
J. Andrés Fernández-Sarmiento, DVM, Juan M. Dominguez, DVM, PhD, María M. Granados, DVM, PhD, Juan Morgaz, DVM, PhD, Rocío Navarrete, DVM, PhD, José M. Carrillo, DVM, PhD, Rafael J. Gómez-Villamandos, DVM, PhD, Pilar Muñoz-Rascón, DVM, Juana Martín de las Mulas, MD, PhD, Yolanda Millán, DVM, PhD, Montserrat García-Balletbó, MD, PhD, and Ramón Cugat, MD, PhD

Investigation performed at the Department of Animal Medicine and Surgery, University of Córdoba, Córdoba; the Department of Animal Medicine and Surgery, Cardenal Herrera University, Valencia; the Department of Comparative Pathology, University of Córdoba, Córdoba; and the Department of Orthopaedic Surgery and Traumatology, Hospital Quirón, Barcelona, Spain

Background: The use of plasma rich in growth factors (PRGF) has been proposed to improve the healing of Achilles tendon injuries, but there is debate about the effectiveness of this therapy. The objective of the present study was to evaluate the histological effects of PRGF, which is a type of leukocyte-poor platelet-rich plasma, on tendon healing.

Methods: The Achilles tendons of twenty-eight sheep were divided surgically. The animals were randomly divided into four groups of seven animals each. The repaired tendons in two groups received an infiltration of PRGF intraoperatively and every week for the following three weeks under ultrasound guidance. The tendons in the other two groups received injections with saline solution. The animals in one PRGF group and one saline solution group were killed at four weeks, and the animals in the remaining two groups were killed at eight weeks. The Achilles tendons were examined histologically, and the morphometry of fibroblast nuclei was calculated.

Results: The fibroblast nuclei of the PRGF-treated tendons were more elongated and more parallel to the tendon axis than the fibroblast nuclei of the tendons in the saline solution group at eight weeks. PRGF-treated tendons showed more packed and better oriented collagen bundles at both four and eight weeks. In addition to increased maturation of the collagen structure, fibroblast density was significantly lower in PRGF-infiltrated tendons. PRGF-treated tendons exhibited faster vascular regression than tendons in the control groups, as demonstrated by a lower vascular density at eight weeks.

Conclusions: PRGF was associated with histological changes consistent with an accelerated early healing process in repaired Achilles tendons in sheep after experimental surgical disruption. PRGF-treated tendons showed improvements in the morphometric features of fibroblast nuclei, suggesting a more advanced stage of healing. At eight weeks, histological examination revealed more mature organization of collagen bundles, lower vascular densities, and decreased fibroblast densities in PRGF-treated tendons than in tendons infiltrated with saline solution. These findings were consistent with a more advanced stage of the healing process.

Clinical Implications: Based on the findings in this animal model, PRGF infiltration may improve the early healing process of surgically repaired Achilles tendons.

Injured tendons heal slowly because of less blood supply and reduced metabolism compared with many other tissues. The use of growth factors has been proposed as a strategy to improve tendon healing. Platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), vascular-endothelial growth factor (VEGF), epidermal growth...
factor (EGF), basic fibroblastic growth factor (bFGF), and insulin-like growth factor type-I (IGF-I), among others, have been recognized to play key roles in tendon healing. Although these growth factors potentially could be used therapeutically to accelerate the complex process of tendon healing, it seems unlikely that a single growth factor will give a positive result in vivo. Some authors have suggested that the interaction of various growth factors with a natural balance of anabolic and catabolic functions, present in the right concentration at the right time, would be necessary to optimize the tissue environment and to favor the tendon healing process.

Autologous plasma, generally called platelet-rich plasma (PRP), is being used to treat musculoskeletal injuries. Platelets contain a complex pool of growth factors, interleukins, and other biologically active proteins, such as fibrinogen, fibronectin, and vitronectin, which have prominent roles in the healing process. Several clinical studies have demonstrated that applications of PRP can improve the healing response after tendon injuries but there is an intense debate about the effectiveness of these products.

