Equine meniscal degeneration is associated with medial femorotibial osteoarthritis

Keywords: equine; meniscus; osteoarthritis; femorotibial

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SUMMARY

Background: There is limited information available concerning normal equine meniscal morphology, its degeneration and role in osteoarthritis (OA).

Objectives: To characterize normal equine meniscal morphology and lesions and to explore the relationship between equine meniscal degeneration and femorotibial OA.

Study Design: *Ex vivo* cadaveric study.

Methods: Menisci were harvested from 7 normal joints (n = 14 menisci) and 15 joints with OA (n = 30 menisci). A macroscopic femorotibial OA score (cartilage degeneration and osteophytosis) was employed to measure disease severity in each compartment. The femoral and tibial meniscal surfaces were scored for macroscopic fibrillation and tears (1-4). Histological sections (Regions: cranial and caudal horn; body) were also scored for microscopic fibrillation and tears (0-3) and inner border degeneration (0-3).

Results: Partial meniscal tears were present on both femoral and tibial surfaces in all 3 regions and most frequently identified on the femoral surface of the cranial horn of the medial meniscus and body of the lateral meniscus. There was a significantly positive correlation between the global medial meniscal macroscopic scores and osteophyte ($r=0.7$, $p=0.002$) or cartilage degeneration ($r=0.5$, $p=0.03$) scores within the medial femorotibial joint. The global medial meniscal macroscopic score was greater ($p=0.004$) in the advanced OA joints compared with control joints.

Main limitations: The menisci were principally from abattoir specimens without a known clinical history because of the challenge in obtaining a large number of specimens with a clinical diagnosis of femorotibial OA.

Conclusions: This study is the first to describe normal equine meniscal morphology and lesions. Meniscal lesions were identified in all segments and on both articular surfaces. Meniscal degeneration significantly correlated with OA severity in the equine medial femorotibial joint. The relationship between OA and meniscal pathology remains to be elucidated and we speculate that mechano-biological events play a role.

INTRODUCTION

Stifle lameness accounted for 42 % of hindlimb referral lameness in eventing horses[1]. However, this data does not represent all equine athletic disciplines. For example, in racehorses, it is probably much lower, but the exact prevalence is not known. Meniscal injury has been reported to account for up to 20% of all stifle lamenesses [2; 3]. We also recently reported that OA pathology is most frequently observed in the medial femorotibial compartment of the stifle[4]. Equine meniscal lesions have been identified in the cranial horn of the meniscus on arthroscopic examination [3; 5-9] and in all 3 meniscal segments on ultrasonographic examination [3; 6-11]. However, only one third of the femoral surface of the meniscus is visible by arthroscopy [5; 12]. Macroscopic lesions of the remaining meniscal femoral or tibial surfaces and intrasubstance degenerative lesions are not visualized on arthroscopic examination [7]. Approximately half (13/25) of the lesions diagnosed on ultrasonographic examination of equine menisci were not observed on arthroscopic examination [7] thus underpinning the importance of a meniscal ultrasonographic examination. However ultrasonographic visualization of the caudal menisci is limited [9]. Open, large bore magnetic resonance imaging (MRI) technology, accommodating all sizes of equine stifles [13], holds the promise to provide a more accurate diagnosis and improve understanding of equine meniscal disease as MRI is the gold standard for evaluation of meniscal disease in man [14].

The menisci are semilunar structures that enhance the congruency between the femoral condyles and tibial plateau, transfer load and are paramount for femorotibial joint stability [6; 15]. Meniscal tears have been classified in man and include longitudinal (or bucket-handle when they penetrate deeper into the meniscal substance), vertical, horizontal, radial, oblique and complex tears [16]. The incidence of meniscal tears identified on MRI in a study of the relationship of meniscal disease and OA in man was 16% in control patients and increased to 57% in OA patients

[17]. Furthermore, loss of meniscal intra-substance integrity has recently been shown to be correlate with risk factors for cartilage degeneration in man [18]. Knowledge of equine meniscal morphology and lesion types is particularly important to avoid interpretation of normal structural artefacts on ultrasonographic examination leading to a misdiagnosis [7].

