رقم البحث (33)

ROLE OF BOVINE URINARY BLADDER SUB-MUCOSA FOR TENDON RECONSTRUCTION

BY

Ahmed. H. F. AL-Bayati & Adbul-Hadi J. AL-Abedi

Department of Surgery and Obstetrics-College of Veterinary Medicine-University of Baghdad-Iraq

ABSTRACT

This study was carried out on 12 adult healthy donkeys, which were divided randomly into two equal groups, treatment group and control group. In treatment group, a strips (3cmX0.3cm) of decellularized and disinfected scaffolds were prepared from the whole fresh bovine urinary bladders of slaughtered cows at the local abattoir as urinary bladder extracellular matrix (UBM-ECM), were firmly embedded within the groves of the superficial digital flexor tendons (SDFTs) of the forelimbs. While, the defects of the control group were injected with sterile normal saline instead of UBM. Ultrasonographical and histopathological evaluation was depended along the period of 16 weeks to follow the healing progression of tendon tissue. By the end of the study, the ultrasonographic following-up revealed an increased echogenicity and regular orientation of tendon fibers of treatment group. In comparsion, the presence of hypoechoic areas with randomly arranged collagen fibers was confirmed in the control group. The histopathological sections at 8 and 16 weeks post-surgery, appeared that bovine UBM treated tendons have enhanced cellularity, increased vasculature, improve tendon architecture, suggesting enhanced tendon healing and reconstruction regeneration than those in untreated tendons which were healed by scar tissue formation. Based on the promising results obtained by this study, the ultrasonographical and histopathological findings confirmed that the filling tendon defects with xenogeneic bovine UBM accelerate and improve of tendon tissue healing without adverse immuno response.

Key words: Tendon, Urinary bladder matrix, Urinary bladder sub-mucosa, Bio-implants.

INTRODUCTION

Equine tendon and ligament injuries are generally regarded as having a predictably prolonged convalescent period (taking a full year for recovery often with poor healing quality) with high probability for re-injury, in part due to relatively poor blood supply to the affected tissues (1). As a result, treatment of tendon injuries is challenging, in addition, classic conservative and/or surgical interventions methods which were used to accelerate or enhance tendon healing have significant limitations with unclear outcomes (2). In the last few decades, biomaterials have become critical components in the development of effective new medical therapies for wounds care (3,4), and many new tissue engineered materials have been introduced: artificial polymers, biodegradable films and biomaterials derived from mammalian (human, porcine, bovine and equine) tissues, using a combination of principles of engineering and biology. Many biological and synthetic scaffolds have been developed during the last 15^{th} years and both of positive and negative results have been reported in clinical applications for tendon and ligament injuries. So, limitations of previous generations of biologically derived materials are overcome, many new and impressive applications for biomaterials are being examined (5).

Biological scaffold materials composed of ECM have been shown to facilitate the constructive remolding of many different tissues in both pre-clinical and clinical applications in animals and human field (6). They are a well-defined 3D surface proteins microstructure, allowing host cell integration and natural porosity, which provide much larger space for host cell attachment, proliferation, migration and assists gas and metabolite diffusion. These properties allow biological scaffolds interact quickly with host tissue and induce new tissue formation faster (7). These scaffolds can be obtained from a variety of mammalian tissues, including; heart valves, blood vessels, skin, nerves, skeletal muscle, tendons, ligaments, small intestinal submucosa (SIS), urinary bladder and liver (8). Allografts and xenografts have become increasingly popular for tendons and ligaments repair to overcome the limited availability and donor site complications encountered with the use of autograft tissue (9). So, to minimized the risk of host rejection in the case of allografts and xenografts, a non-collagen components should be removed while retaining its natural collagen structure and mechanical properties. Therefore, these scaffolds are processed through cascade steps, including general cleaning, removal of lipids or fat deposits, disruption of cellular and DNA materials, crosslinking and sterilization. The final scaffolds are composed mainly of naturally occurring collagen fibers, predominantly type-I collagen and several of them have a surface chemistry

and native structure that is bioactive and promotes cellular proliferation and tissue ingrowth (7,10).

Due to the fact that the studies on uses of bovine UBM in animals and in human field are absent, this study was planned to assess the efficacy of bovine UBM ultrasonographically and histopathologically, in acceleration and enhancement of tendon healing, after experimentally induced-tendon injury, via tendon tearing of SDFT of equine species.

MATERIALS AND METHODS

Experimental Animals

Twelve apparently healthy adult donkeys (**7** female and **5** male), ranging in age from (2-3) years and weighing (80 to 100) kg, were recruited for this study. All animals were evaluated clinically by a physical and clinical examination including the ultrasonographical evaluation, before initiation of the experiment, to rule out pre-existing tendonitis or any abnormalities of the SDFTs.

Preparation of Urinary Bladder Matrix

The whole fresh urinary bladders were harvested from a slaughtered cows at the local abattoir and UBM-ECM will be prepared as a decellularized scaffold, as described by (11,12,13). The excess adipose tissue and collagenous connective tissue was removed mechanically from outside of the bladder by scissors. The intralumenal water pressure was used to expand and stretch the bladder to facilitate the removal of all urinary bladder layers except the sub-mucosal layer. The bladder was then bisected on one side from the opening to the apical region forming a rectangular-shaped sheet. The serosal side of the bladder was placed downward to remove of the mucosal layer and then the luminal side of the bladder was placed downward to remove the tunica serosa and tunica muscularis layers by genital mechanical delamination, using the sharp edge of the knife, and finally prepared a flattened rectangular sheet (Fig.1). The remaining tissue (sub-mucosal layer) was then soaked in phosphate buffered saline (PBS) (pH7.4) containing of penicillin (100IU/ml), streptomycin (100ug/ml) and amphotericin (100µg/ml) and then represented UBM. The risk of host rejection (immuno-rejection) of UBM was minimized by disruption of cellular and DNA materials, the following removal of the appropriate tissue layers, the remaining tissue was treated with a (0.1%) peracetic acid (PAA) and (4%) ethanol solution for two hours at room

temperature on a shaker. Traces of peracetic acid were removed and the pH was returned to approximately 7.4 by rinsing the ECM at room temperature, with shaking, in PBS one time, then in water twice, and then again in PBS one time. Each rinse lasted 15mins. The resulting decellularized ECM scaffolds were terminally sterilized by immersion in 0.1% PAA solution titrated to pH 7.0 at room temperature for five hours, and finally, the disinfected and decellularized scaffold was maintain in sterile PBS containing antibiotics and antifungal drugs and preserved at 4ċ (6).

