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The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the horse

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SUMMARY

Objective: Equine models of osteoarthritis (OA) have been used to investigate pathogenic pathways of OA and evaluate therapeutic candidates for naturally occurring equine OA which is a significant clinical disease in the horse. This review focuses on the macroscopic and microscopic criteria for assessing naturally occurring OA in the equine metacarpophalangeal joint as well as the osteochondral fragment-exercise model of OA in the equine middle carpal joint.

Methods: A review was conducted of all published OA studies using horses and the most common macroscopic and microscopic scoring systems were summarized. Recommendations regarding methods of OA assessment in the horse have been made based on published studies.

Results: A modified Mankin scoring system is recommended for semi-quantitative histological assessment of OA in horses due to its already widespread use and similarity to other scoring systems. Recommendations are also provided for histological scoring of synovitis and macroscopic lesions of OA as well as changes in the calcified cartilage and subchondral bone of naturally occurring OA.

Conclusions: The proposed system for assessment of equine articular tissues provides a useful method to quantify OA change. It is believed that addition of quantitative tracing onto plastic and macroscopic measurement as recently described would be an improvement for overall assessment of articular cartilage change.

Introduction

Spontaneous osteoarthritis (OA) is a common problem in the horse. Equine degenerative arthritis was reported as an entity and the pathologic changes were compared to human OA in 1930[1]. Equine OA was reviewed in 1962[2] and macroscopic and microscopic changes were described for OA in the distal limb joints of the horse in 1973[3]. OA can occur early in equine athletes, or later in older horses[4–6]. An arthroscopic grading system has also been used for clinical disease in the carpus[7] and fetlock joints[8] and biomarkers related to macroscopic and histologic cartilage lesions have been evaluated[9,10].

A number of experimental models of OA have been developed and used in the horse[11–38] (Table I). Unlike the dog, complete surgical transection of the anterior cruciate ligament (ACL) alone arthroscopically in horses did not result in progressive OA in two cases (Trotter GW, unpublished data, 1996). However in a published study involving one of the authors (MH) arthroscopic and radiographic assessment of naturally occurring ACL deficient horses resulted in severe OA and the horses were Grade 2 or more lame[39]. The most commonly published model is the arthroscopically created osteochondral fragment-exercise model developed at Colorado State University (CSU)[25–34]. This model evolved with two major changes since its inception: (1) burring back an osteochondral lesion that was larger than the fragment; (2) not flushing tissue debris from the joint post-defect creation. This model is clinically relevant to horses and has relevance to human OA. Unlike many other *in vivo* models, no instability is created. The model induces progressive OA, yet it does not produce a severe lameness[25–34,40]. Macroscopic and histological evaluations are presented in this paper.

The various methods of describing macroscopic changes that have been reported are listed in Table II[10,25–34,41–45]. Histological grading (microscopic changes) systems for articular cartilage[25–34,47] have used modifications of the Mankin grading system[48,49] and the Osteoarthritis Research Society International (OARSI) scoring

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Table I
Experimental models of OA that have been described in the horse

Type of model	Specific name
Intra-articular injection of chemicals	Filipin[11,12] Sodium monoiodoacetate[13–15] Amphotericin[16] *E. coli* lipopolysaccharide[17–19] Interleukin-1[20] Polyvinylalcohol foam particles[21] Carrageenan[22]
Instability	Carpal fracture[23] Cutting collateral & collateral sesamoidean ligaments in metacarpophalangeal joint[24]
Osteochondral fragmentation and exercise	Carpal osteochondral fragment-exercise model[25–34]
Trauma	Single impact on medial femoral condyle[35] – progresses to focal defects and OA
Disuse	Lower limb cast immobilization[36–38]

system[35] (Table II). It has also been pointed out that validation studies are necessary when developing alternative methods for scoring[50].

Anatomy and joint pathology

The metacarpophalangeal (MCP) joint is the most common site for spontaneous OA followed by the carpal joints. The most published equine model is the osteochondral chip fragment-exercise model in the middle carpal joint. A major difference in these joints compared to the human knee is that the articular cartilage is only 1 mm thick compared to human knee articular cartilage being 2–3 mm. Other aspects pertinent to the horse are that the metacarpophalangeal and carpal joints have close fitting articular surfaces that can quickly develop linear erosions and wear lines in association with osteochondral fragmentation. Some of these are referred to as "kissing" lesions and are directly mechanical whereas others are presumably associated with articular debris being trapped. In addition, when osteochondral defects are created adjacent to the synovial membrane in the carpus and metacarpophalangeal joint, synovial adhesions occur and can result in replacement of the superficial cartilage surface with layers of connective tissue, obscuring the lamina splendens and interfering with cartilage nutrition and homeostasis. Subchondral bone sclerosis and subsequent remodeling creating focal osteonecrosis are also common in this species as OA progresses. The most recently described post-traumatic OA model[35] uses the medial femorotibial joint where the articular cartilage thickness is similar to the human knee[51].

Scoring of alterations in joint structures

Macroscopic scoring

Macroscopic scoring systems are described here for both spontaneous OA in the MCP joint OA as well as the experimental carpal joint OA model. These models have been the most frequently reported and, therefore, scoring recommendations are presented for these two entities. Macroscopic examination is usually done without any ink staining of the surface though its use has been reported in spontaneous OA[45] as well as a stifle model[35]. The macroscopic scoring technique recommended for spontaneous OA is depicted in Table III and illustrated in Fig. 1.

The macroscopic scoring system uses the descriptive terms partial- and/or full-thickness erosion, wear lines and palmar arthrosis. Wear lines have been documented to be vertical clefts within the articular cartilage that can be present even in mildly osteoarthritic joints[48]. They have recently been shown to reduce the mechanical integrity of the articular cartilage surface[52], thus leading many to the conclusion that they are due to a weakening of the collagen matrix complex, ultimately resulting in randomly-oriented articular cartilage fibrillation. Wear lines have also been correlated with reduced prognosis in cases of osteochondral fragmentation in racehorses[8]. The wear lines were scored, and horses with increased scores had a significantly reduced chance of returning to performance at their previous level. It is therefore presumed that the presence of wear lines indicates early pathologic change that is worth noting and grading in gross assessment of joint surfaces.