We postulate that equine meniscal and articular cartilage degeneration are interlinked by mechanobiological events. The objectives of the present investigation were: 1) to characterize normal equine meniscal morphology and lesions and 2) to explore the association between meniscal degeneration, aging and equine OA.

MATERIALS AND METHODS

Specimens

The study protocol was approved by the institutional Animal Care and Use Committee. Menisci investigated (Table 1) were from the stifle joints of adult horses (n = 21), characterized in a previous imaging investigation of stifle OA[4] and stored in a tissue bank. Additional menisci from donated horses were included: (n = 2). One joint was included per animal. The joints were either placed in saline soaked gauze and frozen at -20°C or processed immediately following euthanasia.

Macroscopic assessment

Menisci were thawed in water. The macroscopic changes were scored by consensual agreement of 2 individuals, blinded to the pathology (articular cartilage degeneration and osteophytes) previously assessed in that joint compartment [4]. Exceptionally, 1 pair of menisci was evaluated by only one individual, at the end of the study. The macroscopic changes (fibrillation and tears) in each of the 3 regions (cranial horn; body and caudal horn, Figure 1) of either the tibial and femoral meniscal surfaces were scored (details in Figure 2; modified from Pauli et al.[19]) following the application of India Ink. The tibial or femoral surface macroscopic meniscal score was the cumulative scores of the three regions, whereas the global meniscal macroscopic score was the sum of the scores on both surfaces.

Histologic assessment

The menisci were placed in 10% formaldehyde for 2 hours and transferred to EDTA 20% for 2 weeks, to facilitate sectioning. Each meniscus was then laid over a protractor with the femoral surface uppermost and the cranial border aligned with the angle 0. Three slices (≈ 0.5cm thick) were cut at 30, 90 and 150° (Figure 1) and embedded in paraffin. Five µm sections were cut and stained with HEPS (hematoxylin, eosin, phloxine and saffron). All slides were digitalized with a LeicaDM 4000B microscope and Panoptiq™ v.1.4.3 computer software.

HEPS stained sections from macroscopically normal appearing menisci of selected horses of different ages were first examined to describe normal meniscal histological features at different ages. The histologic lesions (fibrillation, disruption, lack of tissue) were scored (details in Figure 2; modified from Pauli et al.[19]). Each section was graded independently by 2 observers, one a board certified pathologist. Histologic changes on both the femoral and tibial surfaces and inner border were assessed. The tibial or femoral surface histologic meniscal score was the sum of this parameter score and inner border score from the 3 regions, whereas the global meniscal histologic score was the cumulative scores recorded in the 3 sections.

A paired t-test was employed to assess if there were differences in the global macroscopic meniscal scores between the lateral and medial menisci. A Wilcoxon test was used to detect differences between the regional macroscopic meniscal scores and total tibial or femoral surface macroscopic scores between the medial and lateral menisci. The same test was used for the global histologic meniscal score comparison between the lateral and medial menisci. The regional macroscopic and histological meniscal scores within the medial or lateral menisci were assessed to identify differences with a Friedman test for non-parametric values and then with Tukey post-hoc tests when needed.

Assessment of meniscal lesion association with femorotibial compartment OA

In order to assess the association between meniscal degeneration and OA, global femorotibial compartment macroscopic OA scores, calculated in a prior recently published study [4] were employed. Specimens where the femorotibial joint OA had been scored were included for this arm of the study (Table 1). Briefly, the articular cartilage changes (fibrillation and erosion; 0-3) and osteophytes (0-3) were scored in the cranial, middle and caudal regions of the femoral condyles and tibia. The regional scores were summed to provide a total femorotibial compartment macroscopic cartilage (0-9) or osteophyte score (0-9) or a global femorotibial compartment macroscopic OA score (all cartilage and osteophyte scores summed) (Table 1). The menisci were also categorized into arbitrary groups, based on their corresponding global femorotibial compartment macroscopic OA score [4]: control (a score of < 5; no osteophytes); moderate OA ($\geq$ 5 to < 20) and advanced OA (a score of ≥ 20) to further elucidate the association between meniscal degeneration and OA lesions.