Experimental design

The experiment animals were divided randomly into two equal groups (six animals/group). In treatment group, the palmar surface of the middle third of the metacarpal region of right forelimbs was prepared for aseptic surgery. Under the effect of tranquilizer by intravenous administration of acepromazin maleate (0.1mg/kg B.W), and then under the effect of general anesthesia (using of intramuscular injection of a combination of Ketamin-Xylazine at a dose rate of 2.2mg/kg and 1.1mg/kg, B.W. respectively), a 5cm incision was made along the course of the palmar surface of the metacarpal region, including the skin, subcutaneous fascia and tendon paratenon. The tendon was isolated and 3cmX0.3mm longitudinal intratendenous tearing and depth of 0.2mm, was induced by a partial removal of tendon tissue to make a groove within the tendon core. The bioimplant was trimmed to fit the tendon defect and moistened by soaked in worm sterile PBS for one hour prior of implantation. The implant was firmly embedded within the groove which then closed with simple interrupted suturing pattern using non-absorbable sutures (Nylon 2-0) (Fig.2). In control group, the same procedure was repeated as in treatment group, but the defect of the tendon was injected with 3ml sterile normal saline instead of UBM.

The incision was closed in layers and the limb was casted by applying of compression bandage immediately post-treatment and remained for four weeks with changing every four days. Twice daily, hand walking was begun on the day following implantation and continued on an increasing basis for 45days. All animals were injected intramuscularly with a mixture of streptomycin/penicillin for five days. The animals were housed in semi opened stables in the farm animal of the Veterinary Medicine College, Baghdad University along the period of the experiment, receiving free water and food (concentrated and alpha-alpha hay).

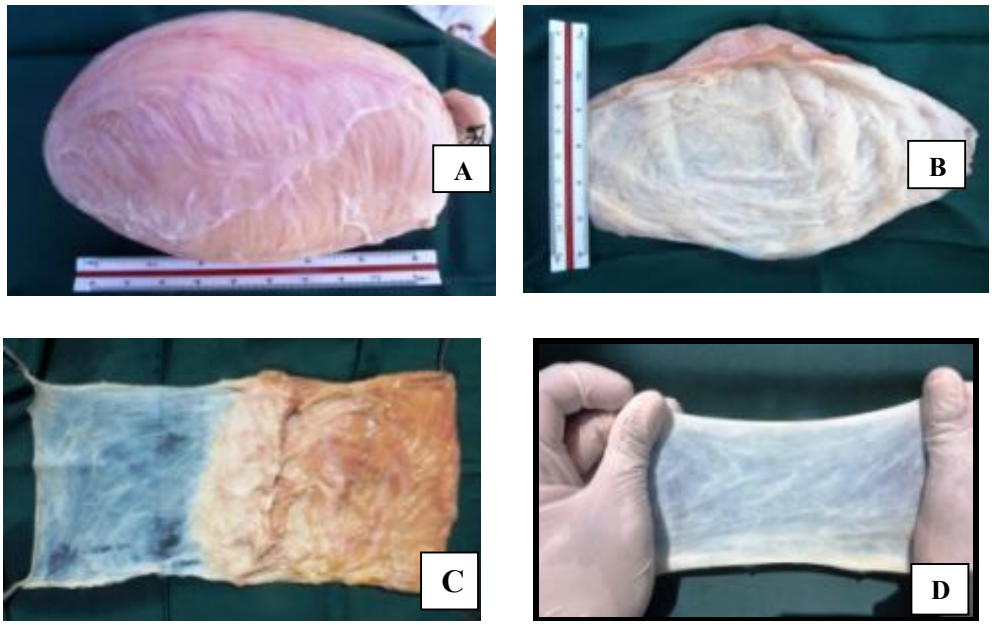

Fig.1: Shows the bovine urinary bladder (A). Longitudinal opening of urinary bladder (B). Mechanical separation of mucosal and seromuscular layers from the submucosa (C). U.B sheet (D).

