Evaluation of iatrogenic hemarthrosis of the metacarpophalangeal joint as a method of induction of temporary reversible lameness in horses

Carter E. Judy, DVM, and Larry D. Galuppo, DVM

Objective—To determine whether iatrogenic hemarthrosis of the metacarpophalangeal joint could be used as a model for temporary reversible joint pain in horses.

Animals—8 adult horses.

Procedure—Each horse was evaluated on a treadmill before and after injection of 1 metacarpophalangeal joint with 10 mL of autogenous blood. Horses were evaluated subjectively and objectively by use of a computerized force measurement system at intervals until lameness abated. The mean force difference between injected and noninjected limbs at all time periods after injection was compared with the difference between limbs at baseline. From each horse, synovial fluid samples collected before and 24 hours and 30 days after injection were analyzed for total protein concentration and cell type and number. Venous blood samples were collected before and 6 and 24 hours after injection for assessment of plasma cortisol concentration.

Results—For 24 hours after injection, the mean force difference between injected and noninjected limbs was significantly increased over baseline. The greatest force difference was detected after 2 and 4 hours. Baseline and 24-hour force data were not significantly different. Compared with baseline values, synovial fluid protein concentration and nucleated cell and RBC counts were increased significantly at 24 hours after injection but were not different at 30 days after injection. No significant changes in plasma cortisol concentration were detected at any time point.

Conclusions and Clinical Relevance—In horses, iatrogenic hemarthrosis of the metacarpophalangeal joint appears to induce temporary reversible lameness with a mild to moderate degree of synovitis. (*Am J Vet Res* 2005;66:1084–1089)

In horses, experimental induction of lameness has been used in investigations to evaluate the efficacy of joint pain treatments and is a valuable means with which novel treatments can be assessed. To date, many methods of inducing lameness in horses have been used. Techniques such as the injection of lipopolysaccharide,[1-3] amphotericin B,[4] sodium monoiodoacetate,[5] polyvinyl alcohol foam particles,[6] and carrageenan[7] into synovial structures have been used to create synovitis. Surgical techniques, such as osteochondral fragment creation[8-10] and disruption of the collateral ligament and lateral collateral sesamoidean ligaments,[11] have been used to create osteoarthritis. Temporary tendonitis induced via injection of collagenase has been used to evaluate specific nerve blocks in relation to tendon injuries,[12] and mechanical techniques, such as the application of sole pressure, have also been used to induce observable lameness.[13-15] Although these methods are successful in producing lameness in horses, many are irreversible and others require the use of expensive materials that are foreign to the horse's body and have the potential to produce permanent injury.

In humans, hemarthrosis is known to be a cause of considerable pain and if left untreated can result in synovitis and osteoarthritis.[16-18] Hemarthrosis has also been reported[19] as a cause of persistent, recurrent lameness in a horse. It was hypothesized that a single injection of autogenous blood into the metacarpophalangeal joint of horses would cause a temporary synovitis and result in a lameness that could be quantified by use of a computerized in-shoe pressure measurement system.[20,a]

The objective of the study of this report was to determine whether iatrogenic hemarthrosis of the metacarpophalangeal joint could be used as a model for temporary reversible joint pain in horses. We used an objective measurement system[a] to evaluate the severity and duration of lameness associated with iatrogenic hemarthrosis to determine whether the experimental procedure would result in degrees of pain similar to those identified in horses with naturally occurring mild joint diseases, such as traumatic hemarthrosis, synovitis, capsulitis, and collateral ligament desmitis. It was hypothesized that iatrogenic hemarthrosis of the metacarpophalangeal joint of horses would result in lameness that could be consistently quantified by an in-shoe force measurement system and that the force difference between injected and noninjected limbs would dissipate over time.