Palmar arthrosis lesions occur on the distal palmar aspect of the third metacarpal condyle at the site of maximal articulation with the proximal sesamoid bones, and represent a spectrum of osteochondral damage[53]. Change in the subchondral bone often precedes macroscopic changes in the cartilage. This area is distinct in its rapid progression of damage compared to other sites within

Table II
Macroscopic scoring and microscopic grading systems for horse described in the literature

Macroscopic scoring	
Joint	Description
Middle carpal joint (CSU system)	Four grades of damage based on all articular surfaces lesions as well as assessment of synovial membrane for inflammation and hypertrophy which are used to define a total joint pathology score[25–34]
Metacarpophalangeal joint	Five grades distal metacarpus based on arthroscopic system in six locations[10]
Metacarpophalangeal joint	Three grades of wear lines, erosion & palmar arthrosis on distal metacarpus[41]
Metacarpophalangeal joint	Macroscopic scoring of proximal first phalanx and using India ink[42,43]
Metacarpophalangeal joint	Uses adaptation of OARSI scoring system[44] product of lesion grade, lesion stage and relevance of based on weight bearing/non-weight bearing location (modified by citing score % or area in mm^2 distal metacarpus)[44,45]
Medial femorotibial joint	Uses combination of ICRS systems and modification of WORMS[46] system on medial femoral condyle[35] (also India ink and tracing to map lesions)
Grading of microscopic changes	
Tissue	**Parameters scored**
Synovial membrane	Five grades ranging from (normal–severe) for each of the following changes: cellular infiltration, vascularity, intimal hyperplasia, subintimal edema and subintimal fibrosis[25–34] A cumulative score is also calculated
Articular cartilage	CSU system: Chondrocyte necrosis, chondrone formation, fibrillation, focal cell loss and Safranin O–Fast Green staining and each graded 0–4[25–34] Bristol system: Modified HHGS Structure (Grades 0–4), cells (0–3) and staining with Haematoxylin and Safranin O (0–4)[47] Guelph system: Modified OARSI system with six grades (surface intact, surface discontinuity, vertical clefts, erosion, denudation and deformation with histologic subgrades)[35]
Bone (osteochondral sections)	Osteochondral lesions (graded 0–4), subchondral bone remodeling (0–3) and osteochondral splitting (0–3)

Table III
Macroscopic staging technique to describe gross changes on the third metacarpal condyle of horses[34,38]

Overall macroscopic change		
Lesion	Grade	Description
Wear lines	0	None
	1	1 or 2 partial-thickness wear lines/joint surface
	2	3–5 partial-thickness or 1–2 full-thickness wear lines/joint surface
	3	>5 partial-thickness or >2 full-thickness wear lines/joint surface
Erosions	0	None
	1	Partial-thickness erosion, <5 mm in diameter
	2	Partial-thickness erosion, >5 mm in diameter
	3	Full-thickness erosion
Palmar arthrosis (osteochondral lesions distal palmar aspect of metacarpus[53]) [Fig. 1(C)]	0	None
	1	Partial-thickness erosion, <5 mm in diameter
	2	Partial-thickness erosion, purple discoloration, >5 mm in diameter
	3	Full-thickness erosion, purple discoloration, >5 mm in diameter

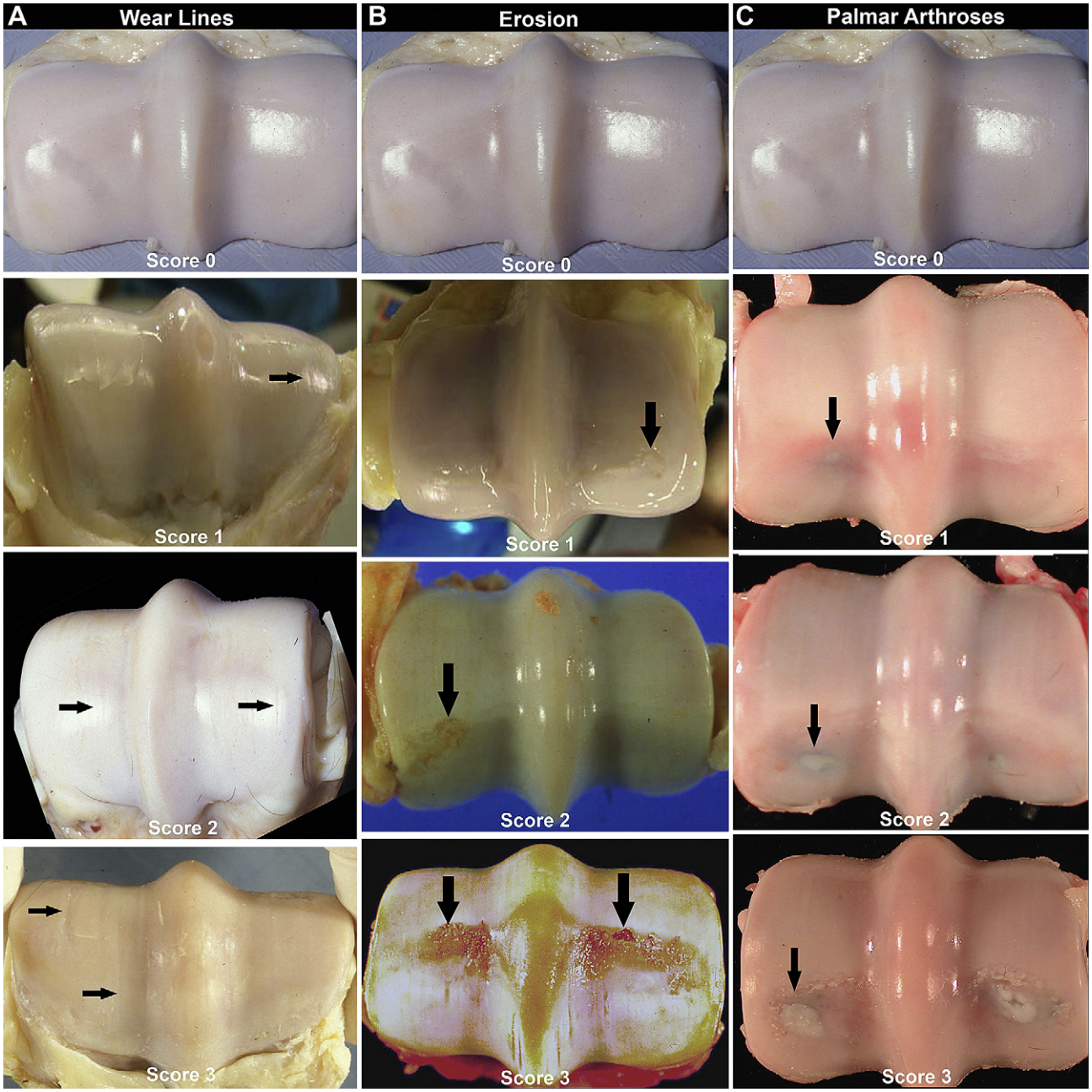

Fig. 1. Gross images of the distal third metacarpus showing the spectrum of gross changes with spontaneous OA of the metacarpophalangeal joint. (A) Demonstration of the spectrum of wear line damage in the third metacarpal condyle. Images for Scores 1 and 2 show partial-thickness wear lines (arrows) and the Score 3 image shows full-thickness wear lines (arrows). Note in Score 3 there are multiple partial-thickness wear lines in addition to the full-thickness wear lines. (B) Arrows demonstrate the progression and severity of articular cartilage erosion on third metacarpal condyles. (C) Arrows demonstrate the severity of palmar arthrosis on the third metacarpal condyle.

