Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 4| Issue 3|JUL-SEPT|2014|96-108

Research Article
Biological Sciences

EFFECIVENESS THE BONE MARROW AFTER TENOTOMY AND TENORRHAPHY OF SDFT IN DONKEYS

Abdalbari A. Al-faris & Hameed Abed Kate

Department of Surgery and Obstetrics

University of Basra / Collage of Veterinary Medicine, iraq

***Corresponding Author Email: vetedu2000@yahoo.com**

ABSTRACT

Eight healthy male donkeys were used , they were divided into two groups (control and Bone Marrow treated group) , were anesthetized with (Ketamine3 mg/kg B.W and Xylazine 0.5 mg/kg B.W) administered intravenously. The biopsies were taken from each group at 30 and 60 days postoperative for histopathological examination. Histologically the tendon of control group was reveals vacuolated and slightly new blood vessels and present oedema ,that is on 30 days , but on 60 days there was vacuolated tendon cells with infiltration of inflammatory cells. In BM treated group we shows marked increase in neo blood vessels on 30 days which become moderate on 60 days post operation, in the same time collagen bundles are increasing on 30 days, which become regular and arranged in files on 60 days post operation.The sonography observation in control group on 30 and 60 days showing moderate fluid in the SDFT and absence of normal fibers and short croppy fibers. In BM group on 30 days the images indicates mild fluid in the SDFT with slower demonstrated evidence of healing on ultrasound while on 60 days showibg corresponding longitudinal view fiber alignment grade and also shows normal tendons and ligaments demonstrate a homogenecity echogenic. The conclusion, that appear all results were recorded in BM group was the best from control group due to short time to healing of SDFT because the functions and advantages of HA.

KEY WORDS

Tendon, sonography, bone marrow, Donkeys

INTRODUCTION

The normal bone marrow has three primary components: osseous matrix, red marrow, and yellow marrow. The osseous components of the marrow are the trabeculae of cancellous bone, which provide the supporting framework for the red and yellow marrow elements. The red or cellular marrow is hematopoietically active, producing RBCs, WBCs, and platelet precursors. Hematopoietically inactive yellow marrow is composed of fat cells. These two types of marrow differ in their chemical composition (1).

Bone marrow has been found to be one of the most reliable sources of adult stem cells and have been used in treating several conditions (2). In equine species, bone marrow (BM) is one of the most studied and used sources for obtaining adult stem cells (3).

Tendon healing is a slow process. The tendon has low vascularity and is under tension during the healing process. After the injury, the cellularity is also not raised sufficiently to restore the structure and function of the tendon. Therefore, there is a need to increase the rate and quality of tendon healing. Stem cell therapy is one of the available options with encouraging results (4).

The use of autologous mesenchymal cells from BM to repair experimental injuries of tendons and ligaments has been amply described in experimental animals (5).

This study was conducted to evaluate the effects of autologous BM on tendon regeneration of SDFT in donkeys.

MATERIALS AND METHODS

A total of 8 donkeys of both sexes, age of 2–3years with body weight of 70–130 kg (mean ±SE ,weight 97.77 ± 8.11 kg), were used in this study. Donkeys were purchased from different localities of Basra city. These animals were examined clinically and by ultrasonography to ensure that they present no preexisting tendon damage and to exclude the presence of any locomotor disorders. Animals were kept at the Veterinary animal house, Basra University, Iraq, and were fed on a straw and bran; they had free access to water. Two weeks before doing of the experiment, all animals were dewormed. During the entire experimental period, all animals were kept under similar management and feeding practices.

Study design

Donkeys were divided randomly into two groups (each group four animals), one of them was used as a control group, and tendons were tenectomized and lifted for healing without applying any material to serve as controls. While in second group as BM treated group, the Bone marrow from tuber coaxe of same donkey was put in the site of severed tendon in the first treatment group.

Sedation was inducted via intravenous injection of xylazine Hcl (at 0.5 mg/kg.) Then, the animals were generally anesthetized using the ketamine hydrochloride at the dose (3 mg/kg) intravenously administered into the jugular vein. (6).

The anesthetized animals were positioned in lateral recumbency, with the limb selected for tenorrhaphy uppermost and fixed in extension to obtain the correct angle for the introduction of the instruments. The metacarpal region of the limb was aseptically prepared for surgery. A tourniquet was placed above the carpus to minimize hemorrhage.

Surgical Procedure

In the control group, a 10-12-cm mid-metacarpal linear skin incision was made over the plantar aspect of the right forelimb, and the paratenon was longitudinally incised for exposure of SDFT. 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. A stay suture was done at the proximal side of the SDFT, under the carpal joint, using nyoln thread No 2.0 (Daclon, S.M.I co) to prevent the tendon from slipping upward and this was considered as the first stitch of tenorrhaphy. The superficial digital flexor tendon was then transverse cutting with the scalpel. The cut ends of the tendon were approximated by Bunnell-technique suture using nylon suture material size 2.0, and Skin closure was accomplished using silk suture material size 2.0 (silk, S.M.I co.,Hunningen-Belgium) in a routine manner (Figure 1). The site of operation was casting with window, removed after 30 days from operation in the last two periods, while in the first period within 14 days after operation for the removal of skin suture and assessment of clinical parameters.