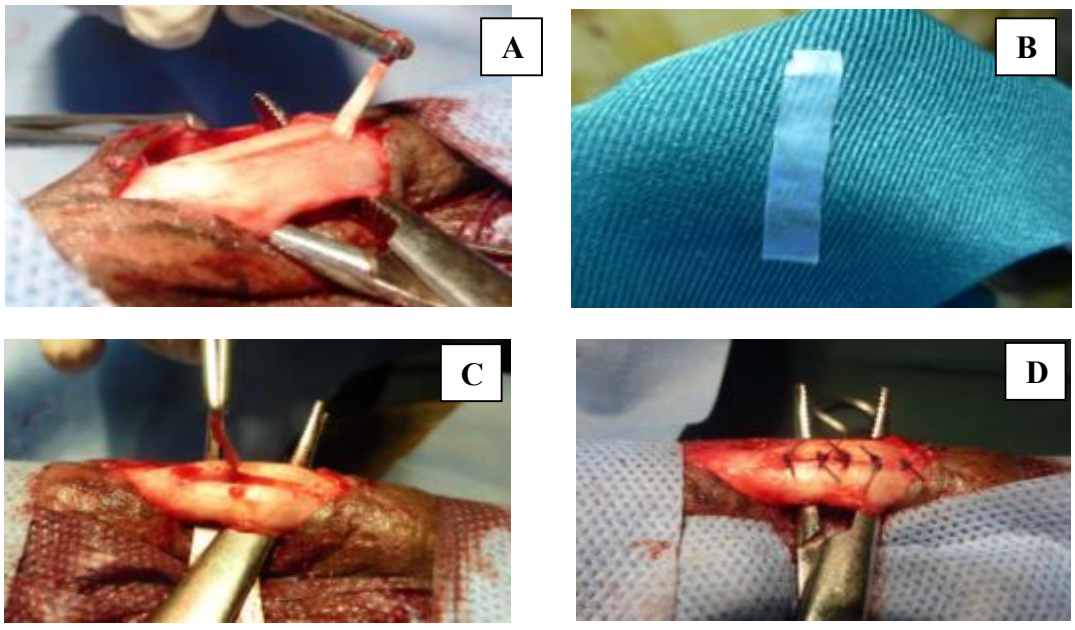

Fig.2: Shows inducing of longitudinal linear tearing of the SDFT (A), trimmed bioimplant to fit longitudinal tendon tearing (B), placement of UBM strip inside the tendon defect (C), and closing of the tendon defect with simple interrupted suturing using (2-0) Nylon suture (D).

Ultrasonographical evaluation

The tendons were evaluated using Welld Ultrasonographic Machine (WED-9618V, China) with a linear (7.5)MHz transducer, for monitoring the tendons post-surgery, on day of treatment and bimonthly till week 16th post-UBM implantation. Ultrasonographic images were recorded prior to tendon injury, served as control images. All the ultrasonographical examinations were completed with the animals were sedated and restrained in stocks. The ultrasound images were used to measure the echogenicity and the fibers alignment in the core lesion (14). The echogenicity was determined based on a tendon echogenicity score (TES) of 1 to 4, suggested by (15), as following; **1**=Slightly less echogenic 25% than normal. **2**=Half echogenic and half anechoic 50%. **3**=Mostly anechoic 75%. **4**=Completely anechoic 100%.

The evaluation of changes in the fibers alignment, based on the liner arrangement of the echoes in the longitudinal images, was scored as fibers pattern score (FPS) between (0 and 3): **0** =Parallel fibers pattern in 76-100% of fibers, **1** =Parallel fibers pattern in 51-75% of fibers. **2** =Parallel fibers pattern in 26-50% of fibers, **3** =Parallel fibers pattern in 0-25% of fibers (16).

Histopathological evaluation

The histopathological evaluation was performed for all animals of the study at (8) and (16) weeks post-operation (six animals/group and three animals for each period). The palmar metacarpal region of treated and untreated animals was carefully dissected to isolate the lesion area and determine correlated structures. The samples were collected from the lesion of tendon, using standard histological procedures, a one cm^3 segment was obtained and fixed in (10%) neutral formalin solution, and embedded in paraffin, sectioned longitudinally and transversally in (5-7) micron sections on a rotary microtome and staining with hematoxylin and eosin stains (17).

Statistical analysis

The statistical analysis system (SAS) (18) program was used to effect study factors in traits. Least significant difference (LSD) was used to significant compare between means. The level ($P<0.05$) is considered significant.

RESULTS

Ultrasonographical evaluation

Ultrasonographical following-up of tendon healing through the longitudinal and transversal ultrasound images of treated and untreated animals which shown in figures (3&4), was performed two weeks intervals post-surgery and until the end of the study, revealed the presence of tendon lesions variable in size, shape and position, ranged from hypo to hyperechoic along the study, with different degree of fibers disruption and alignment, being generally located in the lesion of the SDFT. The lesion was clear directly post-surgery, but the severe lesion was noticed between day (5 to 7) post-surgery, which contained an area of decreased echoic intensity along the lesions (TES and FPS scores as 3), as well as, swelling of the SDFT. There was also edema of the peritendinous tissue which enhanced the ability to discern the underlying SDFT by physically separating it from the skin. Two weeks post-surgery, most severe lesions were identified and the ultrasound examination confirmed significant SDFT lesions characterized by presence of uniform hypo to anechoic areas with loss of the linear pattern of tendon fibers, being generally located in the core of the SDFT, but without differences among the treatment and control groups. The TES scores ranged from grade 2 to 3 (Mean=2.5 for treatment group and 2.7 for control ones). The same grades were confirmed for both groups in the FPS scores (Mean=2.6 for treatment group and 2.8 for control ones)

Tendon repair was initiated in the treatment group at week fourth post-implantation of UBM, , which was indicated by its swelling appearance of SDFT with TES scores (Fig.5), ranged from (2 to 3, Mean=2.0), and the FPS scores were initially higher (from grades 2 to 3, Mean=2.2) (Fig.6), while, the improvement become lower than scores for the control group (graded 2 and 3, Mean=2.5 and 2.6, respectively). At the eighth week, ultrasound determination of TES and FPS scores ranged from (1 to 2, Mean=1.5), but these scores improved and still different from those of the control ones (between grade 2 and 3 for each TES and FPS, Mean=2.3 and 2.4), as compared to the examination at week fourth. The ultrasonic images of treatment group revealed the presence of hypo and hyperechoic area at the site of defect, while, most of the defect of control group was hypoechoic in appearance. At the end of the study, (16) weeks post-surgery, the TES and FPS values were lower than those measured in the control group. The ultrasound evaluation has revealed an increased echogenicity (TES grade 1) earlier in the treatment group which characterized by the presence

of hyperechoic areas, and the orientation of fibers was correctly parallel to the axis of the tendon (FPS grade 1 and 2, Mean=1,2), when compared to the control ones, the echogenecity was ranged from hypo to hyperechoic lesion (TES and FPS graded as 2 and 3, Mean=2.0 and 2.2 respectively). The tendons of the treatment group appeared to be almost completely repaired and have a significant amount of tendon-like tissue formation in the SDFTs implanted with UBM than that in the control group, except one animal in treatment group which showed minimal change from the pre-implantation and adhesion with the surrounding tissues. In contrast, most animals of the control group showed tendon ultrasound images that revealed fibrosis during the healing process.

