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Flexor Tendon Injury: Avascular or Vascularized Region Suture? Biomechanical and Histopathological Study in Rabbits*

Lesão do tendão flexor: sutura na região avascular ou vascularizada? Estudo biomecânico e histopatológico em coelhos

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Abstract

Objectives The present study aims to analyze the mechanical and histopathological aspects of flexor tendon healing focusing on the suture placement site in a vascular or in an avascular region.

Methods A total of 83 rabbits were submitted to a Kessler-type central suture in the vascularized tendon region (TN group) and in the avascular tendon region (FC group). The operated limb was immobilized for 3 weeks. The animals were sacrificed in the immediate postoperative period, and at 2, 3 and 6 weeks after the procedure. The mechanical properties studied were: maximum load, stress at maximum load, modulus of elasticity, energy at maximum load, and energy per area. The contralateral tendon was used as control. The histopathological study was descriptive.

Results The analysis of the mechanical properties showed similar behavior in both groups, with stabilization or discrete increased values between the immediate period and 3 weeks after the procedure, and marked increased values at 6 weeks. Histopathology demonstrated that the healing process was similar in the TN and FC groups.

Conclusion Central suture placement in the vascularized or avascular fibrocartilaginous region results in no differences in the biomechanical and histopathological aspects of flexor tendon healing in rabbits.

Keywords
- tendon injuries
- rabbits
- sutures

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Resumo

Objetivos Analisar os aspectos mecânicos e histopatológicos da cicatrização do tendão flexor com interesse no local de colocação da sutura, na região vascularizada ou avascular.

Métodos Um total de 83 coelhos foram submetidos à sutura central tipo Kessler na região de tendão vascularizado (grupo TN) e na de tendão avascular (grupo FC). O membro operado foi imobilizado por 3 semanas. Os animais foram sacrificados no período imediato, e a 2, 3 e 6 semanas de pós-operatório. As propriedades mecânicas estudadas foram: carga máxima, tensão na carga máxima, módulo de elasticidade, energia na carga máxima e energia por área. O tendão contralateral foi utilizado como controle. O estudo histopatológico foi descritivo.

Resultados A análise das propriedades mecânicas demonstrou comportamento semelhante em ambos os grupos, com estabilização ou discreto aumento no período imediato, com 3 semanas e aumento acentuado com 6 semanas. A histopatologia demonstrou processo de cicatrização semelhante nos grupos TN e FC.

Conclusão A colocação da sutura central na região vascularizada ou fibrocartilaginosa avascular não apresenta diferenças em relação aos aspectos biomecânicos e histopatológicos na cicatrização do tendão flexor profundo dos dedos do pé do coelho.

Palavras-chave

- traumatismos dos tendões
- coelhos
- suturas

Introduction

In the treatment of zone II flexor tendon injuries, central suture placement in the tendon palmar region to limit vascular injury is a classic approach.[1,2] However, experimental studies have concluded that the dorsal positioning of the central suture results in greater resistance compared to palmar suturing.[3–7] The controversy between placing the central suture in the dorsal tendon region and obtaining greater resistance, but possibly impairing blood supply to the tendon, or placing the suture in the palmar region to preserve irrigation, but achieving less resistance, persists at the surgical practice.[8]

The present study aimed to analyze the mechanical and histopathological aspects of flexor tendon healing focusing on suture placement at the vascular or avascular region.

Material and Methods

The study design was previously approved by the Ethics Committee on Animal Experimentation of the institution.

A total of 83 male Norfolk rabbits, weighing between 1,500 g and 2,000 g, aged ~ 90 days old, were used.

The animals were divided into groups according to the location of the suture: normal tendon group (TN) sutured at the vascularized region; and fibrocartilage group (FC), sutured at the avascular region. Each group was subdivided into four subgroups according to the postoperative follow-up time, which were immediate, and of 2, 3, and 6 weeks (►**Tables 1** and **2**).

The deep digital flexor tendon was used. This tendon is located in the ankle region, within the osteofibrous channel, and it is surrounded by synovial tissue, where it presents an elliptical nodule, in which the region presenting epithelium and blood vessels is referred to as normal tendon in this investigation, whereas the whitish, avascular region is referred to as fibrocartilaginous tendon.[9–11] The tendon injury was performed through medial surgical access at the ankle region and complete cross-section of the tendon at the center of the nodule. The contralateral tendon was used as control.

