*An anatomical and histological study of the equine proximal manica flexoria*

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**Summary**

**Objectives:** The main aim was to describe the gross and histological appearance of the manica flexoria (MF) and to identify any differences between the fore and hind limbs. An additional aim was to relate the findings to diagnostic and surgical anatomy of the MF.

**Methods:** Measurements of the MF were made on cadaver limbs from horses free from pathology within the digital flexor tendon sheath (DFTS). Histological sections, stained with Haematoxylin and Eosin and Alcian-PAS, were evaluated based on three micro-anatomical zones from dorsal to palmar/plantar. The prevalent tenocyte morphology, number and distribution of blood vessels and nerves were described in each zone. Fore and hind limb measurements were compared using a Students T-test.

**Results:** Proximally, the MF attaches to the DFTS via a reflection of areolar tissue. The fibrous MF is longer in the fore (32.0±4.2mm) than the hind (29.4±3.8 mm) limb (p=0.04), with the areolar portion longer in the hind (22.9±5.3 mm) compared to the fore (16.7±4.3 mm) limb (p=0.0005). Histologically, degenerate blood vessels were prevalent in the palmar/plantar region and were associated with chondrocyte-like tenocytes, indicative of fibrocartilagenous metaplasia.

**Clinical significance**: The study has provided a detailed anatomic description of the MF relevant for interpretation of diagnostic and surgical evaluation. Fibrocartilaginous metaplasia occurs on the palmar surface of the MF.

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**Introduction**

The proximal manica flexoria (MF) is a collar of tendinous tissue originating from the medial and lateral borders of the superficial digital flexor tendon (SDFT) within the proximal aspect of digital flexor tendon sheath (DFTS). Within the proximal aspect of the DFTS, the MF and the SDFT create a ring through which the deep digital flexor tendon (DDFT) passes. The proposed function of the MF is maintenance of the flexor tendons in alignment as they pass over the palmar/plantar aspect of the metacarpo(tarso)phalangeal joint (1). The proximal MF is distinct from the distal or digital manica flexoria, a similar tendinous band positioned on the dorsal surface of the DDFT in the mid-pastern region of the DFTS (2). Tearing of the MF is recognized as a relatively common cause of non-septic tenosynovitis in horses (3-5) . Injury is more prevalent in the hind limbs, with cob and pony breeds over-represented (3, 5) . MF tears can occur at the medial or lateral attachment to the SDFT; however, the medial border appears to be affected more commonly than the lateral border (3, 5). If the tear is complete, the MF tissue frequently recoils to form a mass of tissue within the proximal aspect of the DFTS, making it difficult to identify the origin of the tear at the time of tenoscopy, which is frequently months after the injury occurs. In addition to the physical presence of torn tendinous fibres, the release of extracellular matrix components and inflammatory cytokines results in tenosynovitis and effusion of the DFTS. Chronic tears may be accompanied by adhesions between the DFTS and the MF (3, 5). The gold standard technique for diagnosis of MF tears is tenoscopic examination, although ultrasonography and contrast radiography contribute to the diagnosis in some cases (6).

The aim of this study was to improve our knowledge of the MF through a detailed description of the gross and histological appearance in normal horses. The first objective was to describe the normal appearance of the MF as well as the relationship of the MF to related structures of the DFTS, in order to inform diagnostic imaging and surgical approaches to the MF. The second objective was to describe the histological appearance of the MF at selected sites from medial to lateral. Based on the high prevalence of MF tears in the hind limbs of horses, we hypothesized that anatomic differences would exist between the manica flexoria in the fore and the hind limbs, and potentially between the medial and the lateral aspects.

**Materials and Methods**

Limbs were obtained from equine cadavers following euthanasia for reasons unrelated to distal limb musculoskeletal pathology. Written informed consent was gained from owners. Limbs were collected as ipsilateral pairs or single limbs depending on the availability and the reasons for euthanasia Limbs were transected at mid-radius or mid-tibia in the fore and hind limbs, respectively, to ensure that the flexor tendons remained intact below the musculotendinous junction. Following transection, samples were collected either within 6 hours of death or the limbs were frozen at -200C and were defrosted at room temperature for 24 hours prior to dissection and sampling. There was no evidence of tenosynovitis or MF tears in any of the limbs used for the study.