Histopathological evaluation

Eight weeks post-surgery, the microscopical examination of the tendon sections of control group showed immature fibrous C.T infiltrate by few MNCs around suture materials and few B.Vs with scant proliferation of young tenoblasts (Fig.7A,B). While the sections of the treated tendons have expressed early stage of consolidation phase of tendon remodeling. The implanted UBM made a scaffold to proliferate tendon tissue without inflammatory reaction. The sections appeared a moderate proliferation of tenoblasts producing dense collagen fibers mixed with MNCs infiltrate and dilated B.V.s were seen in the peritendon. In other sections, more B.Vs, high density of collagen fibers and more tenoblasts with few MNCs are also seen in the endotenon of treated tendon (Fig.7C,D).

At 16weeks post-surgery, the histopathological sections of untreated tendons were showed a dense collagen fibers with few cellularity and few MNs around blood vessels in the epitenon (Fig.8 A,B). While, the sections of treated tendons appears the presence of mature fibrous C.T. and large number of tenocytes extended between collagen fibers and B.Vs with MNCs in the endotenon area (Fig.8 C,D). The sections also appeared that there were no signs of adverse inflammatory reaction or rejection post-implantation of UBM along the study.

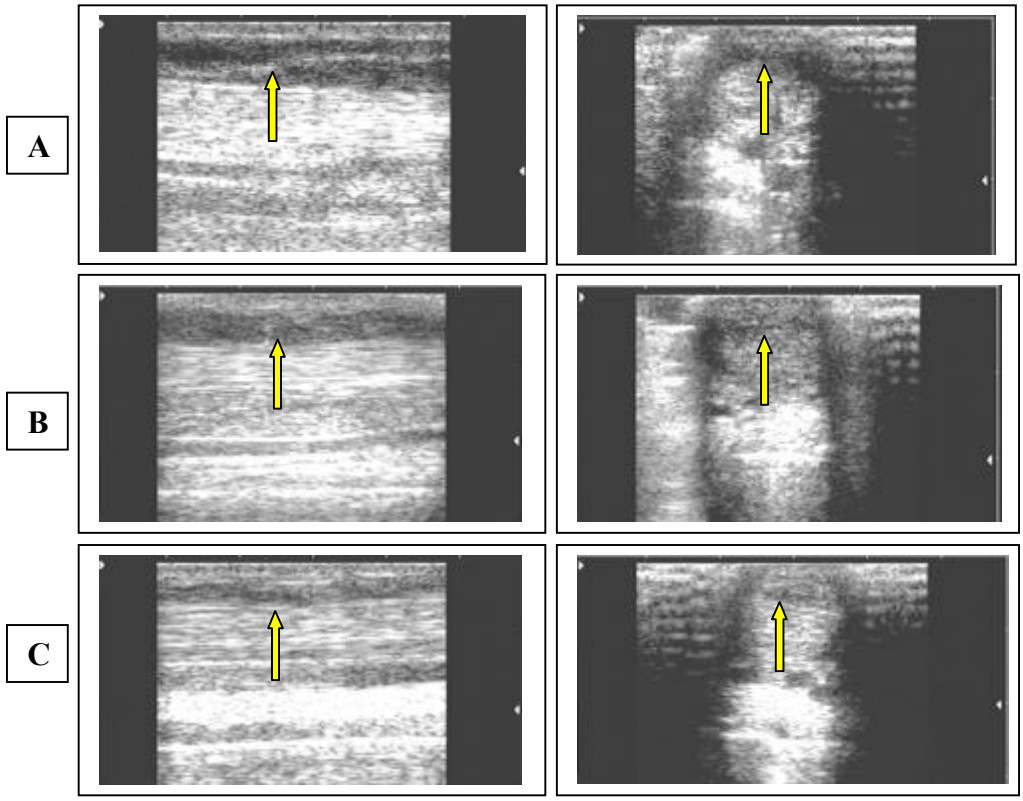

Fig.3: Shows the sequential longitudinal & transversal ultrasound images of SDFT of control group, at day of surgery (A), **8** weeks (B) and **16** weeks (C) post-surgery.

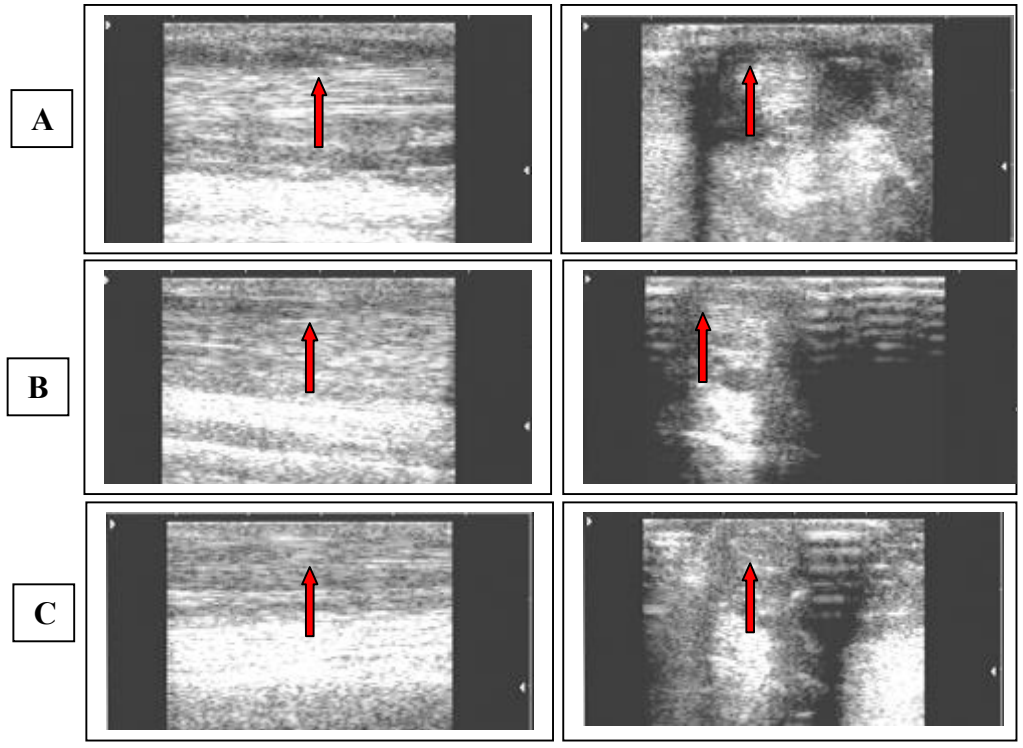

Fig.4: Shows the sequential longitudinal transversal ultrasound images of treatment group, at day of surgery (A), 8 weeks (B), and 16 weeks (C) post-UBM implantation.

