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Effect of bone marrow on superficial digital flexor tendon healing in sheep

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Summary

The present study was to compare the effectiveness of bone marrow transplantation on the healing of experimentally transected superficial digital flexor tendon in sheep. Eight adult sheep, aged between 9 months to one year were used. The animals were divided into two equal groups (control and allograft). An transverse cutting has been made in the superficial digital flexor tendon of the middle third of the metatarsal bone under the effect of sedation and local anesthesia. The tendons were sutured with Bunnell suture by using polypropylene thread (2.0 usp) in the control group. While in allograft groups 2-3ml allograft bone marrow was injected at the site of suture tendon which was induced by similar procedure as in control group. The incision was closed as a routine manner.

Gross examination showed adhesions between the tendon and adjacent structures which were severe in the control group , less than that in allograft. Histopathological observations of biopsies harvested after (15,30 and 60 days) showed dense immature collagen fibers which was characterized by regular direction, uniform of their cellular elements filled the gap space of tendon injury with more adhesion in late stages of the control group. In allograft treated group inflammatory cells and arrangement fibrous connective tissue with collagen fibers appear more than in the control group in the same period with less adhesion.

Keywords : Tendon . Bone marrow . Super Digital Flexor Tendon Healing

Introduction

Tendon tissue is formed by an intricate network of macromolecules that constitute the extracellular matrix and interact with the cellular components composed of tenocytes [1]. The major constituent of the extracellular matrix is collagen fibers type 1 and noncollagenous fibers glycoprotiens and protoglycan . The interaction between the components is important for the function of the tendon, the conduction and with standing of tensile loads , particularly when subjected to the heavy stress [2] .

The flexor tendons of the deep digital flexor (DDFT), and superficial digital flexor (SDFT), which run down the back of the leg joint from the level of the knee or hock. The SDFT ends at the pastern(second phalanx).

The DDFT ends on the lower surface of the coffin bone(third phalanx). Injury to the SDFT is more commonly known as "bowed tendon", at the back of the knee, in the

region of the hock and at the level of the fetlock and upper pastern joint, the tendons are enveloped by a fluid filled sheath, Several strong, short, annular ligaments help to keep the tendons in place in areas of high movement such as joints[3 and 4]..

Many therapeutic approaches have been used to improve tendon healing, including physical therapy, the use of steroid and non steroid anti-inflammatory drugs (NSAIDs), surgical intervention and the use of support bandage which have been shown to alleviate the load on the tendon [5,6 and 7].

The aim of the present study was to illustrate the effect of bone marrow tissue on tendon healing. And the feasibility of bone marrow aspiration puncture is performed on sheep and mainly to verify the effectiveness of allograft implant of bone marrow in the treatment of tendons affection. These animals are then placed in a cast following surgery to try and reduce the amount of motion in the affected area .

Materials and methods

Eight apparently healthy endogenous sheep ageing, 9 months to1 year, of both sexes and weighing 20-25 Kg were chosen. animals were preconditions in the animals facility for at least 21 days before the initiation of the study. They were kept in controlled environments throughout the study, for the observation and the adaptation the sheep were subsequently housed in pairs in the(3*2.5) meters -pens for the entire experiment. All sheep vaccinated against enterotoxaemia by co-Baghdad, in a dose of 5ml.also dewormed with ivermectin at a dose 0.2mg/kg B.W. Both types of drug administrated subcutaneously.

The sheep were divided randomly into equl two groups.

1-First group (control group).