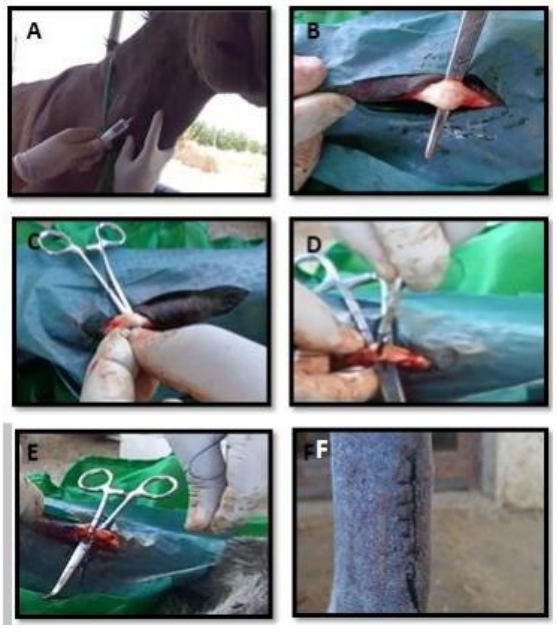

Fig.1: SDFT tenorrhaphy(A) I/v injection of anesthetized material(B) Mid metatarsal incision including the skin, s/c and paratenon(c) for exposure of the SDFT and curved artery forceps were inserted under the superficial tendon to expose it,(C) A stay suture was done at the proximal side of the SDFT and this was considered as the first stitch of tenorrhaphy,(D) The transverse cutting of SDFT with the scalpel , (E)The cut ends of the tendon were approximated by Bunnell-te1chnique suture using nylon, (F) Skin closure was accomplished using silk suture material.

In autologous bone marrow treated group, the same technique as that of the control was performed, except the application of bone marrow (2-3ml) to the sites of tendon anastomosis that was taken from iliac bone of the same animal.

Postoperative care

Donkeys were confined in a stall rest for 4 weeks and monitored daily for changes of clinical signs.

Broad-spectrum antibiotic represented by Penicillin-streptomycin* (Eech one ml containe Procaine penicillin 200gm , Dihydrostreptomycin suulphte 250 mg) in a F dose of 8 mg and 10 mg/Kg B.W. was respectively administered intramuscularly for seven days. Also OTC spray (oxytetracycline Hcl 4.2 gm , Gention violet 420 mg).

Skin incisions were checked daily and final evaluation was performed at the time of stitches removal in (14) days postoperatively.

Procedure for Bone Marrow aspiration from the tuber coxae

An approximate area of 10 cm of the skin overlying the iliac crest was prepared for the procedure by shaving and then using Povidone antiseptic solution. A fenestrated drape was used to protect the prepared surgical site from contamination. The bony prominence of the iliac crest was then palpated and a 1(cm) incision was positioned on top of the bone. A surgical artery clip was used to blunt-dissect the soft tissues between the skin and the bone. The intramedullary cavity of the iliac crest was penetrated with a commercially available Jamshidi biopsy needle) using constant pressure and a screwing motion. Routinely, 2-3 mL of bone marrow was aspirated; the aspirate was collected in a 20 ml syringe (Figure 2).

The needle in one hand and drive the needle into the bone marrow, it was prefer 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 (2).

This technique is used in autologous bone marrow groups provide the amount of marrow which should be put in sever tendon injury immediately to prevent coagulation of the samples.

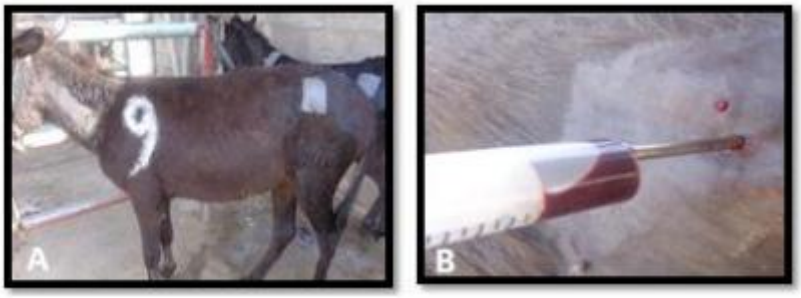

Fig. 2: Bone marrow aspiration (A) preparation of area,
(B) Aspiration of bone marrow from tuber coxae using Jamshidi needle and syringe 20 ml.

Histopathogical examination

The specimens were collected from the anastomotic site of the tendon and slightly above and lower to the site for a period of 30 and 60 days postoperatively for three groups were immediately fixed in 10% buffered formalin, routinely processed, sectioned and stained with Hematoxylin and Eosin (H&E) as well as Van gieson stain (Bancroft and Gamble, 2008). A professional pathologist +microscopically evaluated the vascularization, cellularity, collagen fibers alignment, inflammatory cells and granulation tissues.

Ultrasonographic Evaluation

Ultrasonographic examinations of SDFT were carried out just before the surgical interference, and at 30 and 60 days postoperatively using 6.5 MHz mechanical linear scanner (BMD-3000V, Biomed, USA). For this purpose, donkeys were prepared by clipping and shaving of the hair at the area that needed to be examined. Scanning of the limb was done from just distal to the carpal joint to the level of the proximal sesamoid bones.

Ultrasound transmission gel was liberally applied at the site to prevent air trapping between transducer and the skin surface. Longitudinal and transverse scans were obtained for the examined tendon at each specific time point to observe the different echogenic texture, fibers alignment as healing indicators and any other soft tissue changes, if any.

RESULTS

Histological section on 30 days reveals vacuolated with swelling of cells and slightly new blood vessels and there is empty space of edema (Fig. 3 and 4). On 60 days, there was vacuolated tendon cell with infiltration of leukocyte and presence of fibroblast as well as there is new blood vessels are formed when staining with van Gieson (Fig.5 and 6).

In BM group, on 30 days, shows collagen fibers and marked increase in neo blood vessels (Fig. 7, 8), but in 60 days, there is moderate increase in neo blood vessels and collagen fiber arranged in files (Fig, 9 and 10).