A Spearman correlation coefficient was employed to correlate the global medial and lateral meniscal macroscopic and histological scores with the total femorotibial compartment macroscopic osteophyte and cartilage scores to identify correlations between meniscal lesion scores and OA in each femorotibial compartment**.** Kruskal-Wallis tests were employed to detect differences between global medial and lateral meniscal macroscopic and histologic scores in groups categorized by OA status (control, OA moderate, or advanced OA). Post-hoc tests[20] were performed on the statistically significant findings to reveal the direction of the differences.

Association of meniscal degeneration with age

Meniscal specimens with a known age were assessed (specimens 2-7, 11, 12, 14-17, 19, 21-23, n=32 menisci, Table 1). A mixed ANCOVA was employed on the global macroscopic and histologic meniscal scores with age as co-factor, laterality as fixed factor and horse ID as random factor to determine the association of meniscal degeneration and age. A value $p=0.05$ was considered significant.

RESULTS

Normal menisci

Histologic appearance: The menisci were wedge-shaped in cross-section, with a concave femoral surface, a flat tibial surface and a superficial lamellar layer, which stained slightly orange with HEPS (Figures 1 & 2). The central part of the meniscus was eosinophilic and the inner third was also occasionally lightly eosinophilic (Figure 2). Matrix fibres were oriented radially at the femoral and tibial meniscal surfaces. In each sample, the lamellar layer was subjectively more cellular and cells were spindle-shaped in appearance compared to the cells of the central zone, that had a heterogenous orientation (Figure 2). The cells of the inner border were round.

Meniscal lesions: location and frequency

A landscape of macroscopic meniscal lesions was available for study spanning from mild fibrillation of the surfaces or inner border, to tears and partial loss of tissue (Figure 3). Surface fibrillation was often present alone, but also occurred in association with partial tears. The meniscal tears were principally oriented longitudinally along the circumferential meniscal fibers (Figure 3 a) or obliquely on the femoral surface (Figure 3 b & c). Macroscopic lesion scores and the corresponding score percentage per region (cranial horn, body, caudal horn) of the femoral or tibial surfaces are illustrated in Figure 4. The only score 4 meniscal lesion was found on a lateral meniscus (Figure 4 a) and score 3 lesions (Figure 4 c-d) were most prevalent in the cranial horn of the medial meniscus and in the body of the lateral meniscus. On the tibial surface, score 3 was the most severe lesion encountered and was most prevalent in the caudal horn of the medial meniscus and in the body of the lateral meniscus (Figure 4).

Comparison of the global macroscopic meniscal scores and the tibial or femoral surface total macroscopic scores between the medial and lateral menisci: Global macroscopic meniscal scores (median, range) were higher (10, 6-14, p=0.04) in the medial meniscus than those of the lateral menisci (8, 6-16) (Figure 4 a). The tibial surface total macroscopic scores of the medial meniscus were higher (5, 3-8, p=0.02) compared with the lateral (4, 3-6), but no difference was detected for the femoral surface scores.

Comparisons of regional macroscopic meniscal scores within the medial or lateral menisci: The regional macroscopic scores (median, range) of the femoral and tibial surfaces of the medial meniscus were higher in the meniscal body (femoral: 2, 1-3; tibial: 2, 1-3) compared to the caudal (femoral: 1, 1-3; tibial: 2, 1-3; p=0.01) and cranial horns (femoral: 2, 1-3; tibial: 1, 1-3; p=0.04) respectively. No significant difference was detected between regional scores in the lateral meniscus.

<u>Histologic appearance:</u> A variety of lesions were observed and spanned from surface changes and undulation to complete meniscal tissue disruption. The majority of the lesions affected the lamellar layer, with some penetration to the central zone. Representative lesions and corresponding scores are provided in Figure 2. At the femoral surface, the highest median histologic score was 2 in the cranial horn of the medial meniscus and 1 in the body of the medial meniscus on the tibial surface. The highest median histologic score recorded at the inner border was 2 in both the body and caudal horn of the medial meniscus.

Comparison of global histologic meniscal score and tibial, femoral surface or inner border total histologic scores between the medial and lateral menisci: The global histologic medial meniscal scores (median, range) were higher (12, 0-18, p=0.01) compared with the lateral meniscus (4, 0-21) (Figure 4 b). The inner border and tibial surface total histologic scores were higher (p=0.008 and 0.02 respectively) in the medial (inner border: 5, 0-9; tibial surface: 2, 0-9) compared to the lateral meniscus (inner border: 1, 0-9; tibial surface: 1, 0-6) (Supplementary item 2). No other differences were identified.