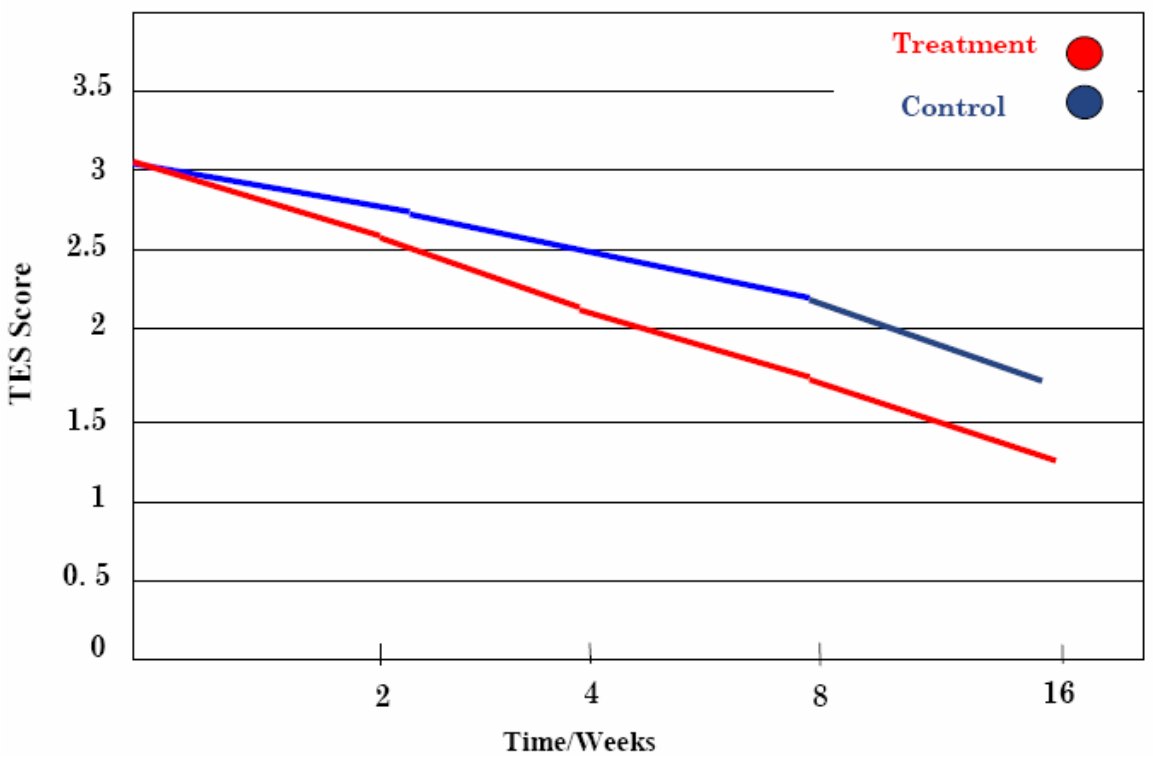

Fig.5: Shows means of TES scores for experimental groups in the different analyzed moments, post.

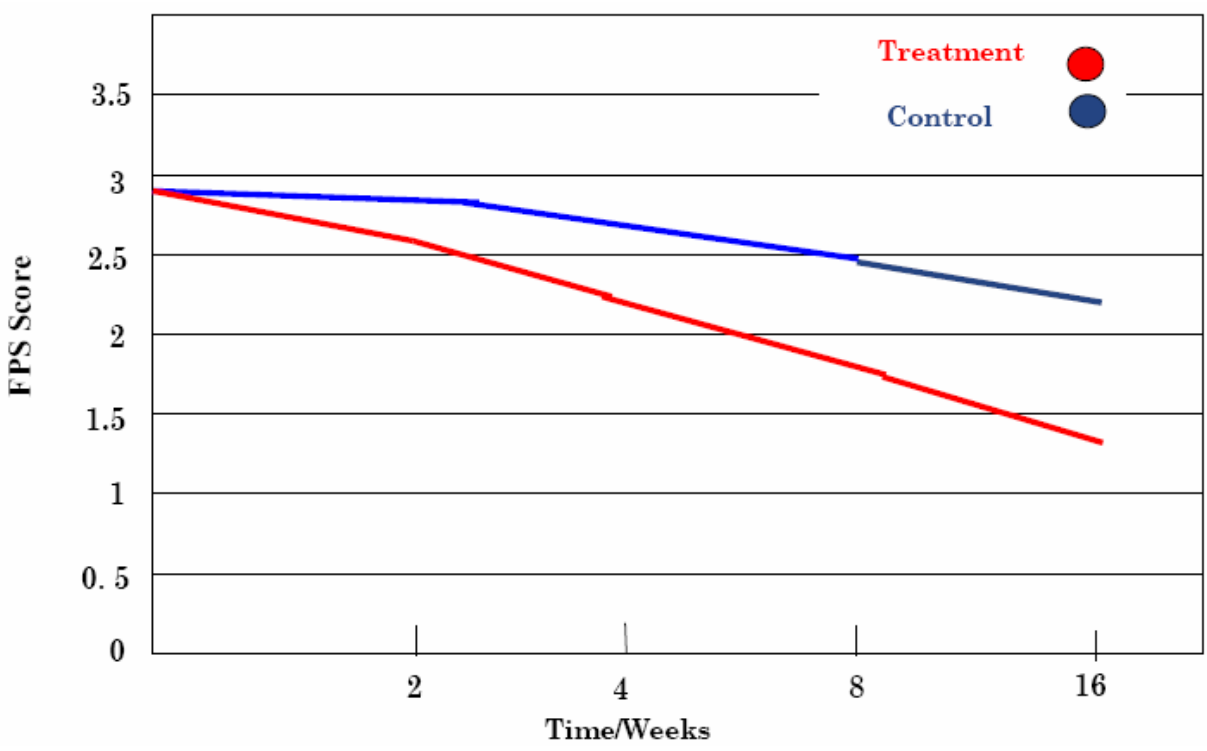

Fig.6: Shows means of FPS scores for experimental groups in the different analyzed moments, post-surgery.