Table IV
Macroscopic staging system to describe gross changes in the induced osteochondral chip fragment-exercise OA model

Macroscopic scoring of total erosion (Fig. 2)	
Score	Description
0	No gross fibrillation/fissuring
1	Very superficial erosion with articular cartilage swelling
2	Partial-thickness erosion
3	Partial- and full-thickness erosion
4	Extensive full-thickness erosion to the level of subchondral bone

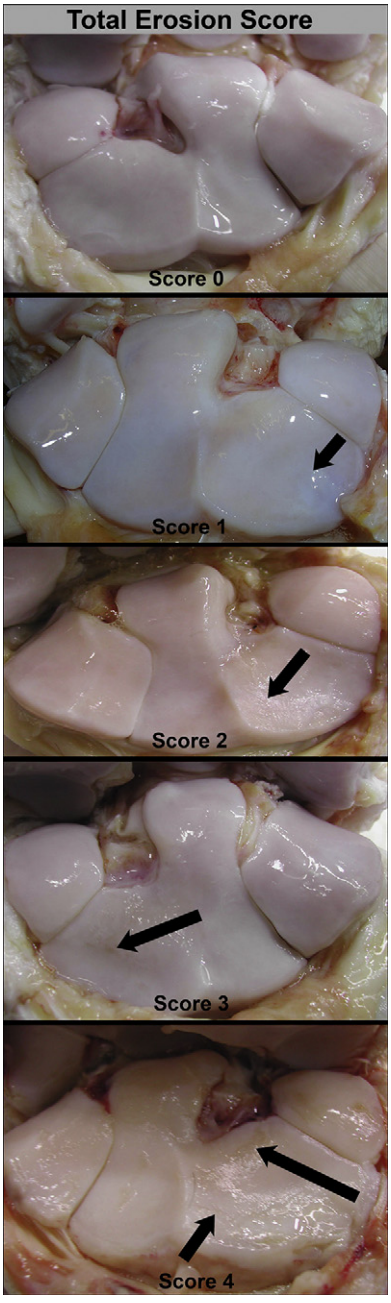

Fig. 2. Examples of erosion scores based on gross observation from the osteochondral fragment model. Score 0, shows no gross fibrillation; Score 1, shows very superficial erosion with articular cartilage swelling indicated by arrow; Score 2, shows gross partial-thickness erosions depicted by an arrow; Score 3, shows gross full-thickness erosion depicted by an arrow; Score 4 shows both gross full-thickness erosion and cartilage fibrillation lines depicted by arrows.

the joint, and represents a consistent spectrum of change in molecular, cellular, matrical and tissue-level properties[6,41,53–59]. Since palmar arthrosis lesions are the earliest events to occur in MCP joints undergoing athletic work, their gross characterization is necessary in any study. Although the scoring of these lesions is similar to gross scoring of articular cartilage erosion in other locations in the joint, these lesions are scored separately since they represent a specific pathologic process within the joint. It is also critical that scoring is done in a consistent location.

A different scoring system for the induced osteochondral chip fragment-exercise OA model is used (Table IV and Fig. 2)[26–34]. Both middle carpal joints are aseptically prepared and opened. The articular surface is examined for location of lesions as well as lesion character. Pitting, ulcerations and discoloration of the articular cartilage of each carpal bone are scored grossly on a subjective ordinal scale of 0–4 (where 0 is normal and 4 is severe change) for

Table V
Microscopic grading system for articular cartilage histology

Articular cartilage (Fig. 3)		
Outcome parameter	Score	Description
Chondrocyte necrosis*	0	Normal section without necrosis
	1	No more than one necrotic cell located near the articular surface per 20× objective
	2	1–2 necrotic cells located near the articular surface per 20× objective
	3	2–3 necrotic cells located near the articular surface per 20× objective
	4	3–4 necrotic cells located near the articular surface per 20× objective
Cluster (complex chondrone) formation	0	No cluster formation throughout section
	1	Two chondrocytes (doublets) within same lacunae along superficial aspect of the articular cartilage section
	2	2–3 chondrocytes (doublets & triplets) within same lacunae along superficial aspect of the articular cartilage section
	3	3–4 chondrocytes within same lacunae along superficial aspect of the articular cartilage section
	4	Greater than four chondrocytes within same lacunae along superficial aspect of the articular cartilage section
Fibrillation/fissuring	0	No fibrillation/fissuring of the articular cartilage surface
	1	Fibrillation/fissuring of the articular cartilage restricted to surface and superficial zone
	2	Fissuring that extends into the middle zone
	3	Fissuring that extends to the level of the deep zone
	4	Fissuring that extends into the deep zone
Focal cell loss*	0	Normal cell population throughout the section
	1	A 10–20% area of acellularity per 20× field
	2	A 20–30% area of acellularity per 20× field
	3	A 40–50% area of acellularity per 20× field
	4	A greater than 50% area of acellularity per 20× field
SOFG stain uptake	0	Normal staining
	1	Less than 25% loss of staining characteristics
	2	25–50% loss of staining characteristics
	3	50–75% loss of staining characteristics
	4	Greater than 75% loss of staining characteristics

* Chondrocyte necrosis is used to grade presence of lacunae with necrotic nuclei still present compared to focal cell loss which is most likely an extension of the pathologic change but lacunae or nuclei are no longer present.