A total of 45 limbs were obtained from horses with an age range of 1 day to 24 years (median 10.0; mean 10.3 years). The specimens included 18 paired fore- and hind limbs and nine single limbs (4 fore, 5 hind). Thoroughbred (TB) and TB cross breed horses accounted for 26 of the limbs with 19 limbs from cob and pony type breeds.

*Dissection protocol*

In all cases, limbs were examined in a non-weight bearing position, as would be the case during tenoscopic surgery. All measurements of the manica flexoria and the distances to related structures were made in triplicate using electronic calipers (Draper Vernier Caliper 150mm, Hampshire, UK). Skin was removed from the proximal MC/MT to the level of the mid-pastern. The proximal and distal borders of the proximal MF were identified. Prior to further dissection, the distance (mm) between the distal border of the MF and the proximal and distal borders of the PAL and the PSBs were measured. The palmar/plantar annular ligament (PAL) was transected at its lateral border axial to the lateral proximal sesamoid bone (PSB) to allow entry into the DFTS and examination of the intra-thecal structures. A suture was placed at the distomedial border of the MF to allow orientation of the sample throughout the processing.

The MF and approximately 5cm of SDFT and DDFT proximal and distal were resected en bloc. The medial side of the MF, as identified by the suture, was marked with permanent ink to allow preservation of the orientation during processing for histological evaluation. The proximal-distal length of the main tendinous portion of the MF was measured axially and at the lateral and medial borders. The proximal-distal length of the proximal non-tendinous areolar portion of the MF was measured axially (Figure 1). Other measurements included the width of the MF at the distal border and the dorsopalmar/plantar thickness at the proximal and distal borders.

Following measurement, the resected tissue was fixed en bloc in 10% neutral buffered formalin. After fixation, six sections of each MF were obtained according to the sampling scheme demonstrated in Figure 2. Two longitudinal samples were obtained from the axial one third of the MF. Transversely oriented samples, one proximal and one distal, were obtained from the medial and the lateral border of the MF, incorporating the junction with the SDFT. Each tissue section was 0.4 cm wide. The six tissue sections for each limb were included in a single cassette and were processed routinely for histology. The longitudinal samples could be identified by their distinct size and shape. The medial and lateral samples could be easily differentiated by the permanent ink marking medially; however, the proximal and distal samples could not be differentiated. Therefore, only one medial and one lateral sample from each horse was evaluated. Two serial, 6µm thick sections from each tissue sample were stained: one with Haematoxylin and Eosin (H&E) and one with Alcian-Periodic Acid Schiff (Alcian-PAS) for identification and evaluation of microscopic structures and to highlight carbohydrate and proteoglycan content, respectively.

For each MF sample, three tissue sections (medial, lateral and longitudinal) were examined under light microscopy. Longitudinal sections were examined qualitatively to determine the orientation of collagen fibres (longitudinal, transverse, or random) and to document the presence of nerves and blood vessels. The transverse sections were examined quantitatively to distinguish differences in tissue characteristics (collagen, nerves and blood vessels) between the axial, middle and abaxial areas, with the abaxial areas incorporating the MF-SDFT junction on either the medial or the lateral side. Within each histological section and each region (axial, middle, abaxial), three micro-anatomical zones, with a laminar arrangement from dorsal to palmar/plantar were defined and examined (Figure 2). The micro-anatomic zones of the MF within the transverse sections were defined as: the dorsal zone (20% width:E1); the central zone, (50% width:E2); and the palmar/plantar zone (30% width:E3)(7, 8).

For each medial or lateral transverse MF section evaluated, nine representative photomicrographs were obtained: one per zone (E1-3) for each region (axial, middle, abaxial). The microphotographs were taken at 10x magnification using a Zeiss Primo Star trinocular microscope equipped with an AxioCam ICc1 microscope camera (Oberkochen, Germany). Once collected, the photomicrographs were coded so that the primary author was blinded to the image location. The primary author (BSc, BVM&S, Diploma European College of Veterinary Surgeons) was trained by an experienced pathologist (DVM, PhD) to recognize the different tenocyte and blood vessel morphologies, prior to undertaking the data collection for the study. For each photomicrograph the following was recorded: the predominant tenocyte cell type, the number and type of vascular structures (blood vessels) and the number of nerves. The counting was repeated twice by the primary author with the numbers averaged.