Materials and Methods

Horses—Eight adult horses obtained from the university research herd were studied. The University of California's Animal Use and Care Committee approved the experimental protocol for use of iatrogenic hemarthrosis as a potential model for temporary reversible joint pain in horses. From preliminary results in 3 horses that were not used in the

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From the Veterinary Medical Teaching Hospital (Judy) and the Department of Surgical and Radiological Sciences (Galuppo), School of Veterinary Medicine, University of California, Davis, CA 95616-8747. Dr. Judy's present address is the Alamo Pintado Equine Clinic, PO Box 249, Los Olivos, CA 93441.
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Address correspondence to Dr. Galuppo.

study, it was determined that this method was safe and additional pain management was not required. However, should any horse have persistent lameness and signs of pain at any stage of the study, nonsteroidal anti-inflammatory medication (ie, phenylbutazone at a dosage of 2.2 mg/kg, PO, q 12 h) would be administered for pain control. Each horse was weighed, and a complete physical examination was performed. Horses were evaluated for lameness during trotting in hand in a straight line on asphalt and lunging in circles to the right and left. Examinations were videotaped for evaluation.

Procedure—Prior to application, each in-shoe sensor was calibrated with a known force of 5.63 kg/cm^2 (80 lb/in^2) by use of a sensor calibration system.[b] A calibrated sensor was applied to a prefabricated shoe between two 0.056-cm-diameter plastic plates and secured with porous white tape.[c] A left and right shoe-sensor combination was fabricated for each horse, and each horse was assigned its own sensor pair. Shoe-sensor combinations were secured to shod horses with adhesive tape,[d] and a converter[e] was secured to each metacarpophalangeal joint region with interdigitating hook-and-loop wrap and elastic tape.[f] Each shoe-sensor combination was attached to the converter[e] and connected to a compatible microcomputer[g] via a 30-foot cord and a 16-bit receiver card, according to the manufacturer's instructions.

Horses were moved to a motorized equine treadmill[h] and exercised at a speed of 4 m/s. The vertical ground reaction force and temporal hoof strike data of both forefeet were acquired simultaneously by use of a data acquisition software package.[i] The time of acquisition was 8 seconds for a total of 400 frames (50 frames/s). The treadmill was stopped, and horses were allowed to rest briefly as the acquired data were translated. Three sets of data for both forelimbs were acquired in a similar fashion for each horse.

After acquiring the baseline kinetic data, 1 metacarpophalangeal joint region and the left jugular vein of each horse were aseptically prepared. A lateral collateral sesamoidean ligament approach was used,[21,22] and 1 mL of fluid was aspirated from the joint by use of an aseptic technique. Ten milliliters of whole blood was aspirated from the jugular vein and injected into the joint (without the addition of anticoagulants) immediately after removal of the joint fluid sample using the same needle and portal. In horses with identified lameness, the sound limb was injected. In horses with no identifiable lameness, iatrogenic hemarthrosis was randomly assigned to be performed in either the left or right forelimbs. Baseline lameness was assessed subjectively and objectively immediately after the injection.

During the 12 hours after administration of the injection, all horses were evaluated by use of the aforementioned protocol every hour for the first 4 hours and then every 2 hours for the next 8 hours. Horses were then evaluated every 24 hours until lameness was no longer evident as determined via findings of subjective lameness grading and objective hoof strike assessments. At each evaluation time point, 3 sets of vertical ground reaction force and temporal hoof strike data were acquired for each horse.

The maximum force (N) for each forelimb for 10 successive hoof strikes was recorded. All videotaped gait examinations were blindly evaluated for lameness by 1 investigator (CEJ). Observed signs of lameness were graded according to the guidelines of the American Association of Equine Practitioners[j] as follows: grade 0 = sound, grade 1 = lameness inconsistently observable under special circumstances, grade 2 = lameness consistently observable under special circumstances, grade 3 = lameness consistently observable at a trot in a straight line, grade 4 = lameness observable at a walk, and grade 5 = minimal weight bearing in motion or at rest.

Samples of synovial fluid (1 to 2 mL) were aspirated from each injected metacarpophalangeal joint at 24 hours and 30 days after the experimental treatment. Each sample was placed in a 3-mL EDTA collection vial with the majority of the EDTA solution removed. These samples were submitted for cytologic evaluation in which total nucleated cell count, total protein concentration, and cell type were recorded. Samples of venous blood obtained from the left jugular vein (10 mL placed in standard blood collection vials) were collected immediately prior to and 6 and 24 hours after administration of the joint injection for assessment of plasma cortisol concentration. A CBC was performed immediately prior to injection.

