Characterization of experimentally induced post-traumatic osteoarthritis in the medial femorotibial joint of horses

Courtney J. Bolam, DVM; Mark B. Hurtig, DVM, MVSc; Antonio Cruz DVM, MVM, MSc; Beverly J. E. McEwen, DVM, PhD

Objective—To study osteoarthritis in the equine medial femorotibial (MFT) joint after a single traumatic injury.

Animals—10 mature horses.

Procedure—In vitro explant cultures were used to determine injury threshold for stifle joint cartilage. Contusive impacts were applied to the medial femoral condyle (MFC), and horses were followed for 84 (n = 5) and 180 days (5). Synovial fluid samples were collected every 14 days for determination of sulphated glycosaminoglycan (sGAG) concentrations. Radiographic and lameness evaluations were performed. Gross and histologic descriptions, and immunohistochemistry, cartilage sGAG content determination, and cartilage aggregate modulus determination were performed at the MFC impact site (MFCi), MFC nonimpact site (MFCn), and medial tibial plateau (MTP).

Results—Synovial fluid sGAG concentration decreased significantly on days 14, 28, 42, and 56 in all horses. Macroscopic and microscopic articular lesions developed within all MFT joints. No radiographic abnormalities were observed. Mild lameness was evident in several horses. No significant differences were found between short-term and long-term cohorts of horses with respect to histologic scores and TUNEL results. On immunohistochemistry, MFCi was positive for COL2–¾Cshort. International Cartilage Repair Society scores differed significantly between short-term and long-term cohorts of horses. In all horses, sGAG concentrations were significantly decreased at the MFCi, compared with the MFCn.

Conclusions and Clinical Relevance—Use of contusive impacts on the MFC of horses results in cartilage lesions that are similar to those described clinically, supporting trauma as a contributing factor in the natural pathogenesis of osteoarthritis. (Am J Vet Res 2006;67:433–447)

Osteoarthritis is the most common cause of lameness in horses, resulting in substantial athletic and economic loss. The large mass and athletic ability of horses can create remarkably high biomechanical forces within joints, which may lead to irreversible destruction of the articular cartilage, synovial membrane, SCB, joint capsule, and supportive ligaments. Although the initiating event is not always clearly defined, biochemical, genetic, age-related, hormonal, metabolic, neurologic, and biomechanical factors can all contribute to disease progression.

Acute and chronic trauma are considered important etiologic factors in osteoarthritis of humans; however, the pathophysiologic process of PTOA is complex. Although the widespread consensus is that mechanical damage can initiate PTOA, little agreement exists as to whether the primary injury is to the articular cartilage, SCB, associated soft tissues, or combination of these structures.

Proponents of primary bone injury suggest that trabecular microfractures lead to SCB sclerosis and increased stresses that must be borne by the articular cartilage. The cartilage matrix undergoes cyclic damage and ineffective repair, leading to a progressively more catabolic state in which osteoarthritis is the end result. Proponents of primary articular cartilage injury suggest that damaged cartilage loses its inherent biomechanical properties, which compromises articular lubrication and congruency, leading to stress concentrated and eventual SCB injury.

Although study results indicate that these alterations in cartilage and underlying bone can begin independently, the roles...
of these 2 tissues in the initiation and progression of osteoarthritis are complex and likely intertwined.

A direct correlation between the severity of acute injury and the risk of PTOA has not been demonstrated, but the initial stages of PTOA are difficult to detect and quantify. Arthroscopic evaluation of injured human knees suggests that occult injuries are more common than overt chondral or osteochondral fractures. The use of magnetic resonance imaging has revealed SCB edema (or bone bruises) in the femoral condyle and tibial plateau of human knees with anterior cruciate ligament injuries. Although cartilage overlying these sites usually appears normal arthroscopically, chondrocyte apoptosis and necrosis have been observed on histologic examination and are associated with an increased risk of PTOA.

Without serial assessments that use sophisticated diagnostic techniques, osteoarthritis may go undiagnosed until it is advanced and the historical link between injury and clinical signs may be lost. Therefore, surrogate markers of osteoarthritis have been investigated. Products of type II collagen and proteoglycan anabolism and catabolism appear to be useful as markers of joint disease in equine synovial fluid. Intra-articular amounts of bone-specific alkaline phosphatase, cartilage oligomeric matrix protein, and a myriad of inflammatory mediators have also been investigated in horses. Results have varied with species, breed, disease process, and joint under investigation. Although results of some studies revealed increased concentrations of sGAGs in synovial fluid of horses with osteoarthritis, others failed to identify any alterations and still others identified decreased synovial fluid sGAG concentrations.

In humans, the risk of developing PTOA varies among individuals and joints. Although a single articular trauma can initiate progressive PTOA, high-speed activities that use repetitive high amounts of articular impact (contusion) and torsional loading are more commonly implicated. In athletic horses, high-intensity training can cause insidious injury and a persistent reparative response within the SCB. During this so-called adaptive remodeling, subchondral trabecular bone develops focal thickening and increased density (sclerosis). Substantial degeneration has been observed in the cartilage overlying sclerotic SCB, but whether this cartilage damage preceded or followed the bony alterations is unknown. However, if this bone formation becomes disunited from concurrent bone resorption, nonadaptive remodeling ensues, which results in focal weakening and necrosis of the SCB with subsequent collapse or fracture of the overlying articular surface.

While SCB injury and subsequent remodeling are found prominently in joint disease of the equine carpal and fetlock joints, these lesions are unreported in the stifle joint. Osteoarthritis of the equine stifle joint has frequently been described as a sequel to joint instability secondary to soft-tissue injury or joint incongruity secondary to osteochondrosis or subchondral cystic lesions. The pathogenesis of subchondral cystic lesions in the MFC of horses may be related to the creation of osteochondral defects of an appropriate size and shape. In adult horses, these defects more likely result from mechanical trauma than from persistent developmental defects. However, the nature and magnitude of the insult and whether it is a single event or requires repeated injury remain unknown. Osteoarthritis of the stifle joint may also occur secondary to articular cartilage damage without the development of a subchondral cystic lesion. Although osteochondrosis may cause this damage in young horses, the cumulative effect of repeated trauma is more likely the cause in mature animals.

Several species have been used to investigate the role of articular contusion as an initiating factor in PTOA. The study of PTOA in vivo provides the advantage of evaluating acute and long-term effects of articular contusion on the joint and the entire animal. Although changes suggestive of early osteoarthritis following a single articular impact have been documented, advanced PTOA seldom results. In these studies the type of injury, energy, mass, area of contact, orientation, rate of loading, and magnitude of the impact all played an important role in the initiation and progression of osteoarthritis after a single injury. In rabbits and dogs, a 22- to 25-MPa injury to the femoropatellar joint initially created subtle lesions that did progress to osteoarthritis in 6 months. Previous work performed in our laboratory in canine and ovine femorotibial joints revealed that impact injuries of 15 to 25 MPa progressed in a predictable manner to PTOA, although subchondral cystic lesions did not develop.