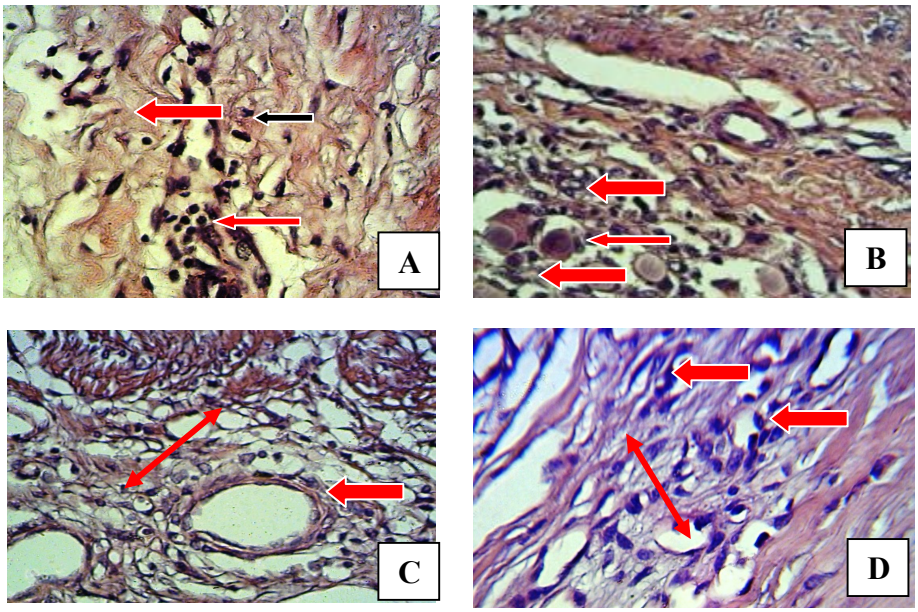

Fig.7: Histopathological sections of control group, **8**weeks post-surgery, shows immature fibrous C.T (thick arrow) infiltrate by few MNCs and few B.Vs (thin arrow) with scant proliferation of young tenoblasts (black arrow) (**A**), with fibrous C.T (thick arrow) infiltrate with slight MNcs around suture materials (thin arrow) (**B**). The sections of treatment group appears, at the same period, moderate proliferation of tenoblasts producing dense collagen fibers (double arrow) mixed with MNCs infiltrate and dilated B.V.s seen in the peritendon (thick arrow) (**C**). More B.Vs, high density of collagen fibers (double arrow) and more tenoblasts with few MNCs are seen in the endotenon of treated tendon (thick arrow) (**D**) (H&E40X).

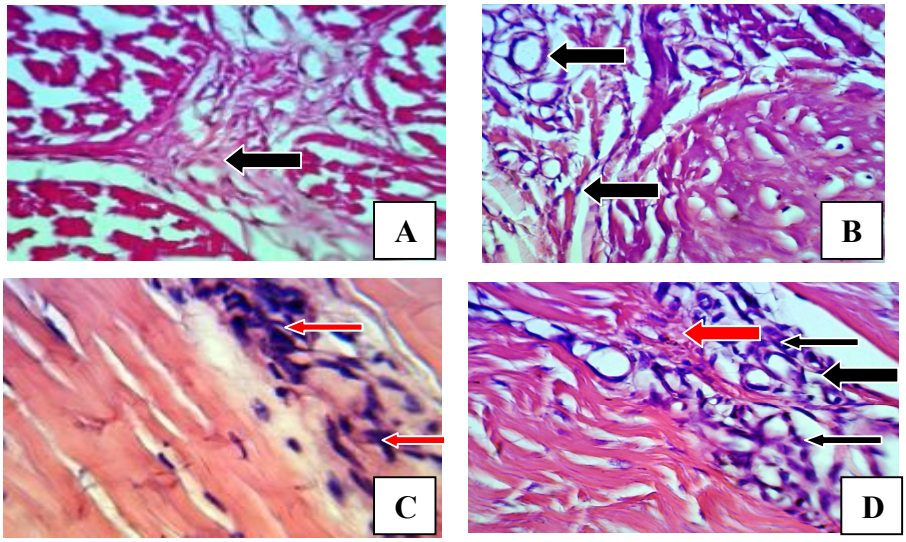

Fig.8: Histopathological sections of control group**, 16**weeks post-surgery, shows a dense collagen fibers with few cellularity and few MNCs around blood vessels (thick arrow) (**A,B**). The sections of treated tendons appears the presence of large number of tenocytes between collagen fibers (arrows) (**C**), and mature fibrous C.T. (red arrow) with large number of tenocytes extended between tendon fibers (thin arrows) and presence of B.Vs with MNCs in the endotenon area (thick arrow) (**D**) (H&E & V.G 40X,100X).

DISCUSSION

Tendon injury is significant affection in equine species due to its common occurrence (in both horse and human), the lack of a highly effective treatment, the requirement for lengthy rehabilitation and the risk of reinjury (19,20). So, incomplete healing of tendon injuries can lead to marked dysfunction and disability with compromised joint biomechanics, debilitating pain and returning to the same level of activity (21,22). There has been great interest in research of therapies that influence the quality or the speed of tendon repair like; low level therapeutic laser, pulsed magnetic therapy and ultrasound waves (23). Injection of platelets rich plasma (PRP), bone marrow or pure stem cells isolated from equine adipose tissue has been suggested to increase the rate and quality of healing of tendons and ligaments injuries (24,25).