Comparisons of regional histologic meniscal scores within the medial or lateral menisci: The medial meniscus regional femoral surface histologic score (median, range) was higher (p=0.003) in the cranial horn (2, 0-3) compared to the body (0, 0-2) and caudal horn (0, 0-2) and its regional tibial surface histologic score was higher (p=0.009) in the body (1, 0-3) compared to its cranial horn (0, 0-3). No other differences were detected.

Association between meniscal lesion scores and OA in each femorotibial compartment

Significant correlations were identified in the medial femorotibial joint alone. Both global medial meniscal macroscopic and histologic scores were positively correlated with the total femorotibial compartment osteophyte scores ($r=0.7$, $p=0.002$ and $r=0.6$, $p=0.04$ respectively; Figure 5 b & d). The global medial meniscal macroscopic score was also positively correlated with the total femorotibial compartment macroscopic cartilage score ($r=0.5$, $p=0.03$; Figure 5 a).

Comparisons of meniscal pathology between OA groups

The global medial meniscal macroscopic and histologic scores (median, range) were greater ($p=0.004$ and $p=0.01$ respectively) in the advanced OA joints (macroscopic: 12, 12-14; histologic: 17, 16-18) compared to control joints (macroscopic 8, 6-9; histologic: 4, 2-12,). No other significant associations were detected.

Meniscal degeneration and age

The global meniscal macroscopic and histologic scores increased with age ($p<0.0001$) (Figure 6).

DISCUSSION

The findings of the present study provide valuable insight into equine meniscal disease. First, a detailed description of macroscopic and histologic lesions of the equine meniscus is provided. Second, we observed that meniscal macroscopic and histologic degeneration scores were higher in the medial meniscus compared to its lateral counterpart, confirming previous clinical reports [3; 5-8; 10]. Third, the medial meniscal macroscopic lesion scores were higher in its body whereas the histologic scores were higher in the body of the meniscal tibial surface, but also in the cranial horn on the femoral surface. Fourth, the meniscal degeneration scores correlated positively with the macroscopic osteophyte and cartilage degeneration scores within the medial femorotibial joint, suggesting a link to OA, similar to that observed in man [15].

The meniscal tears were oriented longitudinally in the direction of the circumferential meniscal fibres, or obliquely on the femoral surface. The macroscopic score 3 lesions we described are similar to longitudinal tears in man [16] whereas the score 4 lesions are comparable to complex tears [16].

The data presented here provides additional evidence that the equine medial meniscus is more frequently affected by pathology than its lateral counterpart [3; 5-7; 10], similar to man [15]. These results confirm and extend those of Adrian et al. [7] who reported that more than half (25/47) of the medial menisci examined ultrasonographically had lesions, compared to less than one fifth (6/34) of the lateral menisci. However, no gold standard histopathological confirmation was available in the latter study.

There are few studies on equine femorotibial or meniscal biomechanics published in the English veterinary literature [21-24]. In contrast, this is a well studied area in man and it is known that the medial meniscus withstands greater forces than its lateral counterpart and is the most frequently injured [15]. The total axial forces generated in a human limb at a walk are at least 2 to

3 times body weight[25]. The knee joint transmits 65-73% of these forces with the remaining transferred by surrounding soft tissues[26]. Furthermore, 85% of the peak force is transferred through the medial side, depending on the valgus angle of the knee and this side can bear up to 201% of body weight at maximum axial load[26]. Although these findings cannot be directly extrapolated to horses, the commonality of medial meniscal lesions in both suggests similarities in etiology related to biomechanical loading events in the femorotibial joint compartment. Caution should however be exercised when extrapolating the findings from human bipeds to equine quadrupeds. To our knowledge, the forces transmitted through equine femorotibial joints in vivo have been not been measured or reported in the English veterinary literature. Information from quadruped dogs reveal that the fore limbs support 63% of body mass during standing and at all walking speeds[27]. This information may also apply to the horse at similar gaits. The commonality of medial meniscal lesions in humans, horses and dogs suggests similarities in etiology related to biomechanical loading events in the femorotibial joint compartment, but requires further study.