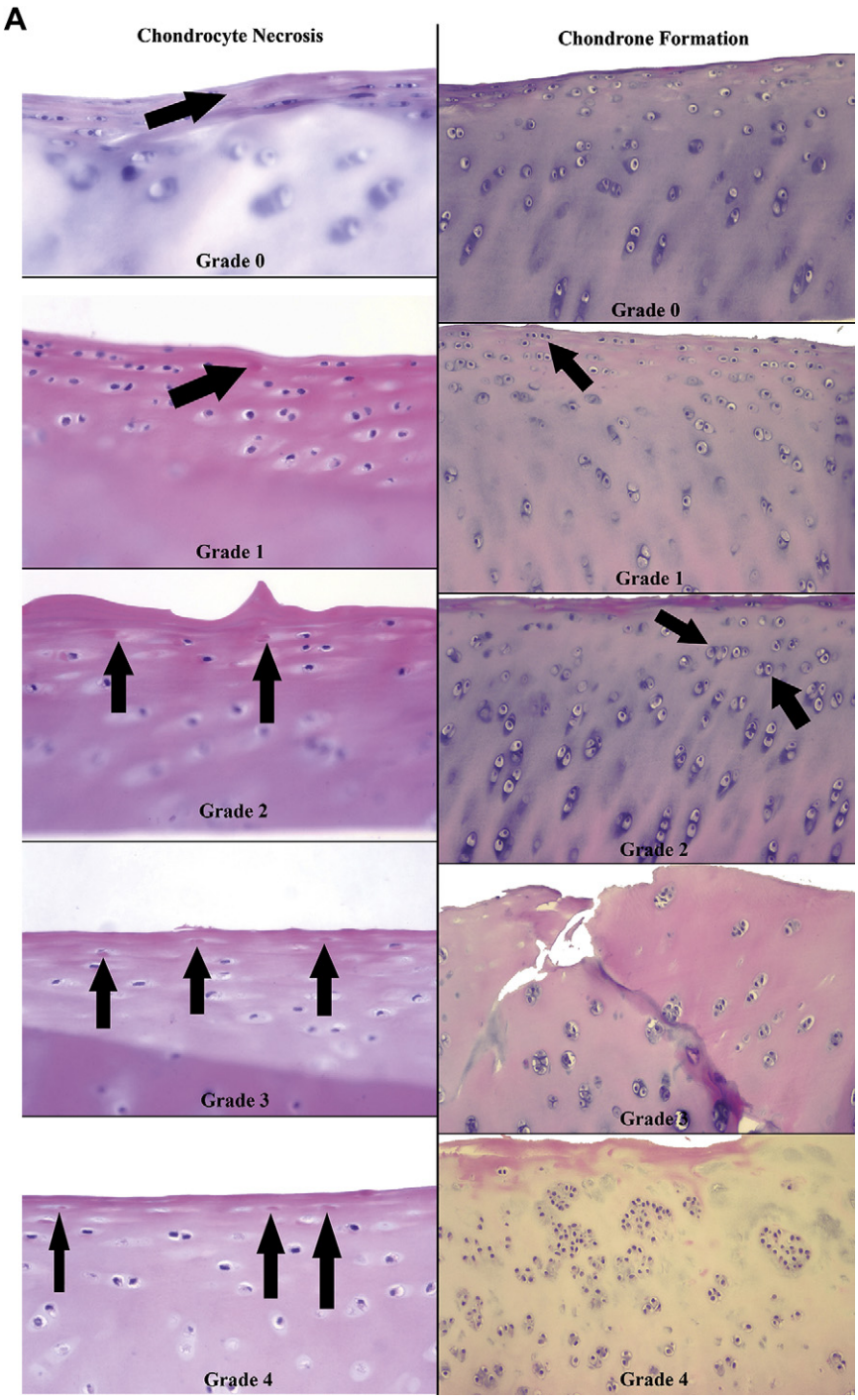

Fig. 3. Examples of H&E and SOFG stained articular cartilage sections that are 5 mm in length for each of the graded outcome parameters used in the carpal osteochondral fragment model. The entire section is graded and the final score represents the entire section taking into account local areas of more severe pathology i.e., change. (A) Chondrocyte necrosis and chondrone formation. Chondrocyte necrosis (40×): Score 0 represents a normal section without chondrocyte necrosis, arrow shows a normal area of eosin uptake (red/pink stained area) that is common close to the surface and should not be confused with a necrotic chondrocyte. Scores 1–4, arrows represent necrotic cells which are typically located near the articular surface. A Score 1 would typically represent 1–2 necrotic cells in the section typically in the superficial layer, where a Score 2 would represent 2–3 necrotic cells typically in the superficial layer, and Scores 3 and 4 would represent increased numbers of necrotic cells that are not only present at the surface but in deeper layers as well. Cluster (complex chondrone) formation (20×): Scores 1 & 2 have arrows representing cluster formation (doublets) more superficial than normal. Scores 3 and 4 have increased numbers of chondrocytes within a single lacuna that extends deep in the cartilage section. (B) Fibrillation, focal cell loss and SOFG stain uptake. Fissuring (20×): Score 1 has fissuring/fibrillation restricted to surface and superficial zone highlighted with an arrow; Score 2 has fissuring that extends into the middle zone; Score 3 has fissuring that extends to the level of the deep zone and Score 4 has fissuring that extends into the deep zone. Focal cell loss (20×): Scores 1–3 have arrows depicting increasing areas that are devoid of chondrocytes to a point where few cells remain as shown in the Score 4 example. SOFG stain uptake (20×): Score 0, has an example of normal loss of staining in the superficial zone depicted by an arrow; Scores 1–3 have arrows depicting areas of abnormal staining. Score 4 has virtually complete loss of staining in all zones.

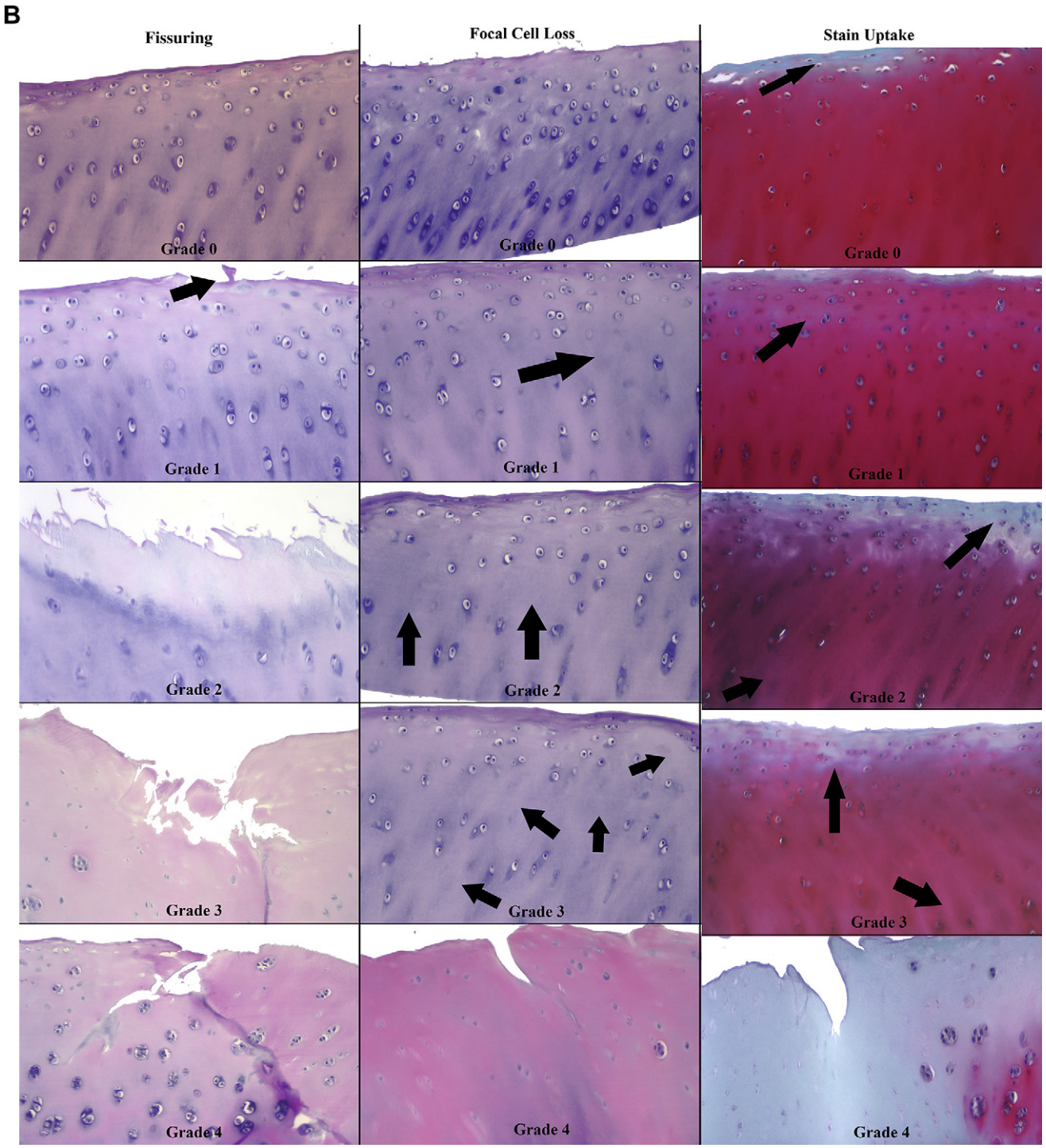

Fig. 3. (*continued*).

partial- and full-thickness cartilage erosions. A total erosion score is also assigned using the same subjective ordinal scale; this score accounts for the overall gross degeneration of the entire articular surface. The condition of the synovium is evaluated for hypertrophy and inflammation again using a 0–4 scale where 0 represents normal and 4 represents severe pathologic change. Details are recorded and photographs taken.