Utrasound image in control group, on 30 days, showing the presence of moderate fluid in SDFT and absence of normal fiber pattern, and short, croppy fiber (Fig. 11) and the hole where the tendon fibers are injured over time this area of fiber damage will fill in fluid (Fig. 12). On 60 days, there is moderate fluid and the large anechoic (black) area within the SDFT (Fig. 13)and the black area is a core lesion are a common section where the Centre of the tendon has a visible hole. (Fig. 14)

The bone marrow group, on 30 days, the Ultrasound image indicates mild fluid in the SDFT (Red), with slower to demonstrate evidence of healing on ultrasound (Fig. 15 and 16). On 60 days, there is corresponding longitudinal view fiber alignment grade (Fig.17) showing normal tendons and ligaments demonstrate a homogenecity echogenic (evenly white) appearance on ultrasound when viewed on cross-section) fiber pattern is evaluated by placing the transducer parallel to the tendon ligament fiber (Fig. 18)

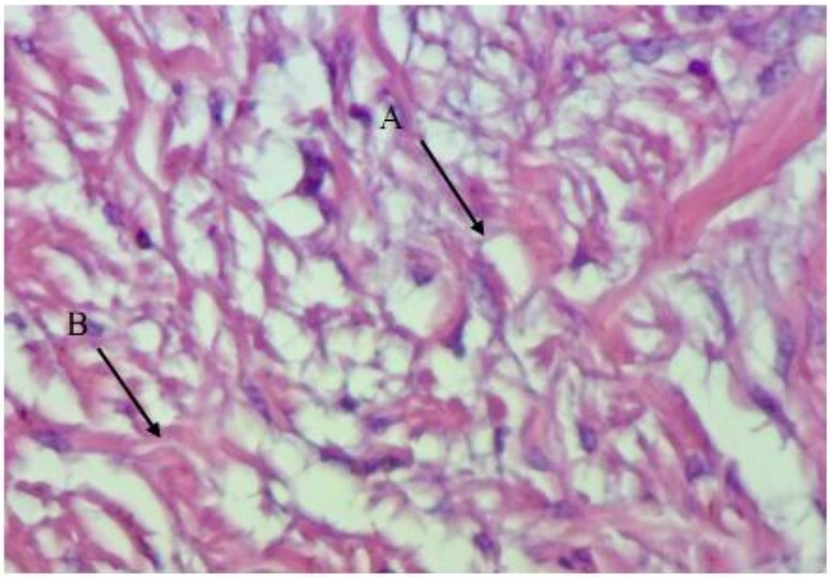

Fig. 3: Histopathlogical section in the SDFT of control group at 30 days postoperatively showing (A) Edema, (B) collagen fiber (Van-Gieson stains 10 X).

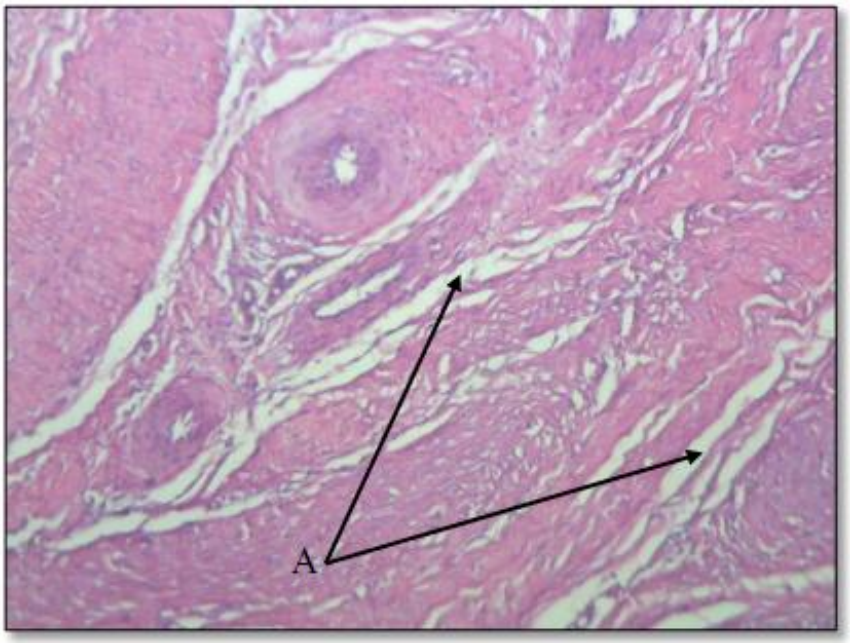

Fig. 4: Histopathlogical section in the SDFT of control group at 30 days postoeratively showing, (A) edematous fluid (H&E stains 10 X).

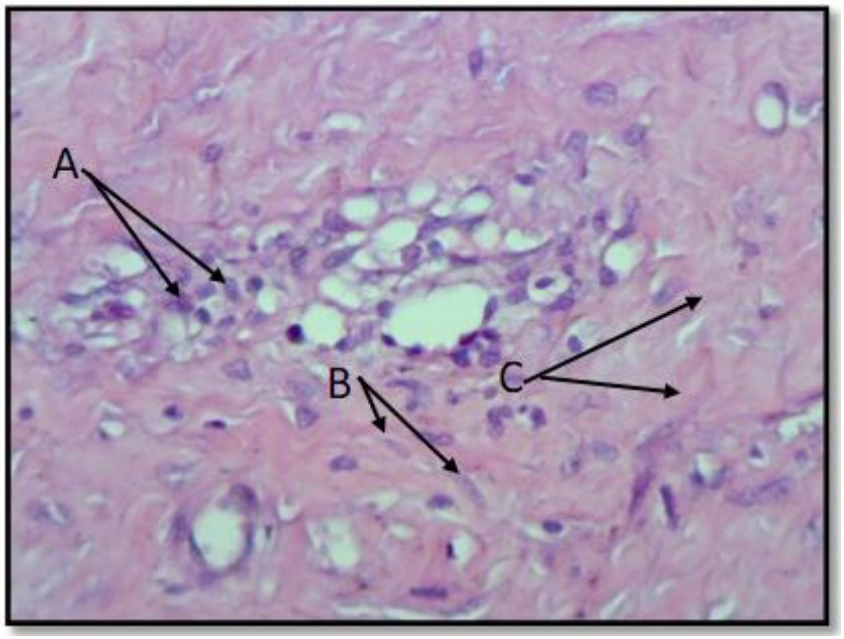

Fig. 5: Histopathlogical section in the SDFT of control group at 60 days post-surgery (A) leuckocyte, (B) fibroblast, (C) collagen fiber (Van-Gieson stains 40 X)