The purpose of the present study was to evaluate the effect of plasma rich in growth factors (PRGF), which is one type of leukocyte-poor PRP, on tendon healing. We compared the histological appearance of acutely surgically injured and repaired Achilles tendons that had been treated with PRGF with that of repaired tendons that had been infiltrated with saline solution in sheep. Our hypothesis was that weekly infiltrations of PRGF onto the injured tendons would accelerate the healing response and would improve the histological properties of these tendons.

Materials and Methods

Experimental Model of Divided Achilles Tendon

The present study was approved by the Bioethical Committee on Animal Research at our institution. We strictly adhered to the guidelines for the use of laboratory animals proposed by the National Institutes of Health. We used twenty-eight skeletally mature female Merino sheep weighing between 45 to 55 kg for our study. A veterinary surgeon performed general and orthopaedic physical examinations on each animal to guarantee that all sheep were free from lameness.

With use of sterile technique, the right hindlimb of the anesthetized sheep was prepared for surgery. A lateral skin incision was made over the Achilles tendon. The peritenon was incised longitudinally (Fig. 1-A). A full-thickness transverse tendon division of the Achilles tendon was performed with use of a scalpel, 5 cm proximal to its insertion into the calcaneus (Fig. 1-B). The tendon was repaired with use of a three-loop pulley pattern with a nonabsorbable synthetic monofilament (polypropylene) (Premilene USP 1; B Braun Aesculap, Melsungen, Germany) (Fig. 1-C). The peritenon was sutured with a continuous synthetic monofilament (polyglyconate) (Monosyn USP 3-0; B Braun Aesculap). The wound was closed in layers and was covered with a sterile dressing.

The repaired tendon was protected during the postoperative period with use of a custom system that allowed the animal to bear weight on the operatively treated hindlimb without subjecting the Achilles tendon to macromovements. A tarsal transarticular external skeletal fixation system protected the repaired tendon while the animal was bearing weight on the operatively treated limb. The bars on the tibia and metatarsus were joined together with two connecting bars, forming a triangular configuration (Fig. 1-D).

The surgical approach of the Achilles tendon through a lateral skin incision allowed precise and reproducible measurements of the tenotomy area (Fig. 1-A). The tenotomy point was 5 cm proximal to the calcaneal tuberosity. This point was measured with use of a ruler (Fig. 1-B). A three-loop pulley pattern was used to repair the tenotomy (Fig. 1-C). The tarsal transarticular external skeletal fixation system protected the repaired tendon while the animal was bearing weight on the operatively treated limb. The bars on the tibia and metatarsus were joined together with two connecting bars, forming a triangular configuration (Fig. 1-D).

Plasma Rich in Growth Factors (PRGF): Preparation and Infiltration on Repaired Area

PRGF was prepared with use of the PRGF-Endoret system (Biotechnology Institute [BTI], Vitoria, Spain). Disposable BTI extraction and fractioning tubes were used in a custom-designed bench-top centrifuge. Each 5-mL extraction tube contained 0.5 mL of sodium citrate solution (3.8%) as anticoagulant. Twenty milliliters of blood were collected from the jugular vein of each animal and were divided into four tubes that were centrifuged at 630 times gravity for eight minutes according to the method reported by Anitua et al. Blood for PRGF preparation was given for five days after surgery.

![Fig. 1](http://example.com/fig1.jpg)

The surgical approach of the Achilles tendon through a lateral skin incision allowed precise and reproducible measurements of the tenotomy area (Fig. 1-A). The tenotomy point was 5 cm proximal to the calcaneal tuberosity. This point was measured with use of a ruler (Fig. 1-B). A three-loop pulley pattern was used to repair the tenotomy (Fig. 1-C). The tarsal transarticular external skeletal fixation system protected the repaired tendon while the animal was bearing weight on the operatively treated limb. The bars on the tibia and metatarsus were joined together with two connecting bars, forming a triangular configuration (Fig. 1-D).
was collected just prior to surgery and was processed intraoperatively. The upper layer of the centrifuged plasma was removed with use of a pipette and was discarded. The remaining 0.5 mL of plasma fraction that lies just above but not including the interphase zone ("buffy coat") was retrieved with use of a sterile pipette and was placed in a fractioning tube. This plasmatic fraction is the PRGF according to the manufacturer’s instructions. The plasma separation procedure was done in a laminar flow cabinet in accordance with the PRGF-Endoret system manufacturer instructions. The 0.5 mL of PRGF from each of the four tubes were combined to obtain a total volume of 2 mL of PRGF per animal. The platelets were activated by adding 0.1 mL of 10% calcium chloride just prior to the injection of PRGF in the injured tendon. The time delay between blood collection and PRGF application was less than one hour.