The predominant tenocyte morphology in each sample was assessed for each zone (E1- E3) in each region (axial, middle, abaxial). Tenocyte morphology was categorized into three types. Type 1 tenocytes were cigar shaped, fibroblast-like cells with the nucleus oriented parallel to the collagen fibres and inconspicuous cytoplasm. Type 2 tenocytes were ovoid-shape occurring singly or in pairs. Type 3 tenocytes formed clusters of round, chondrocyte-like cells (Figure 3) (7). Based on the predominant cell type, each zone E1-3 within each photomicrograph was assigned a score based on the following scale: 1 = more than 70% of type 1 tenocytes; 2 = 30 to 50 % of type 2 tenocytes; 3 = focal presence of aggregates of type 3 tenocytes; and 4 = diffuse prevalence of type 3 tenocytes, occasionally forming an “isogenous group-type” cell clustering.

The blood vessel classification was as follows: 1= blood vessels exhibiting normal structure including a patent lumen and clear intimal cell layer; 2= blood vessels exhibiting partial luminal occlusion and severe thickening of the vessel wall, intimal proliferation with vessel wall degeneration expressed by increased Alcian-PAS staining affinity and a faded outline; 3= “ghost” blood vessel in which the lumen and wall were replaced by a concentric lamellar and dense aggregate of Alcian material (9). For each micro-anatomic zone (E1-3), the total number of vascular structures was counted, and the proportion of vessels in each category was calculated. Nerves were identified and counted. The intensity of Alcian staining in each image was subjectively assessed as: 1= absent or mild; 2=moderate; or 3=strong (9).

**Data evaluation**

Descriptive analysis was performed for the anatomical findings. Paired fore limb and hind limb gross anatomical measurements were compared using a Students T-test. Unpaired limbs were not included in this analysis. Cob and pony limbs were compared to Thoroughbred-type limbs using a one-tailed Student T-test, with all limbs included in this analysis. A general histological description of the different sections is provided with observations regarding collagen fibre orientation. Tenocyte morphology and blood vessel type in each of the zones, E1-3, were described with a qualitative description of prevalent cell types in each zone. Observations of age related differences are provided.

**Results**

*Gross Anatomy*

The MF consists predominantly of a cupola shaped section of tendinous tissue attached throughout its length to the SDFT via the medial and lateral borders. The medial and lateral borders of the MF are not distinct as regards the gross appearance of the fibre pattern between the MF and the SDFT tissue; however, the MF tissue was markedly thinner than the adjacent border of the SDFT. The distal aspect of the MF tapers into or emerges from the lateral and medial margins of the SDFT. The MF is thickest proximally, becoming progressively thinner from proximal to distal. The distal border of the MF consisted of very thin tissue (<1mm) which had a distally tapering attachment to the SDFT. The MF had no direct attachments to the DFTS except at the proximal border. At the proximal border of the MF there was a reflection of loose areolar connective tissue, which attaches the MF to the dorsal surface of the SDFT and the adjacent lining of the DFTS (Figure 1).

The dorsal surface of the MF was apposed with the inter-sesamoidian ligament and dorsal lining of the DFTS, although the structures were separated by synovial fluid. The palmar/plantar MF surface was adjacent to the dorsal surface of the DDFT and separated from it by the synovial fluid that surrounds both the dorsal and palmar/plantar surfaces of the MF. The DDFT had synovial reflections that insert onto the dorsal abaxial surface of the SDFT adjacent to the areolar connective tissue that is proximal to the proximal border of the MF. There were no attachments between the DDFT and the MF (Figure 1).