In the study reported here, the threshold for progressive cartilage injury in the equine MFC was defined by ex vivo investigation. Subsequent in vivo studies define the clinical, macroscopic, histologic, and biochemical consequences of a 60-MPa injury to the equine MFT joint. We undertook development of the use of contusive chondral injury in the equine stifle joint to provide insight into the role that mechanical injury plays in the initiation and progression of osteoarthritis in horses. We hypothesized that degenerative joint disease and SCB cysts would result from a focal impact injury to the equine MFC and progress to osteoarthritis. The sGAG concentration in MFT joint synovial fluid was examined as a surrogate indicator of early cartilage degeneration.

**Materials and Methods**

**Determination of cartilage injury threshold—Stifle joints** (n = 6) were obtained from 3 mature horses euthanized for reasons other than musculoskeletal disease, endotoxemia, or sepsis. Horses were euthanized after sedation (xylazine, 1.1 mg/kg, IV) with sodium pentobarbital (125 mg/kg, IV). Joints were dissected, and the MFC was exposed, inspected for any macroscopic abnormalities, and then secured on a metal tabletop. A handheld, spring-driven impactor with a 6.5-mm-diameter nonporous tip was used to create focal impact injuries of 30, 50, 60, and 80 MPa on the weight-bearing surface (Figure 1).

The impactor consists of a stainless-steel barrel housing a spring-loaded weighted rod. A trigger allows it to be primed by pulling back on the arm, which is then released, accelerating it against the tip positioned on the articular surface. Impact magnitude is proportionate to spring compression and is measured...
by a 227.3-kg load cell connected to a computer equipped with data acquisition software and an acquisition rate of 5,000 Hz. Cartilage sections were kept moist with lactated Ringer’s solution during impact trials and data collection.

Assessment of chondrocyte viability was done daily for 3 days by use of previously described staining, culture, and chondrocyte counting methods. Areas that were within 100 µm of the deep or lateral edges of the section were easily damaged by cutting and handling and therefore were not assessed. Results were recorded as proportions of live and dead cells. All experiments were initiated within 24 hours of euthanasia.

Assessment of impact injury in horses—This study was approved by the University of Guelph Animal Care Committee and was in accordance with the Canadian Council on Animal Care. Ten mature (4 to 15 years old; mean ± SD, 7.4 ± 4.23 years old) Standardbreds (7 mares and 3 geldings) were enrolled in this prospective study. All horses were examined for signs of lameness by 1 observer (CJB), and a detailed examination of the stifle joint, including palpation, was performed. Throughout the study, lameness scores were assigned in accordance with the American Association of Equine Practitioners guidelines.

Standard radiographic views (lateral, cranial, caudal, and plantarolateral-caudomedial oblique projections) were obtained of both stifle joints. Horses with recognized lameness, pathologic changes of the stifle joints, or radiographic changes were excluded from the study. By use of a block design, horses were assigned a treatment and cohort. Treatments were 60-MPa contusive impact injuries in the right or left MFT joint. The 2 cohorts were a short-term (84 days; horses 1 through 5) and long-term group (180 days; horses 6 through 10).

Surgical protocol

All horses had routine preoperative examinations and laboratory tests to ensure they did not have undiagnosed systemic disease processes. Penicillin G sodium (20,000 U/kg, IV) and phenylbutazone (4.4 mg/kg, IV) were administered 1 hour before surgery. Horses were premedicated with xylazine (0.5 to 1 mg/kg, IV) and 10% guaifenesin (500 mL, IV). General anesthesia was induced with ketamine (2.2 mg/kg, IV) and maintained with halothane in 100% inspired oxygen by use of a circle system for the duration of the surgery. Preparation for surgery, each horse was placed in dorsal recumbency and the area surrounding each stifle joint was clipped, aseptically prepared, and draped. Synovial fluid (3 mL) was obtained from the MFT joint and processed as already out-
duced, allowing documentation of the section of abnormalities. Horses were allowed to recover in padded recovery rooms and given assistance to stand as needed.

Postoperative care consisted of penicillin G sodium (20,000 U/kg, IV, q 6 h) for 24 hours followed by trimethoprim-sulfamethoxazole (24 mg/kg, PO, q 12 h) for 7 days and phenylbutazone (4.4 mg/kg, PO, q 12 h) for 3 days followed by a declining dose for 5 days. Butorphanol (20 mg, IM) was administered if needed to control immediate postoperative pain. Routine physical examinations, including surgery site and lameness assessment (at a walk), were performed daily for the first 10 days. Horses were confined to box stalls for the first 5 days and then allowed a small paddock turnout for an additional 5 days. Following that, horses were turned out for the remainder of the study. Skin sutures were removed between days 14 and 21 after surgery.

Postoperative synovial fluid collection and analysis

On postoperative days 14, 28, 42, 56, and 84, synovial fluid was collected from the impacted MFT joints of all horses while they were sedated (detomidine [3 to 5 mg] and butorphanol [10 mg, IV]). A medial approach, between the medial patellar ligament and the medial collateral ligament, and an 18-gauge, 3.8-cm-long (1.5-inch) needle were used.

In the long-term cohort, the same intervals were used with additional samples obtained at 112, 140, 168, and 180 days. Samples were placed in EDTA-coated tubes for cytologic examination (WBC count, differential cell count, and total protein determinations) and Eppendorf tubes for biochemical analysis. Samples for biochemical analyses were centrifuged at 13,500 × g for 30 minutes; the supernatant was decanted and stored as aliquots at –80°C.

Clinical evaluation

On the day prior to euthanasia, standard radiographic views of both stifle joints were repeated. All horses were evaluated for lameness at a walk and a trot by 2 observers (CJB, MBH). Flexion tests of the hind limbs were performed and lameness scores assigned. The impacted stifle was also assessed for the presence of periarticular swelling or edema, synovial effusion, and other abnormalities.

Postmortem assessments

The short-term and long-term cohorts of horses were euthanatized on days 84, and 180, respectively. Horses were euthanatized after sedation (xylazine, 1.3 mg/kg, IV) with sodium pentobarbital (125 mg/kg, IV). The impacted stifle joint was immediately harvested en bloc. Synovial fluid was collected from the MFT joint and processed as already out-

Figure 1—Photograph of the handheld impactor device. Notice the interchangeable tip (white arrowhead) with a 6.5-mm-diameter tip as used in this study, trigger device (black arrowhead), and arm (white arrow). Bar = 40 cm.
coelastic materials.\textsuperscript{65,66} Creep and stress-relaxation behavior solution for biphasic viscoelastic cartilage surface was determined by use of the indentation transducer that produced characteristic signals in contact measured with a 21-gauge needle in line with a 100-g force transducer that produced characteristic signals in contact measured with a 21-gauge needle in line with a 100-g force transducer. The aggregate modulus of the cartilage surface was determined by use of the indentation creep and stress-relaxation behavior solution for biphasic viscoelastic materials.\textsuperscript{65,66}

Biomechanical creep indentation testing Mechanical properties of the MFCi, MFCn, and MTP were determined by use of a custom-made weighted beam apparatus equipped with a 2-mm porous indenter tip that applied 1.27 MPa over 10 minutes (Figure 2). The displacement (ie, creep) was measured with a Hall effect transducer, and data were collected at 1,000 Hz. Cartilage thickness was determined by 3 observers (CJB, MBH, and BJEM). Two histologic sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM). Two histologic sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM). Two histologic sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM). Two histologic sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM). Two histologic sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM).