The repair was performed using a Kessler-type suture using 4–0 monofilamentar nylon suture (Nylon 4.0, agulha triangular 2,0 cm; Point Suture; Fortaleza; Ceará, Brasil) placed in the vascularized region in the TN group, and in the avascular region in the FC group, supplemented by a circumferential,

Table 1 Normal tendon (TN) group: subgroups, postoperative follow-up time, number of animals, and studied features

Subgroup	Immobilization (weeks)	Follow-up (weeks)	Number of animals ($n = 42$)	
			Biomechanical	Histopathology
TN_0*	0	0	6	0
TN_2	2	2	6	4
TN_3	3	3	6	6
TN_6	3	6	6	8

The subgroup number indicates the follow-up time in weeks.
*This subgroup was not submitted to histopathological analysis.

Table 2 Fibrocartilage (FC) Group: subgroups, postoperative follow-up time, number of animals, and studied features

Subgroup	Immobilization (weeks)	Follow-up (weeks)	Number of animals ($n = 41$)	
			Biomechanical	Histopathology
FC_0*	0	0	6	0
FC_2	2	2	6	5
FC_3	3	3	6	5
FC_6	3	6	6	7

The subgroup number indicates the follow-up time in weeks.
*This subgroup was not submitted to histopathological analysis.

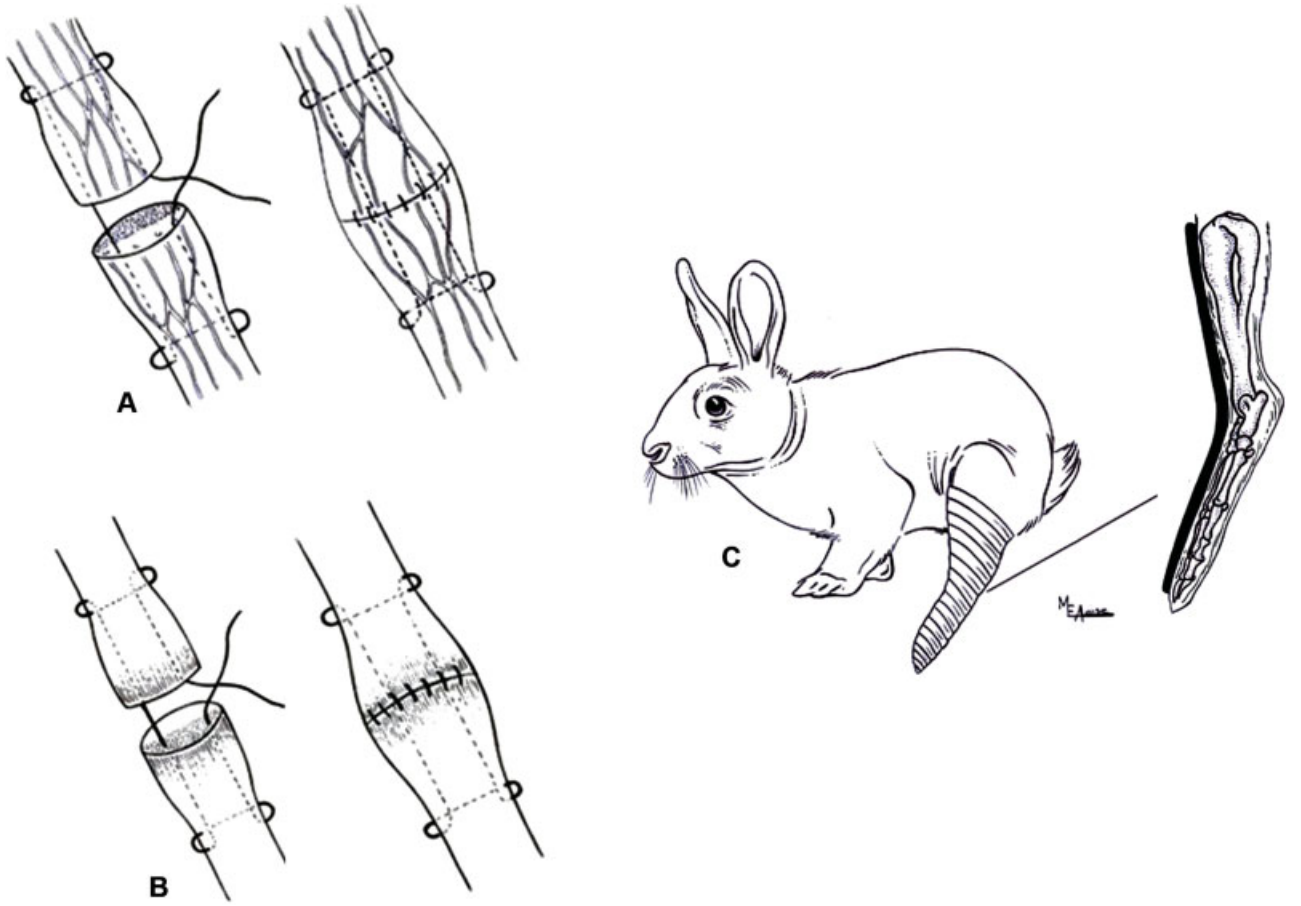

Fig. 1 (A) Suture used in the normal, vascularized tendon (TN) group. (B) Suture used in the avascular fibrocartilage (CF) group. (C) Animal with immobilized operated pelvic limb.

6–0 monofilamentar nylon suture (Mononylon Ethilon 6.0, agulha cilíndrica 1,5 cm, Ethicon, São Paulo, São Paulo, Brasil). The operated end was immobilized with a plastic splint holding the ankle and fingers in extension (►**Fig. 1**).

After euthanasia, the tendons were removed in 4-cm fragments, fixed in sinusoidal metal claws at a 20 mm distance from the central healing site. The claws were assembled axially in a universal mechanical test machine with a 1,000 N (EMIC-Equipamentos e Sistemas de Ensaio Ltda., Modelo DL 10000, Curitiba, Parana, Brasil) load cell, and the load application speed was of 30 mm/minute. The section area of the tendon was determined by measuring the elliptical area of the non-operated contralateral tendon nodule region; this value was used to calculate the material mechanical properties of the operated and nonoperated tendons.