2-Second group allograft treated group, is using of the bone marrow from metacarpal of other sheep and put at the site of the severed tendon

In the control group,straight incision approximately 10 cm long was performed through the skin of the posterior aspect of metatarsal area of the right hind limb then carried down to the subcutaneous tissue , bleeding was carefully arrested .The skin edges were reflected laterally to expose the dorsal surface of the tendon . Blunt dissection was performed to separate the SDFT from the deep digital flexor tendon. When this has been achieved, curved artery forceps were inserted under the superficial tendon to expose it (Fig-1) . A stay suture was done at the proximal side of the SDFT, under the hock joint, using polypropylene thread No 2.0 to prevent slipping the tendon upward and this was considered as the first stitch of tenorrhaphy. The superficial digital flexor tendon then was transverse cutting with the scalpel . The cut ends of the tendon were approximated by Bunnell-technique suture using polypropylene suture material size (2.0) (Fig-2) . The subcutaneous tissue was sutured by simple continuous pattern using polydixenome size (4.0) and skin was closed by routine technique. The site of operation was casting with window, removed after 30 days from operation in last two period, while in first period directly before biopsy was taken.

In allograft the treatment group the similar steps were performed as described in the control group with the exception of the application of bone marrow (2-3ml) on the sites of tendon anastomosis which was taken from metacarpal bone of the other animal. The animal restrained in lateral position after the injection of xylazine in a dose 0.5mg\kg B.W. The metacarpal bone of the forelimb is prepared for aseptic technique. Incision approximately 5 cm. long was made through the skin of the right metacarpal bone. Small pore in cortical bone was made to introduce the aspiration needle.

It was preferred to leave the sleeve on the needle to avoid bending and to drive it with the hub of needle in the palm of my hand for security and stability .Aspiration of bone marrow and the first 2-3ml of marrow

aspired from any needle site provides the best opportunity to capture the osteoprogenitor cells in their highest concentration [8]. This technique is used in allograft groups to provide the amount of marrow which should be put in severed tendon injury .

The clinical signs that appeared on the animal as the dysfunction of the hind limbs movement , which continued about crouched the legs on the ground , and developing the hock joint deviation outside of the limb during walking and swelling the site operation were recorded.

Animals were sedated and anaesthized locally, then longitudinal incision was made in the skin parallel to the original incision, blunt dissection to the sequential tissue layers to reach the lesion, once this was achieved, it was examined grossly and the changes were recorded. These examinations were associated with 15, 30, and 60 days post operative in two experimental groups.

Biopsies of 1 cm3 were harvested from the anastomotic site of the tendon and slightly above and lower to the site for a period of 15, 30 and 60 days post operative for two groups, then were fixed in 10% neutral buffered formalin for 72 hrs. After which the section was prepared routinely. The slides were stained with Hematoxyline-Eosin stain ,some section was stained with Van-gieson stain to demonstrate the connective tissue [9].

a

Fig. 1: Superficial digital flexor tendon prepared for transaction (a)

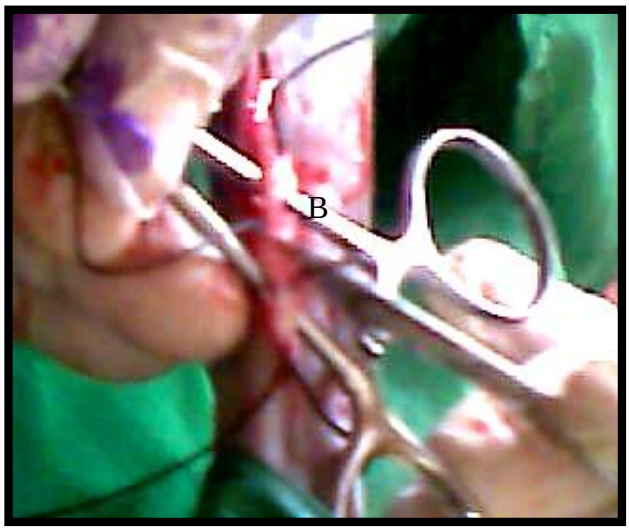

Fig. 2: Approximation of the severed tendon with Bunnell suture using polypropylene suture material (B)

Results

Soon after the animals had recovered from sedation we noticed the following they stand and walked with an obvious lameness which continued for 5-7 days, then they recovered gradually on 8^{th}-10^{th} days and animals showed normal gait.

Macroscopically severe adhesion was observed between the superficial digital flexor tendon and surrounding structures not showing smooth and shinning appearance in the control group after 60 days follow up. While in allograft group , the adhesion was less than the control group at the same period given above.