Cartilage sGAG concentration Cartilage tissue specimens for sGAG analysis were taken (Figure 2), weighed, and digested by incubation with an equal volume of papain\textsuperscript{1} (1 mg/mL) in papain digest buffer (0.69 g of sodium phosphate monobasic, 0.0326 g of N-acetyl cysteine, and 0.076 g of EDTA tetrasodium salt in a volume of 100 mL [pH, 6.5]) at 65°C overnight. These specimens were subsequently frozen at −80°C until further biochemical analysis.

Histologic examination and scoring Osteochondral sections were obtained from the MFC and MTP (Figure 2), fixed in neutral-buffered 10% formalin overnight, decalcified in 50% citric-formic acid, and then embedded in paraffin. Five-micron-thick sections were cut and stained with H&E and toluidine blue. Representative sections from each site were examined and scored independently by 3 observers (CJB, MBH, and BJEM). Two histologic scoring systems were used. The modified Mankin system\textsuperscript{67} and a new method developed by the OARSI\textsuperscript{a} were used (Appendix). The modified Mankin system allocated points for abnormalities in cellular organization (0 to 6), detail (0 to 4), and matrix staining (0 to 4). To create scores for the entire MFT joint, scores for the MFC and MTP were summed. The OARSI scoring system has an open-ended scale and estimates the grade and stage. The grade estimates the depth and severity of cartilage loss, whereas the stage is proportional to the area of each lesion. Stage was determined by the extent of the lesion in histologic sections cross-referenced to the digital photographs of the India ink–stained MFC. To create scores for the entire MFT joint, scores for the MFCi, MFCn, and MTP were summed.

Cartilage sections for TUNEL and COL2–¾C\textsubscript{short} immunohistochemistry were deparaffinized in xylene and ethanol (Figure 2). Slides for TUNEL were pretreated with testicular hyaluronidase\textsuperscript{6} and proteinase K\textsuperscript{7} and stained by use of a commercially available kit.\textsuperscript{7} Negative and positive controls for TUNEL were included in the kit, but confirmatory studies were done with nuclease-pretreated normal equine cartilage and liver sections. Slides for COL2–¾C\textsubscript{short} immunohistochemistry were pretreated with 0.5% hydrogen peroxide in methanol\textsuperscript{8} and digested with chondroitinas\textsuperscript{e} and treated with a blocking agent tissue block.\textsuperscript{7} Sections were then incubated with the COL2–¾C\textsubscript{short} antibody\textsuperscript{6} at 37°C, followed by a biotinylated secondary antibody\textsuperscript{6} at room temperature (approx 19°C to 21°C). Streptavidin-horseradish peroxidase conjugate\textsuperscript{7} and diaminobenzidine substrate\textsuperscript{6} were added to produce the brown end product. Negative controls were also prepared. All slides were examined microscopically at a magnification of 200X and compared with controls. The location and degree (mild, moderate, or marked) of staining were assessed.

Synovial membrane from the MFT joint was excised and fixed in formalin (Figure 2). Fixed synovial membrane was embedded in paraffin. Slides were made from 5-µm-thick sections; stained with H&E; and examined microscopically for evidence of WBC infiltrate, villous hypertrophy, capsular fibrosis, and increased vascularity.

Synovial fluid sGAG concentration All synovial fluid samples were thawed at room temperature and analyzed as a batch. Each sample was digested overnight by incubation with papain\textsuperscript{1} as described for cartilage. The following morning, the digested cartilage samples were thawed and all digested samples (synovial fluid and cartilage) were evaluated simultaneously by use of a previously described microplate 1,9-dimethylmethylene blue dye assay.\textsuperscript{6} Results were reported as CSC micrograms per microliters of synovial fluid and as CSC micrograms per milligram of cartilage.
Radiographic assessment

All pre- and postoperative radiographs of the impacted stifles were assembled and examined by a board-certified radiologist for signs of degenerative joint disease. This observer was blinded to the status of horses.

Statistical analysis—A 2-way ANOVA (partial block design with location blocked on the horse) was used to compare mean cartilage sGAG concentration at the MFCi, MFCn, and MTP and between short-term and long-term cohorts of horses. An ANOVA for repeated measures with the Dunnett comparison was used to evaluate mean synovial fluid sGAG concentration over time. Biomechanical data were compared by use of a t test with length of treatment as a class effect. When no differences were found for thickness or modulus, these data were pooled and compared between experimental sites and normal controls for this laboratory (data not shown). The Wilcoxon-Mann-Whitney U test was used to compare the ICRS scoring system between short-term and long-term cohorts of horses. A Student t test was used to compare the modified Mankin, OARSI, and modified WORM scoring systems between the short-term and long-term cohorts of horses. Pearson correlation coefficients were determined for comparison of the histologic scoring systems (modified Mankin and OARSI) as well as the macroscopic scoring systems (ICRS and modified WORMS) following application of a multiple ANOVA to remove the cohort effect. Correlation plots of the residuals were also assessed to ensure that consideration of the mean correlation was appropriate (both positive or both negative). Agreement between observers (interclass correlation coefficient) for the histologic scoring systems was determined by use of a 2-way mixed-effect model. Analyses were performed by use of computer software. Values of P < 0.05 were considered significant.

Results

Determination of cartilage injury threshold—Mild uptake of ethidium bromide was observed, indicating chondrocyte membrane injury in the superficial zone of the articular cartilage that received 30-MPa injuries. This did not differ from controls at any time point. Impact injuries of 50 and 60 MPa created macroscopic fissures and extensive superficial zone cell injury (Figure 3). Middle- and deep-zone chondrocyte injury was evident where fissures penetrated these zones. At 80 Mpa, the impactor tip elevated the cartilage off the SCB, leaving no viable cells.

Assessment of impact injury in horses—Preoperatively, no horse had palpable distention of either

Surgical and clinical observations

Two horses in the short-term cohort required butorphanol (20 mg, IM, once) for supplementary analgesia in the immediate postoperative period. As a result of the size of the instrument portal, all horses had moderate extravasation of arthroscopic irrigating fluid (ie, lactated Ringer’s solution) and periarticular edema for the first 7 to 10 days. All horses were lame at a walk on the impacted limb for the first 72 hours after surgery. One horse disrupted the instrument portal.
portal incision during recovery from anesthesia, but this healed by the second intention following stent placement as well as cefiotaxim (2.2 mg/kg, IV, q 12 h) and phenylbutazone (2 g, PO, as needed) treatment for an additional 5 days. This complication prevented collection of synovial fluid from this horse on day 14.

Incisional complications were encountered for 2 horses in the long-term cohort. One horse spontaneously developed a subcutaneous periarticular abscess 21 days after surgery, which resolved with drainage. The other horse developed a discrete, subcutaneous seroma adjacent to the instrument portal 4 months after surgery.

At the time of euthanasia, grade 1 (on a scale of 5) hind limb lameness was present for 2 horses in the long-term cohort. In the short-term cohort of horses, lameness ranged from grade 1 to 2. All other horses were sound. Hind limb flexion increased the lameness for 1 horse in the short-term cohort from grade 0 to 1. In the long-term cohort of horses, 1 horse had moderate effusion in the femoropatellar and MFT joints, whereas 2 other horses had mild periarticular thickening. Findings on stifle joint palpation were unremarkable in all other horses.