Articular cartilage pieces, 5 mm^2, are obtained from the radial carpal bone and the radial facet of the third carpal bone as well as osteochondral sections obtained from the radial carpal and third bone for histological processing as described[28–30]. A synovial membrane specimen that is approximately 3×4 mm is harvested from the middle carpal joint dorsal to the radial carpal bone, with efforts not to include the fibrous joint capsule. This scoring system is illustrated in Fig. 2 and described in Table II.

A technique utilizing India ink to detect spontaneous osteoarthritic cartilage changes has been described[45] and could be used for macroscopic evaluation; however it has not been used in the models

Table VI
Microscopic grading system for synovial membrane histology in the carpal osteochondral fragment model

Synovial membrane (Fig. 4)		
Outcome parameter	Grade	Description
Cellular infiltration (lymphocytes and some plasma cells)	0	No mononuclear cells in the section
	1	Occasional small areas of mononuclear cells throughout the section
	2	Mild presence of mononuclear cells in 25% of the section
	3	Moderate presence of mononuclear cells in 25–50% of the section
	4	Marked presence of mononuclear cells in greater than 50% of the section
Vascularity	0	Normal
	1	Slight increase in vessels in focal locations throughout the section
	2	Mild increase in number and dilatation of vessels in focal locations throughout the section
	3	Moderate increase in number and dilatation of vessels in up to 50% of the section
	4	Marked increase in number and dilatation of vessels in greater than 50% of the section
Intimal hyperplasia	0	None
	1	Villi with 2–4 rows of intimal cells within the section
	2	Villi with 4–5 rows of intimal cells over 25–50% of the section
	3	Villi with 4–5 rows of intimal cells over 50% of the section
	4	Villi with 5 or greater rows of intimal cells over 50% of the section
Subintimal edema	0	No edema
	1	Slight edema detected within section
	2	Mild edema within 25% of the section
	3	Moderate edema within 25–50% of the section
	4	Marked edema in greater than 50% of the section
Subintimal fibrosis	0	Normal
	1	Slight increase in fibrosis within the section
	2	Mild increase in fibrosis in 25% of the section
	3	Moderate increase in fibrosis in 25–50% of the section
	4	Marked increase in fibrosis in greater than 50% of the section

presented here. In the stifle joint India ink staining has been used[35] to delineate subtle surface fissures, matrix depletion and thinning as described by others[46,48]. India ink particles are prevented from entering in intact cartilage surface with an unaffected proteoglycan-rich matrix but have a high affinity for articular cartilage with surface fibrillation and proteoglycan depletion[46,48]. The technique used by Cantley *et al.* was semi-quantitative. A quantitative technique for evaluating OA on the basis of India ink staining and digital imaging techniques has been described in the equine metacarpophalangeal joint but is applicable to the proximal aspect of the first phalanx only[43]. Tracing India ink stained area onto plastic applied to the medial femoral condyle was used by Bolam *et al.*[35] to overcome the problems associated with area measurements digitally of a curved surface and provides staging of progression. A similar technique could be used in the equine carpus and is currently being explored.

- We recommend different macroscopic scoring systems for spontaneous OA in the MCP joint as well as experimental carpal joint OA; these are presented in Tables II and III. Future consideration of tracing India ink stained areas onto plastic as described in the medial femoral condyle[35] is recommended.

Microscopic scoring of cartilage alterations

Recommendation of sectioning and staining to be used

Cartilage sections in the CSU model are harvested from three locations (radial facet of the third carpal bone, fourth carpal bone and distal radial carpal bone) and are fixed in 10% phosphate buffered formalin, embedded in paraffin, cut into 5 μm sections and mounted on coated glass slides. Duplicate sections are stained with haematoxylin and eosin (H&E) or stained with haematoxylin and 0.1% aqueous safranin O for approximately 6 min in counter stain with 0.1% aqueous fast green. Staining procedures must be very consistent between slides especially with the safranin O–fast green (SOFG) stain and normal equine cartilage and trachea are used as a control between batches. Alternatively, frozen sections can be used and are stained with a haematoxylin/safranin O combination at Bristol[39]. Osteochondral sections are first decalcified in 10% formic acid (Stevens Scientific Decalcifying Solution). That can be monitored by the oxalate endpoint determination method[60]. Following decalcification samples are imbedded in paraffin and cut into 5 μm sections and mounted on coated glass slides. H&E staining and safranin O are the same as detailed above.

- For simple histological scoring of osteochondral sections we recommend the use of formic acid decalcification, embedding in paraffin, and cutting into 5 μm sections with H&E as well as safranin O staining of different sections. Frozen sections of cartilage are an alternative.

Grading criteria

The histological and histochemical grading system used in the equine osteochondral fragment model is as presented in Table V and illustrated in Fig. 3.

The total histologic grade will range from 0 to 16 and SOFG staining can be scored 0–4 for each layer or 0–4 overall (Total score of 20).

The above system is relevant for the osteochondral fragment model, but when knee injury and OA are modeled in the horse, the system described by Pritzker *et al.*[44] that uses a product of extent (area affected) and Grade (depth of the lesions) has been proposed as an alternative in an equine femorotibial joint model[35]. This system has also been recently validated for the equine stifle joint (M. Hurtig personal communication). For the sake of brevity, only the osteochondral fragment model has been described here.