The mechanical properties studied were: maximum load (N) and energy at maximum load (N.10-3), referred to as structural properties, and stress at maximum load (MPa), modulus of elasticity (MPa), and energy per area (N. 10-3/mm2), referred to as material properties, depending, accordingly, to the section area for calculation. The histopathological aspects were assessed by light microscopy with hematoxylin and eosin staining, Masson trichrome staining and picrosirius red staining under polarized light.

The statistical study of the mechanical property variables, according to the central suture site (TN and FC groups), to the experimental moments (immediate, 2, 3, and 6 weeks), and to the side (operated and contralateral control) were performed by nonparametric analysis of variance (ANOVA) for a two-factor model in repeated measures complemented by the respective multiple comparison tests.[12] Results were discussed at a 5% significance level.

Results

Mechanical properties

►**Tables 3** to **7** show the median, the minimum and the maximum values for the mechanical properties studied in both groups (TN and FC), in the operated and control sides during the four experimental moments, accompanied by comparison letters (statistical analysis).

►**Figs. 2** and **3** illustrate the results obtained for each mechanical property.

The ruptures always occurred at the tendon suture or at the healing site in both experimental groups. In untreated intact tendons, used as control, the ruptures always occurred at the segment between the claw and the nodule.

Histopathological Analyses

The tendon healing process was similar in both experimental groups; differences were only observed between subgroups, being related to the temporal evolution of the phenomena.

Table 3 Descriptive measures (median, minimum and maximum values) of maximum load according to experimentation time, suture type (normal tendon and fibrocartilage) and side (operated or control side)

Time (weeks)	Suture	Side	
		Control	Operated
0	TN	189 (138–254) **aAβ**	25 (22–29) **aAα**
	FC	149 (119–200) **aAβ**	29 (20–39) **aAα**
2	TN	172 (149–228) **aAβ**	29 (19–33) **aAα**
	FC	171 (119–185) **aAβ**	32 (16–35) **aAα**
3	TN	195 (119–240) **aAβ**	29 (15–40) **aAα**
	FC	139 (134–196) **aAβ**	32 (20–48) **aAα**
6	TN	174 (156–293) **aAβ**	91 (68–119) **aBα**
	FC	208 (185–300) **aBβ**	89 (55–98) **aBα**

Suture: TN (normal tendon) / FC (fibrocartilage); smal letters: suture comparison, timing fixation; capital letters: timing comparison, fixation suture and side; greek letters: side comparison, fixation timing and suture; equal letters: similarity; different letters: difference.