Synovial fluid analysis

Synovial fluid samples were recovered for each horse at every time point with the exception of the day 14 sample from 1 horse. Appearance of the samples ranged from serosanguineous to colorless. Blood contamination and low viscosity samples were observed more frequently in the early postoperative period; however, synovial fluid appeared normal by 6 to 8 weeks after surgery. Leukocyte and total protein concentrations were below 2.0 X 10^9 cells/L and 20 g/L, respectively, by 4 weeks after surgery.

Postmortem assessments

A broad spectrum of macroscopic abnormalities was recognized in the MFT joint of all horses (Figure 4). Synovitis, characterized by mild synovial membrane hypertrophy and discoloration, was present. In all horses, the articular surface of the MFC and MTP was roughened. Observers described this as a stippled or cobblestone appearance. Loss of surface reflectivity was evident and correlated with uptake of India ink.

In the short-term cohort of horses, India ink uptake in partial- and full-thickness erosions and fissures was observed. In all but 1 horse, the MFCi was readily identified as a discrete, depressed area that was stained with ink on the axial weight-bearing articular surface of the MFC. The cartilage remained attached to the underlying bone but was fibrillated, folded, and thin. For 1 horse in the short-term cohort, the MFCi was not discernible from the MFCn. No abnormalities of the medial meniscus, lateral femorotibial, or femoropatellar joints were identified. Small osteophytes were seen in 2 horses on the abaxial surface of the MFC. No horse developed osteochondral fragments or overt articular instability.

In the long-term cohort of horses, the degree and distribution of India ink uptake were more extensive and local full-thickness defects were documented in all horses. Cartilage at the MFCi was typically elevated, resulting in an undermined flap of thickened soft articular cartilage and exposed calcified cartilage. Cartilage within 2 to 3 mm of the MFCi was invariably flattened and thin. Mild fibrillation of the free border of the medial meniscus was documented in 4 of 5 of the long-term cohort of horses.

### Table 1—The ICRS score and modified macroscopic WORM data from the MFT joint of experimental horses.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Short-term cohort (n = 5)</th>
<th>Long-term cohort (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICRS score*</td>
<td>1A (n = 1)</td>
<td>None</td>
</tr>
<tr>
<td>Flat-thin areas</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>PT lesions</td>
<td>2 (3)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>FT lesions</td>
<td>3B (3)</td>
<td>3B (4)</td>
</tr>
<tr>
<td>SCB exposed</td>
<td>None</td>
<td>3C (2)</td>
</tr>
<tr>
<td>Blister</td>
<td>None</td>
<td>3D (1)</td>
</tr>
<tr>
<td>Worst score for each horse</td>
<td>1A (1), 2 (1), 3B (3)</td>
<td>3B (2), 3C (2), 3D (1)</td>
</tr>
<tr>
<td>Overall mean (SD NA)</td>
<td>3A</td>
<td>3C</td>
</tr>
<tr>
<td>Modified WORMS1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartilage (0–6)</td>
<td>3 [4], 5 (1)</td>
<td>2.5 (2), 3 [1], 5 (2)</td>
</tr>
<tr>
<td>Cysts (0–3)</td>
<td>0 (5)</td>
<td>0 (5)</td>
</tr>
<tr>
<td>Bone attrition (0–3)</td>
<td>0.5 (2)</td>
<td>0 (2), 2 (3)</td>
</tr>
<tr>
<td>Osteophytes (0–7)</td>
<td>0 [3], 2 (2)</td>
<td>0 (1), 2 [3], 3 (1)</td>
</tr>
<tr>
<td>Meniscus (0–6)</td>
<td>0 (5)</td>
<td>0 (1), 1 (4)</td>
</tr>
<tr>
<td>Synovitis (0–3)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Loose bodies (0–3)</td>
<td>0 (5)</td>
<td>0 (5)</td>
</tr>
<tr>
<td>Bursae (0–3)</td>
<td>0 [4], 1 (1)</td>
<td>0 (4), 1 (1)</td>
</tr>
<tr>
<td>Total (34 possible)</td>
<td>5 (2), 6 (1), 8 (2)</td>
<td>12 (1), 9 (2), 7.5 (1), 5.5 (1)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6 ± 1.5</td>
<td>9 ± 2.4</td>
</tr>
</tbody>
</table>

*In the ICRS scoring system, the grade of the most severe lesion is the final score, where 0 = normal, 1A = minor softening, 1B = fissures, 2 = lesions involving < 50% of cartilage thickness, 3A = lesions involving > 50% of cartilage thickness, 3B = calcified cartilage, 3C = exposure of SCB, 3D = cartilage blisters, 4A = mild SCB damage, and 4B = SCB eburnation. In the WORM scoring system, the entire MFT joint including bone and all soft tissue structures is accessed, where 0 = normal and increasing values indicate more degenerative change.

NA = Not applicable. PT = Partial thickness. FT = Full thickness. Blister = A focal area of swollen cartilage.
term cohort of horses. The lateral femorotibial and femoropatellar joints had minor abnormalities that were limited to loss of articular surface (ie, sheen), and mild fibrillation was observed in 3 horses. Small to moderately sized (3- to 5-mm-long) osteophytes were seen for 4 of 5 horses in the long-term cohort on the abaxial surface of the MFC. Again, no osteochondral fragmentation or overt articular instability was observed.

ICRS scores
The ICRS scoring system was used to grade each lesion on the MFC and MTP; according to convention, the final grade represents the most severe lesion (Table 1). In all horses except 1 of the long-term cohort, the most severe lesion was at the MFCi but the MTP was always damaged. Scores ranged from 1A to 3D. All but 1 horse in the short-term cohort had more than 1 lesion within the MFT joint that could be scored. When ICRS scores of the short-term and long-term cohorts of horses were compared, they were significantly \((P = 0.044)\) different.

Modified WORMS
Scores ranged from 5 to 12 out of a possible 34 (Table 1). The modified WORMS increased between the short-term and long-term cohorts of horses, but this difference was not significant \((P = 0.11)\). When the ICRS and modified WORM scoring systems were compared, no significant \((r^2 = 0.01; P = 0.77)\) correlation was found between them.

Biomechanical creep indentation testing
Complete data sets were available for only 6 of 10 horses (Table 2) because of technical difficulties in measuring extremely thin degenerate cartilage at the MFCi. Normal aggregate modulus and thickness for the MFC cartilage are 2.4 \(\pm\) 3.4 MPa and 1.99 \(\pm\) 0.53 mm. The MTP has a similar aggregate modulus and cartilage thickness in the central nonmeniscus-covered weight-bearing area. The MFCi had an articular surface that was significantly \((P = 0.008)\) less yielding when a load was applied and thinner \((P < 0.001)\) than normal controls and the MFCn. The high modulus for 2 horses in the short-term cohort and 1 horse in the long-term cohort corresponds to a thin layer of degenerate cartilage over the SCB. In the MFCn, the loss of mechanical properties was less dramatic, although the cartilage was thinner than normal. The same was true in the MTP cartilage. The high modulus value of this site for 1 horse in the short-term cohort corresponded to a focal erosion. The aggregate modulus of the MFCn was not significantly \((P = 0.244)\) different from that of normal controls, although the MFCn thickness was significantly \((P = 0.025)\) different.