The gross measurements of MF length, width and relationships to adjacent anatomic structures from the paired ipsilateral fore and hind limb are summarized in Table 1. There was no significant difference between the mean medial-lateral MF width in the fore (32±0.9mm) and the hind (32±1.2mm) limbs (p=0.06). The tendinous MF was significantly longer in the fore limb compared to the hind limb at the medial, axial and lateral sites (Table 1). In contrast, the proximal, non-tendinous reflection of areolar tissue was significantly longer in the hind limb compared with the fore limb (p=0.01). The measurements relating the position of the MF to the PAL indicated that there was no significant difference in overlap between the fore and hind limbs (p=0.29) (Table 1). No significant differences were identified between Cob and pony breeds compared to non-Cob type breeds with regard to the gross anatomical measurements of MF length (medial p=0.42, axial p=0.22, lateral p=0.35, areolar portion p=0.08). MF width was significantly less in Cob and pony breeds compared to non-Cob type breeds (p<0.001).

Histological processing was performed on 35 limb samples; however, when assessed for quality, 11 had to be removed due to processing artefacts such as folded tissue, microtome sectioning artefact and poor stain uptake. A subset of 24 limbs (11 fore limb, 13 hind limb) was available for histopathological examination and no evidence of morphological alteration ascribable to freeze/thaw artefacts was evident in any of the samples examined. These unpaired samples represented different breeds (Cob and pony n=10; TB or TB type n=14) and ages (mean 10.6, median 11, range 1 day – 22 years). Subjectively, the MF samples from younger animals (<10 years) appeared softer in texture offering less resistance to routine microtome cut during histopathological processing.

The qualitative evaluation, of the longitudinal samples (n =24) demonstrated that the collagen bundles were regularly orientated from proximal to distal. On the transverse sections, a clear distinction could not be identified between the collagen fibre orientation of the SDFT and the MF. The quantitative evaluation of the transverse histological sections revealed variations in predominant cell morphology between the zones E1-3. On the dorsal aspect of the MF (E1), type 1 tenocytes were the predominant cell type. Zone E2 consisted of 70% type 1 and 30% type 2 tenocytes. The palmar/plantar surface of the MF (E3) contained some type 2 tenocytes, while having the highest concentration of type 3 chondrocyte-like tenocytes (Table 2). Subjectively, Zone E3 with a predominance of type 3 tenocytes, had high intensity Alcian blue staining most commonly recorded, compared to zone E1 in which staining was most commonly graded as mild. Subjectively, there was no visible difference in the Alcian blue staining between the medial and lateral samples or between the forelimb and hind limb samples Statistical analysis was not possible due to the small sample size.

Blood vessel analysis revealed that type 1 blood vessels were the predominant type in all zones, but made up about 90% of all blood vessels in zones E1 and E2 compared to 70% in E3. Zone E3 had the highest percentage of type 2 and 3 blood vessels (15% each). The abaxial histological sections of the MF contained the greatest number of blood vessels overall (mean 26/photomicrograph) whilst the photomicrographs obtained from the axially located samples had the least (mean 9/photomicrograph). Overall, examination of the sections revealed that type 3 tenocytes and type 3 blood vessels were commonly found together.

In the longitudinal sections, blood vessels were oriented from proximal to distal with a tortuous appearance, making accurate quantification difficult. Nerves were encountered infrequently with a mean of 0.5 (0-2) per photomicrograph within zone E2. Nerves were identified at the proximal aspect of the longitudinal samples, close to the border with the areolar connective tissue, and within the abaxial region of the transverse samples. Nerves were more frequently identified in the lateral than in the medial transverse samples.

Overall, zone E1 contained normal (type 1) BVs and tenocytes (type 1). Zone 2 similarly consisted of predominantly type 1 BVs and a mix of type 1 and 2 tenocytes. Zone E3 had a predominance of tenocytes with a chondrocyte phenotype, with increased proteoglycan within the extracellular matrix and degenerate blood vessels.

**Discussion**

This study describes the gross anatomic features and anatomic relationships of the equine proximal MF to the adjacent SDFT, DDFT and palmar/plantar annular ligament which are relevant for clinical assessment of the structure either during diagnostic imaging or surgical procedures. Measurements of the MF demonstrated that the fibrous MF was significantly longer and the areolar portion significantly shorter in the fore limb compared to the hind limb. The histological investigation of the proximal MF demonstrated dorsopalmar/plantar variation in tenocyte and blood vessel morphology. In particular, we noted an increased prevalence of tenocytes with a fibrocartilaginous morphology on the palmar/plantar aspect of the MF which was consistent with regional fibrocartilaginous metaplasia. The fibrocartilaginous appearance of the palmar/plantar section of the MF tissue suggests that the palmar MF adjacent to the DDFT may be subjected to compressive loading at during fetlock hyperextension.