Microscopical examination showed proliferation of fibrous connective tissue that filled the gap between the two ends of the tendon, characterized by irregular direction and different shape of their cells (Figure -3). The line of the tendon injury was irregular which is red in color section (Figure -4).

Histological section reveaed regular highly cellular F.C.T. and showed the

healing in the two ends of the tendon, with more collagen fibers (Figure -5). In Van-Gieson it is very clear the collagen fiber filled of the tendon cutting line as a red color (Figure -6).

There was a wide area of F.C.T. connected the two edges of tendon fibers, as immature attached to the tendon fiber (Figure -7) the tendon fibers when stained by Van-Gieson stain appear a yellowish-red in color (Figure -8).

Histological section of the treated group with allograft reveals irregular cellular F.C.T. and mononuclear cells infiltration (Figure -9). In addition irregular collagen fibers were also seen attached to the tendon fibers (Figure -10).

The histological changes showed more proliferation of immature F.C.T. with irregular arrangement of mononuclear cells (Figure -11), and the line tendon healing with highly arrangement of the F.C.T. as well as the myofibroblastic attachment appeared as in (Figure-12).

Sections tendon on 60 days post operation showed irregular proliferation with high cellular its F.C.T. and a wide area of tendon fibers in healing region (Figure -13). In Van-Gieson stain showed clearer appearance of the irregular F.C.T. take yellowish-red in color filled the gap (Figure -14).

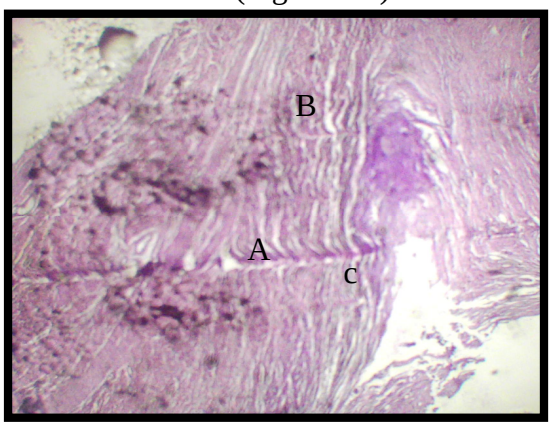

Fig-3: Histopathlogical section in the tendon of control group at 15 days post surgery (A) Irregular highly cellular F.C.T , (B)Mononuclear cells infiltration, (C)line of tendon healing .(H&E20X).

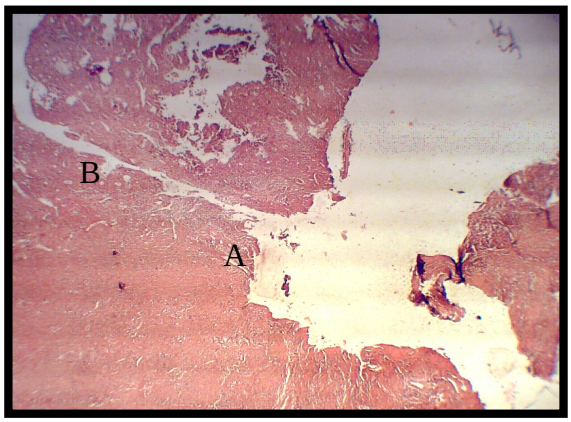

Figure :4: Histopathlogical section in the tendon of control group at 15 days post surgery (A) line of tendon suture (B)proliferation of F.C.T stained with light red color Van-Gieson stains 20 X).