Table 4 Descriptive measures (median, minimum and maximum values) of maximum load energy (N. 10^{-3} m) according to experimentation time, suture type (normal tendon and fibrocartilage), and side (operated or control side)

Time (weeks)	Suture	Side	
		Control	Operated
0	TN	514 (291–638) **bAβ**	53 (14–75) **aAα**
	FC	282 (209–522) **aABβ**	66 (46–108) **aABα**
2	TN	376 (320–580) **aAβ**	43 (18–76) **aAα**
	FC	383 (216–463) **aABβ**	53 (18–101) **aAα**
3	TN	374 (184–524) **aAβ**	34 (12–54) **aAα**
	FC	248 (176–347) **aAβ**	51 (28–99) **aAα**
6	TN	412 (308–899) **aAβ**	139 (87.5–239) **aBα**
	FC	507 (260–1043) **aAβ**	107 (78–169) **aBα**

Suture: TN (normal tendon) / FC (fibrocartilage); smal letters: suture comparison, timing fixation; capital letters: timing comparison, fixation suture and side; greek letters: side comparison, fixation timing and suture; equal letters: similarity; different letters: difference.

Table 5 Descriptive measures (median, minimum and maximum values) of stress at maximum load (MPa) according to experimentation time, suture type (normal tendon and fibrocartilage), and side (operated or control side)

Time (weeks)	Suture	Side	
		Control	Operated
0	TN	22 (16–32) **aAβ**	3.1 (2.7–3.4) **aAα**
	FC	17 (14–22) **aAβ**	3.4 (2.2–4.7) **aAα**
2	TN	21 (18–27) **aAβ**	3.5 (2.2–3.8) **aAα**
	FC	20 (13–23) **aAβ**	3.5 (1.9–4.2) **aAα**
3	TN	22 (15–27) **aAβ**	3.7 (1.9–4.3) **aAα**
	FC	18 (14–32) **aAβ**	4.1 (2.9–7.8) **aABα**
6	TN	21 (18–40) **aAβ**	11.9 (7.5–13.6) **aBα**
	FC	25 (20–29) **aAβ**	9.9 (4.5–13) **aBα**

Suture: TN (normal tendon) / FC (fibrocartilage); smal letters: suture comparison, timing fixation; capital letters: timing comparison, fixation suture and side; greek letters: side comparison, fixation timing and suture; equal letters: similarity; different letters: difference.

Table 6 Descriptive measures (median, minimum and maximum values) of modulus of elasticity (MPa) according to experimentation time, suture type (normal tendon and fibrocartilage), and side (operated or control side)

Time (weeks)	Suture	Side	
		Control	Operated
0	TN	119 (95.5–193) **aAβ**	16 (15–49) **aAα**
	FC	99 (87–120) **aAβ**	17 (10–22) **aAα**
2	TN	114 (89–131) **aAβ**	31 (2.8–41) **aAα**
	FC	114 (72–144) **aAβ**	30 (14–37) **aAα**
3	TN	164 (121–219) **aAβ**	35 (12–43) **aAα**
	FC	135 (62–265) **aAβ**	37 (14–123) **aAα**
6	TN	161 (96–195) **aAβ**	72 (55–91) **aBα**
	FC	119 (96–261) **aAβ**	71 (40–148) **aBα**

Suture: TN (normal tendon) / FC (fibrocartilage); smal letters: suture comparison, timing fixation; capital letters: timing comparison, fixation suture and side; greek letters: side comparison, fixation timing and suture; equal letters: similarity; different letters: difference.