- The histological and histochemical grading system depicted in Table V is recommended for use in the equine osteochondral fragment model. Future consideration should be given to the

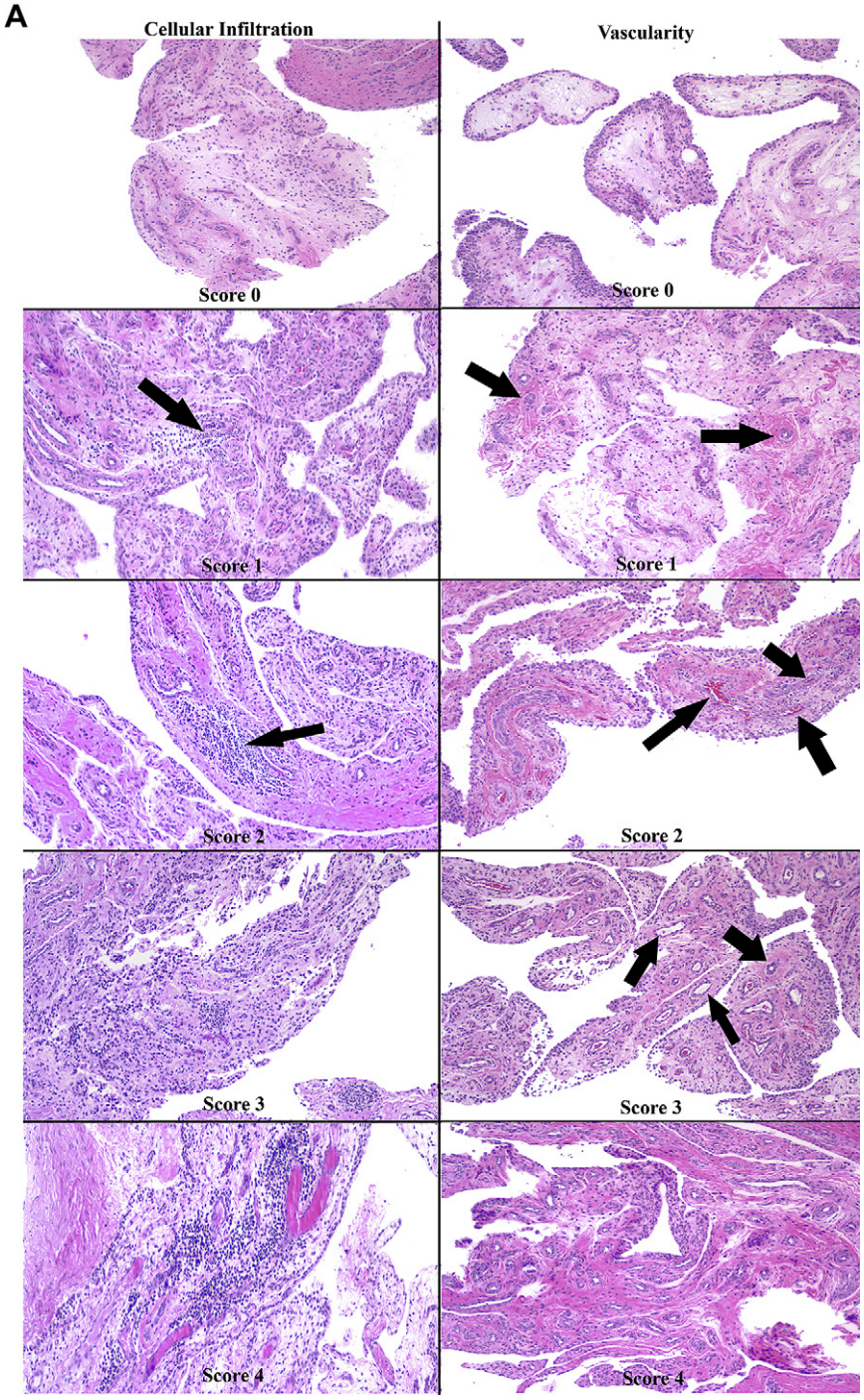

Fig. 4. Examples of H&E stained sections representing a similar surface area of synovial membrane for each scored outcome parameter used in the carpal osteochondral fragment model. (A) Cellular infiltration and vascularity. Cellular infiltration (10×): Score 0, no inflammatory cells are observed within the section; Score 1, slight inflammatory infiltrates are detected and consist of mononuclear and segmented cells. An example of such an area is depicted by the arrow; Score 2, a mild area of mixed inflammatory infiltrates is highlighted by the arrow. Higher scores represent greater concentration of infiltrates over a larger surface area. Vascularity (10×): Score 1 has examples of increased vessels depicted using arrows; Score 2 and higher also have increases in vessel numbers as well as dilatation of some vessels depicted by arrows. Higher scores represent greater involvement throughout the section. (B) Intimal hyperplasia, subintimal edema and subintimal fibrosis. Intimal hyperplasia (10×): Score 0 represents an example of no intimal hyperplasia depicted by white arrow, Score 1 shows villi that have areas ≈4 rows of intimal cells and 2 rows depicted by arrows; Scores 3 & 4 have increasing amount of intimal cells. Subintimal edema (10×): Scores 1–4 have examples of edema often recognized by the 'gun blue' appearance (arrows) of the tissue as well as the lack of subintimal organization which is especially prominent in the Score 4 example. Subintimal fibrosis (10×): Scores 1 & 2 have arrows depicting areas of increased fibrosis which often begins around the vasculature; the higher Grades have evident fibrosis over a greater region of the section.

