Quantitative effect of tenorrhaphy on intrinsic vasculature of the equine superficial digital flexor tendon

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Objective—To compare effects of the locking-loop suture pattern (LLP) and 3-loop pulley (3LP) suture pattern for tenorrhaphy on the intrinsic vasculature of the superficial digital flexor tendon (SDFT) of horses in vitro after surgery.

Sample Population—16 forelimbs obtained from 8 mature horses.

Procedure—Tenotomy and subsequent tenorrhaphy was performed in anesthetized horses. Following systemic administration of heparin, horses were euthanatized and the limbs were removed and placed under tension to load the flexor tendons. The intrinsic vasculature was then perfused with a mixture of barium sulfate and water. Four-millimeter sections of the SDFT were prepared for microangiographic analysis. Mean vessel density was calculated for each section by use of a grid consisting of 1.5-mm^2 vascular assessment squares (VAS). Comparisons were made among the control, LLP, and 3LP groups.

Results—Mean ± SD vessel density was 3.11 ± 0.38, 1.47 ± 0.47, and 2.01 ± 0.63 perfused vessels/1.5 mm^2 for control, LLP, and 3LP groups, respectively. Significant differences in vascular density were detected between the control and 3LP groups, control and LLP groups, and LLP and 3LP groups.

Conclusions and Clinical Relevance—Use of the LLP and 3LP pattern has deleterious effects in vitro on the intrinsic vasculature of the SDFT. However, the 3LP pattern was less disruptive to the intrinsic vasculature, compared with the effects for the LLP. Use of the 3LP tenorrhaphy suture pattern in clinical situations may result in less damage to the intrinsic vasculature of the SDFT of horses during convalescence. (*Am J Vet Res* 2004;65:279–282)

Traumatic lacerations of the tendons in horses are 1 of the most widespread and potentially devastating injuries currently faced by equine practitioners.[1,2] Lacerations of flexor tendons often have a guarded prognosis and may severely impact the future use of equine athletes.[1-3] It has been documented[2-5] that tenorrhaphy, when possible, is the most advantageous treatment for transected flexor tendons and increases the likelihood of returning a horse to riding status. There are several options for tenorrhaphy suture patterns, including the single **locking-loop pattern (LLP)**, multiple locking-loops techniques, and the **3-loop pulley (3LP)** pattern. The resistance to gap formation, breaking strength, and ease of placement of these suture patterns has been documented in other studies.[4,6,7] Furthermore, it has been reported[8,9] that the equine **superficial digital flexor tendon (SDFT)** contains a highly anastomotic vascular system within the tendon, and disruption of this network can presumably cause a disease process similar to that of naturally developing tendonitis.

Tenorrhaphy should be accomplished in a manner that causes minimal disruption to the intrinsic vasculature and provides maximal strength. Use of the LLP in the digital flexor tendon of chickens allows for vascular perfusion through the tenorrhaphy zone.[10] However, to the authors' knowledge, the effects of this pattern on the intrinsic vasculature of the equine SDFT have only been anecdotally addressed in the literature.

In 1 study[7] that used slow-motion videotapes to evaluate tenorrhaphy patterns under small loads, it was found that the LLP compresses and distorts the tendon when placed under load, whereas conversely, the 3LP pattern only minimally affects the structure of the tendon. Along this line, it seems intuitive that if tenorrhaphy sutures compress the tendon and distort the normal anatomic relationships, they may also impinge on the intrinsic vascular system.

The objective of the study reported here was to determine the effects of the LLP and the 3LP suture pattern on the intrinsic vasculature of the equine SDFT immediately after tenotomy. The intratendinal perfusion technique used was similar to methods successfully used to document the anatomic relationships of the intrinsic vascular systems of the equine SFDT and deep digital flexor tendon in other studies.[8,11] Our hypothesis was that the LLP would constrict many delicate intratendinal vessels and thereby decrease vascular perfusion at the tenorrhaphy site, whereas use of the 3LP pattern would allow for better vascular perfusion through the tenorrhaphy zone.