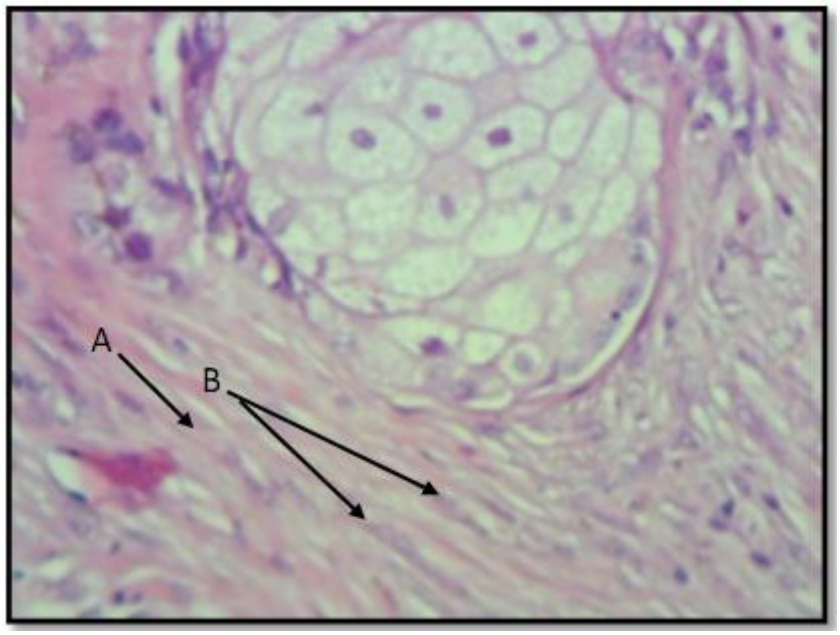

Fig. 6: Histopathlogical section in the SDFT of control group at 60 days postperatively showing (A) collagen fiber, (B) fibroblaste (H &E stains 40 X).

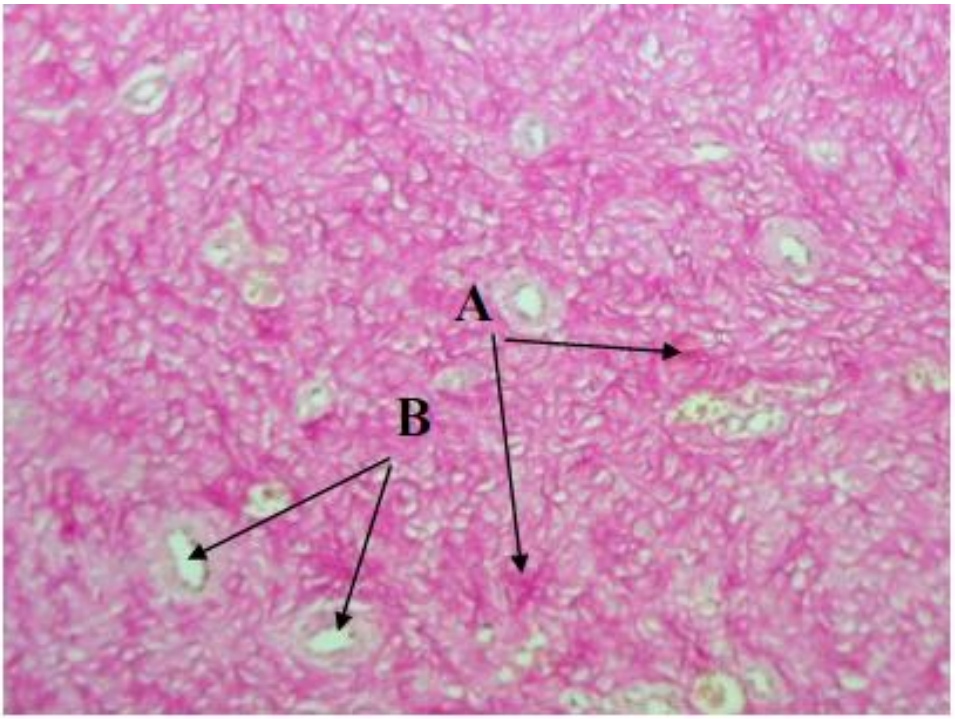

Fig. 7: Histopathological section in the SDFT of the bone marrow treated group at 30 days postoperatively showing (A) collagen fibers (B) marked increase in neo blood vessels (Van-Gieson stains X10)