After 2 weeks of postoperative evolution, when the space between the tendon stumps was large, filling occurred mainly by fibrin exudate and by the proliferation of granulation tissue. If the space between the tendon stumps was smaller, filling was predominantly composed of granulation tissue and interstitial edema, irregular foci of fibrin deposits, and discrete mononuclear inflammatory infiltrate permeated by a few eosinophils. This granulation tissue, in the central region of the space, proliferated from the vessels of the interstitium of the tendon stumps with an irregular deposition of still immature collagen fibers. In some samples, the presence of a small space between the stumps was accompanied by the deposition of collagen fibers in parallel disposition to the longitudinal axis of the tendon, showed by the Masson trichrome stain; these fibers were delicate and refractive when stained with red picrosirius and examined under polarized light. In peripheral zones, the granulation tissue originated from peritendinous tissues, forming adhesions with the surrounding dermis and synovial tissue; collagen deposition in these areas was irregular. In the middle of the repair process, foreign body granulomas were identified surrounding the sutures and interrupting the continuity of the regular deposition of collagen.

Table 7 Descriptive measures (median, minimum and maximum values) of energy per area (N.mm/mm^2) according to experimentation time, suture type (normal tendon and fibrocartilage), and side (operated or control side)

Time (weeks)	Suture	Side	
		Control	Operated
0	TN	63 (35–76) **bAβ**	6.3 (1.7–8.3) **aAα**
	FC	33 (25–60) **aAβ**	7.7 (5.0–12.8) **aAα**
2	TN	44 (39–69) **aAβ**	5.1 (2–8.9) **aAα**
	FC	44 (26–55) **aAβ**	6.3 (2.1–11.0) **aAα**
3	TN	44 (27–57) **aAβ**	4.1 (1.5–6.6) **aAα**
	FC	38 (21–55) **aAβ**	7.1 (4.2–15.8) **bAα**
6	TN	48 (34–108) **aAβ**	20 (11–25) **aBα**
	FC	56 (39–87) **aAβ**	13 (7–18) **aBα**

Suture: TN (normal tendon) / FC (fibrocartilage); smal letters: suture comparison, timing fixation; capital letters: timing comparison, fixation suture and side; greek letters: side comparison, fixation timing and suture; equal letters: similarity; different letters: difference.

In the animals sacrificed 3 weeks after the surgery, there was a reduced interstitial edema in the granulation tissue, absence of fibrin deposits, presence of a minimal mononuclear inflammatory infiltrate, and a denser deposition of collagen compared with the results obtained in animals euthanized 2 weeks after the procedure.

In the 6th postoperative week, most of the granulation tissue was replaced by a dense deposition of collagen, similar to the pattern observed in the tendinous tissue of the stumps, in an arrangement parallel to the longitudinal axis of the tendon; when stained with picrosirius and examined under polarized light, the reparative collagen was characterized by more delicate, slightly less refracted fibers when compared with the tendinous tissue of the stumps. Foreign body granulomas involving sutures and peripherally dermal and synovial tissues adhesions were observed at all experimental times with a progressive increase in the density of cicatricial collagen.

►**Fig. 4** shows the results observed 2, 3, and 6 weeks after the surgery.

Discussion

The mechanical and morphological behavior of the sutured tendons was similar in most of the studied parameters in animals from the TN and FC groups, indicating that there were no differences in tendon healing regarding the location of the central suture point, either in the normal vascularized tendon region or in the avascular fibrocartilaginous tendon region.

Mechanical assays performed immediately after the repair (TN0 and FC0 subgroups) assessed the initial strength