Data reduction and statistical analyses—For each horse, the mean maximum force difference between the injected and noninjected limb was calculated. This value was derived by calculating the mean value of the force differences between 10 hoof strike measurements for each limb in each of 3 trials. Because mean maximum force values recorded for lame horses differed between the initial lame limb and injected limb at each time period, the difference in maximum force between the injected and noninjected limb was calculated as an absolute value for each hoof strike. For each horse, maximum force values were compared between forelimbs for each trial by use of a paired 2-tailed Student *t* test. Values of $P < 0.05$ were considered significant. Lameness was considered detectable if there was a significant difference between the maximum force values for the left and right forelimbs. The forelimb with experimentally induced lameness was determined to be the limb that had a lower maximum force value in reference to the opposite forelimb.

At each time point, mean maximum force difference between injected and noninjected limbs was derived by determining the mean value of maximum force differences for all 8 horses. The mean maximum force difference at each time period was calculated by subtracting the force difference for each trial from baseline measurements for each horse. The mean force difference from baseline was normalized for each horse's individual weight by dividing the mean maximum force difference (converted to kilograms) by each horse's individual weight, which yielded unitless data. An ANOVA for repeated measures was used to evaluate the interaction of trial with the mean maximum force difference from baseline corrected for individual horse's weight. Significance was set at a value of $P < 0.05$.

The accuracy of the pressure measurement system was evaluated by calculating a Pearson correlation coefficient between lameness grades determined via the blinded clinical evaluation for lameness and force measurements of the lame forelimb.

An ANOVA for repeated measures was performed on findings of synovial fluid analyses, including values of total protein concentration and counts of total nucleated cells, RBCs, neutrophils, and small and large mononuclear cells in synovial fluid samples obtained at baseline and 24 hours and 30 days after joint injection. A similar analysis was performed for plasma cortisol concentrations measured at baseline and 6 and 24 hours after joint injection. Significance was set at a value of $P < 0.05$. All analyses were performed by use of a commercial statistical software package.[k]

Results

The mean ± SD weight for the 8 horses was 477 ± 63 kg. All horses were instrumented and trotted without difficulty on the treadmill according to protocol. Horses did not appear to resent the placement of the equipment, and all horses trotted as they had during previous sessions on the treadmill without instrumentation. There was no change in gait detect-

ed between horses with or without instrumentation. All equipment functioned normally for the duration of the study. The software[i] facilitated data evaluation by producing a graphic representation of ground reaction force and time for the left and right forelimbs (**Figure 1**).

Prior to injection (baseline), 7 of the 8 horses were lame in a forelimb on the basis of findings of clinical (mean lameness grade, 3.0 ± 0.3) and computerized analysis (mean force difference, 560.2 ± 530.8 N). Four horses were lame in the right forelimb, and 3 were lame in the left forelimb. The 4 horses that were lame in the right forelimb were injected in the left metacarpophalangeal joint, and the 3 horses that were lame in the left forelimb were injected in the right metacarpophalangeal joint. The horse that was not lame was injected in the right metacarpophalangeal joint. The source of lameness was not localized by clinical assessment or diagnostic anesthesia in any of the lame horses. Results of all CBCs were within standard reference ranges. No horses required treatment with analgesics during or after the procedure.