The sheep were randomly divided into four groups of seven animals each. In the sheep in the two experimental groups, 2 mL of PRGF were infiltrated intratendinously into the divided tendon stumps with use of a 23-gauge needle just before suturing of the peritenons. The injections were repeated every week for the following three weeks. The repaired tendons in the other two groups (controls) were injected with 2 mL of saline solution plus 0.1 mL of 10% calcium chloride as described above. The infiltrations during the postoperative period were carried out in properly sedated animals with use of sterile technique under ultrasonographic guidance. The animals in one PRGF-treated group and one saline solution-treated group were killed at four weeks, and the remaining animals were killed at eight weeks.

**Histological Procedure**

After each animal was killed, both Achilles tendons were harvested and were fixed in 10% neutral buffered formalin. Contralateral (non-operatively treated) tendons were studied as normal controls. Each operatively treated tendon was cut lengthwise on the dorsal midline with use of a scalpel blade. A 1-mm-thick slab of tissue that included the entire repaired area was excised. This slab of tissue also contained intact tendon fibers on either side of the repaired area. The margin adjacent to the intact tendon fibers was yellow-inked for histological identification. Two paraffin blocks were obtained from each operatively treated tendon. From each block, six 4-μm-thick consecutive paraffin sections were cut. Sections were mounted in slides, and three were stained with hematoxylin and eosin and three were stained with Masson trichrome.

The stained sections were digitalized with use of a photomicroscope (Axioskop; Carl Zeiss, Oberkochen, Germany) with an attached digital camera (DS-5M camera head; Nikon, Tokyo, Japan) coupled with a digital imaging controller (DS-Li camera control unit, Nikon) that allowed us to observe, focus, and capture histological images. Slides were sampled in a systematic randomized manner by superimposing a dotted transparent template onto each slide. Several dots spanning the whole tissue section were marked over the glass of the slide with use of a permanent marker. The regions of interest were chosen during the histological examination beside a previously marked dot. In Masson trichrome-stained sections, nine histological images per slide were captured with a magnification of ×200. In hematoxylin and eosin-stained sections, nine histological images per slide were captured with a magnification of ×200 and nine histological images per slide were captured with a magnification of ×630. These histological images were transferred to a computer equipped with image-analysis software (Image Pro Plus, version 6.0; MediaCybernetics, Rockville, Maryland) to perform quantitative measurements. In order to blind the evaluation of the histological images, all of the captures were encoded with use of an identification number and were randomly evaluated by three independent pathologists.

**Morphology of Fibroblast Nuclei**

The morphometry of fibroblast nuclei was studied with the aid of image-analysis software on images captured at a magnification of ×630 and stained with hematoxylin and eosin. The outlines of fibroblast nuclei were traced with use of the cursor of the image software (Fig. 2-A). The following quantitative parameters were calculated from each nuclear profile: area (μm²), perimeter (μm), major axis (μm), minor axis (μm) and roundness [(perimeter²)/4 x π x area]. The nuclear aspect ratio (defined as the ratio of the minor axis to the major axis) was also calculated, with values approaching zero suggesting a spindle shape and with the value of 1.00 representing a perfect circle. The nuclear orientation angle was defined as the angle between the major axis of the nucleus and the longitudinal tendon axis, with values of 0° representing a nucleus that is perfectly aligned toward the longitudinal axis. When this value increases, the nucleus becomes more angled until it approaches 90°, where it lies perpendicular to the long axis of the tendon.