Materials and Methods

Sample population—The forelimbs of 8 mature horses (ie, 16 forelimb SDFTs) were selected for use in the study. The horses were being sent to slaughter, and none had a his-

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tory or evidence of injury to the SDFT. All horses were subjected to lameness evaluation, palpation of the tendons, and ultrasonography of the tendons prior to inclusion in the study. An institutional animal care and use committee approved this project.

Surgical procedure—Horses were medicated with xylazine hydrochloride (1.0 mg/kg, IV), and anesthesia was induced with a mixture of ketamine hydrochloride (2.2 mg/kg, IV) and diazepam (0.1 mg/kg, IV). Anesthesia was maintained by administration of halothane and oxygen or a mixture of guaifenesin (5% solution, 1 L), ketamine (1.0 g), and xylazine (500 mg). Horses were positioned in lateral recumbency, and hair over the palmar aspect of the mid-metacarpal region of each forelimb was clipped.

The SDFTs were assigned randomly to the various treatment groups. Five tendons were assigned to the LLP tenorrhaphy group, 5 were assigned to the 3LP tenorrhaphy group, and 6 served as control tendons. For the tenorrhaphy groups, a palmarolateral or palmaromedial approach was used (10-cm longitudinal skin incision centered over the SDFT immediately distal to the palpable communicating branch of the palmar nerves). The SDFT was then exposed by use of blunt and sharp dissection with curved Mayo scissors. A 5-cm incision was then made in the paratenon, and curved Kelley forceps were used to isolate and elevate the SDFT. The SDFT was sharply transected, and an LLP or 3LP tenorrhaphy pattern was placed in the tendon by use of No. 2 polydioxanone.[a] The 3LP tenorrhaphy pattern was placed such that sutures were 0.5 cm from the cut ends of the tendon and spaced approximately 0.5 cm apart. The LLP tenorrhaphy pattern was placed centrally with longitudinal entrance - exit sites of the sutures spaced approximately 0.5 cm apart. A surgeon's knot and 4 throws were used for all tenorrhaphy patterns. The same investigator (CLC) performed all tenotomies and tenorrhaphies. We did not perform a surgical approach to the SDFT for the control specimens.

Tenorrhaphy zones were defined as the area encompassed by the most proximal suture entrance-exit site to the most distal suture entrance-exit site. Mean ± SD size of the tenorrhaphy zone for the 3LP tenorrhaphy group was 28.8 ± 3.35 mm, whereas mean size of the tenorrhaphy zone for the LLP tenorrhaphy group was 24.0 ± 5.66 mm.

Following tenotomy and tenorrhaphy, each horse was systemically administered heparin sodium (200 U/kg, IV). Ten minutes were allowed to elapse after heparin administration, and horses were then euthanatized with pentobarbital sodium (87 mg/kg, IV). Both forelimbs of each horse were then immediately amputated at the mid-radius proximal to the musculotendinous junction of the SDFT. To preserve the normal blood supply to the SDFT and allow as much intratendinal perfusion as possible, muscular, osseous, and paratenon attachments were left undisturbed except at the tenorrhaphy site.

Holes were created at the toe and mid-radius by use of a 1.27-cm drill bit. A galvanized carriage bolt (1.11 cm in diameter) was inserted through the hole in the mid-radius and secured to the toe via a cable and tensioning device. Both treatment groups and the control group were then placed in extension with the limbs under dorsal tension (approx 30 kg). This load created a gap of 0.5 cm at the tenorrhaphy site in the LLP group but no measurable gap in the 3LP tenorrhaphy group.