The mean ± SD change in the subjective degree of

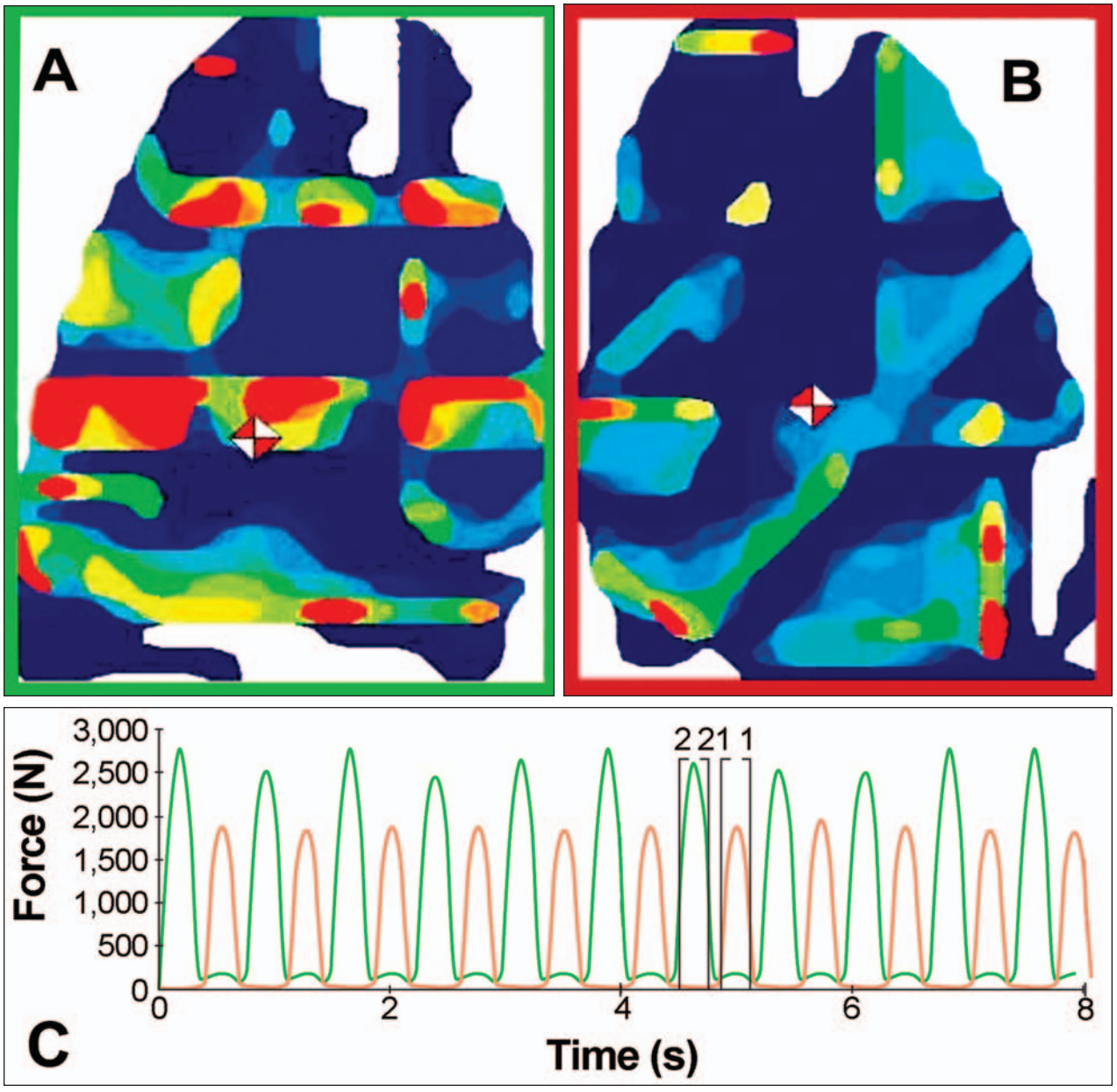

Figure 1—Representative results of an analysis of forelimb hoof strike data (generated by computer software[i]) obtained by use of a computerized in-shoe pressure measurement system from a horse exercised at a speed of 4 m/s on a motorized equine treadmill after injection of 1 metacarpophalangeal joint with autogenous blood to induce lameness. A—In-shoe sensor pressure mapping of 1 hoof strike of the noninjected forelimb. B—In-shoe sensor pressure mapping of 1 hoof strike of the injected forelimb. In panels A and B, the red and white square represents the center of force for that force mapping image. C—Graphical representation of ground reaction force (N) versus time (seconds) for noninjected (green line) and injected (red line) forelimbs during 11 consecutive hoof strikes. Notice that the red line has a consistently lower peak than the green line, indicating that less cumulative force is being placed on the injected forelimb, compared with that being placed on the noninjected forelimb. The bracketed peaks marked 1 and 2 represent the entire portion of the stride in which the force mapping images in panels A and B were generated.