Current studies regarding tendons and ligaments regeneration, emerged the field of tissue engineering through focusing mainly on ECM (ECM devices) reconstruction (4,7). Bladder lamina propria, commonly called UBM or bladder submucosa matrix (BSM) have been used as biomaterials for various reconstructive procedures due to their biocompatibility and regenerative potentials (26). The present study regard as the first randomized controlled clinical trial reporting the effectiveness of bovine UBM scaffold in tendon reconstruction of equine species. It demonstrated that the filling of tendon defect with UBM scaffold significantly improve the healing process of the tendon tissue. Through the ultrasound evaluation, it has revealed an increased echogenicity and the orientation of fibers was correctly parallel to the axis of the tendon earlier in the treatment group within (8-16)weeks post-implantation of UBM when compared to the control ones. According to correlation made previously by (27), a dark hypoechoic region within the normally highly echoic tendon is indicative of a tendon lesion. The low echo signal is related to fluid accumulation within the tendon and disorganized collagen fibrils in tendinopathic granulation tissue, while high signal (hperechogenicity) is related to decrease in the size of tendon injury, aligned collagen fibrils and may replace the damaged tissue by normal tissue or mature scar tissue.

The histopathological following-up revealed the enhancing of host cells infilteration, angiogenesis and restore of normal tissue artiticthture after local implantation of bovine UBM in the tendons defects, as well as, promote tissue regenration without evidence of fibrosis (as occured in untreated tendons), biodegraded and well biocompatability. The histopatholgical results of this study are in close to other studies that used UBM of different species to replace

the defects or enhancing the healing of other tissues rather than tendon ones. Porcaine UBM was used in the study of (28) and (6), to replace the achillis tendon and the urethral defects in human respectively, although, (12), treated osteoarthritis with a powdered procain UBM. This study suggested that UBM can provide a good supporting for tendon tissue healing process, but the mechanism by which the bioscaffold promote tendon healing remain to be elucidated. The current information about the mode action of ECM is largely based on pre-clinical data, mainly from researches focusing on procaine derived SIS and UBM. In the current study, it is proposed that the improvement in the injured tendons tissue may related to the role of UBM-ECM components these include structural and functional molecules; collagen, fibronectin, laminin, glycosaminoglycans and growth factors such as; transforming growth factor-beta (TGF-β), basic-fibroblast growth factor (b-FGF), platelets derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). These structures have an important effects upon the host response and the remodeling events that determine the eventual clinical outcome (29,9).

Many studies indicated histopathologically that UBM permits early remodeling and vascularization of injured tissue which can be potentially increase resistance to infection and adhesion formation. These facts explain why no infections and adhesions were recorded by this study. At the same time, (30), concluded in a study designed to determine the efficacy of UBM in collagenase-induced superficial digital flexors (SDFs), that UBM does not appear to be an effective treatment for collagenase-induced SDF tendonitis. They suspected that may be differences in clinical tendonitis that might render the treatment more effective in the clinical setting. Researchers have demonstrated that these matrices may act as a scaffold to support cell ingrowth and granulation tissue formation. They have receptors that permit fibroblasts to attach to the scaffold, stimulate angiogenesis and act as a chemo-attractant for endothelial cells. As a result, they markedly provide adequate microenvironment for cells that allows attachment, migration, proliferation and differentiation (31,5,32). (33), demonstrated that there are other potential advantages of the use of ECM grafts include; the capability to decrease the *in vivo* mechanical forces on the tendon repair during postoperative healing, to prevent repair gap formation or failure, to allow host cell infiltration and ideally even enhance the biology of healing, and to be replaced by organized host tissue over time.

The absent of signs of bioscaffolds rejection in the present study, as described by (9), which included; the post-operative infection, chronic immune response, aggravated of pain, implant failure and ongoing tendon tissue lysis, may be related to the procedure of removing

of any non-collagen components (acellularization) from the xenogeneic UBM scaffold. The interaction between scaffold surface and host cells is a key aspect of the use of scaffolds for tendon reconstruction. As a result, the biocompatibility and the inflammatory response associated with foreign body rejection is the major concern about both biological and synthetic scaffolds (10). So, the procedure of acellularization treatment aims to decrease the bio-burden and the risk of inflammatory or foreign body reactions, to reduce antigenicity by removing of any non-collagen components. Acellularization may also enhance host cell infiltration with phenotypically appropriate cells and possibly prevent transmission of infectious genomic vectors (34,35).

In conclusion, the present study revealed that ultrasound seems to be an important guidance to follow-up of tendon healing. This study also confirmed that the intralesional implantation of tendon with heterogeneic UBM-ECM was a simple, safe, had no side effects and result in evidence of the formation of a tissue organizationally similar to normal tendon tissue comparsion to the untreated tendons. This technique provides preliminary support for the clinical use of bovine UBM in treatment of equine tendon injuries.

REFERENCES

Dyson, S.J. (2003). Superficial flexor tendonitis in event horses, show jumpers, and dressage horses. Diagnosis and Management of Lameness in the Horse., W. B. Saunders, Pp:639-643.

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):1-11.

Coons, D.A. and Alan Barber, F. (2006). Tendon graft substitutes-rotator cuff patches. Sports Med. Arthrosc., 14:185-190.

Aurora, A.; McCarron, J.; Iannotti, J.P. and Derwin, K. (2007). Commercially available extracellular matrix materials for rotator cuff repairs: state of the art and future trends. J. Shoulder Elbow Surg., 16:171-178.

Longo, U.G.; Lamberti, A.; Maffulli, N. and Denaro, V. (2010). Tendon augmentation grafts: a systematic review. British Medical Bulletin, 94:165-188.

Rosario, D.J.; Reilly, G.C.; Salah, E.A.; Glover, M.; Bullock, A.J. and MacNeil, S. (2008). Decellularization and sterilization of procaine urinary bladder matrix for tissue engineering in the lower urinary tract. Regen. Med., 3(2):145-156.

Chen, J.; Xu, J.; Wang, A. and Zheng, M. (2009). Scaffolds for tendon and ligament repair: review of the efficacy of commercial products. Expert. Rev. Med. Devices, 6:61-73.