Perfusion technique—The median artery was located and catheterized with a 14-gauge polypropylene catheter[b] proximal to the musculotendinous junction of the SDFT. A mixture of 300 mL of barium sulfate and 200 mL of water was infused into the artery at a pressure of 120 mm Hg, as regulated by a manometer. Neutral-buffered 10% formalin (75 mL) was added to the final 250 mL of perfusate. The perfusion procedure required approximately 3 h/limb. Lateromedial and dorsopalmar radiographs were taken of the initial 2 control specimens to ensure perfusion was adequate. These radiographs were deemed unnecessary for specimens of the treatment groups, because all tendons used as control specimens contained vessel densities within control limits in the area outside the tenorrhaphy zone. Following perfusion, limbs were maintained in extension and refrigerated for a minimum of 12 hours at 7.2°C to allow the perfusate to cure.

Each SDFT was harvested by transection proximally at the musculotendinous junction and distally at the metacarpophalangeal joint. Each SDFT was meticulously dissected from the limb, and the paratenon was stripped from the tendon circumferentially. The SDFTs were then anchored by inserting sutures at the ends and attachment to a quartered section of polyvinyl chloride pipe, which was followed by immersion in neutral-buffered 10% formalin to prevent shrinkage and distortion during transit to the laboratory for microangiographic analysis.

Microangiographic protocol—The SDFTs were manually measured and transected in 4-mm transverse sections. For the tenorrhaphy groups, SDFTs were sectioned beginning at the tenotomy site and continuing in proximal and distal directions. Tendons in the control group were sectioned beginning at an area immediately distal to the carpometacarpal joint and continuing in a distal direction to an area immediately proximal to the metacarpophalangeal joint.

The 4-mm transverse sections were submitted for soft-tissue radiography by use of high-detail film.[c] Tenorrhaphy sutures were removed from the tendons prior to radiographic imaging. Radiography was performed by use of a high-definition x-ray cabinet[d] at 31 kVp and 3 mA for 4 seconds. Tendons were radiographed separately by placing the 4-mm sections (arranged from proximal to distal) directly on the film jacket.

Microangiographic films were then transferred to a stereomicroscope,[e] and digital images were obtained[f] and stored in a photoediting program.[g] A ruler was placed under the microscope and photographed with each tendon to calibrate the images to scale and overlay a **vascular assessment square (VAS)** grid of 1.5-mm^2 squares.

Calculation of vessel density—Digital images obtained for each 4-mm section were used to calculate vessel density. Perfused vessels were manually counted, and perfused vessel density was determined for each section. The VAS grid was positioned over each 4-mm section and calibrated to the image such that the grid encompassed the entire section (**Fig 1**). This allowed use of peripheral vessels when determining vessel density in each 4-mm section. Vascular perfusion within the tenorrhaphy site was determined by use of the number of perfused vessels per VAS within the tenorrhaphy zone. The tenorrhaphy zone was defined as the area encompassed by

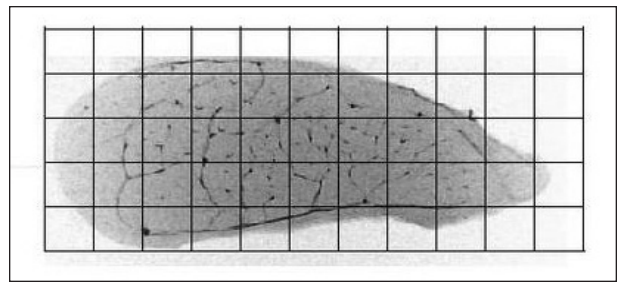

Figure 1—Digital image of a 4-mm section of the superficial digital flexor tendon obtained from a control specimen with a vascular assessment square (VAS) grid overlay used for determining vessel density per 1.5 mm^2.

the most proximal suture entrance-exit site to the most distal suture entrance-exit site.

Vessel density was determined in 4-mm sections proximal to the tenorrhaphy zone in the treatment groups to ensure they were perfused similar to control specimens, but these sections were not included in the statistical analysis. Manual counting was performed by use of a photoediting program[g] by the same investigator (CLC) for all sections. The investigator was aware of the source of the specimens during the counting procedure because it was obvious as to which sections were being displayed during the counting process.