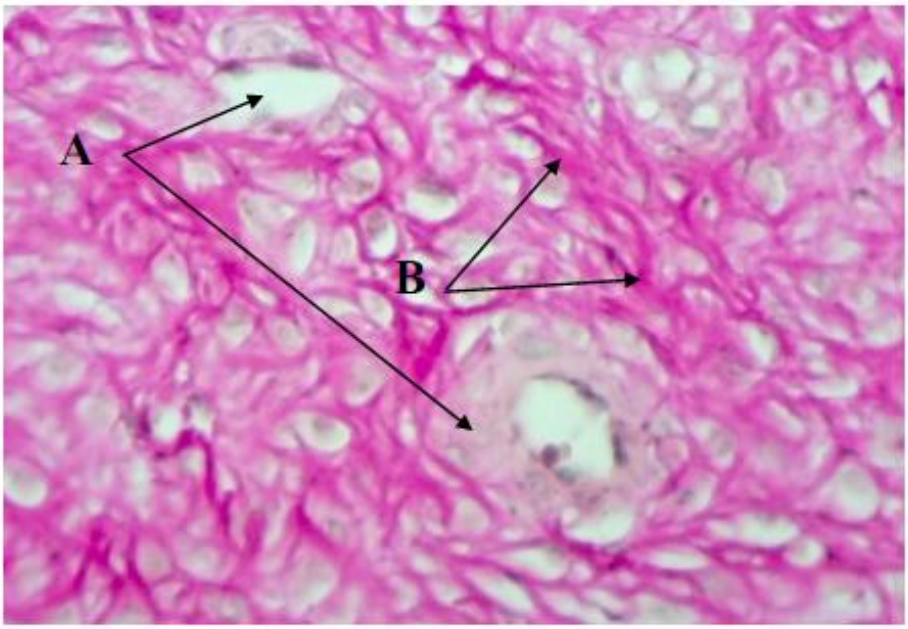

Fig. 8: Histopathological section in the SDFT of the bone marrow treated group at 30 days postoperatively showing (A) marked increase in neo blood vessels, (B) collagen fiber (Van-Gieson stains X40)

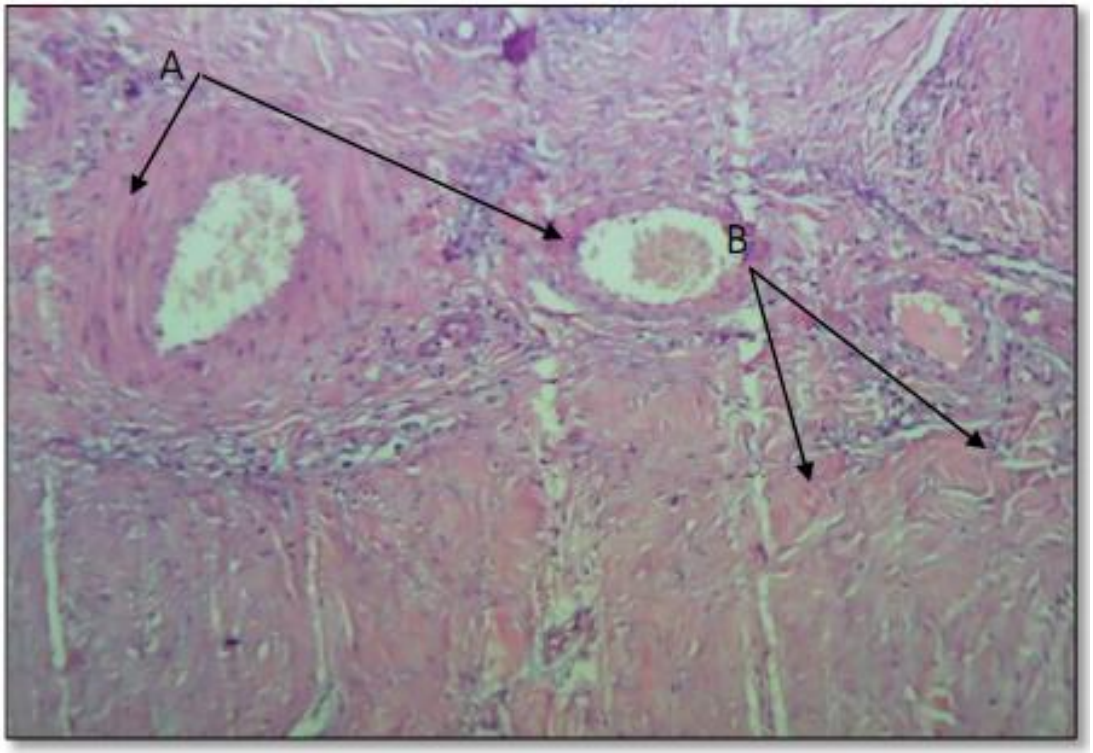

Fig. 9: Histopathological section in the SDFT of the bone marrow treated group at 60 days postoperatively showing (A) moderate number of blood vessels (B) collagen fiber arranged in files (H&E stains X10).

