Microvasculature of the suspensory ligament of the forelimb of horses

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Objective—To determine the microvascular anatomy of the suspensory ligament of the forelimb of horses.

Sample—17 cadaveric forelimbs from 9 adult horses with no known history of forelimb lameness.

Procedures—The median artery of the forelimb was cannulated proximal to the antebrachiocarpal joint and injected with contrast medium for CT evaluation of the gross vasculature (n = 2) or India ink to evaluate the microvasculature (12). Routine histologic evaluation was performed on an additional 3 forelimbs to confirm the microvascular anatomy.

Results—The vascular supply of the suspensory ligament of the forelimb originated from branches of the medial and lateral palmar and palmar metacarpal vessels as well as the proximal and distal deep palmar arches. An abundant, longitudinally oriented microvascular supply was evident throughout the length of the suspensory ligament without distinct variation among the proximal, midbody, and distal regions. The intraligamentous blood supply originated from a periligamentous vascular plexus that surrounded the suspensory ligament throughout its length. Histologic findings indicated the presence of a periligamentous connective tissue plexus, which contained vessels that penetrated and anastomosed with an extensive network of intraligamentous vessels throughout the length of the suspensory ligament.

Conclusions and Clinical Relevance—The suspensory ligament of the equine forelimb had an abundant intraligamentous microvascular supply throughout its entire length. The absence of an obvious hypovascular area suggested that regional variations in healing rates of the suspensory ligament are not associated with the microvascular anatomy. (Am J Vet Res 2013;74:1481–1486)
been described. To our knowledge, a detailed study to describe the microvascular anatomy of the forelimb SL of horses has not been performed. Therefore, the purpose of the study reported here was to use results of contrast-enhanced CT, microvascular injection (ie, Spalteholz tissue-clearing technique), and routine histologic evaluation to describe the blood supply of the SL of the forelimb of horses. It was hypothesized that zones of hypovascularity would exist in the midbody and distal regions of the SL. The presence of hypovascular zones in these areas could provide a possible mechanistic explanation for the poor healing and high rate of recurrence for injuries to the midbody region and distal branches of the SL.

Materials and Methods

Seventeen cadaveric forelimbs were used for the study. The forelimbs were obtained from 9 adult horses that were euthanized for reasons unrelated to the study and had no history of forelimb lameness or gross pathological lesions in the forelimbs used. The distal aspect of the limbs was harvested at the junction of the middle and distal third of the radius. The horses ranged in age from 4 to 18 years (mean, 8.7 years; median, 9 years) and included 4 mares and 5 geldings. Breeds represented included Arabian, Arabian cross (n = 2), Quarterhorse (2), Paint (2), Standardbred (1), and Thoroughbred (2). The forelimbs were frozen and stored at –20°C until analysis, at which time they were thawed prior to injection of the contrast media or India ink.

Contrast-enhanced CT—To identify the major vessels that supply the SL, the median artery of 2 forelimbs was cannulated just proximal to the carpus and injected with 60 mL of contrast medium. The limbs were then imaged with a 16-slice CT scanner at a slice thickness of 0.625 mm. The CT images were processed with computer software to highlight and identify the major vascular supply to the SL. Regions of interest were automatically selected on the basis of the radiopacity (Hounsfield units) of contrast medium for vasculature and manually corrected on a slice-by-slice basis to outline the vessels.

Microvascular perfusion by the Spalteholz tissue-clearing technique—To identify the microvascular anatomy of the SL specimens, the median artery of 12 forelimbs was cannulated just proximal to the carpus, and 120 to 180 mL of India ink was injected under constant manual pressure. Complete vascular filling was confirmed by the presence of India ink in the dermal capillaries following a small incision at the coronary band of the hoof. The limbs were then frozen at –20°C for a minimum of 48 hours. Five-millimeter-thick sections of the limb were then cut in either the sagittal (n = 7) or transverse (5) plane from the middle carpal joint to the metacarpophalangeal joint with a band saw and fixed in neutral-buffered 10% formalin. Tissue clearing was performed by means of the modified Spalteholz technique as described.