The anatomic study of the MF identified a number of consistent anatomical features of clinical relevance. During ultrasonographic examination, the MF tissue should be directly associated with the medial and lateral borders of the SDFT, whereas the synovial reflections of the DDFT should be visualized more proximally at the level of the areolar reflection of the MF. Although the tissue of the MF is markedly thinner than that of the SDFT, a discernible border was not observed between the SDFT and the MF. The lack of clear demarcation between the MF and the SDFT is relevant for tenoscopic exploration and surgical procedures in which the MF may require resection. Definition of the MF and SDFT junction is easiest when viewing the distal tapering margin of the MF arthroscopically (personal observation), as the tissue curves obliquely from the SDFT margin. The consistent measured width (~30mm), as well as the anticipated length of the MF is important as a guide to the amount of tissue that the surgeon can anticipate resecting in order to remove the majority of the MF. The proximal border of the tendinous MF is identified by the reflection of areolar tissue which is easier to transect than tendinous MF, particularly if the damaged MF has become fibrotic and thickened. The common overlap of the distal MF border and the proximal PAL margins in the non-weight-bearing limb has potential implications for PAL desmotomies performed tenoscopically or through a semi-open procedure, since damage to the MF has been reported during tenoscopic PAL desmotomy (10).

The actual function of the MF remains a mystery. The fact that this structure can be resected with no known consequences for normal locomotion is interesting and may indicate a lack of clear function during locomotion. It has been suggested that the MF functions to maintain the DDFT and SDFT in alignment during hyperextension of the limb (1, 11) . In the non-weight bearing limb, the proximal border of the PAL and the distal border of the MF were very closely apposed. The study revealed that the tendinous MF is significantly longer in the fore than in the hind limb whilst the proximal reflection of areolar tissue is longer in the hind limb. The kinematics of the fore and hind limbs are known to differ with the fore limb bouncing and the hind limb sliding which results in more hyperextension of metatarsophalangeal joint compared to the metacarpophalangeal joint (12, 13). In addition, there is evidence that the horizontal hoof velocity is greater in the hind as compared to the fore limb at gallop (14). Therefore, there may be a greater requirement for the MF to stretch and slide along the DDFT in the hind as compared to the forelimb, potentially leading to a greater propensity for injury.

Fibrocartilaginous metaplasia is a well-recognized physiological adaptation of tendinous tissue placed under compressive and/or shear forces which enables increased resistance to compression, and in many cases this is an advantageous adaptation to load bearing (15-18). Tendons that wrap around a bone are subjected to changes in position and are the most likely to undergo fibrocartilaginous metaplasia (15, 17). Rabbit tendons surgically re-routed to be placed under compression rather than tension undergo fibrocartilaginous metaplasia. Following reversal of the tendon re-routing procedure, the histological changes associated with fibrocartilaginous metaplasia were reversed (19) indicating further that the alteration in tissue morphology relates to changes in loading patterns. In horses, fibrocartilaginous metaplasia has been characterized most thoroughly in the DDFT as it passes over the navicular bone (8, 9). It has also been reported in the SDFT within the fetlock canal (20) and in the metacarpo/tarsophalangeal joint collateral ligaments (21). The current study has identified the combination of tenocytes with a cartilaginous phenotype, increased proteoglycan within the extracellular matrix and degenerate blood vessels in the palmar/plantar MF which is consistent with fibrocartilaginous metaplasia (8, 22). The presence of fibrocartilagenous metaplasia within the MF may indicate that the MF becomes compressed during locomotion in this palmar/plantar region. The histological appearance of fibrocartilaginous metaplasia could indicate the normal adaptation of the MF to the forces placed upon it, or could be analogous to the fibrocartilagenous metaplasia that precedes DDFT tears in the region of the navicular bone (8, 22).