Cartilage sGAG concentration
Despite variation in cartilage sGAG concentration at all sites, a significant \((P < 0.001)\) difference was found between the MFCi and MFCn for horses of each cohort (Table 3). This difference is beyond normal site-to-site variation in the MFC (data not shown). A significant \((P < 0.001)\) difference was found between the MFCi and MTP but not between the MFCn and MTP \((P = 0.11)\). No significant \((P = 0.28)\) difference was found between short-term and long-term cohorts of horses at either the MFCn or MTP.

Histologic examination and scoring
In all horses, the MFCi was readily identified, and taken together with lesions in the MFCn, all horses had structural changes consistent with osteoarthritis. The superficial chondrocyte zone and its birefringence with polarized light were absent in the entire MFC and most of the MTP. Diffuse delamination of the superficial zone that exposed deeper regions of fissures was observed, but no evidence of subchondral cystic lesion development was found.

In the short-term cohort of horses, the damaged articular cartilage at the MFCi typically had pale matrix staining and remained in situ, although it was commonly detached from the SCB in a horizontal manner at the level of the tidemark. Cartilage fragmentation, areas of hypocellular matrix, chondrocyte clusters (chondrones), and fissures within the superficial and middle zones were present. Similar fissures were present in the MFCn and MTP. Duplication of the tidemark and incursion of subchondral vasculature into calcified cartilage were frequently present on both joint surfaces, but no other abnormalities of the SCB were seen. Evidence of repair tissue was limited and consisted of scant pockets of poorly differentiated fibroblasts of unknown origin (Figure 5).

In the long-term cohort of horses, the same spectrum of lesions was found but more extensive and deeper fissures were present. Cartilage at the MFCi was elevated from the calcified cartilage or absent. This layer was never breached to expose SCB. Remaining cartilage fragments were markedly hypocellular, contained only chondrocyte clusters, and had little matrix staining. Tidemark duplication was present, and SCB

---

**Table 2**—Mean ± SD (range) of biomechanical creep indentation testing data from the MFC and MTP of experimental horses.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Short-term cohort ((n = 5))</th>
<th>Long-term cohort ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MFCi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulus (MPa)</td>
<td>9.45 (\pm) 3.22 (7.63–11.40)</td>
<td>7.39 (\pm) 5.53 (2.60–15.18)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>0.41 (\pm) 0.24 (0.19–0.69)</td>
<td>0.39 (\pm) 0.22 (0.03–1.87)</td>
</tr>
<tr>
<td><strong>MFCn</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulus (MPa)</td>
<td>2.57 (\pm) 0.96 (1.07–3.70)</td>
<td>2.65 (\pm) 0.66 (2.62–3.31)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.20 (\pm) 0.52 (0.57–1.68)</td>
<td>1.08 (\pm) 0.73 (0.25–2.26)</td>
</tr>
<tr>
<td><strong>MTP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulus (MPa)</td>
<td>5.14 (\pm) 6.62 (2.02–15.0)</td>
<td>2.48 (\pm) 0.82 (1.73–3.36)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.18 (\pm) 0.41 (0.76–1.76)</td>
<td>1.54 (\pm) 0.82 (0.37–2.62)</td>
</tr>
</tbody>
</table>

**Table 3**—Mean ± SD (range) of sGAG concentrations from the MFC and MTP of experimental horses.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Short-term cohort ((n = 5))</th>
<th>Long-term cohort ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MFCi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sGAG (mg/mg)</td>
<td>37.0 (\pm) 20.8 (15.2–65.4)</td>
<td>20.7 (\pm) 9.1 (9.4–34.1)</td>
</tr>
<tr>
<td><strong>MFCn</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sGAG (mg/mg)</td>
<td>52.0 (\pm) 12.9 (30.4–63.2)</td>
<td>54.7 (\pm) 10.6 (44.8–72.0)</td>
</tr>
<tr>
<td><strong>MTP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sGAG (mg/mg)</td>
<td>63.2 (\pm) 7.8 (55.2–72.5)</td>
<td>61.8 (\pm) 8.5 (54.4–70.0)</td>
</tr>
</tbody>
</table>

Values with different superscript letters are significantly \((P < 0.05)\) different.
Figure 5—Photomicrographs of sections taken at the junction of the MFCi and MFCn or adjacent to lesions in the MTP that demonstrate the spectrum of pathologic changes. Histologic sections are shown at low (20X; panels on left) and high (100X; panels on right) magnification. Notice that the MFC (A and B) and MTP (C and D) of a horse in the long-term cohort have the typical destruction of articular cartilage at the MFCi. This corresponds to an OARSI score of 31 and a modified Mankin score of 18. Notice that the MFC (E and F) and the MTP (G and H) of a horse in the short-term cohort have a focal partial-thickness MFCi zone with deep radiating fissures. The MFCn area has delamination of the superficial cartilage zone (F). The latter finding is also present in cartilage from the MTP (H). This corresponds to an OARSI score of 24 and a modified Mankin score of 15. H&E stain; left panels, bar = 1000 µm; right panels, bar = 200 µm.
abnormalities were limited to a mild increase in secondary osteons in the subchondral plate. Evidence of cartilage repair was limited to matrix flow around the perimeter of the MFCI, poorly differentiated fibroblasts lining the acellular matrix, and exposed calcified cartilage in several horses (Figure 5).

Synovial membrane and joint capsule were normal with the exception that mild villous hypertrophy was found in 2 horses in the long-term cohort and increased vascularity was identified in 2 horses in the short-term cohort and 2 horses in the long-term cohort. One horse in the short-term cohort had a small population of plasma cells identified deep in the intimal layers of several villi, but no other WBC infiltrates were seen.

Modified Mankin and OARSI scores were slightly increased for horses in the long-term cohort, compared with horses in the short-term cohort, but this difference was not significant (modified Mankin, \( P = 0.42 \); OARSI, \( P = 0.40 \); Table 4). Interobserver agreement was considered good \( \left( r^2 = 0.42 \right) \) for the modified Mankin scoring system and \( \left( r^2 = 0.73 \right) \) for the OARSI scoring system, but correlation between these 2 scoring systems was poor \( \left( r^2 < 0.01; P = 0.88 \right) \).

The most severe but smaller lesions were at the MFCI, whereas the MFCn and MTP had diffuse but less-severe cartilage degeneration. The OARSI system allowed different degrees of pathologic change in the MFC to be described, whereas the modified Mankin score required a single score for the entire condyle, which was dictated by the MFCI.

**TUNEL**

The TUNEL-positive (apoptotic) cells were present in the superficial cartilage layer of all horses at the MFCI, MFCn, and MTP. No discernible difference was found between short-term and long-term cohorts of horses. At the MFCI, TUNEL-positive cells were concentrated beneath the impact zone, extending through the middle zone to the deep zone. The depth and concentration of TUNEL-positive cells decreased with increasing distance from the impact zone. Empty chondrocyte lacunae without a nucleus were also a prominent feature in the MFCI (Figure 6).