In addition to the laterality of meniscal lesions, we also analyzed regional site prevalence of lesions and their severity. We observed that equine meniscal lesions arise in all 3 meniscal segments in agreement with others [7], but also on both the femoral and tibial surfaces. When the site prevalence of medial meniscal lesions was studied more closely, score 3 macroscopic lesions were most commonly located in the cranial horn of the medial meniscus on the femoral surface. This site prevalence may, in part, be explained by the results a recent equine meniscal biomechanical study [22] that identified a caudal translocation of equine menisci occurring from full extension to full flexion of the stifle joint. The least movement occurred at the cranial horn of the medial meniscus [22]. The investigators speculated that this lack of movement induced meniscal trapping between the femur and tibia in hyperextension that could contribute to the high prevalence of lesions at this site. In contrast, in both man [28] and dogs [29], meniscal tears have been predominantly diagnosed in the caudal horn. The cranial meniscal horns are more movable than the posterior horns in man [29] and this may explain some of the species differences. The forces on the caudal meniscal horn are also known to increase substantially throughout flexion of both equine and human femorotibial joints [15; 23]. The increased incidence of caudal horn injuries has been ascribed to this caudal translocation of load in man [15].

The change in equine meniscal conformation from a C-shape to an L-shape described by Fowlie et al. [22] that arises during stifle flexion may place the meniscal inner border under tension and could explain the fraying frequently observed at this location in all 3 meniscal segments in the present study. Furthermore, Bonilla et al. [23] also reported that the center of the equine tibial plateau, that has no meniscal tissue cover, sustained increased stress loads throughout stifle flexion and could contribute to fraying of the meniscal inner border or formation of meniscal body tears.

Meniscal tears were also observed on the equine meniscal tibial surface in the present study and have not been described previously in horses. We speculate that this pathology may be related to the subchondral bone resorption we recently identified at the medial tibial plateau in equine femorotibial OA joints [4]. These tears could potentially be visualized by ultrasound examination or MRI but would not be identified arthroscopically.

The presence of osteophytes is considered pathognomic for the presence of OA in equine joints [30]. Both the macroscopic and histologic meniscal scores were positively associated with the presence of osteophytes in the medial femorotibial joint underpinning a likely association between both events in this joint. It is well known in man [15; 31] and has recently been shown in horses that the medial femorotibial compartment is the most commonly affected by OA in the stifle joint [4]. It is also increasingly recognized that meniscal injuries contribute to femorotibial OA [17], though it has never been studied in horses. A large percentage (44%) of patients with meniscal tears diagnosed on arthroscopic examination had accompanying cartilage lesions [31] Meniscal degeneration also increases with OA severity in man [17; 18]. The correlation we observed between equine meniscal degeneration and OA does not imply causation and further studies will be required to establish where the earliest changes arise: in the meniscus or the articular cartilage or both concurrently. As both the meniscus and articular cartilage are tightly interlinked anatomically and biomechanically, loss of biomechanical function of either tissue through a single event trauma or as a result cyclical stress induced injury will impact the other. Similarly, biological events such as cellular activation of the pro-inflammatory/protease cascades in either tissue or the joint may upregulate degradation of their extracellular matrices. A recent 3 year longitudinal study, employing quantitative MRI (3T), imaged human patients with posterior meniscal horn lesions but no radiographic OA or MRI cartilage lesions at study entry [32]. The investigators detected elevated cartilage relaxation times, reflecting matrix degeneration, adjacent to the meniscal lesions at the medial tibial plateau at 2 years, but not in matched controls. This finding supports the argument that meniscal lesions may contribute to, or be one of the first signs, of degenerating cartilage. These recent findings suggest meniscal lesions contribute to the development of OA in the femorotibial joints. However, in contrast Badlani et al. [33] also found no significant difference between patients with or without medial meniscal tears and the development of OA, over a 2 year period.