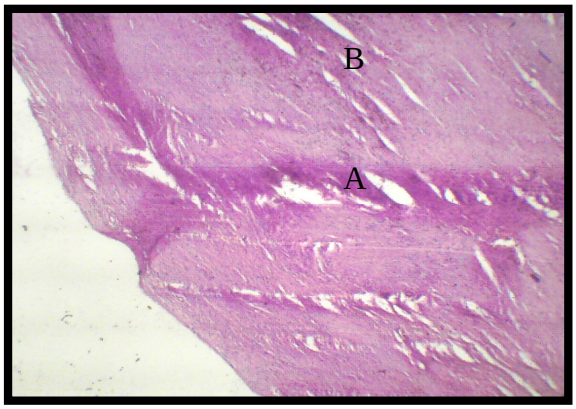

Figure :5: Histopathlogical section in the tendon of control group group at 30 days post surgery (A) line of tendon healing, (B irregular highly cellular F.C.T.(H&E20X

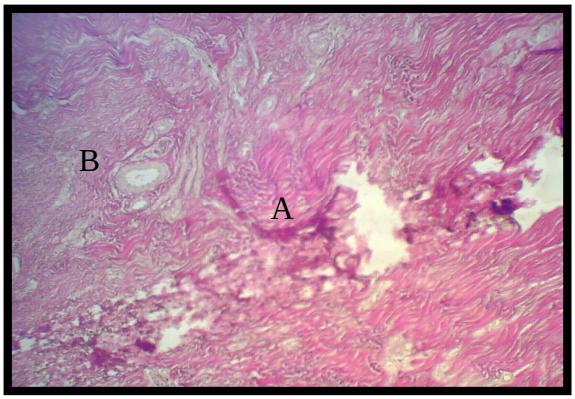

Figure :6: Histopathlogical section in the tendon of control at 30 days post surgery (A) line of tendon healing ,(B)irregular tendon fiber (Van-Gieson stains 20 X).

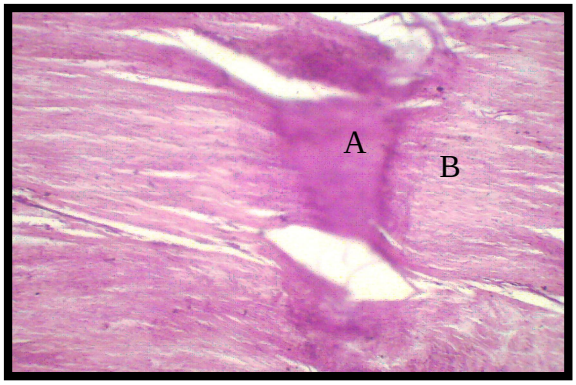

Figure -7:: Histopathlogical section in the tendon of control control group at 60 days post surgery (A) immature F.C T. attached healing to the tendon fiber ,(B)tendon fiber .(H&E20X).

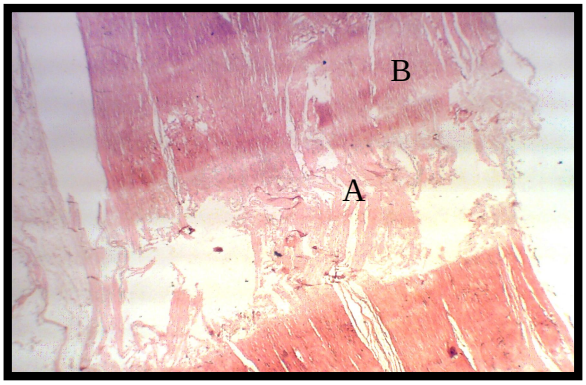

Figure :8: Histopathlogical section in the tendon of group at 60 days post surgery (A) line of tendon , (B) proliferation of F.C.T stained with light red color crossing through F.C.T (Van-Gieson stains 10 X).

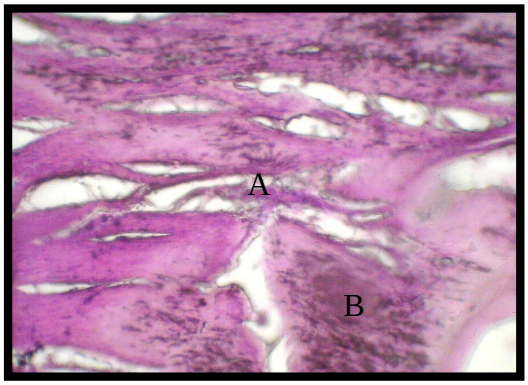

Figure:: 9 Histopathlogical section in the tendon of allo-graft group at 15 days post surgery (A) Irregular highly cellular of F.C.T ,(B)Mononuclear cells infilteration .(H&E40X)