Perfused vessels were marked so that no vessel was inadvertently counted twice, and the count was recorded for each VAS grid. Peripheral areas of the tendon sections that only partially filled the VAS grid squares were counted in the same manner as for fully filled grid squares. This allowed perfused peripheral vessels to be used in calculations of vessel density. Vessels that coursed parallel to the long axis of the tendon and bordered > 1 VAS grid squares were included in the count of the grid square that contained the largest portion of the vessel, as determined by subjective assessment of the investigator. In contrast, perfused vessels that were perpendicular to the long axis of the tendon were included in the count of each VAS grid square in which they appeared.

Statistical analysis—Tendons obtained from 8 horses were randomly assigned to the treatment groups, with multiple sections for each tendon; hence, the data were analyzed as a completely randomized design with subsamples. This analysis used a general linear model procedure with differences in treatment groups examined by use of least-squares means.[12] Results were considered significant at a value of $P < 0.05$.

Results

The 3LP tenorrhaphy group consisted of thirty-six 4-mm sections (mean ± SD, 7.6 ± 0.8 sections/tendon). The LLP tenorrhaphy group consisted of thirty 4-mm sections (mean, 6.0 ± 1.4 sections/tendon). The control group consisted of seventy-seven 4-mm sections (mean, 12.8 ± 1.3 sections/tendon). For control specimens, only the sections located at the mid-metacarpal region were used for analysis.

Vessel density for the control group ranged from 2.49 to 3.97 vessels/VAS (mean ± SD, 3.11 ± 0.38 vessels/VAS). Vessel density for the 3LP tenorrhaphy group ranged from 0.69 to 3.19 vessels/VAS (mean, 2.01 ± 0.63 vessels/VAS), whereas vessel density for the LLP tenorrhaphy ranged from 0.63 to 2.26 vessels/VAS (mean, 1.47 ± 0.47 vessels/VAS).

Perfused vessel density differed significantly among all 3 groups. The LLP treatment group had a significantly lower vessel density through the tenorrhaphy zone (1.64 fewer vessels/VAS), compared with the vessel density for the control group. The 3LP tenorrhaphy group had a significant reduction in perfused vessel density through the tenorrhaphy zone (1.10 fewer vessels/VAS), compared with the vessel density for the control group. The LLP tenorrhaphy group also had a significant reduction in perfused vessel density through the tenorrhaphy zone (0.54 fewer vessels/VAS), compared with the vessel density for the 3LP tenorrhaphy group.

Discussion

The superficial layers of the intrinsic vasculature were grossly visible after perfusion and dissection. Superficial areas of the SDFT appeared to contain numerous small vessels that were highly anastomotic and longitudinally oriented. Consistent with observations in another study,[7] the LLP caused gross constriction of the area of the tendon in which it was placed in the study reported here. The 3LP pattern flared and spread the tendon ends but did not appear grossly to cause the constriction seen with the LLP tenorrhaphy.

Microangiographically, the LLP and 3LP pattern appeared to decrease perfusion through the tenorrhaphy zone. For both treatment groups, the reduction in perfusion began in the 4-mm section proximal to the most proximal entrance-exit site of the suture. This compromise, although not included in the tenorrhaphy zone, corresponded with the area that was grossly constricted by the sutures. The areas of reduced perfusion within the tenorrhaphy zone were closely related to entrance-exit sites of the sutures. The more peripheral areas of the tendon remained perfused for a greater distance toward the tenotomy site than for the central region of the tendon. The 4-mm sections that encompassed the transected ends of the tendon contained only a scant amount of perfused vessels for both treatment groups, and these vessels were located most often at the periphery of the tendon. Perfused vessel density had values consistent with the control group at the second or third 4-mm section distal to the most distal suture entrance-exit site.

Even with external coaptation, tenorrhaphies gap, to some degree, during the convalescent period in horses.[4,5,7] This provides evidence that even with rigid immobilization, the tenorrhaphy zone (and therefore the suture pattern) is under load, and this could theoretically result in constriction of the tissue by the sutures. It is not known whether actual reduction of perfused vasculature in the initial postoperative and convalescent periods in clinically affected horses would be similar to that found for the experimental loads described here.