**COL2-\( \alpha \)C\(_{short}\) Immunohistochemistry**

Positive staining for the COL2-\( \alpha \)C\(_{short}\) epitope was identified in the articular cartilage of all horses at the MFCI; no differences in staining results were found between short-term and long-term cohorts of horses.

### Table 4

<table>
<thead>
<tr>
<th>Scoring systems</th>
<th>Sites</th>
<th>Short-term cohort (n = 5)</th>
<th>Long-term cohort (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OARSI</td>
<td>MFCi</td>
<td>4.17–5.50</td>
<td>4.33–5.50</td>
</tr>
<tr>
<td></td>
<td>Stage (1–4)</td>
<td>1 (5)</td>
<td>1 (5)</td>
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<tr>
<td></td>
<td>Score (1–6)</td>
<td>4.17–5.50</td>
<td>4.33–5.50</td>
</tr>
<tr>
<td>MFCn</td>
<td>Grade (1.0–6.5)</td>
<td>2.33–3.33</td>
<td>2.33–2.67</td>
</tr>
<tr>
<td></td>
<td>Stage (1–4)</td>
<td>3 (5)</td>
<td>3–4</td>
</tr>
<tr>
<td>MTP</td>
<td>Grade (1.0–6.5)</td>
<td>2.00–3.00</td>
<td>2.33–4.00</td>
</tr>
<tr>
<td></td>
<td>Stage (1–4)</td>
<td>2–4</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>Score (1–6)</td>
<td>4.00–12.00</td>
<td>4.86–16.00</td>
</tr>
<tr>
<td>Total</td>
<td>15–24</td>
<td>17–31</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>21 ± 3.4</td>
<td>23 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Mankin</td>
<td>MFC</td>
<td>4.00–6.00</td>
<td>5.67–6.00</td>
</tr>
<tr>
<td></td>
<td>Structure (0–6)</td>
<td>3.00–4.00</td>
<td>3.33–4.00</td>
</tr>
<tr>
<td></td>
<td>Cell abnormalities (0–3)</td>
<td>2.00–3.00</td>
<td>2.67–3.00</td>
</tr>
<tr>
<td></td>
<td>Matrix staining (0–4)</td>
<td>9.33–13.00</td>
<td>11.67–13.00</td>
</tr>
<tr>
<td></td>
<td>Score (1–6)</td>
<td>3.00–4.00</td>
<td>3.33–4.00</td>
</tr>
<tr>
<td>MTP</td>
<td>Structure (0–6)</td>
<td>3.00–4.00</td>
<td>3.00–4.00</td>
</tr>
<tr>
<td></td>
<td>Cell abnormalities (0–3)</td>
<td>0.33–1.67</td>
<td>0–1.00</td>
</tr>
<tr>
<td></td>
<td>Matrix staining (0–4)</td>
<td>0–0.67</td>
<td>0–1.67</td>
</tr>
<tr>
<td></td>
<td>Score (1–6)</td>
<td>3.67–6.00</td>
<td>3.00–6.33</td>
</tr>
<tr>
<td>Total</td>
<td>13.50–18.67</td>
<td>15.67–18.00</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>16 ± 2.1</td>
<td>17 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

*Values are the mean of scores reported by the 3 persons reviewing the slides. The final scores were rounded to the nearest whole number. \( \) Mathematic product of the grade and stage.
as comparable to naturally occurring osteoarthritis.48-50 That a single traumatic event can result in lesions that line (time 0).

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Mild to moderate lameness.48-50 Radiographic findings, mal joint effusion, few palpable abnormalities, and many horses with stifle joint osteoarthritis have mini-
es and is believed to be the cumulative effect of repeat-

increasing distance from the impact zone.

The stain was concentrated around the periphery of the impact zone and decreased in intensity with increasing distance from the impact zone.

Synovial fluid sGAG concentration
Baseline synovial fluid sGAG concentration was established by use of perioperative samples (time = 0). A significant decrease in mean synovial fluid sGAG concentration was documented at days 14, 28, 42, and 56 (P = 0.003, 0.002, < 0.001, and 0.005 for each time point, respectively), compared with baseline (Figure 7). On day 84, the decrease was no longer significant (P = 0.85) and concentrations gradually increased to approach baseline. On day 168, synovial fluid sGAG concentrations were increased above baseline, although not significantly (P = 0.51).

Radiographic assessment
No radiographic evidence of degenerative joint disease was found in any horse before or after surgery. No radiographic evidence of SCB cyst development was observed.

Discussion
In our study, we experimentally induced a broad spectrum of pathologic change in the MFT joint, of which the most substantial finding was cartilage loss and thinning. More severe erosions were present for horses in the long-term cohort, compared with the short-term cohort, suggesting that the osteoarthritis was progressive; however, the widespread loss of the superficial cartilage layer for horses in the short-term cohort indicates that these joint surfaces are likely to degenerate further, with little chance for recovery. The collagenous network of the cartilage is critical to its biomechanical properties, and superficial loss of this layer is thought to be the initial step toward irreversible, progressive cartilage degeneration.49

Damage to the articular cartilage of the weight-bearing surface of the MFC has been described in horses and is believed to be the cumulative effect of repeated trauma.49-50 Data of our study support the concept that a single traumatic event can result in lesions that are comparable to naturally occurring osteoarthritis.49-50

Many horses with stifle joint osteoarthritis have minimal joint effusion, few palpable abnormalities, and mild to moderate lameness.49-50 Radiographic findings, when present, are generally vague.49,50 By the time stifle joint osteoarthritis creates detectable clinical signs, relatively advanced cartilage lesions are found that are likely to progress quickly, leading to a guarded prognosis in affected horses.49-50 This is consistent with our data and observations.

The use of horses has some advantages in the study of osteoarthritis and cartilage repair. The large size of horses permits arthroscopy as well as serial arthrocentesis, and the thick cartilage of the MFT joint is similar to that of the human knee. Others have studied induced osteoarthritis in horses through the creation of instability or osteochondral incongruity in joints with thin cartilage and more-limited arthroscopic access. In our study, an advantage of the use of conclusive impact was that a small amount of cartilage was damaged and articular incongruities that were induced were minor and unlikely to have resulted in high amounts of local stress, which have been documented with large full-thickness chondral, osteochondral, or subchondral defects.50 Without ongoing injury, the progression of osteoarthritis beyond the initial lesion could be modified by use of chondroprotective drugs, thus assessing their efficacy as treatment for osteoarthritis.

One requirement for the establishment of experimentally induced osteoarthritis in our study was the identification of the required injury threshold. In other species, much lower forces (15 to 35 MPa) have been used.49,50,51 In experiments in which isolated cartilage is used, damage has been produced with as little as 7 to 15 MPa because cartilage is highly susceptible to mechanical injury when separated from SCB.49,50 In our study, the cartilage-calcified-cartilage-SCB continuum was intact when the injury was applied in the ex vivo and in vivo experiments.