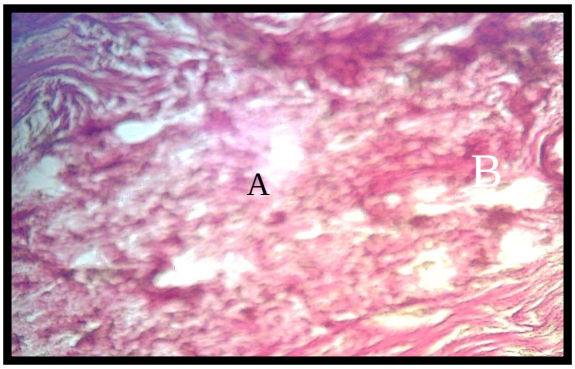

Figure :10: Histopathlogical section in the tendon of allograft group at 15 days post surgery (A) proliferation F.C.T stained yellowish red color (Van-Gieson stains 20 X)

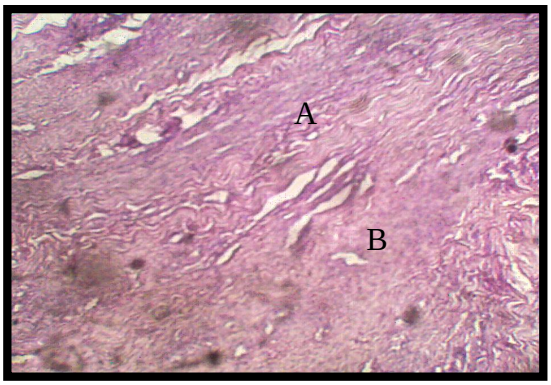

Figure :11: Histopathlogical section in the tendon of allograft group at 30 days post surgery (A) Irregular F.C.T ,(B)Mononuclear cells infilteration .(H&E20X) light red color (C) myofibroblast (Van-Gieson stains 10 X).

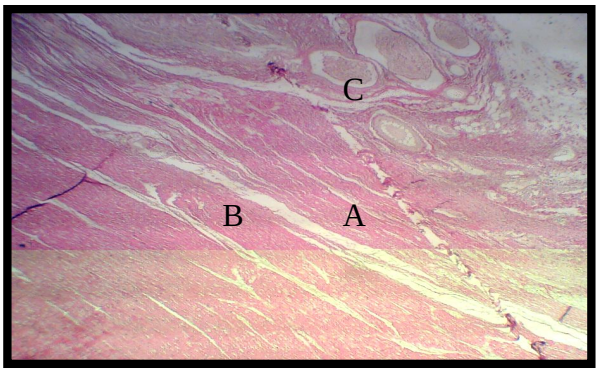

Figure 12: Histopathlogical section in the tendon of allograft group at 30 days post surgery (A) line of tendon healing ,(B)proliferation of F.C.T stained with

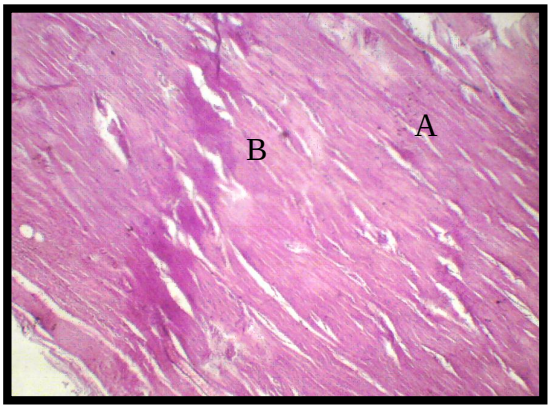

Figure :13 Histopathlogical section in the tendon of allograft group at 60 days post surgery (A) Irregular highly cellular F.C.T ,(B) line of tendon healing .(H&E20X