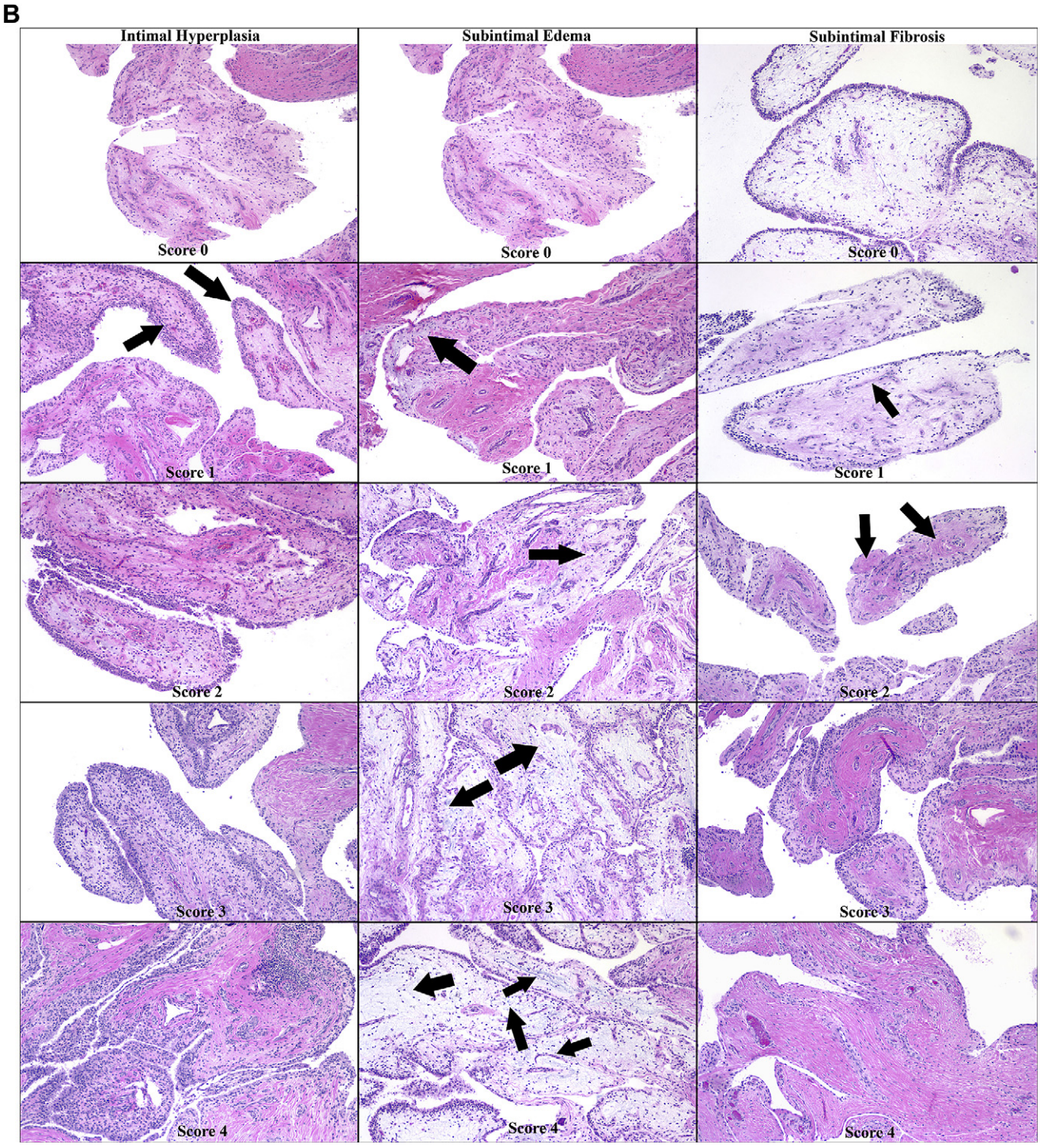

Fig. 4. (*continued*).

system Pritzker *et al.*[44] as an alternative (recently used in the equine femorotibial joint model).

Microscopic scoring of synovial alterations (grading of synoviopathy)

Recommendation of staining and sectioning

A 3–4 mm of synovial membrane has been harvested at gross examination from a villous area. The harvested tissue is fixed in 10% formalin for 48 h and then embedded in paraffin prior to 5 μm sectioning, mounting and staining with H&E.

Grading criteria

These sections are evaluated and graded blindly for cellular infiltration, vascularity, intimal hyperplasia, subintimal edema and subintimal fibrosis[25] (Table VI). These changes are illustrated in Fig. 4.

- For evaluation of synovial membrane changes we recommend 5 μm thick paraffin embedded sections stained with H&E and graded blindly for cellular infiltration, vascularity, intimal hyperplasia, subintimal edema and subintimal fibrosis as detailed in Table VI.

Table VII
Proposal for microscopic grading system for osteochondral lesions in spontaneous OA of the metacarpophalangeal joint

Grade	Description
Osteochondral lesions [Fig. 5(A)]	
0	No visible changes in cartilage or bone
1	Minor disruption of subchondral bone matrix. Lesion occupies <25% of histologic condylar surface, and extends no more than 1–2 mm deep to the normal chondrosseous junction. Some of the matrix within the lesion is pale staining and occasional marrow spaces contain debris. Tidemark is reduplicated. Fibrin may be present in the subchondral bone layer. No apparent superficial cartilage fibrillation
2	More severe disruption of subchondral bone matrix. Areas of matrix are fragmented to comminuted. Lesion occupies 25–50% of histologic condylar surface and extends approximately 2–4 mm deep to the normal osteochondral junction. A significant amount of the subchondral bone matrix is pale staining, and there is significant debris within marrow spaces. The tidemark is reduplicated and often disrupted. Moderate amounts of fibrin present in the subchondral bone layer. Cartilage overlying lesion is thickened with superficial cartilage erosion and fibrillation. Reparative fibrocartilage is also apparent in the superficial cartilage layers
3	Complete collapse of osteochondral tissue. Lesion occupies >50% of histologic condylar surface and extends 43 mm deep to the normal osteochondral junction. Pale staining subchondral bone matrix and fibrin are abundant. The tidemark is reduplicated and often disrupted. Superficial thickening of cartilage and/or fibrocartilage present and may be completely detached from thickened deeper cartilage
4	Obvious loss of osteochondral tissue leaving an ulcer (not observed)
Subchondral bone remodeling [Fig. 5(B)]	
0	No visible changes in cartilage or bone
1	Advancement of the subchondral bone into the calcified cartilage layer with scalloped subchondral bone margins (arrowheads), but not crossing any tidemarks
2	Subchondral bone advancement through the calcified cartilage layer, crossing one or more tidemarks, but below the most superficial tidemark front
3	Subchondral bone advancement through the calcified cartilage layer and disruption of the tidemark front
Osteochondral splitting [Fig. 5(C)]	
0	No visible changes in cartilage or bone
1	Involve the tidemark and subchondral bone, but are simple linear defects
2	Have fragments and debris within the split and connections between splits
3	Involve articular cartilage and displacement of calcified cartilage fragments into the underlying subchondral bone

Microscopic scoring of bone alterations

Recommendation of sectioning and staining

Osteochondral sections are decalcified and embedded in paraffin and cut into 5 μm sections and mounted on coated glass slides. Sections are stained with H&E.

Grading criteria

Description of microscopic features in osteochondral sections has been limited to studies with spontaneous OA in the equine metacarpophalangeal joints[37,49]. Microscopic grading of osteochondral lesions is depicted in Table VII and illustrated in Fig. 5.

- A proposal for microscopic grading of osteochondral lesions in spontaneous OA of the metacarpophalangeal joint is presented and input for modification relevant to other joints is anticipated.

Evaluation of sources of variability

Intra- and inter-observer validation study

Validation of synovial membrane and articular cartilage scoring systems (H&E & SOFG) for the CSU osteochondral fragment model was carried out during the first 4 years of model development through the agreement of one board certified pathologist specializing in musculoskeletal tissue and three board certified equine surgeons specializing in orthopaedics. Each of four observers would score a section and then the group would discuss the score and agree on an overall score for the particular section.

More recently representative sections of synovium and articular cartilage for each level of all outcome parameters were randomly and blindly presented to each of the authors for grading. These results were then analyzed using PROC GENMOD in SAS (www.support.sas.com) to perform linear regression for repeated measures. The analyses investigated differences by tissue (synovium or cartilage), specific outcome variables, observers and their interactions. The results indicated no significant differences with respect to tissue or outcome variables although one of six observers provided scores that were systematically different from the median (for all observers) or the lead observer. Thus differences were not detected between five and six reviewers when individuals were compared to mean values or those of the lead evaluator. The data was also analyzed to calculate an intraclass correlation (ICC) for all the authors. This method of agreement indicated 0.82 [95% confidence interval (CI) = 0.77–0.86] ICC value with a value of 1 indicating perfect homogeneity and −1 indicating extreme heterogeneity. When a group ($N = 4$) of untrained individuals were asked to evaluate the slides based on the outlined grading scheme there level of agreement was 0.92 (95% CI = 0.84–0.96) when their individual scores were compared to their mean scores. It is clear that independent agreement can be obtained from multiple evaluators using the above described grading criteria even if they have not been tutored.