**Histological Study of Collagen Fibers**

The histological appearance of the collagen fibers was studied on images captured at a magnification of ×200 and stained with Masson trichrome. A 5-point semiquantitative grading scale was designed to evaluate the packing and orientation of collagen fibers (see Appendix). This semiquantitative grading scale was a modification based on the grading scales described in three different publications.

**Fibroblast Density**

The fibroblast density was determined with use of a counting frame that was superimposed onto each histological image; these images were captured at a magnification of ×630 and were stained with hematoxylin and eosin (Fig. 2-B). The number of fibroblasts per area was calculated according to the formula:

\[ \text{fibroblasts/mm}^2 = \frac{N}{630^2} \]

where N was the number of fibroblasts counted within the counting frame, and 630 was the area of the counting frames (in our study, 0.004 mm²).

**Vascular Density**

Vascular density was quantified with use of histological images that were captured at a magnification of ×200 and stained with hematoxylin and eosin. The number of blood vessels in each image area was divided by the area of the histological image captured at a magnification of ×200 (in our study, 0.142 mm²).

**Evaluation of the Inflammatory Cell Infiltration**

The infiltration of inflammatory cells was analyzed with use of images captured at a magnification of ×200 and stained with hematoxylin and eosin. As the different inflammatory cell subtypes were not quantified, the inflammatory cell infiltration was defined on the basis of the density of the main inflammatory cell subtype observed in the histological sections (lymphocytes). The density of inflammatory cell infiltration was characterized with use of a 5-point semiquantitative grading scale. A value of 0 was assigned if inflammatory process was absent, a value of 1 was assigned when there was slight inflammatory cell infiltration, a value of 2 was assigned when there was moderate inflammatory cell infiltration, a value of 3 was assigned when there was strong inflammatory cell infiltration, and a value of 4 was assigned where there was severe inflammatory cell infiltration homogeneously spread in the entire field of view.

**Statistical Analysis**

Descriptive statistics for each study group (including the mean, standard deviation, and frequencies for categorical data) were calculated. The nonparametric Kruskal-Wallis test and the Mann-Whitney U test were used to analyze the significance between groups for quantitative parameters (morphology of fibroblast nuclei, fibroblast density, and vascular density). Categorical data (packing of collagen fibers, orientation of collagen fibers, and inflammatory cell infiltration) were analyzed with use of the chi-square test to determine if the infiltration with PRGF or saline solution yielded significant differences in the frequency distribution between study groups. Values were presented as mean and the standard deviation (SD). The level of significance was set at p < 0.05.

**Source of Funding**

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Representative images demonstrating computer analysis of the histopathological captures. The nuclear profiles of the fibroblasts were traced manually with use of the cursor of the image software that had been loaded onto a digital tablet, and morphometric parameters of the fibroblast nuclei were calculated (Fig. 2-A). To determine the fibroblast density, a four-squares frame was superimposed onto each image, and the nuclei (asterisks) that were included inside each square without touching the exclusion borders (red borders) were counted. The number of fibroblast nuclei was divided between the total area of the four squares to obtain the fibroblast density (Fig. 2-B).
any financial interest or other relationship with any commercial company related to this study.

**Results**

All animals reached the end of the study without incident. Morphometric data on fibroblast nuclei are shown in Table I. Fibrocyte nuclei in normal Achilles tendons had a spindle shape, as demonstrated by a low value for the nuclear aspect ratio (0.07 ± 0.01) (see Appendix). The PRGF-treated tendons showed lower values of the nuclear aspect ratio than did the saline solution-treated tendons at both four and eight weeks. Nuclei were significantly more elongated in PRGF-treated tendons than in saline solution-treated tendons at eight weeks (p = 0.008) (Fig. 3-A). Fibrocyte nuclei in normal tendons were highly aligned with respect to the longitudinal tendon axis (mean nuclear orientation angle, 2.7 ± 0.9). Fibroblast nuclei tended to be more parallel to the longitudinal axis in PRGF-treated tendons than in tendons infiltrated with saline solution. This orientation was reflected by a lower value for the nuclear orientation angle, which was significantly lower at eight weeks compared with the saline solution group (p < 0.001) (Fig. 3-B). Other morphometric parameters of fibroblast nuclei (major axis, minor axis, roundness, area, and perimeter) showed a more normal appearance in the PRGF groups than in the saline solution groups, with significant differences between the groups in terms of the major axis, roundness, and perimeter at eight weeks (Table I).