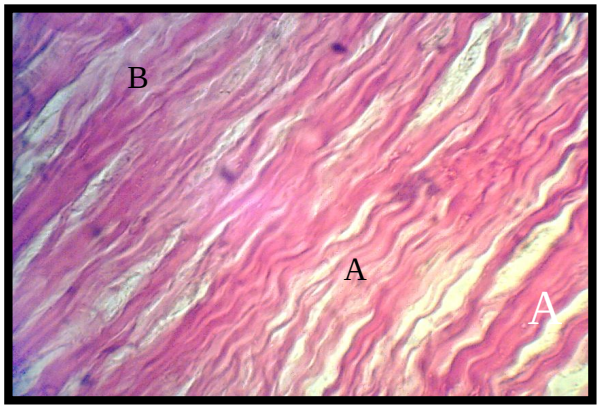

Figure :14: Histopathlogical section in the tendon of allograft group at 60 days post surgery (A)proliferation of F.C.T stained with light red color (B) myofibroblastic attachment (Van-Gieson stains 20X

Discussion

In the present study, the severed tendon was repaired by end to end anastmosis, the repair of tendon is usually accompanied with scar formation which may be desirable for the healing of the tendon but can be a disaster as far as the restoration of function that agrees with [10].In addition, accurate apposition allows direct healing and revascularization across the injury site, which agrees with [11].

The results of the clinical follow up including pain post operatively which made all sheep in recumbent position for 2 days. This pain may be originated from a combination of mechanical factor (break down of collagen) and chemical irritant in addition to neurotransmitters that may generate pain in this condition with the advancement of time the pain subsided and animals gain its normal gait, this agrees with [12].

Adhesion formation which was noticed grossly between the tendinous and surrounding soft tissue in the present study after tenorraphy, poses a major clinical problem because this will lead to the loss of gliding function. This may be due to disruption of the sheath at the time of surgery allowing granulation tissue and tenocytes from surrounding tissue to invade the repair site. Exogenous cells predominant over endogenous tenocytes, permitting the surrounding tissue to attach the repair site and resulting in adhesion formation, specifically, in the control group but in the treated group the adhesions were lesser. This finding is in agreement with a study done by [13].

The histopathological findings of the control group through the 15 days revealed that proliferation of the fibroblast and migration with production of collagen fibers- type-III- immature, this information agrees with [14]. After this period the remodeling phase commences, which last till approximately 10 weeks with decreased cellularity and collagen and glycosaminoglycan synthesis. The remodeling phase is very important to return it to begin to the normal structure of the tendon after their healing of tendon, because in this stage the repair tissue changes from cellular to fibrous, and the metabolism of tenocyte remains high during this period. The synthesization of collagen- type-I is continuous during this stage of maturation. This disagree with [15]), but agree with [16].

Histopathological changes in the allograft showed the regular and mononuclear cells with a wide region and decreased in tendon line healing as well as uniform of their cellular elements in the last stage of the present study, these results agree with [17 and 18)

Cytokine and chemokine activity is essential for tendon healing and functional improvement. The interlukine-12 (IL-12) is produced by macrophages and dendritic cells, and may be associated with later

stages. This factor (IL-12) is very important and helpful for the healing of damaged tendon. In the present investigation the mononuclear cells and certain kinds of cytokines may be sufficient to repair degenerated tissues.

The results achieved in this study meet with the findings of several researches who found that bone marrow enhanced the process of healing. Some of them revealed that bone marrow enhances the function of inflammatory cells such as polymorphonuclear leukocytes (PMN), phagocytosis, production of interleukin (IL-I), transforming growth factor-1 (TGF-1) and platelet-derived growth factor (PDGF). As a result, bone marrow promotes granulation and organization, this agrees with [19]. One of the most interesting effects of stem cells on tendon healing, bone healing, as well as products the permitted a substantial decrease in treatment frequency with minimal scar formation [20].