Local environmental factors within the tendon have also been associated with the development of fibrocartilaginous metaplasia (23). Mesenchymal stem cells cultured in an environment of hypoxia and compression develop a cartilaginous rather than tendinous phenotype, consistent with the changes observed in fibrocartilaginous metaplasia (24). The current study identified that degenerate or “ghosted” blood vessels, with intimal damage and luminal occlusion with concentric fibrous material, are found within areas of the MF, particularly those with a predominance of type 3 tenocytes. Previous studies have demonstrated an increased number of ghosted blood vessels in areas of fibrocartilaginous metaplasia suggesting that lack of vasculature and a shift to a more cartilaginous phenotype contribute to the tendon susceptibility to injuries (8, 9). Possibly, a reduction in or damage to the number of functional blood vessels due to compressive and shear forces applied to the tendon may create relative hypoxia. The hypoxia and morphological distance from vasculature is known to provide a favorable microenvironment for chondroplasia which may be stimulated by the up-regulation of specific cytokines, such as hypoxia inducible factor, and mediators of apoptosis (23, 25).

There is evidence that fibrocartilaginous metaplasia may constitute a necessary underlying pathophysiological change predisposing to tendon tearing. In humans, fibrocartilaginous metaplasia has been suggested as the aetiology for tears of the rotator cuff tendons in the shoulder, the patellar tendon and the anterior tibialis tendon (26, 27).  The authors hypothesize that histological characteristics of fibrocartilaginous metaplasia identified in the current study may contribute to MF tears in the horse, although further work comparing MF tissue from horses in different age and injury status groups is required. Subjectively, the histological processing of samples in the current study was more difficult in the older compared to the younger horses. In the equine population, tears of the MF have been reported in middle aged and older animals (5), therefore, one hypothesis for MF tears could be fibrocartilaginous metaplasia within the MF with alterations in biomechanical properties that increase the propensity for failure.

Although the study has presented some clear anatomic and histological features of the equine proximal MF, there are limitations associated with the study. The age range was large and the exercise history of the horses was not available, therefore, histological features that have potentially been associated with age, could relate to exercise history. The freezing and thawing of limb specimens prior to harvesting the histological samples may have resulted in damage to the cells; however, previous work of a similar nature (8) used similar methodology. In addition, evaluation of the tissue sections by one of the authors (ER) indicated that freeze-thaw artefact did not alter the tenocyte or blood vessel phenotype. In addition, there was difficulty in histological processing of some sections due to the “cartilage like” tissue consistency that reduced the quantity of specimens suitable for examination, preventing statistically robust conclusions, in particular, regarding differences between medial and lateral portions of the MF. The difficulty in histological sectioning could have been assisted by softening the samples in phenol and alcohol for a number of days prior to sectioning (28).

**Conclusions**

This study has provided a detailed description of the equine proximal manica flexoria, defining the tendinous and the areolar portions, and the variation noted in the length of these structures between the fore and hind limbs. The identification of fibrocartilaginous metaplasia on the palmar surface of the MF has provided a potential area for further investigation of the aetiology of MF tears.

**Figure Legends**

 **Figure 1**. Gross appearance of the manica flexoria (MF) with the associated superficial and deep digital flexor tendons. a) The view from the dorsal aspect of the MF showing the portion which apposes the palmar/plantar wall of the digital flexor tendon sheath. b) Lateral view of the MF with the probe inserted between the MF and the deep digital flexor tendon, extending beyond the proximal areolar portion. The opening through the areolar portion of the MF occurs at the site of dissection from the dorsal DFTS wall. c) Palmar surface of the MF. The SDFT has been split to demonstrate the complete MF and its attachments. MF= manica flexoria, SDFT= Superficial digital flexor tendon, DDFT= deep digital flexor tendon.

**Figure 2.** Schematic representation of anatomic relationship of the manica flexoria (MF, blue) and area of histopathological sampling. a) A schematic transverse representation of the MF representing the three anatomic regions evaluated histologically [axial (A), middle (M), and abaxial (AB)] and the three zones from dorsal to palmar/plantar (E1, E2, E3) within each sample. b) Dorsal view of a sample MF demonstrating the location of the histological sections. The black asterix is the site of the two longitudinal sections. The black arrowheads show the sites of the medial and lateral transverse sections that incorporated the junction between the MF and the superficial digital flexor tendon.