Articular cartilage of humans,73 dogs,52,52 ox,52 and rabbits32,35 can be damaged by impact forces of approximately 25 MPa. This closely agrees with thresholds established in our laboratory for the MFC of dogs and sheep.5 In our preliminary work, it was apparent that equine cartilage was more resistant to impact injury than cartilage from other species. We failed to identify pathologic changes in cartilage of the MFC at values below 50 MPa, whereas at other sites, such as the equine third carpal bone, even higher thresholds of injury were found (data not shown). It is important to note that the initial damage was confined to the superficial cartilage zone and areas of mechanical disruption. Short-term tissue cultures did not demonstrate progression of cell injury into the deeper zones or surrounding uninjured tissue; therefore, in vivo experiments were required to prove that this injury is progressive, leading to irreversible degeneration in the surrounding and opposing articular surfaces. Four adjacent 6.5-mm-diameter impact injuries of 60 MPa constituted a critically sized defect that did not heal spontaneously. This is consistent with previous work that suggests that a 15-mm-diameter lesion may be a critically sized defect in weight-bearing locations of the MFT joint.49

Application of impact force to a curved articular surface required that the impactor tip be perpendicular

Figure 7—Mean ± SD synovial fluid sGAG concentrations of horses versus time. *Significantly (P < 0.05) different from baseline (time 0).
Desjardins et al. for implanting small osteochondral assay as an inexpensive, easily measured, semi-invasive technique (for the medial femoral trochlea). Although it is conceivable that these lesions may have induced some degree of lameness, this was not documented clinically in any of our horses. Therefore, it is reasonable to assume that the load experienced by the impacted stifle joint would be within physiologic limits, thus minimizing the effect of altered weight bearing on the development and progression of PTOA.

Our surgical approach using arthroscopic guidance through a large instrument portal was practical but shared some of the risks of a traditional arthrotomy. Our incision was similar to that used by Desjardins et al. for implanting small osteochondral grafts and reduced the postoperative inflammation, lameness, and need for exercise restriction associated with stifle arthroscopy. The lameness in our horses was mild and transient in nature, and inflammation resolved by 4 weeks after surgery as judged by synovial fluid analyses. Incisional complications were more frequent in the long-term cohort of horses, which was operated on first, and may reflect slightly longer operative times and inexperience with the procedure. A randomization of the short-term and long-term cohorts of horses might have resulted in no differences between groups.

Lesions created in the contralateral stifle joint consisted of cartilaginous defects (the largest of which was 8 × 10 mm) that were treated by use of mosaic arthroplasty (for the MFC) and SCB microfracture techniques (for the medial femoral trochlea). Although it is conceivable that these lesions may have induced some degree of lameness, this was not documented clinically in any of our horses. Therefore, it is reasonable to assume that the load experienced by the impacted stifle joint would be within physiologic limits, thus minimizing the effect of altered weight bearing on the development and progression of PTOA.

The clinical diagnosis of osteoarthritis is always difficult, and findings of our study emphasize the insidious nature of this disease. By 3 months after injury, irreversible degenerative changes were present, reiterating the need for early diagnosis and intervention. To address this, we elected to monitor synovial fluid sGAG concentrations using 1,9-dimethylmethylene blue dye assay as an inexpensive, easily measured, semi-invasive biomarker of joint injury.19 Immunoassays that identify specific epitopes of the degradative or synthetic osteoarthritic pathways may prove more valuable in evaluating the balance between these opposing forces.33,34,54 We documented a significant decrease in synovial fluid sGAG concentration on days 14 to 56 after surgery. This agrees with the findings of Todhunter et al.35 and Oke et al., who also documented decreased synovial fluid sGAG concentrations in horses with naturally occurring osteoarthritis. Synovial fluid sGAG concentrations reflect the metabolic capacity of the chondrocytes.31 The initial decrease in synovial fluid sGAG concentrations documented in our study can be attributed to the chondrocyte death induced by contusive injury. Over time, as the osteoarthritis progressed, undamaged cells became hypertrophic and synovial fluid sGAG concentrations finally increased to above baseline values.

Results of studies in other species reveal that synovial fluid sGAG concentration increases with concurrent proteoglycan loss from cartilage.5,33-34 In our study, depletion of cartilage sGAG at all sites was documented as expected, as preexisting proteoglycan was lost secondary to disseminated articular structural changes.5,33,34 Once depleted, the proteoglycans of osteoarthritic cartilage are not replenished.33,34 In our study, mechanical injury appears to have resulted in substantial chondrocyte damage, resulting in acellular areas of matrix. These damaged chondrocytes attempted repair through increased synthesis of matrix constituents, while simultaneously increasing the number of active chondrocytes via cloning. However, this upregulated synthesis is uncoordinated, producing proteoglycan fragments that cannot be properly assembled and are subsequently degraded and lost into the synovial fluid.43-45 Later in the disease course, enzymatic degradation of proteoglycans becomes important.33,34,56 Over time, viable chondrocytes become irreversibly catabolic, then apoptotic, and finally die, resulting in a loss of a critical number of chondrocytes. Remaining cells were unable to effectuate repair, and the areas of inert, partially attached acellular matrix we observed at the MFC 3 months after surgery became detached and were lost by 6 months. We assume that this degenerate, poorly attached tissue was unable to withstand normal load bearing. Cartilage biomechanical properties mirrored the loss of sGAG at the MFC and reflected extremely thin, degenerate cartilage overlying hard SCB. The MFC was less degenerate, and the creep indentation method used may not have been sensitive enough to measure subtle loss of biomechanical properties.

The TUNEL-positive chondrocytes, pale-staining (ghost) chondrocytes, and empty chondrocyte lacunae were found in all sites in both cohorts of horses. Such cells were most frequent in the superficial zone but also numerous at the MFCi and SCB, where fissures penetrated the deeper cartilage zones.12,13 Use of TUNEL identifies apoptotic and late-stage necrosis cells, so their presence in the long-term cohort of horses indicated that an ongoing loss of cells had occurred. No phagocytic cells are present within articular cartilage; therefore, damaged cartilage is remodeled slowly, and it is unknown how long necrotic debris, apoptotic bodies, and chondrocyte ghosts may persist.12 Large areas of tidemark duplication were seen in the MFC, whereas it was often focal in the MTP. Duplication of the tidemark is a common finding in osteoarthritic cartilage and is considered evidence of reactivation of endochondral ossification.33,34,19 This results in cartilage thinning, SCB stiffening, and subsequent continued injury of the overlying cartilage.12,13 Vascular penetration through the calcified cartilage into the deep zone of the cartilage has been described after impact injury and was occasionally identified in the MFC of horses in our study.

The cleavage of type II collagen by collagenases linked to progressive osteoarthritis results in the pro-
duction of one-quarter- and three-quarter-length fragments. The antibody used in our study identified the collagenase-specific cleavage site (COL2–¾C_short), providing evidence of type II collagen degradation. Although the techniques used in our study identified only the presence of degraded collagen within the articular cartilage, it is plausible that this marker would also be present in the synovial fluid and might be used for early diagnosis of PTOA.