Gap lengths attained in our study for a dorsally applied tension of approximately 30 kg were comparable to those obtained by application of approximately 4.5 kg of direct isolated tension on the SDFT.[6] However, it is speculated that in vivo loads are greater than those achieved in vitro, even with external coaptation.

It has been reported[13] that transected digital flexor tendons in rabbits without tenorrhaphy have a variable loss of perfused vasculature at the transected ends of the tendons. In that study, the authors also determined that the Bunnell criss-cross tenorrhaphy pattern caused consistent failure of vascular filling at the tenorrhaphy zone and that adhesions formed by 1 week after surgery from extratendinal tissue with an influx of highly vascularized peritendinous tissue. In horses, similar fibrovascular reactions involving SDFT defects are evident during the convalescent period.[4] On the basis of results of the study reported here, it appears that certain tenorrhaphy patterns, especially the LLP, may predispose clinically affected horses to developing such a reaction during the healing period.

The amount of perfused intrinsic vasculature found in the study reported here, primarily for the LLP group,

may have been sufficiently reduced to cause a clinically important area of ischemia within the tenorrhaphy zone. Theoretically, this would widen the tendon gap in a clinically affected horse from only the transected ends of the tendon to also include the tenorrhaphy zone. It has been reported[14] that placement of constricting suture patterns, such as the Bunnell pattern, in the SDFT of rabbits will result in degenerating tendon cells at 1 week after surgery, and the constricted area will be devoid of viable cells by 4 weeks after surgery. In that study in rabbits, authors reported that such constricting suture patterns lead to local tendon death, even in unloaded models. When the tenorrhaphy zone is composed of nonperfused tissue, it may need to heal through extrinsic processes. This may predispose the repair to delayed healing and increase the possibility of many complications, such as adhesions or severe fibrosis, all of which could contribute to eventual lameness.

Our study focused on the effects of tenorrhaphy on the intrinsic vasculature immediately after tenorrhaphy and the fact that in vivo perfused vessel density could conceivably change during the convalescent period. However, it has been reported[14] in rabbits that the area of the SDFT encompassed by Bunnell suture patterns does not become incorporated into the healing area of the tendon, even at 8 weeks after surgery.

The difference in the range of perfused vessel density between the 3LP and LLP tenorrhaphy groups may have been attributable to the fact that the 3LP tenorrhaphy group contained more perfused vessels at the extremities of the suture pattern than the LLP group. The most severe area of reduction for tendons of the 3LP tenorrhaphy group involved the sections closest to the tenotomy site, whereas the LLP group appeared to have a more consistent reduction in perfusion throughout the tenorrhaphy zone and a vast majority of the perfused vasculature was located peripheral to the suture entrance-exit sites. Although both patterns significantly reduced vascular perfusion within the tenorrhaphy zone, it is evident that the single LLP, even under minimal experimental loads, reduces perfusion to a significantly greater degree than does the 3LP pattern.

We cannot extrapolate the findings of this study to include multiple locking-loop patterns because double or triple locking-loop tenorrhaphy suture patterns may be able to more uniformly distribute the loads encountered across the tendon and thereby reduce the amount of constriction caused by each suture. On the basis of issues raised by the study reported here, we believe additional studies are required to determine the effects of tenorrhaphy in horses on the intrinsic vasculature in vivo during the convalescent period.

[a]PDS II, Ethicon Inc, Somerville, NJ.
[b]Abbocath-T, Abbott Laboratories, Abbott Park, Ill.
[c]Kodak X-Omat AR 8 X 10 film, Eastman Kodak Co, Rochester, NY.
[d]Cabinet x-ray system, Faxitron Series, Hewlett-Packard, Palo Alto, Calif.
[e]2000 model Olympus research stereomicroscope SZX12, Olympus Industrial America Inc, New York, NY.
[f]Sony 10X digital Mavica camera, Sony Co, New York, NY.
[g]Microsoft photoeditor, Microsoft Corp, Redmond, Wash.

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