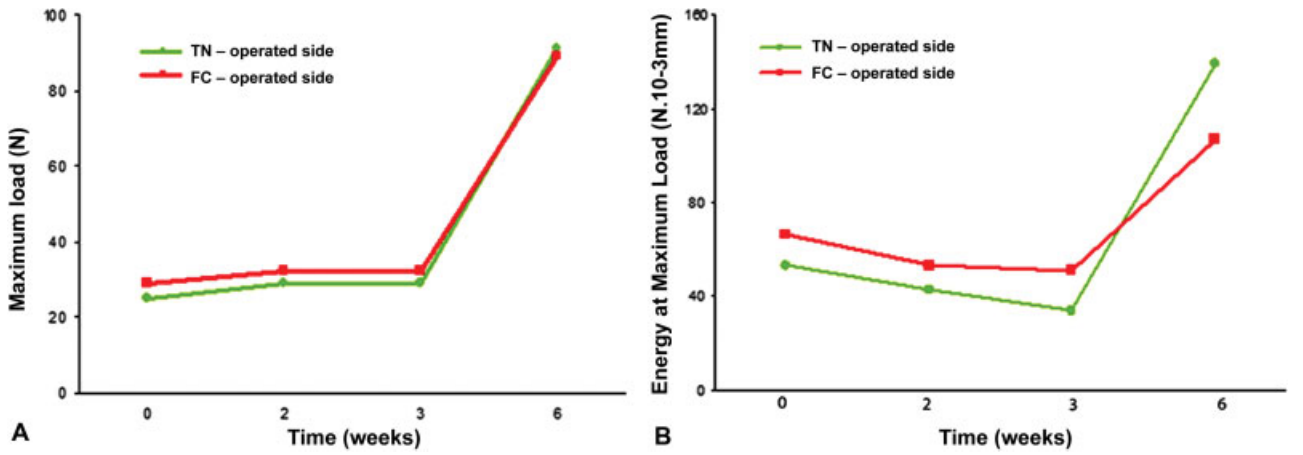

Fig. 2 Structural biomechanical properties. (A) Median maximum load values (N) in the TN and FC groups, operated side, during the four experimental moments; (B) Median energy at the maximum load values (N.10-3m) in the TN and FC groups, operated side, during the four experimental moments.

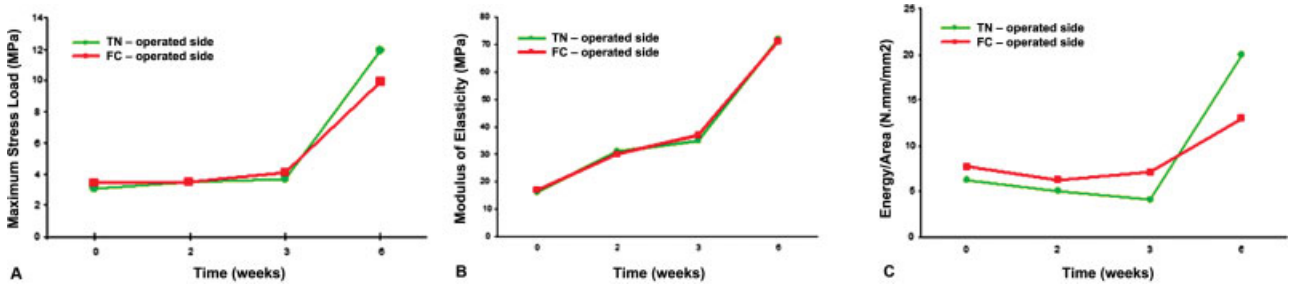

Fig. 3 Material biomechanical properties. (A) Mean of the stress at maximum load (MPa) in the TN and FC groups, operated side, during the four experimental moments; (B) Modulus of elasticity (MPa) in the TN and FC groups, operated side, during the four experimental moments; (C) Median energy per area values (N.mm/mm2) in the TN and FC groups, operated side, during the four experimental moments.

