraneural injection of either medical-grade 98% ethyl alcohol or formaldehyde in the palmar digital nerves of horses resulted in substantial, but partial, heel analgesia that persisted for at least 112 days. We found no advantage of formaldehyde over alcohol, and because formaldehyde resulted in greater soft tissue inflammation, we do not currently recommend it. Over the 4 months of this study, mean relative analgesic potency following injection of alcohol, as determined by measuring PVF following experimental induction of heel pain, was 53.8% and mean relative thermal analgesic potency was 43.2%, which likely indicated clinically relevant reductions in heel pain. Ideally, lameness score would be reduced to 0 (ie, no lameness), but in some clinical settings, a decrease in lameness score by 1 lameness grade (eg, from grade 2 to grade 1), in conjunction with adjunctive treatments such as NSAID administration and therapeutic trimming and shoeing, could be sufficient to make a horse serviceably sound. We did not determine how long analgesic effects observed in the present study would persist, but in a study of human patients with trigeminal neuralgia, perineural injection of alcohol resulted in some degree of pain relief for as long as 46 months.

Although significant differences were not found between the alcohol and formaldehyde treatments in the present study, other than with regard to skin sensitivity scores, subjective evaluation of the data suggested that alcohol may have induced less complete and less prolonged analgesia than formaldehyde. On the other hand, subjective examination of histologic specimens indicated that nerve alignment was better preserved and scarring was less extensive with alcohol injection, compared with formaldehyde injection. Importantly, the potential for reinnervation was not evaluated in the present study. Although the hist-
topathologic changes we saw may mean that reinnervation would be more likely with alcohol, as opposed to formaldehyde, and that painful neuroma formation would be less likely, research is needed to determine whether this is the case.

In the present study, histologic examination was performed on all 5 palmar digital nerves injected with alcohol but on only 2 nerves injected with formaldehyde and 2 nerves that underwent sham surgery. Considering that horses would not be euthanized at the end of the study, we did not want to perform biaxial, bilateral neurectomies in these horses and believed we could obtain the information we wanted as to the mechanism of axon damage and degree of inflammation and fibrosis with this limited number of samples. Alcohol injection induced minimal inflammation, as determined on the basis of circumferential of the palmar region and histopathologic evidence of mild inflammation, whereas formaldehyde caused greater soft tissue swelling (as determined by palmar region circumference) and greater histopathologic evidence of perineural fibrosis and inflammation. The degree of fibrosis seen with alcohol injection permitted a neurectomy to be performed without difficulty, which would allow neurectomy to be performed if clinical lameness persisted after injection of the nerve, whereas, subjectively at least, the degree of difficulty in isolating the nerve and dissecting the nerve free from surrounding fibrotic tissue was greater with formaldehyde, potentially affecting the ease with which subsequent neurectomy could be performed.

In the present study, skin sensitivity scores, a measure of heel skin desensitization, were not significantly different among the surgical control, negative control, and alcohol-injected heels, even though lameness in response to experimentally induced heel pain was substantially less with alcohol injection. Possible explanations for this include differential loss of sensation in response to alcohol injection on the basis of afferent fiber types within the nerve, contributions to skin sensation from branches of the nerve proximal to the injection site, or both. Cutaneous peripheral afferent nerve fibers consist of small unmyelinated C fibers, medium-diameter myelinated Aβ fibers, and large myelinated Aδ fibers. These different nerve fibers carry different types of information to the CNS. In humans, it has been determined that C fibers and Aδ fibers transmit nociceptive information, including thermal and mechanical input. In contrast, cutaneous Aβ fibers are responsive to nonnoxious stimuli such as touch, vibration, and pressure. In our study, the difference in the alcohol-injected heels between sensitivity to touch and sensitivity to the application of heat may have been caused by differences in the fiber types’ susceptibility to injury. The larger Aβ fibers may have been spared more than the smaller Aδ and C fibers, allowing tactile information to be transmitted but making the heels less sensitive to thermal stimulation after alcohol injection. The formaldehyde-injected heels had a decrease in skin sensitivity to touch and a decrease in sensitivity to thermal stimulation. One potential explanation for this difference is the substantial inflammatory response induced by formaldehyde, which affected a larger area of the palmar region than was seen following alcohol injection. Alternatively, these differences could reflect differences in nerve injury severity or mechanism after intraneural injection of formaldehyde, compared with alcohol.

Our method of applying bolts through custom-fitted shoes allowed for consistent, temporary induction of heel pain that permitted serial comparative evaluation for each heel and did not require euthanasia. Application of the bolt could be repeated reliably, despite the expected differences in the horses’ hooves and the changes over time resulting from hoof growth, trimming, and resetting of the custom horseshoes. We controlled for these changes by adjusting the bolt tightness as necessary at the time the shoes were reset on the basis of responses for the negative control (ie, untreated) heel. Some horses became more lame when the bolt was tightened in the negative control heel, which we attributed to irritation of the heel over time and changes secondary to hoof trimming. Horses did develop a subclinical, although measurable by means of the force plate, increase in sensitivity to the bolt with time in the negative control and surgical control heels. This presumably represented soreness at the site of the bolt pressure in these heels with repeated application. This finding was not associated with formaldehyde or alcohol injection because it was seen in both limbs and had an imperceptible effect on baseline lameness measurements.

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b. Dehydrated alcohol, American Regent Inc, Shirley, NY.
c. Formaldehyde (40% by volume), Fisher Scientific Inc, Fair Lawn, NJ.
de. Elastikon elastic tape, Johnson & Johnson, Skillman, NJ.
f. Heat lamp analgesia meter, Columbus Instruments International Corp, Columbus, Ohio.
g. Histology/Immunohistochemistry Lab, Comparative Pathology and Mouse Phenotyping Shared Resource, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

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References