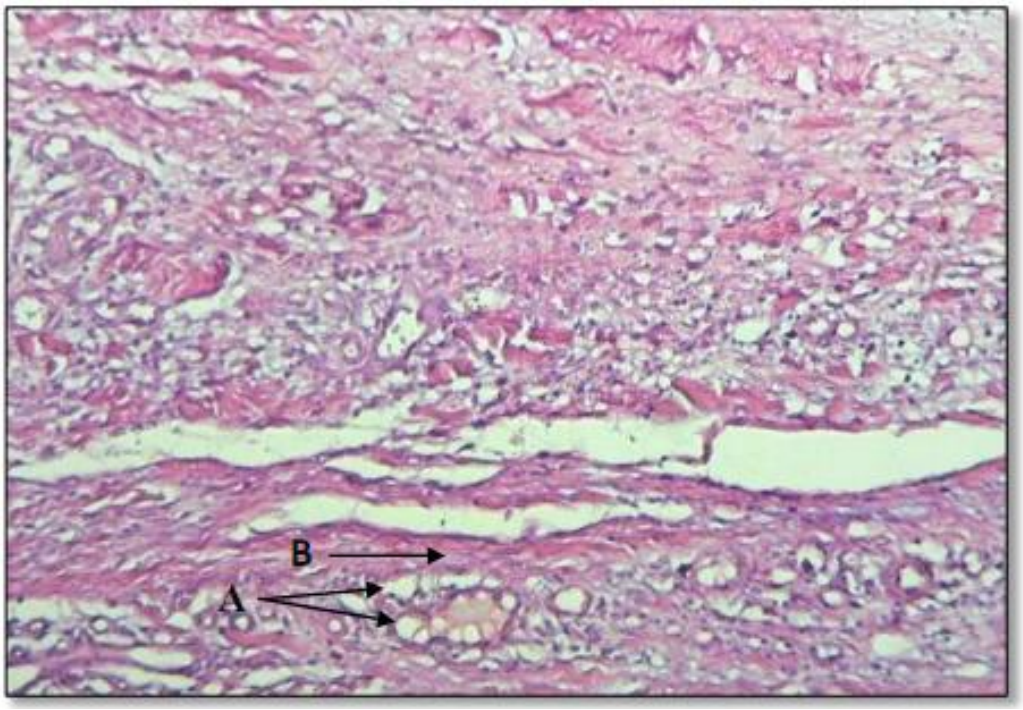

Fig. 10: Histopathological section in the SDFT of the bone marrow treated group at 60 days postoperatively showing (A) blood vessels, (B) fibrosis (Van-Gieson stains X10).

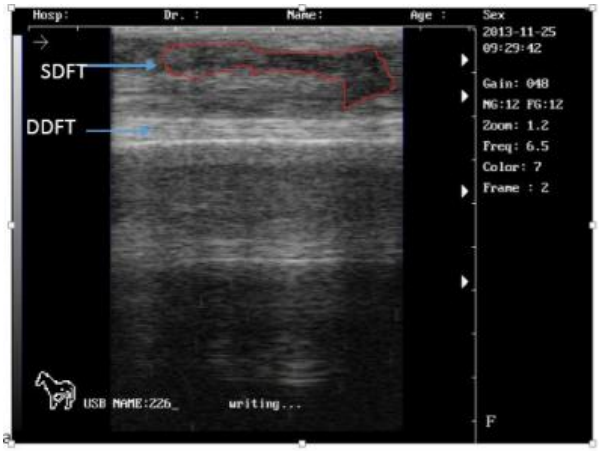

Fig. 11: Ultrasound longitudinal image for the tendons of control group at 30 post operatively showing the presence of marked fluid in SDFT and absence of normal fiber pattern, and short, croppy fiber.

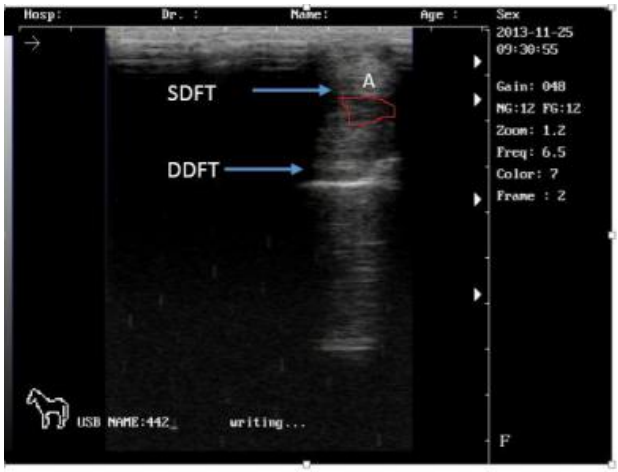

Fig. 12: Cross section of tendons of control group at 30 days post operatively indicates the hole where the tendon fibers are injured (Red),over time this area of fiber damage will fill in fluid (A).

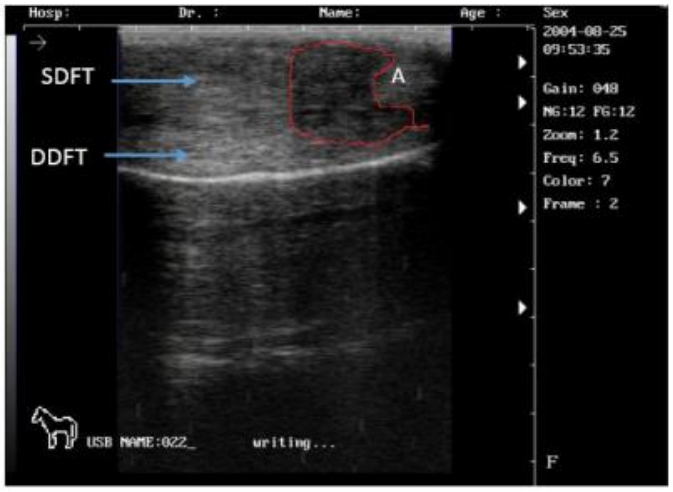

Fig. 13: Ultrasound longitudinal image for the tendons of control group at 60 days post operatively indicates moderate fluid and the large anechoic (black) area within the SDFT (Red).core lesion (A).

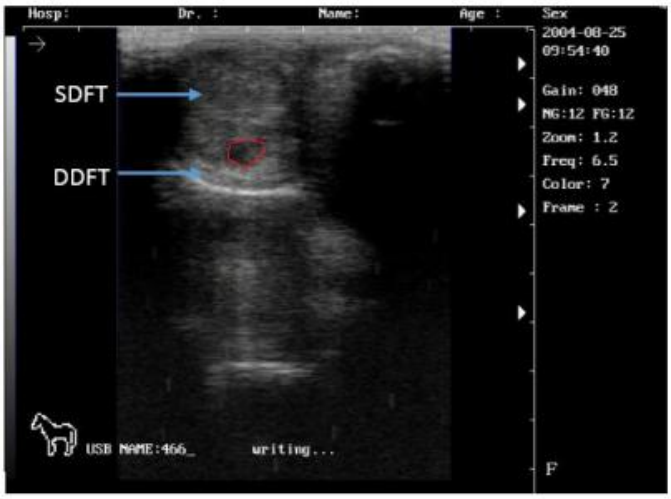

Fig. 14: Cross section of tendons of control group at 60 days post operatively indicates, the black area is core lesions area common section where the Centre of the tendon has a visible hole (Red).