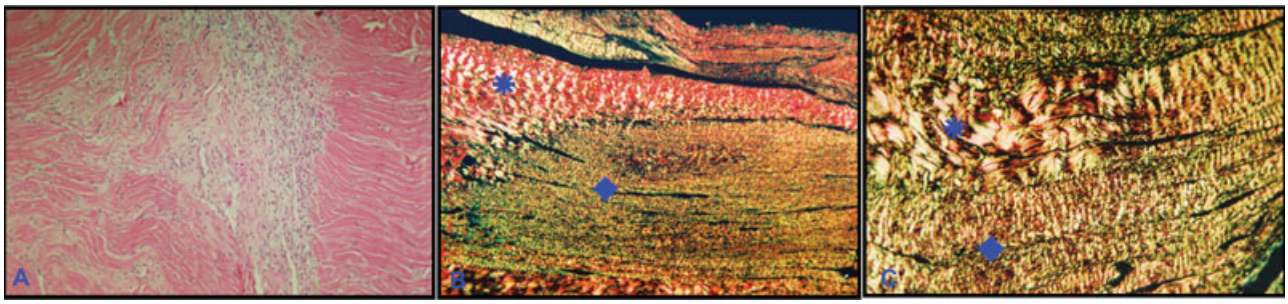

Fig. 4 (A) 2 weeks after the procedure – the space between the stumps is filled by granulation tissue with mild interstitial edema and mononuclear inflammatory infiltrate; at the left of the space, there is granulation tissue from the interstitium of the stump tendinous fibers (hematoxylin and eosin [H&E] – original magnification, 100X); (B) Three weeks after the procedure – the tendinous stump present dense collagen with intense refringence (*), in continuity with less refringent and deposition of more delicate collagen fibers, in the space between the tendon stumps (*) (picrosirius red under light polarized - original magnification, 100X); (C) Six weeks after the procedure – there is a distinction between the denser and refringent stump collagen (*) and the cicatricial collagen, more delicate, less refringent and parallel to the longitudinal axis of the tendon (•) (picrosirius red under light polarized – original magnification, 100X).

of the suture. Soejima et al[4] and Stein et al[13] performed similar studies using linear mechanical assays in isolated tendons. Soejima et al[4] found a superior mechanical profile in the dorsal surface suture, while Stein et al[13] observed no differences comparing the suture sites. Similar studies, however, using curvilinear mechanical tests, showed a superior mechanical profile in dorsal surface sutures.[3,5–7,14] It is possible to suppose that the linear test performed in the present investigation cancels out the mechanical effect of greater stress transmission on the dorsal surface compared with the palmar aspect of the flexor tendon. In addition, continuous circumferential peripheral suture, which may increase the resistance of the central repair by up to 50%, may have equalized repairs performed in normal and fibrocartilaginous tendon regions.[15]

The mechanical tests performed 2, 3, and 6 weeks after the procedure evaluated the resistance of the tendon scar. The mechanical properties studied in the TN and FC groups did not present significant statistical differences, except for the energy per area item 3 weeks after the surgery, in which the FC group presented higher values compared with the TN group. These results indicate that there was no difference in the tendon healing process regarding the location of the central suture. Theoretically, the placement of the central suture at the vascularized tendon region (TN group) could damage the blood supply and impair healing. However, the immobilization of the operated limb for 3 weeks postoperatively probably favored the extrinsic healing mechanisms and decreased the possible deleterious effect of the suture at the vascularized tendon region. The presence of adhesions to the dermal and synovial tissues observed in the histological analysis in both groups and at all experimental times in the peripheral region of the tendon scar suggests the predominance of extrinsic healing mechanisms, a fact also observed by Wada et al.[16]

At 3 weeks, the energy per area was higher in the FC group. Energy per area can be characterized as the ability of impact absorption and, in viscoelastic materials, such as tendons, it is related to the amount of the said material and the arrangement of its collagen fibers. It is possible to imagine that, at 3 weeks, the scar presented by the animals of the FC group had a greater volume of and irregularly disposed collagen, which would justify their greater capacity of impact absorption. However, it was an isolated finding because, at 6 weeks, the values were similar in both groups.

The temporal influence over the tendon healing process was similar in both experimental groups, TN and FC, with stabilization of the mechanical property values from the immediate time point up to 3 weeks postoperatively, followed by a marked increase at 6 weeks. Exceptions to this pattern occurred in the FC group regarding stress at maximum load, in which recovery began at 3 weeks, and energy per area, which decreased at the 2nd week and resumed at the 3rd week. In the consulted literature, no experiments varying central suture placement at the vascularized or avascular fibrocartilaginous tendinous region and evaluating the healing process were found. Nessler et al[17] compared the healing process in normal and avascular fibrocartilaginous tendon regions in canine flexor tendons, finding a superior morphological and mechanical performance of the latter. However, their experimental model consisted of a partial lesion, with no suture material and allowing immediate active movement at the postoperative period. In these circumstances, there may have been a predominance of intrinsic tendon healing mechanisms, in which the fibrocartilage, with its irregularly disposed collagen, healed earlier than the normal tendon, which requires a longer time for the alignment of collagen fibers.

Conclusions

An analysis of the mechanical and histopathological results obtained in the present investigation allows us to conclude that the central suture placement in the tendinous vascularized region did not affect the healing process.

Conflicts of Interests
The authors have no conflicts of interests to declare.

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