Histologic evaluation—Three additional forelimbs were processed for routine histologic evaluation. Briefly, 5-mm-thick sections were cut in the transverse axial plane and fixed in neutral-buffered 10% formalin. Selected sections that represented the proximal, midbody, and distal regions of the SL from each limb were further sectioned into 5-µm-thick sections, stained with H&E stain, and evaluated histologically. The histologic vascular anatomy was descriptively compared with the microvascular anatomy determined by the Spalteholz technique.

Results

Contrast-enhanced CT—The principal blood supply to the SL consisted of small branches of the medial and lateral palmar and palmar metacarpal arteries beginning at its most proximal aspect and continuing throughout its length (Figure 1). Branches of these vessels entered the proximal region of the SL at the deep palmar arch. A branch of the medial palmar metacarpal artery entered the midbody region of the SL opposite of where the nutrient artery entered the nutrient foramen of the third metacarpal bone. The distal region and branches of the SL were supplied by branches of the lateral and medial palmar digital vessels and the associated superficial palmar arch as well as branches of the lateral and medial palmar metacarpal vessels and the associated deep palmar arch.

Microvascular perfusion by the Spalteholz tissue-clearing technique—The entire length of the SL had a substantive microvascular supply without distinct...
variation in the intraligamentous vascular density among the proximal, midbody, and distal regions. The uniformly abundant intraligamentous vasculature was consistently visible on all sagittal (Figure 2) and transverse sections (Figure 3) from all 12 limbs evaluated with the Spalteholz technique with minimal variability among specimens. Similarly, an abundant, periligamentous vascular plexus was observed on sagittal and transverse sections that surrounded the SL throughout its length, and those vessels were generally oriented longitudinally, parallel with the long axis of the bone.

Small vascular branches that originated from the palmar metacarpal vessels were observed invaginating into the dorsal aspect of the medial and lateral lobes of the SL beginning at its proximal aspect (Figure 3). The proximal segment of the SL where the periligamentous vessels were most prominent corresponded with the location of the proximal deep palmar arch.21–23 In this area, the palmar aspect of the SL had additional small vessels, which communicated with the proximal deep palmar arch and the medial and lateral palmar vessels.

At the junction of the proximal and midbody regions of the SL, the periligamentous vascular plexus remained visible, with small branches that extended into the SL, particularly on its dorsal aspect (Figures 2 and 3). Near the nutrient foramen of the medullary cavity of the third metacarpal bone, a branch of the medial palmar metacarpal artery extended in a palmar direction with accessory branches that extended into the midbody region of the SL. The periligamentous vascular plexus remained present at the distal region of the SL where it divided into medial and lateral branches. At this level, palmar metacarpal vessels were observed with multiple small branches that supplied the medial and lateral branches of the SL (Figure 3). Small branches from the palmar digital vessels also contributed to the vascularity of the medial and lateral branches of the SL. Additionally, an abundant intraligamentous blood supply was observed in the distal region of the SL.

**Histologic evaluation**—Histologic findings for sections from the proximal, midbody, and distal regions of the SL were consistent with the microvascular anatomy observed in corresponding sections that were evaluated with the Spalteholz technique. Additionally, small arteries and veins were observed within the connective tissue septa in all regions of the SL, which contributed to an abundant intraligamentous blood supply (Figure 4). A periligamentous plexus of connective tissue that contained small arteries and veins surrounded the SL throughout its length. Branches of the medial palmar metacarpal artery, which give rise to the nutrient artery of the third metacarpal bone, also supplied the periligamentous vascular plexus at the midbody region of the SL (Figures 3 and 4). The distal branches of the SL also had an abundant intraligamentous blood supply from the periligamentous vascular plexus.
Discussion