**Figure 3**. Histological features of the transverse sections of the manica flexoria (MF) stained with Alcian-PAS. (a) Panoramic view of the three zones (E1-3) of the MF, magnification 4x. (b-d) Tenocyte types. (b) Type 1 tenocytes: “fibroblast-like” cells oriented parallel to thick collagen bundles (arrow), magnification 40x; (c) Type 2 tenocytes: ovoid cells with a prominent nucleus (arrow), magnification 40x; (d) Type 3 tenocytes: round cells with a “chondrocyte-like” morphology and occasionally arranged in isogenous groups (arrow), magnification 63x. Note the increased amount of Alcian blue extracellular matrix evident in the area of chondrocyte-like morphology (d) as compared to the extracellular matrix in (b). (e-f) Morphology of blood vessels. (e) Initial degeneration of vascular wall (type 2 blood vessel) with a patent lumen still discernible (asterix), magnification 40x. (e) Type 3 blood vessels (“ghosted vessels”) in which the lumen has been occluded by centripetal accumulation of dense PAS and Alcian material (asterix), magnification 10x.

**Table 1**. Summary of the anatomical measurements for the 18 paired ipsilateral fore and hind limb samples of the manica flexoria and their relationship to adjacent structures in the equine palmar/plantar metacarpo/tarsophalangeal region. MF = manica flexoria; PAL – palmar/plantar annular ligament; NSD = no significant difference.

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| **Manica flexoria measurements** | Fore limb(mm ± SD) | Hind limb(mm ± SD) | T-testP-value |
| Tendinous MF medial length | 31.9 ±2.9 | 28.7 ±4.0 | 0.007 |
| Tendinous MF axial length | 32.0 ±4.2 | 29.4 ±3.8 | 0.04 |
| Tendinous MF lateral length | 31.9 ±2.9 | 29.1 ±4.1 |  0.01 |
| Tendinous MF – medial-lateral width | 27.8 ±3.6 | 28.5 ±3.7 |  0.06 |
| Areolar MF - length | 16.7 ±4.3 | 22.9 ±5.3 | 0.0005 |
| **Manica flexoria border relationship distances** |  |  |  |
| Distal MF to distal PSB  | 30.6 ±1.6 | 32.8 ±1.1 |  0.19 |
| Proximal MF to proximal PAL  | 10 .1 ±0.4 | 14.7 ±1 |  0.18 |
| Length of PAL | 43.3 ±3.6 | 43.6 (±3.7) |  0.37 |
| Overlap of MF and PAL | 13.2 ±7.0 | 12.5 ±4.0 |  0.29 |

**Table 2.** Qualitative description of predominant histological features (tenocyte morphology, extra cellular matrix staining, blood vessel type) of the defined zones (E1-3) from dorsal to palmar/plantar on the manica flexoria sections. The table provides the combined histological data from the medial and lateral, fore and hind limb samples. Tenocyte scores: 1 = more than 70% of type 1 tenocytes; 2= 30 to 50 % of type 2 tenocytes; 3= focal presence of aggregates of type 3 tenocyte (Beck et al. 2011). Blood vessel types: 1 = normal structure with patent lumen and clear intimal layer; 2= partial luminal occlusion with severe thickening of the vessel wall, intimal proliferation with vessel wall degeneration shown by increased Alcian-PAS staining; 3 = “ghost” blood vessel with the lumen and wall are replaced by concentric lamellar and dense aggregate of Alcian Blue positive material

|  |  |
| --- | --- |
|  | **Dorsopalmar/plantar histological zone**  |
|  | **E1****dorsal** | **E2** **middle** | **E3****Palmar/ plantar** |
| Predominant tenocyte morphology | Type 1 | Types 1-2 | Types 2-3 |
| Extracellular matrix Alcian-PAS stain intensity (subjective) | Mild | Mild-moderate | Moderate-marked |
| **Blood vessel type** Percent, mean (range) |  |  |  |
| Type 1Type 2Type 3 | **90%**33 (15-55)**10%**4 (0-11)**0%**0 (0-2) | **90%**28 (14-45)**7%**6 (0-14)**3%**2 (0-3) | **70%** 16 (6-25)**15%** 6 (1-9)**15%** 4 (0-7) |

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Figure 1



Figure 2



Figure 3