In the present study, there was a significant effect of age on meniscal degeneration scores. Little is known about meniscal ageing in any species, but age is a known risk factor for the development of OA in man [34]. In a study of the prevalence of meniscal damage in the general population (n=991) and the association of meniscal tears with knee symptoms and radiographic OA, the prevalence of meniscal tear was as low at 19% in women 50-59 years old and high as 56% in men from 70-90 years old. In people with radiographic OA, the prevalence of a meniscal tear was 63% in symptomatic and 60% in non-symptomatic patients. It was concluded that incidental meniscal findings on MRI of the knee are common in the general population and augment with increasing age [14]. These findings will need to be kept in mind as our capacity to image equine menisci improves as it may be a challenge to determine whether all the lesions we detect are actually symptomatic.

It is recognized that this study has some limitations. As many of the samples were obtained from an abattoir, a complete history was not available and it was unknown if clinical signs were associated with the lesions we report, except for 2 horses with a confirmed clinical diagnosis. Additional numbers of specimens would have provided further insight on meniscal changes with age. Moreover, it should be pointed out that meniscal tissue sectioning and slide preparation are challenging, probably related to its very complex and resistant collagen structure and quality histological sections for analysis are difficult to obtain. On the other hand, this is the first study to report normal meniscal morphology and lesions with gold standard post mortem and histological assessments. Future studies including more clinical specimens, with lameness localized to the femorotibial joint by intraarticular anesthesia, and a variety of lesions, could shed additional light on the clinical relevance of the findings we report here.

In summary, equine meniscal lesions were identified in all segments and on both articular surfaces. Meniscal lesions are associated with OA in the medial femorotibial joint and increase with age. The exact relationship between meniscal degeneration and femorotibial OA remains to be elucidated.

TABLE LEGEND:

Table 1: Data on menisci included in the study

Stb = Standardbred, QH = Quarter horse, WB = Warmblood; F = Female, G = Gelding, M = Male; R = Right, L = Left; Med = Medial, Lat = Lateral; ME = macroscopic evaluation

* Data from specimens 21, 22 and 23 were employed for distribution of lesions and meniscal degradation only.

FIGURE LEGENDS

Fig 1: Study Design.

Cr: Cranial, B: Body, Ca: Caudal

Fig 2: Scores.

Macroscopic (a) and histologic (c) meniscal scores with examples (b, d). Arrowheads are pointing at lesions.

Fig 3: Femoral surface meniscal lesions (Score 3).

a) Lesion in the caudal horn of a lateral meniscus extending towards the body. b), c) & d) Lesion in anterior horn of the medial meniscus.

Fig 4: Meniscal lesion laterality and distribution.

Comparison of the medial and lateral global macroscopic (a) and histologic meniscal (b) scores. C) & d) Global macroscopic scores in meniscal segments.

Fig 5: Correlation of meniscal pathological scores with OA lesions.

Fig 6: Association of meniscal degeneration with age

P values indicate that the global medial and lateral meniscal macroscopic and histologic scores significantly increase with age.

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TABLE 1:

Specimen number	Age (years)	Breed	Sex	Stifle	Total FT compartment macroscopic cartilage score		Total compartment macroscopic osteophyte score		Global FT compartment macroscopic OA score
					Med	Lat	Med	Lat	
Horses banked from previous study									
1	-	-	-	R	4	3	4	0	11
2	9	QH	F	L	4	0	0	0	4
3	10	Haflinger	F	R	8	2	10	0	20
4	7	Pony	G	R	3	2	0	0	5
5	27	QH	G	L	9	3	10	0	21
6	4	QH	G	L	0	1	0	0	1
7	21	Appendix	G	L	3	3	5	0	11
8	-	-	-	L	1	1	0	2	4
9	-	-	-	L	1	0	2	1	4
10	-	-	-	R	3	1	6	0	10
11	9	Appendix	F	R	1	1	1	1	4
12	10	QH	G	L	0	1	0	0	1
13	-	-	-	L	1	1	4	0	6
14	8	Pony	M	L	4	0	0	0	4
15	9	QH	F	R	1	1	0	0	2
16	3	Pony	M	R	1	1	0	0	2
17	26	QH	F	L	5	4	9	0	18
18	-	-	-	L	6	6	0	0	12
19	17	QH	G	R	1	2	3	0	6
20	-	-	-	R	3	1	2	0	6
21*	23	QH	F	R	-	-	-	-	-
Horses donated and euthanized because of severe clinical OA									
22*	11	WB	G	L	-	-	-	-	-
23*	14	WB	G	L	-	-	-	-	-