Normal tendons were characterized by highly packaged and perfectly aligned collagen bundles (see Appendix). At four weeks after surgery, the mean value for packing of collagen fibers was significantly greater in the PRGF group than in the saline solution group (3.82 ± 0.51 versus 3.26 ± 0.56; p < 0.001). Similarly, at eight weeks after surgery, the PRGF group also had a significantly higher value for packing of collagen fibers.
in comparison with the saline solution group (4.16 ± 0.49 versus 3.70 ± 0.55; p < 0.001) (Table II). The orientation of collagen fibers was significantly better in the PRGF group in comparison with the saline solution group at both four weeks (3.64 ± 0.63 versus 3.20 ± 0.70; p < 0.001) and eight weeks after surgery (4.21 ± 0.51 versus 3.63 ± 0.62; p < 0.001) (Table II). Most histological fields of view of PRGF-treated tendons showed a regular and dense formation of collagen bundles, with fibers slightly angled to the longitudinal tendon axis at four weeks after surgery. Conversely, most histological views of the saline solution-treated tendons at four weeks showed only a moderate packing of collagen fibers, with disordered orientation (Fig. 4). At

![Collagen fibers structure](image)

**Fig. 4**
Photomicrographs showing the histopathological appearance of the collagen structure. Collagen fibers were more packed in PRGF-treated tendons at both four and eight weeks. Similarly, collagen fibers showed a better orientation in the PRGF group than in the saline solution group. The arrows indicate the longitudinal tendon axis orientation (×200, Masson trichrome; bar = 100 μm).
eight weeks, 21.1% of histological captures in PRGF-treated tendons showed a homogeneous formation of tightly packed collagen bundles, with 25.9% of captures showing collagen fibers that were parallel to the longitudinal tendon axis. Histological captures in the saline solution group exhibited a more delayed stage of collagen maturation at this time (Fig. 4).

Fibroblast density was relatively low in normal tendons (mean, 228 ± 98 fibroblasts/mm²) (see Appendix), although this parameter was considerably elevated in all of the tendons that had been injected with either PRGF or saline solution (Table II). PRGF-treated tendons showed significantly lower fibroblast density than did saline solution-treated tendons at both four weeks (p = 0.008) and eight weeks (p = 0.008) (Fig. 3-C). Histological captures of PRGF-treated tendons showed lower fibroblast density along with an increased maturation of the collagen structure (Fig. 5-A).

Vascularization was barely seen in normal tendons. In contrast, histological evaluation revealed an intense vascular response in all experimental tendons (Fig. 5-B). PRGF-treated tendons exhibited faster vascular regression than saline solution-treated tendons, as demonstrated by a significantly lower vascularization in the PRGF group at eight weeks (p = 0.008) (Table II; Fig. 3-D).

The main inflammatory cell subtype observed in all of the experimental groups was lymphocytes. The inflammatory cell infiltration was significantly lower in the PRGF-treated group than in the saline solution group at both four weeks (1.18 ± 1.04 versus 1.67 ± 0.98; p < 0.001) and eight weeks (0.73 ± 0.81 versus 1.53 ± 0.87; p < 0.001) (Table II). At eight weeks, most histological captures in the saline solution group (92.2%) exhibited at least a slight degree of inflammation, whereas in 46.7% of captures in the PRGF group the inflammatory cell infiltration was absent (Fig. 5-C).