Results of the present study indicated that the SL of the equine forelimb has an abundant microvascular supply, which did not vary substantially among its proximal, midbody, and distal regions. The abundant intraligamentous microvasculature originated from a periligamentous vascular plexus that surrounded the entire length of SL including its distal branches. The vascular supply to this periligamentous plexus originated from branches of the medial and lateral palmar metacarpal arteries as well as branches of the medial and lateral palmar arteries. These findings were consistent with those of other investigators, which suggested that branches of the medial and lateral palmar metacarpal arteries and branches of the medial palmar artery constituted the major vascular supply to the SL. These small branches penetrate the SL and anastomose with a longitudinally oriented network of intraligamentous vessels that pass between the fascicular bundles of collagen fibers. Although the techniques used to identify the microvascular anatomy of the SL in the present study only reflected the structural components of the vascular supply and not the physiologic parameters of blood flow and perfusion, results of other studies indicate an excellent correlation between the microvascular anatomy of connective tissue structures and their vascular response to acute injury. The findings of the present study confirmed previous observations regarding the principal vascular supply of the SL and provided the first detailed description of the microvascular anatomy in each region of the SL.

The intraligamentous microvascular pattern observed for the SL in this study is analogous to the intratendinous microvascular supply for the SDFT of horses, which was described as an extensive, interlac-
ing network of longitudinally oriented, intratendinous microvessels. However, contrary to the findings of the present study, the site of the SDFT that is most commonly injured in horses corresponded to a zone of relative avascularity, and the investigators concluded that this hypovascular zone predisposed the tendon to degenerative injury and contributed to the poor healing response in that area. In human patients, similar hypovascular regions have been identified at common injury sites for various tendons.

Although the results of the present study did not identify any hypovascular areas in the SL, damage to the periligamentous vascular supply of the SL secondary to traumatic or degenerative injury could contribute to a compromised healing response in that area. In human patients, similar hypovascular regions have been identified at common injury sites for various tendons.

Although an area of diminished vascular supply has been implicated in the predisposition of the SDFT to injury, biomechanical factors can also affect the biology of connective tissue healing. A compromised microvascular response secondary to excessive loading has been implicated in the failure to heal of some degenerative tendon lesions in horses. Repetitive stresses on healing tendons can disrupt the microvascular healing response, resulting in the formation of poorly vascularized scar tissue, which fails to adapt and remodel into functional repair tissue. This disruption of the microvascular healing response in tendons is similar to the mechanism of delayed healing or nonunion of fractured bones, in which excessive, repetitive stresses at the fracture site continually disrupt capillary migration into the

Figure 4—Photomicrographs of sections of a forelimb of a horse obtained at the proximal (A and B), midbody (C), and distal (D) regions of the SL. A—Multiple connective tissue septa are present within the SL, which contains cross sections of multiple small arteries and veins (arrows). H&E stain; bar = 50 µm. B—Multiple cross sections of small arteries and veins are shown within a connective tissue plexus (CTP) that surrounds the SL. H&E stain; bar = 50 µm. C—The nutrient artery (NA) of the third metacarpal bone (MCIII) is adjacent and contributes vascular branches to the periligamentous vascular plexus of the SL; intraligamentous connective tissue septa with associated small vessels continue to be present. H&E stain; bar = 100 µm. D—The periligamentous vascular plexus that contributes to the CTP, which enters the SL and provides an extensive intraligamentous blood supply (arrows), is evident. H&E stain; bar = 100 µm. Sectioning artifacts within the SL are present in all panels. NF = Nutrient foramen of MCIII.
repair tissue, resulting in the development of a fibrous or fibrocartilagenous repair tissue instead of bone. Fatigue of the flexor tendons that support the metacarpophalangeal or metatarsophalangeal ( fetlock) joint during high-speed exercise leads to increased stresses on the SL apparatus, which in turn result in acute or degenerative lesions of the SL. Continued mechanical loading of the fatigued or injured tendons could result in a dysfunctional repair response and the production of poorly vascularized scar tissue, which could predispose the SL to reinjury.

Results of the present study do not support an anatomic microvascular supply basis for the regional variations in the healing response of the SL to injury in horses, and the precise relationship between the microvascularity of the SL and the regional variation in its healing response remains to be elucidated. The regional variation in the healing and reinjury rates reported for the SL of horses might be related to topographic heterogeneity in its structural, compositional, and functional characteristics. Additional studies to examine the sequence of biological events following injury to specific areas of the SL are necessary to identify the precise factors responsible for regional variations in outcome.

References