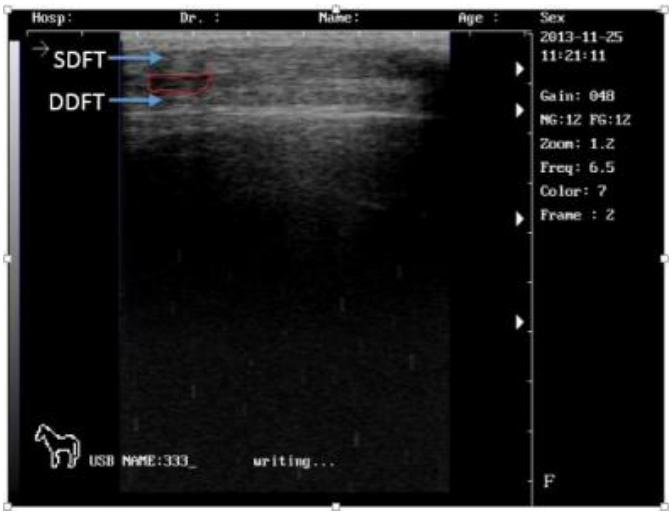

Fig. 15: Ultrasound longitudinal image for the tendons of bone marrow treated group at 30 days post operatively indicates, lesion in a tendon will appear darker (Red), with slower to demonstrate evidence of healing on ultrasound.

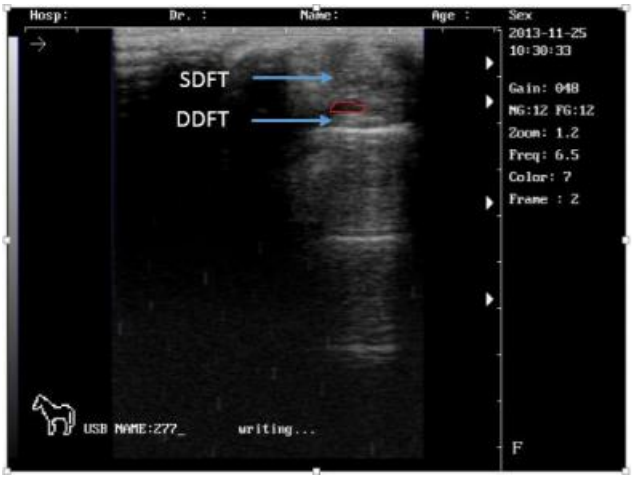

Fig. 16: Cross section of tendons of bone marrow treated group at 30 days post operatively indicates mild fluid in the SDFT (Red), with slower to demonstrate evidence of healing on ultrasound.

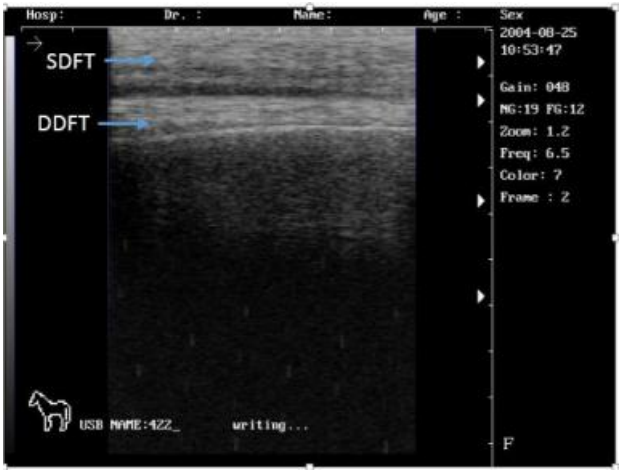

Fig. 17: Ultrasound longitudinal image for the tendons of bone marrow treated group at 60 days post operatively indicates corresponding longitudinal view fiber alignment grade.

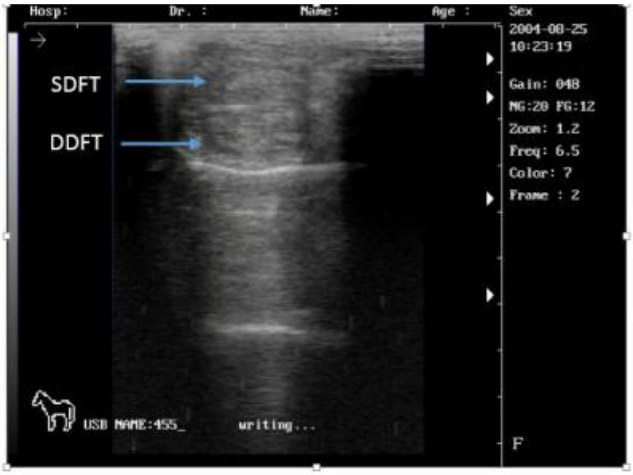

Fig. 18: Cross section of tendons of bone marrow treated group at 60 days post operatively showing normal tendons and ligaments demonstrate a homogenecity echogenic (evenly white) appearance on ultrasound when viewed on cross-section) fiber pattern is evaluated by placing the transducer parallel to the tendon ligament fiber.

DISCUSSION

The results obtained in the present study concerning histological analysis of the tendon show a slight increase in vascularization in control group and marked increase in BM group.

The neovasscularization is important in tendon healing and when it comes to wound healing, it is crucial to have enough angiogenesis because without the restoration of blood flow, oxygen and nutrients cannot be delivered to the healing site. This agrees with (8).