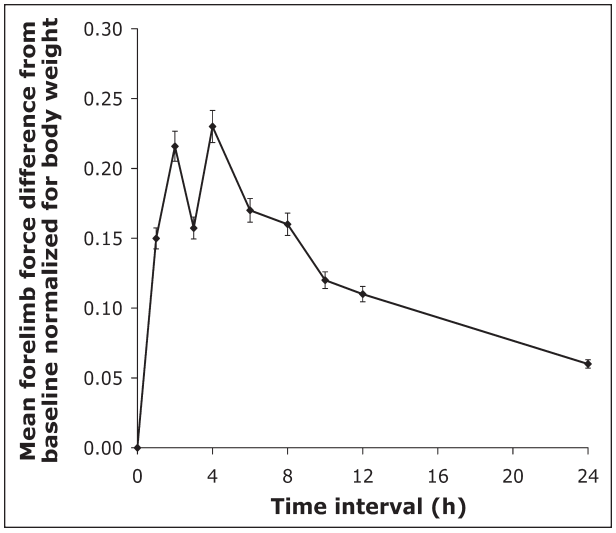

Figure 2—The mean difference from baseline in maximum force between injected and noninjected forelimbs (determined by use of a computerized in-shoe pressure measurement system) of 8 horses that had received an injection of autogenous blood in 1 metacarpophalangeal joint to induce lameness. The x-axis represents the time interval (hours). The y-axis is the mean force difference between injected and noninjected forelimbs from baseline normalized by each horse's weight. Zero force difference represents baseline measurements.

lameness for all horses at all time points was 3.8 ± 1.4 (range, 2.0 to 5.0), when comparing after induction of hemarthrosis with baseline evaluation before injection. The mean change in force as measured by the objective computerized in-shoe pressure measurement system was 750.4 ± 240.1 N (range, 310.1 to 1,070.6 N) in the injected limb after induction of hemarthrosis, compared with baseline values before injection.

In the 8 horses evaluated, lameness was not sustained for more than 24 hours; in most horses, lameness reached peak severity at 4 hours after joint injection, with resolution of the lameness during the following 20 hours. From data collected for the 8 horses, mean difference in maximum force (normalized for individual horse's weight) between injected and noninjected limbs was calculated (**Figure 2**). Significant ($P < 0.05$) differences in the mean maximum force differences between injected and noninjected limbs from baseline measurements were detected between the 2- and 12-hour time points ($P = 0.04$), between the 2- and 24-hour time points ($P = 0.004$), between the 4- and 12-hour time points ($P = 0.03$), between the 4- and 24-hour time points ($P = 0.003$), and between the 6- and 24-hour ($P = 0.04$) time points. There were no significant differences among all other time points including between the baseline and 24-hour time points (for all remaining comparisons, $P > 0.05$). The correlation (r^2) of the grades of the blinded clinical observation of lameness and those of the computerized system was 0.96.

Twenty-four hours after joint injection, the total protein concentration and WBC, RBC, neutrophil, and large and small mononuclear cell counts in samples of synovial fluid obtained from the treated joints were significantly higher than preinjection values. Thirty days after joint injection, the total protein concentration and WBC, RBC, neutrophil, and small mononuclear cell counts in the samples of synovial fluid were significantly lower than the values in samples obtained 24 hours after joint injection. For the large mononuclear cell counts, no significant differences were detected among the samples obtained before and 24 hours and 30 days after joint injection. There were no significant differences in any of the synovial fluid variables assessed between the samples obtained before and 30 days after joint injection. With regard to plasma cortisol concentration, no significant differences were detected among samples collected before and 6 and 24 hours after the joint injection.

Discussion

The objectives of the present study were to determine whether iatrogenic hemarthrosis of the metacarpophalangeal joint could be used to induce temporary, nonimmunogenic, reversible lameness in horses and evaluate the severity and duration of the resultant lameness. Our hypothesis was that this method would induce lameness that was temporary but of sufficient severity to be detectable via clinical observation and use of an adapted in-shoe pressure measurement system.[20]

The findings of our study indicated that injection of 10 mL of autogenous blood into the metacarpophalangeal joint of horses induced lameness that was detectable via clinical observation and use of the in-shoe computerized measurement system. Compared with subjective clinical observation, the objective computerized system was more sensitive in detecting the presence or absence of lameness and in quantifying the severity of the abnormality. The correlation of the computerized system data with assessments by the clinical observer was 0.96. A correlation of 1.0 was not obtained because of the sensitivity of the computerized system to detect lameness when lameness was not severe enough to be seen by the observer. This sensitivity allows for increased detection of subtle changes in gait that would not normally be possible with subjective observation alone.