**TABLE II** Packing of Collagen Fibers, Orientation of Collagen Fibers, and Inflammatory Cell Infiltration in Saline Solution and PRGF-Treated Groups

<table>
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<tr>
<th></th>
<th>4 Weeks</th>
<th>8 Weeks</th>
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<tr>
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<td>Normal Saline Solution</td>
<td>PRGF</td>
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<tr>
<td>Packing of collagen fibers</td>
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<tr>
<td>Percentage of specimens receiving each grade</td>
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<tr>
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<td>—</td>
</tr>
<tr>
<td>Mean grade*</td>
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<td>Orientation of collagen fibers</td>
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<td>Percentage of specimens receiving each grade</td>
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<tr>
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<tr>
<td>Inflammatory cell infiltration</td>
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<tr>
<td>Percentage of specimens receiving each score</td>
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<td>Mean score* (points)</td>
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*The values are given as the mean and the standard deviation.
Platelet-rich plasma is being used with increasing frequency in sports medicine to try to accelerate healing and to allow an earlier return to sports. Despite this interest, there have been only two clinical studies that have evaluated the effectiveness of PRP in the treatment of ruptures of the Achilles tendon in humans: a case-controlled study that showed a faster functional recovery in association with the use of PRP, and a randomized controlled trial that showed no effect. However, several animal studies have shown promising results in association with the use of PRP for the treatment of tendon lesions.

The ratio of the minor axis to the major axis of the nucleus (nuclear aspect ratio) and the angle between the major axis of the nucleus and the longitudinal tendon axis (nuclear orientation angle) provide information about fibroblast nuclei shape and alignment along the longitudinal tendon axis, respectively. In our study, measurement of the nuclear aspect ratio demonstrated that the PRGF-treated tendons had a more elongated nucleus than did the saline solution-treated tendons, suggesting a more mature histological appearance in the PRGF-treated tendons. We observed that fibroblasts in the PRGF-treated groups were more parallel to the longitudinal tendon axis than those in the saline solution groups, as demonstrated by a lower value of the nuclear orientation angle.

Early inflammatory and proliferative phases are defined by a dramatic increase in the fibroblast population. Approximately three or four weeks after the traumatic event, the fibroblast density reaches its peak and then decreases progressively during the remodeling and maturation phases. The neovascularization is prominent during early stages of tendon healing but then progressively disappears. In our study, the PRGF-treated tendons had lower fibroblast density and a faster decrease in neovascularization compared with the tendons that had been infiltrated with saline solution, suggesting that PRGF was associated with histological changes consistent with accelerated early healing.

The collagen structure is closely related to the biomechanical properties of tendons. Packing and orientation of collagen bundles are major features in the collagen structure, and they have been evaluated in several studies. We observed an improvement in both the packing and the orientation of collagen bundles in PRGF-treated tendons, showing a more mature collagen structure compared with saline solution-treated tendons.

Platelets modulate inflammation by secreting chemokines that control chemotaxis of leukocytes and macrophages in injured tissues. The features of the inflammatory response may determine the success of tendon repair. In our study, PRGF-treated tendons showed lower inflammatory cell infiltration than did saline solution-treated tendons at both four and eight weeks. No research has been conducted to investigate the exact effect of PRP on the inflammatory response after acute tendon injury. Further investigations are needed to clarify the role of PRP in the modulation of the inflammatory response during the healing process.

Platelet-rich plasma is a general term that encompasses many different autologous plasma products, even though they are obtained with use of different protocols that may result in different concentrations of platelets, leukocytes, and growth factors and even though these products differ both qualitatively and quantitatively and show different biologic effects. The
PRGF-Endoret system produces a volume of autologous plasma having a platelet concentration above baseline, free of leukocytes and erythrocytes, elaborated by a one-step centrifugation process and using sodium citrate as anticoagulant and calcium chloride as platelet activator.

Scheppull et al.\(^{16}\) and de Vos et al.\(^{11,40}\) reported that the use of PRP did not demonstrate any effect on the healing of human Achilles tendon in clinical studies, but the methodology that they followed for obtaining the autologous platelet concentrate was different from the PRGF methodology that we used in our experimental study. Scheppull et al.\(^{16}\) used a PRP preparation obtained by double centrifugation, which yielded a product with an extremely high concentration of platelets (tenfold above baseline). Previous studies demonstrated that high concentrations of some growth factors, such as TGF-\(\beta\), could be deleterious for tendon healing as TGF-\(\beta\) drives fibrogenesis, potentially stimulates the development of scar tissue, and is associated with the onset of fibrosis\(^{11}\). de Vos et al.\(^{11,40}\) used a PRP product obtained by means of a methodology that collects a high concentration of leukocytes. PRGF is a product free from leukocytes, avoiding the proinflammatory effects of the proteases and cytokines contained in white blood cells\(^{14}\).