Subchondral cystic lesions have been described in osteoarthritis of horses and may occur secondary to mechanical trauma,77 full-thickness articular defects,78 and SCB defects.78 The size of the defect appears to be a critical factor in determining whether cystic lesions develop.75,78 Synovial fluid forced under pressure through a narrow defect in the articular surface may be necessary.79 Injuries created in our study did not produce SCB cysts despite full-thickness cartilage erosions in several horses. This may have been related to an inability of these lesions to act like a 1-way valve. It is also possible that the 60-MPa impact used in our experiment may not have damaged the SCB sufficiently to trigger the resorptive phase of remodeling necessary for the formation of SCB cysts.70,78 Alternatively, this remodeling may not be evident until later in the osteoarthritic process.79 It is also possible that our evaluation methods were not sensitive enough or able to identify pathologic changes in the SCB.

Meaningful, reproducible, and validated assessment of pathologic changes of joints always presents a challenge in studies such as ours. Routine techniques such as viewing the cartilage surface with low-angle incidence light and India ink staining64 were valuable. The loss of surface reflectivity was a predictor of superficial zone loss or delamination. India ink staining proved to be a quick and reliable method for identifying and photographing diffuse lesions. The MFC and MTP of all horses took up India ink, allowing the identification of multiple full- and partial- thickness erosions and fissures on the articular surface areas, which supported the presence of articular pathologic changes.

No widely accepted method for reporting macroscopic lesions exists.82,89 Therefore, our study used 2 systems in an attempt to illustrate the broad spectrum of pathologic changes produced in joints. The ICRS scoring system was designed for intraoperative documentation of cartilage lesions in the human knee and provides a detailed description of an individual lesion (a requisite when repair is contemplated). However, it was unable to capture the subtleties of early osteoarthritic lesions such as diffuse loss of surface reflectivity and partial-thickness cartilage loss. It is an alphanumeric score, making it necessary to convert this score to an ordinal scale so that multiple lesions within a joint surface could be added to generate a comprehensive cartilage score. This proved to be valuable when comparison of short-term and long-term cohorts of horses revealed that significantly deeper lesions were present for horses in the long-term cohort, providing additional evidence for the progression of osteoarthritis.

The modified WORM score was designed to reflect global injury and degeneration with an emphasis on magnetic resonance imaging for detection of subchondral bruising, bone marrow edema, and ligamentous injury. The modification used in our experiment allowed us to assign a single score that reflected an inclusive assessment of soft tissue, cartilage, and bone for all joint surfaces. Although progression of osteoarthritic lesions was apparent, comparison of the modified WORM scores between the short-term and long-term cohorts of horses did not reveal a significant difference.

Two validated histologic scoring systems were also used in our study because the widely used modified Mankin system was unable to accurately portray the lesions we observed. This scoring system confines the observer to specified cellular and structural abnormalities that are assigned a specific order of appearance. This did not accurately describe osteoarthritis resulting from a focal impact lesion because specific structural changes, such as superficial zone loss, took place before fissures were present. Similarly, hypocellular areas of empty matrix were found more frequently than chondrocyte clusters. The OARSI score did not assume a specific pathogenesis, and its more flexible volumetric approach was, in our estimation, a better system. Unlike the modified Mankin system, it does not rely on characterizing the worst lesion, so diffuse or subsidiary lesions can be included in the final score. Interobserver agreement was within the realm of clinical acceptability for both scoring systems; however, it was much better with the OARSI system.

The inability to detect significant differences between the short-term and long-term cohorts of horses by use of these scoring systems, with the exception of the ICRS system, may be related to the small group size. However, it is also possible that the most severe lesions were already established at 3 months, with minimal progression between 3 and 6 months. The lack of a significant correlation between the macroscopic and histologic scoring systems reflects the different criteria used in each.

We were successful in inducing a broad spectrum of macroscopic pathologic changes including, but not limited to, effusion, synovitis, capsulitis, meniscal fibillation, cartilage delamination, cartilage erosion, cartilage elevation or undermining, cartilage fissure formation, osteophyte formation, and exposed SCB within the MFT joint of all horses. These developments are all consistent with the initiation and progression of PTOA.15,23,53,103,112,114,133,32,57,89 Cartilage injury alone appeared to be the predominate lesion. More-severe lesions were identified most frequently for horses of the long-term cohort, although a significant difference between cohorts was identified only by use of the ICRS scoring system.

Results of our study on PTOA in horses are similar to those in studies of other species, although the threshold of cartilage injury was demonstrated to be higher in horses. Our data support the hypothesis that a single traumatic episode can lead to osteoarthritis and that degeneration present at 3 months is irreversible, progressing to partial- and full-thickness cartilage erosion by 6 months. The lesions reported here are primarily unicompartmental and confined to the MFT joint. Three horses in the long-term cohort did
have loss of surface reflectivity and diffuse India ink staining of the troclear groove, indicating that extension to adjacent joint compartments will occur. Methods developed in our study may be useful for assessment of early diagnostic, prevention, and treatment of PTOA in horses and other species.

b. MLP-500, Transducer Techniques, Temecula, Calif.
c. LabVIEW, National Instruments, Austin, Tex.
d. Ioban, 3M Health Care, Saint Paul, Minn.
e. Papain, Sigma Chemical Co, St Louis, Mo.
f. Hyaluronidase, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.
g. Proteinase K, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.
h. ApoPTOAg plus peroxidase detection kit, No. S7101 Chemicon International, Temecula, Calif.
i. Nuclease, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.
j. Hydrogen peroxide (0.5%) in methanol, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.
k. Chondroitinase ABC, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.
l. Protein block, Dako Cytomation, Mississauga, ON, Canada.
m. CCL2–¾Cshort antibody, IBEX, Montreal, QC, Canada.
n.  Biotinylated antibody, Vector Laboratories, Burlington, ON.
o. Streptavidin-HRP, Dako Cytomation, Mississauga, ON, Canada.
p. DAB substrate, Dako Cytomation, Mississauga, ON, Canada.
q. SAS, version 8.0, SAS Institute Inc, Cary, NC.
r. Changoo A. Evaluation of large subchondral bone lesion repair on the equine stifle joint. MS thesis, University of Guelph, Guelph, ON, Canada.
Appendix

The OARSI score is the mathematic product of the grade and stage.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Subgrade</th>
<th>Stage (joint-surface involvement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–Surface intact</td>
<td>1.0–Cells intact</td>
<td>1–&lt; 10%</td>
</tr>
<tr>
<td>2–Surface discontinuity</td>
<td>2.0–Fibrillation through superficial zone</td>
<td>2–10% to 25%</td>
</tr>
<tr>
<td>3–Vertical clefts</td>
<td>2.5–Surface abrasion</td>
<td>3–26% to 50%</td>
</tr>
<tr>
<td>4–Erosion</td>
<td>3.0–Simple clefts</td>
<td>4–&gt; 50%</td>
</tr>
<tr>
<td>5–Denudation</td>
<td>3.5–Branched/complex clefts</td>
<td></td>
</tr>
<tr>
<td>6–Deformation</td>
<td>4.0–Superficial zone delamination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5–Midzone erosion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0–Bone surface intact</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5–Reparative tissues present</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0–Joint margin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.5–Joint margin and internal</td>
<td></td>
</tr>
</tbody>
</table>

* A total score for the MFT joint was determined by summing scores for the MFCi, MFCn, and MTP.