Vasculogenesis differs from angiogenesis, where preexisting and fully differentiated endothelial cells (ECs) respond to angiogenic growth factors (vascular endothelial growth factor (VEGF), fibroblast growth factor-1 (FGF-1), and fibroblast growth factor-2 (FGF-2)) and form new blood vessels form preexisting blood vessels.

In animals models of ischemia, in vitro differentiated endothelial progenitor cells are incorporated into sites of active neovasculogenesis in ischemic tissue, leading to improved perfusion when transplanted after the induction of ischemia, that may be agrees with (9).

We believe that reduced inflammation and degenerative process during the first phase of tendon healing have improved angiogenesis in response to BM administration. This consequently resulted in optimal healing.

Bone marrow derived cells are involved in healing in several fibrotic conditions, this agrees with (10, 11). More specifically, bone marrow can produce

fibroblasts that migrate to the site of injury in fibrotic conditions including both cancer implantation and excisional wounds in the skin. Bone marrow derived fibroblasts are also able to produce type 1 collagen, further supporting a role for these cells in fibrosis, this agrees with (12).

In sonography evaluation, the results of healing the SDFT by detect fine textural abnormalities of these structure and also recorded dynamic evaluation of structures the artifacts that may be mimic tendon, injury and tendon disorder tears or tendonitis as well as tenosynovitis, that these results appear in our study and agree with (13).

Our study in the BM group indicates mild fluid in the SDFT with slower to demonstrate evidence of healing on ultrasound at 30 days post-surgery. While at 60 days we show corresponding longitudinal view fiber alignment, grade also showing normal tendons and ligaments demonstrate a homogeneity echogenic (evenly white). This agrees with (14) who indicate the tissue microenvironments appear to be able to induce cell differentiation and fiber production, as revealed using ultrasound scanning. The correct orientation of fibers strongly suggests that microenvironment and lines of tension and relaxation determine correct tissue morphology.

Our results disagree with (15) they indicate no differences in ultrasonographic parameters between BMMSC treated tendons and their respective controls were found during the 8-week healing period posttreatment despite significant improvement in histologic evaluation in the MSC and AdIGF-MSC-treated tendons at the termination of the study.

REFERENCE

1. Siegel, M.J. (2000). MR imaging of paediatric haematologic bone marrow disease. J Hong Kong Coll Radiol., 3:38–50.
2. Yao, J.; Woon, C.Y.; Behn, A.; Korotkova, T.; Park, D.Y.; Gajendran, V. and Smith, R.L. (2012). The effect of suture coated with mesenchymal stem cells and bioactive substrate on tendon repair strength in a rat model. J Hand Surg Am., 37(8):1639–1645.
3. Gutierrez-Nibeyro, S.D. (2011). Commercial cell-based therapies for musculoskeletal injuries in horses. Vet Clin North Am Equine Pract. 27(2):363–371.
4. Moshiri, A. and Oryan, A. (2012). Role of tissue engineering in tendon reconstructive surgery and regenerative medicine: current concepts, approaches and concerns. Hard Tissue, 1(2):1.
5. Ouyang, H.W.; Goh, J.C.; Thambyah, A.; Teoh, S.H. and Lee, E.H. (2003). Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. Tissue Eng. 9(3): 431–439.
6. Bohart, G. (1997). Anesthesia of horses in the field. In: Current Therapy in Equine Medicine, 4:34-36.
7. Muschler, G.F. (2003): Spine Fusion Using Cell Matrix Composites Enriched in Bone Marrow-Derived Cells. Clin Orthop Rel Res., 407:102-118.
8. Jones, M.K.; Wang, H.; Peskar, B.M.; Levin, E.; Itani, R.M.; Sarfeh, I. J. and Tarnawski, A.S. (1999). Inhibition of angiogenesis by nonsteroidal anti inflammatory drugs: Insight into mechanisms and implications for cancer growth and ulcer healing. Nat Med; 5(12): 1418-1423.
9. Urbich, C.; Heeschen, C.; Aicher, A.; Dernbach, E.; Zeiher, A.M. And Dimmeler, S. (2003). Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation, 108(20):2511-2516
10. Hashimoto, N.; Jin, H.; Liu, T.; Chensue, S.W. and Phan, S.H. (2004). Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest., 113(2): 243-252.
11. Asawa, S.; Saito, T.; Satoh, A.; Ohtake, K.; Tsuchiya, T.; Okada, H.; Neilson, E.G. and Gotoh, M. (2007). Participation of bone marrow cells in biliary fibrosis after bile duct ligation. J Gastroenterol Hepatol, 22(11): 2001-2008.
12. Ishii, G.; Sangai, T.; Sugiyama, K.; Ito, T.; Hasebe, T.; Endoh, Y.; Magae, J. and Ochiai, A. (2005). In vivo characterization of bone marrow-derived fibroblasts recruited into fibrotic lesions. Stem Cells, 23(5): 699-706.
13. Martinoli,C.; Stefano, N. and Derchi, L.E. (1999). Tendon and Nerve Sonography. Radiol Clin North Am., 37(4):691-711
14. Pacini, S.; Spinabella, S.; Trombi, L.; Fazzi, R.; Galimberti, S.; Dini, F.; Carlucci, F. and Petrini, M. (2007). Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. Tissue Eng.,13(12):2949-2955.
15. Schnabel, L.V.; Lynch, M.E.; van der Meulen, M.C.; Yeager, A.E.; Kornatowski, M.A. and Nixon, A.J.

(2009). Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. J Orthop Res., 27(10):1392-1398.

***Corresponding Author:**
Prof.Dr.Abdulbari A.Alfarris
College of veterinary medicine
Basra University
iraq
E-mail:vetedu2000@yahoo.com