Discussion

It is now accepted that the joint is an organ and, consequently, OA involves all it's tissues including articular cartilage, subchondral bone, synovium, fibrous capsule and joint fluid and, in the case of the knee, menisci. The task of this paper was to make recommendations on macroscopic scoring and microscopic grading in equine OA. The range of joints and models used by the authors collectively was wide and consequently we initially had a wide range of scoring (staging) and grading systems. The evaluation systems presented here have been necessarily restricted. We chose to present macroscopic evaluation of spontaneous OA in the metacarpophalangeal joint[53] and in the experimental OA model[25–34]. We had the most collective experience with spontaneous OA in the metacarpophalangeal joint and the carpal osteochondral fragment-exercise model has been the subject of 10 published studies. It is felt that the evaluation systems described here can be extrapolated to other models. With further experience models such as the recently described femorotibial traumatically induced OA model[35] could gain increased usage and modifications of the evaluation system used in that model implemented. It is to be recognized that there are no previous publications giving collective recommendations on macroscopic and microscopic assessments in equine OA. Hopefully, this will serve as a basis for further development and validation of more sophisticated systems.

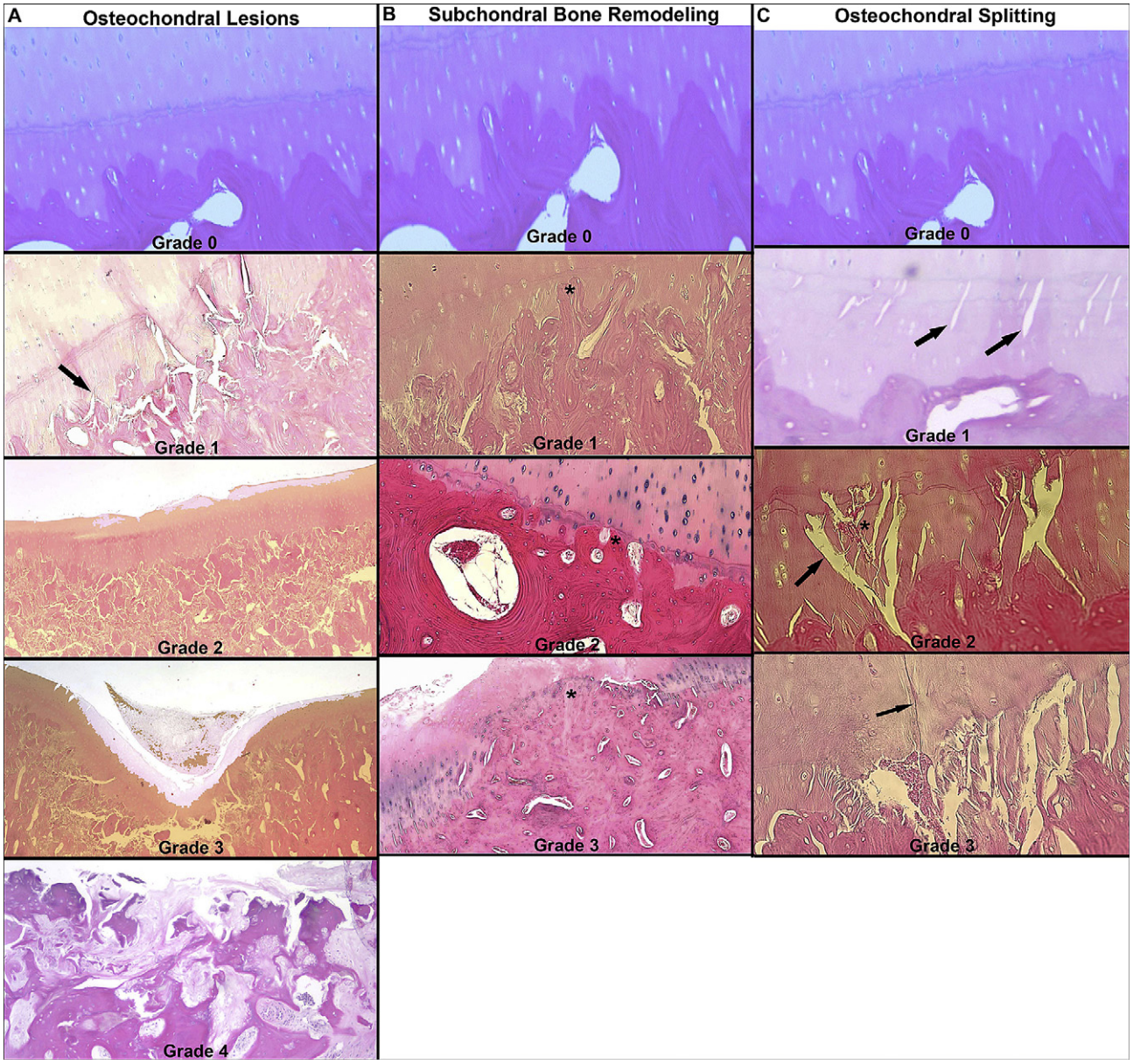

Fig. 5. Illustration of histopathologic grading of osteochondral lesions in spontaneous OA in equine metacarpophalangeal joints. Microscopic images showing the spectrum of damage in the osteochondral area of the third metacarpal condyle. (A) Various osteochondral lesions that rank in severity from mild disruption in the subchondral bone (arrow in Grade 1) all the way up to subchondral bone collapse and complete articular cartilage erosion with fibrosis in the subchondral bone (Grade 4). (B) Microscopic images showing advancement of subchondral bone remodeling through the calcified cartilage (star). (C) The progression of osteochondral splitting which starts in the calcified cartilage and is demonstrated by the arrows.

The assessment systems are also based on the initial case material provided. Osteochondral evaluation of bone as well as articular cartilage is critical with spontaneous OA in the fetlock joint. The system for osteochondral evaluation is therefore presented from spontaneous disease in this joint. On the other hand articular cartilage has been examined in the experimental carpal OA model without calcified cartilage or subchondral bone being included in the sections. In one study the bone changes have been evaluated with this model and details on subchondral bone change in this model are available from this publication[28].

As new techniques develop, both imaging and body fluid biomarkers can add to the information *in vivo* and prior to (or to the exclusion of) post-mortem. With the equine chip fragment model for instance it has been evaluated using radiography, computed tomography (CT) and magnetic resonance imaging (MRI) as well as synovial fluid and serum biomarkers. The results of these studies have been published recently[33,34].

In summary the horse is a suitable model for addressing selected questions related to human OA and there is adequate tissue to harvest for multiple outcome measures. This species requires special facilities as well as trained personnel but control of activity level and other interventions are easily done. This review represents a consensus of the authors to create standardization between laboratories and allow for comparisons to be made between studies in the future.

Disclosures

C. Wayne McIlwraith is employed by Colorado State University.
David D. Frisbie is employed by Colorado State University.
Christopher E. Kawcak is employed by Colorado State University.

Cathy J. Fuller is employed by the University of Bristol.
Mark Hurtig is employed by the University of Guelph.
Antonio Cruz is employed by the University of Guelph.
Thomas Aigner is employed by the University of Leipzig.

Conflict of interest

No author has any conflict of interest related to this work.

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