Badylak, S.F. and Gilbert, T.W. (2008). Immune Response to Biologic Scaffold Materials. Semin Immunol., 20(2):109-116.

Badylak, S.F.; Donald, O.F. and Thomas, W.G. (2009). Extracellular matrix as a biological scaffold material; material and function. Acta. biomaterialaia, 5:1-13.

Longo, U.G.; Petrillo, S.; Maffulli, N.; Lamberti, A. and Denaro, V. (2012). Scaffolds in Tendon Tissue Engineering. Stem Cell International, 8:1-8.

Brown, B.B.S.; Kristina, L.; Janet, R.M.S.; Donna, B.S. and Stephen, F.D. (2006). The basement membrane component of biological scaffold derived from extracellular matrix. Tissue engineering, 12:3.

Rose, M.W.; Wood, J.D.; Byrd, A.S. and Spievacl, A.R. (2009). Effect of a Xenogeneic Urinary Bladder Injectable Bioscaffold on Lameness in Dogs with Osteoarthritis of

the Coxofemoral Joint (Hip): A randomized, Double Blinded Controlled Trial. Intern. J. Appl. Res. Vet. Med., 7(1):13-22.

Eberli, D.; Attala, A. and Yoo, J.J. (2011). One and four layer acellular bladder matrix for fascial tissue reconstruction. J. Mater Sci, Mater Med., 22:741-751.

Dehghan, M.M.; Mehrjerdi, H.K.; Masoudifard, M.; Eslaminejad, M.R.; Sharifi, D. and Vajhi, A.L. (2007). Clinical and Ultrasonographical Findings of Collagenase Induce Tendonitis in the Horses. Iranian J. Vet. Surg., 2(2):47-58.

Genovese, R.L.; Rantanen, N.W. and Hauser, M.L. (1984). Diagnostic ultrasono-graphy of equine limbs. Vet. Clin. North Am. Equine Pract., 2:145-226.

Reef, V.B. (1998). Equine Diagnostic Ultrasound. Philadelphia: W.B. Sounder, Pp:139-186.

Luna, L.G. (1992). Histopathological methods and color atlas of special stains and tissue artifacts. Downers Grove: Johnson, P 767.

SAS. (2004). SAS/Stat Users Guide for Personal Computers. Release 7.0. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).

Smith, R.K.; Kordea, M.; Blunn, G.W. and Goodship, A.E. (2003). Isolation and implantation of autologous Equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. Equine Vet. J., 38(1):99-102.

Pastides, P.S. and Khan, W. (2011). Tendon and Ligament Injuries: The Evolving Role of Stem Cells and Tissue Engineering. British J. of Medicine and Medical Research, 1(4):569-580.

Longo, U.G.; Garau, G.; Denaro, V. and Maffulli, N. (2008). Surgical management of tendinopathy of biceps femoris tendon in athletes. Disabil. Rehabil., 30:1602-1607.

Smith, R.K. and Webbon, P.M. (2005). Harnessing the Stem Cell for the Treatment of Tendon Injuries: Heralding a New Dawn? Br. J. Sport Med., 39:582-584.

Rose, M.W. (2003). Surgical management of superficial digital flexor tendonitis, Diagnosis and Management of Lameness in the Horse. W. B. Saunders, Pp:635-639.

Herthel, D. (2004). Clinical use of stem cells and bone marrow components to stimulate suspensory ligament regeneration. Diagnosis and Management of Lameness in the Horse. W. B. Saunders, Pp:673-674.

Barreira, A.P.B.; Ana, L.I.Z.; Alves, A.L.G.; Mere, E.; Saito, M.E.; Renee, L.A.; Kohayagawa, A.; Menarim, B.C. and Mota, L.S. (2008). Autologous implant of bone marrow mononuclear cells as treatment of induced equine tendonitis. Internal J. Appl. Res. Vet. Med., 6(1):46-54.

Chun, S.Y.; Lim, G.J.; Kwon, T.G.; Kwak, E.K.; Kim, B.W.; Atala, A. and Yoo, J.J. (2007). Identification and characterization of bioactive factors in bladder submucosa matrix. Biomaterials, 28:4251–4256.

Avella, C.S. and Smith, R.K. (2005). Ultrasound evaluation of stem cell treated tendon injuries in the horse: Repair or regeneration. Ultrasound, 17:74-79.

Lee, M.S. (2004). Graft Jacket augmentation of chronic Achilles tendon ruptures. Orthopedics 27:151-153.

Hodde, J.P. Ernst, D.M. and Hiles, M.C. (2005). An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix. J. Wound. Care, 14(1):23-25.

Wallis, W.; Baxter, G.M.; MS, Natasha, M.S.; Werpy, M., Mason. G.L.; Frisbie, D.D. and Jarloev, N. (2010). Acellular Urinary Bladder Matrix in a Collagenase Model of Superficial Digital Flexor Tendonitis in Horses. J. of Equine Veterinary Science, 30(7):365-370.

Cornwell, K. G.; Adam, L. and Kenneth. S. J. (2009). Extracellular matrix Biomaterials for soft tissue repair, Clin. Podiatr. Med. Surg., (26):507-523.

Canonico, S.; Campitiello, F.; Della Corte, A.; Padovano. V. and Pellin, G. (2013). Treatment of Leg Chronic Wounds with Dermal Substitutes and Thin Skin Grafts. Skin Graft, Pp:51-76.

Valentin, J.E.; Badylak, J.S.; McCabe, G.P. and Badylak, S.F. (2006). Extracellular matrix bioscaffolds for orthopaedic applications: a comparative histological study. J. of Bone and Joint Surgery, 88(12):2673–2686.

Ketchedjian, A.; Jones, A. L. and Krueger, P. (2005) Recellularization of decellularized allograft scaffolds in ovine great vessel reconstructions. Annals of Thoracic Surgery, 79(3):888-896.

Lippi, G.; Longo, U. G. and Maffulli, N. (2010). Genetics and Sports. British Medical Bulletin, 93(1):27–47.