Moreover, bioreactors can also expose forming tissue, to specific physical stimuli that may improve tissue growth and maturation, another finding from the study is that although mononuclear progenitor cells are derived from the bone marrow, bone marrow clots very rapidly when exposed to air which is why this dish should be prepared in advance and prompt transfer of the collected marrow to the dish is critical [21], there is additional data indicating that platelet rich plasma may also provide the supportive effect for tendon healing, clinical injury results in a variable disruption of the tendon matrix, which induces an inflammatory response. This response is often short lived, very soon after the injury, fibroplasia is initiated resulting in the scar tissue formation within the tendon. In the later stages of repair, inflammation is no longer an obvious clinical feature, giving rise to a debate whether the disease is a tendonitis or tendinopathy. At present, no treatment is successful in promoting the formation of tendon matrix. It has hypothesized that the best hope for engineering new tendon tissue is the compositional and functional similarities to non-degenerate weight [22]. X-rays diffraction studies have demonstrated that collagen fibril elongation initially occurs as a result of molecular elongation, but as stress increases , eventually leading to slippage of lateral adjoining molecules [23].

The chief findings in the histopathological analysis of the two groups, were related to the initial phases of inflammation and tendon repair events. Vascular changes (hemorrhaging and edema) in acute phase, while mononuclear infiltrations and fibroblasts proliferation and intensification of the ECM were observed in other stages, this consideration is in accordance with tendon healing. The bone marrow derived cells in tendon injury induces the release of angiogenic growth factors after 10 days, while cells proliferation and formation of new vessels are affected in approximately after 30 days. The tendons themselves are composed of longitudinally arranged bundles of fibers, blood supply to tendons is poorly compared to muscles and other tissues [3]. The neovascularization may lead to the improvement of blood supply and play a role in the tissue regeneration at the tendon. In this study there were differences seen between the control and the treated group, in vascularity, collagen density and collagen fiber organization, that these results in agreement with [1,23 and 24].

The limitation of this study showed the control group is in need of a long time for tendon healing but the treated group needs less time than the control group.

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تأثير نخاع العظم على التئام وتر القابضة السطحية في الاغنام

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الخلاصة

صممت هذه الدراسه لمعرفة تأثير استعمال نخاع العظم في التئام وتر القابضة الاصبعيه السطحية المحدثة تجريبياً في الاغنام. استعمل للدراسة الحالية 8 رأس غنم بالغ تراوحت اعمارها 9 اشهرالى 1 سنة، قسمت عشوائياً بالتساوي الى مجموعتين حيث عدت المجموعة الاولى كمجموعة سيطرة والثانية مجموعة معاملة بالرقعة النمطية. استحدث قطع جراحي مستعرض لوتر القابضة الاصبعية السطحية عند الثلث الوسطي للعظم المشطي الثالث في القائمة الخلفية تحت تأثير المهدى والمخدر الموضعي ومن ثم خيط الوتر المقطوع بخياطة Bunnell بأستعمال خيوط البولي بروبيولين قياس 2.0 في مجموعة السيطرة، بينما في مجموعة الرقعة النمطية تم وضع 3-2مل من مادة نخاع العظم النمطي وحقنت في منطقة التقمم بين نهايتي الوتر ومن ثم طبقت نفس الاجرأت التي تمت في مجموعة السيطرة وغلق الجرح بالطرق التقليدية.

اظهر الفحص العياني وجود التصاقات بين الوتر والتراكيب المجاورة والتي كانت اشد في مجموعة السيطرة ، واقل منها في مجموعة الرقعة النمطية . اظهر الفحص النسجي المرضي للخزعات التي تم الحصول عليها بعد 15، 30،و 60 يوم اظهرت وجود ألياف غراوية غير ناضجة تميزت بانتظامها وانتظام مكوناتها الخلوية التي ملأت منطقة القطع للوتر مع كثرة الالتصاقات في المراحل المتقدمة من مجموعة السيطرة، بينما في مجموعة المعالجة بالرقعة النمطية، الخلايا الالتهابية وانتظام النسيج الليفي مع الالياف الغراوية ظهرت اكثر مما موجود في مجموعة السيطرة في نفس الفترة مع قلة الالتصاقات.