While some studies have involved only a single application of PRP\(^{11,16,41}\), we used weekly applications of PRGF during the following three weeks after surgery on the basis of a previous study in sheep by Anitua et al.\(^{22}\). While PRGF was injected into intact tendons without any induced injury in the study by Anitua et al.\(^{22}\), a complete division of the Achilles tendon was surgically induced in the present study. Such intense damage triggers a strong proliferative healing response in the injured area\(^{26,37}\). Anitua et al. reported that the injection of PRGF in intact tendons increased cell density and promoted neovascularization after four weeks of weekly administration in their experimental model\(^{22}\). Our findings suggested that when PRGF was injected into injured tendons, the histological response was different. We observed a decrease in fibroblast density in PRGF-treated tendons, which may be explained by an acceleration in the healing process induced by the PRGF injections. As the healing process advances, a progressive decrease in cell density occurs in the repaired area and the maturation phase of the healing process begins\(^{26,37}\). Thus, PRGF-treated tendons showed a histologically more advanced stage of tendon healing than did saline solution controls at the same postoperative time.

The present study had several limitations. One limitation was that we did not perform any biomechanical testing of the Achilles tendon to assess whether the histological differences that were observed between the PRGF and saline solution groups were mechanically relevant. The main objective of the present study was to histologically evaluate the effect of PRGF on tendon healing. In light of these promising results, future studies are warranted to clarify whether histological improvements in tendon healing are related to superior biomechanical properties. Another limitation of the present study is related to the formulation of PRGF in sheep. The composition of PRGF in humans recently was described by Anitua et al.\(^{42}\), but we are aware of no studies on the concentration of platelets, leukocytes, erythrocytes, or growth factors in the PRGF of sheep. Another concern is that multiple weekly injections were performed in this study and that these injections may elicit an inflammatory response because of the disruption of the peritenon. We considered this unavoidable damage to be the same in both the PRGF groups and the saline solution groups. Hence, differences observed between experimental groups should be attributed to the injected product itself.

In conclusion, we found that PRGF was associated with histological changes consistent with accelerated early healing process in tendons after acute injury and repair of Achilles tendons in sheep. PRGF-treated tendons showed better orientation and a more elongated shape of fibroblast nuclei, suggesting a more advanced stage of healing compared with saline solution-treated tendons. Histological examination of the repaired areas showed a more mature organization of collagen bundles, less inflammatory cell infiltration, faster vascular regression, and lower fibroblast density in PRGF-treated tendons than in saline solution-treated controls.

### Appendix

A table showing the semiquantitative grading scale for the assessment of collagen fibers and photomicrographs showing the normal histological appearance of the Achilles tendon in sheep are available with the online version of this article as a data supplement at jbjs.org.

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J. Andrés Fernández-Sarmiento, DVM
Juan M. Domínguez, DVM, PhD
María M. Granados, DVM, PhD
Juan Morgaz, DVM, PhD
Rocío Navarrete, DVM, PhD
Rafael J. Gómez-Villamandos, DVM, PhD
Pilar Muñoz-Rascón, DVM
Department of Animal Medicine and Surgery, University of Córdoba, Campus de Rabanales, Ctra. Madrid – Km 396, 14014, Córdoba, Spain.
E-mail address for JA. Fernández-Sarmiento: v12fesaj@uco.es

José M. Carrillo, DVM, PhD
Department of Animal Medicine and Surgery, Cardenal Herrera University, Edificio Seminario s/n, 46113, Moncada, Valencia, Spain

Juana Martín de las Mulas, MD, PhD
Yolanda Millán, DVM, PhD
Department of Comparative Pathology, University of Córdoba, Campus de Rabanales, Ctra. Madrid – Km 396, 14014, Córdoba, Spain

Montserrat García-Balletbó, MD, PhD
Ramón Cugat, MD, PhD
Department of Orthopaedic Surgery and Traumatology, Hospital Quirón, Placa d’Alfonso Comín, 5-7, 08023, Barcelona, Spain
References