Because 8 sound horses were not available for use in the present study, the use of clinically lame horses was necessary. To facilitate evaluation of the change in lameness by use of objective measurements and subjective observations, the joint injection was not administered in the limb that was determined to be lame in these horses. In the experimentally treated limb, a mean change in maximum force from the baseline value of 750.4 N was detected 4 hours after injection. This change in weight bearing correlated to a mean change in the subjective lameness grade of 3.8. Although inclusion of sound horses in our investigation would have been preferable, the availability of horses that are not lame is limited in research settings for many reasons.

In the group of horses used in our study, the severity of lameness was greatest at 2 and 4 hours after joint injection, with a slight decrease in the measured lameness at the 3-hour time point. Although the cause of this bimodal peak in severity is not known, it is speculated that the initial degree of lameness (detected with-

in the first 2 hours after joint injection) may have been a result of the increased pressure associated with the addition of 10 mL of blood to the joint and that inflammation within the joint may have peaked at the 4-hour time point. Evaluation of data collected after an injection of sterile balanced ionic solution into the metacarpophalangeal joints of a group of horses would help to determine whether pressure within the joint or another cause was responsible for the initial lameness observed in the horses of this report. Although increased pressure within the joint associated with iatrogenic hemarthrosis may cause an initial lameness response in horses, it would not likely influence the overall results of our study. Such pressure-induced lameness should dissipate rapidly, as the plasma constituents of the small volume of injected blood would likely be resorbed by the synovial membrane shortly after injection. Another potential cause of the change in lameness may be related to variations between horses and the severity of lameness in individuals. However, this is less likely because all 8 horses included in the study responded similarly to the experimental treatment.

Hemarthrosis is known to be an important cause of pain in humans and horses.[16,18,19,23] In horses, hemarthrosis-associated signs of pain have been associated with recurrent lameness, which can be a considerable clinical problem.[19] It was hypothesized that introduction of autogenous blood into a joint of horses would provide an inexpensive method of inducing nonimmunogenic, temporary, reversible lameness that did not cause any long-term deleterious effects. Other methods of experimental induction of lameness used at present result in lameness of various degrees of severity and are associated with long-term and systemic adverse effects.[1,3-15,24-27] Through use of the horse's own blood, the injection of exogenous foreign substances into the joint is eliminated, the cost of the procedure is minimized, the horse's immune system is not sensitized, and long-term adverse effects are seemingly avoided. In the present study, the horses developed mild to moderate inflammation within the experimentally treated metacarpophalangeal joint during the initial 24-hour period after injection, but by 30 days, the synovial fluid variables in all 8 horses had returned to baseline values. This resolution in inflammation along with the reduction in lameness with time suggests that iatrogenic hemarthrosis may be a minimally stressful method for the induction of lameness in horses and that it that may allow animals to be used in future studies without the concern of long-term adverse effects.

The major negative aspect of this method of lameness induction is the duration of lameness. In the 8 horses evaluated in the present study, lameness was not sustained for more than 24 hours; in most horses, lameness reached peak severity at 4 hours after joint injection, with resolution of the lameness over the following 20 hours. This method may be difficult to use when a prolonged predictable lameness is needed. Nevertheless, iatrogenic hemarthrosis of the metacarpophalangeal joint induces temporary reversible lameness in horses; this inexpensive method of lameness induction may be useful as a model for temporary reversible joint pain in horses and may provide researchers with another technique with which to evaluate lameness treatments in equids in the future.

a. F-Scan, Tekscan Inc, South Boston, Mass.
b. Sensor calibration bladder, Tekscan Inc, South Boston, Mass.
c. Porous white tape, Johnson & Johnson Medical Inc, Arlington, Tex.
d. Duct tape, 3M Corp, Saint Paul, Minn.
e. Sensor converter, Tekscan Inc, South Boston, Mass.
f. Elastikon, Johnson & Johnson Medical Inc, Arlington, Tex.
g. 486 IBM Thinkpad, International Business Machines Corp, Armonk, NY.
h. SATO II, equine motorized treadmill, Nordeal Ltd, Kilpedder, Ireland.
i. F-Scan acquisition software, Tekscan Inc, South Boston, Mass.
j. American Association of Equine Practitioners (newsletter). Lexington, Ky: 1983;Mar:12.
k. StatView for Windows, SAS Institute Inc, Cary, NC.

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