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Evaluation of Buffalo Amniotic Membrane Xenograft for Reconstruction of Induced Full-Thickness Tenotomy of Superficial Digital Flexor Tendon (SDFT) in Donkeys: Ultrasound/MRI Imaging-Guided Evaluations

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Abstract

Superfiicial digital flexor tendon (SDFT) is the most frequently injured tendon in equine. This study evaluated the healing efficacy of a fresh buffalo (*Bubalis bubalis*) amniotic membrane (AM) xenograft in repairing experimentally induced full-thickness tenotomy of the superficial digital flexor tendon (SDFT) in donkeys (*Equus africanus asinus*), serving as a model of equine superficial digital flexor tendon (SDFT) rupture. Twelve healthy male donkeys were randomly divided into two groups: the AM-treated group, where the transected SDFT was sutured with Vicryl size 2 and wrapped with AM as a scaffold. The second group was the positive control, where the tendon was sutured without AM. The contralateral limbs served as negative controls. Tendon healing was assessed using ultrasonography and magnetic resonance imaging (MRI). The AM-treated group demonstrated faster resolution of lameness, pain, and swelling compared to the positive control group. Ultrasonography revealed near-normal echogenicity and fiber alignment in the AM-treated tendons, whereas the control group exhibited heterogeneous echogenicity with adhesions. MRI findings showed low signal intensity, normal fiber alignment, and restored crescent-shaped morphology in the AM group, indicating structured remodelling. In contrast, the control group displayed high signal intensity, tendon thickening, and disorganized fibers. These results suggest that fresh buffalo AM xenografts significantly enhance tendon healing by accelerating functional recovery, reducing inflammation, and promoting organized tissue regeneration. The study supports the use of AM as a promising adjunct in tendon reconstruction, improving both structural and functional outcomes in tendon repair. This approach may offer a viable therapeutic strategy for managing SDFT injuries in equines.

Keywords: Amniotic membrane (AM), Buffalo, Donkey, MRI, SDFT, Tenorrhaphy, Ultrasound, and Xenograft.

Introduction

Tendon injuries remain one of the most challenging and economically significant problems in equine practice. They account for 30-50% of all musculoskeletal lameness cases in horses [1,2]. The superficial digital flexor tendon (SDFT) is most frequently injured (≈75% of cases), followed by the deep digital flexor tendon (DDFT) (15%) and suspensory ligament (SL) (10%) [3]. Regarding the affected limb, a staggering 97-99% of tendon injuries occur in the forelimbs [4,5]. Beyond their primary role in force transmission, the equine superficial digital flexor tendon (SDFT), the functional equivalent of the Achilles tendon (AT) in humans,

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serves critical energy-storing functions and enhances locomotor efficiency [6,7,8].

The severity of tendinopathies ranges from localized unilateral partial fiber microdamage to complete bilateral full-thickness ruptures [9]. While the mid-metacarpal region is the most common site of injury, lesions can develop at any point between the musculotendinous junction and the insertion branches [10].

Rupture of the superficial digital flexor tendon (SDFT) is a severe and potentially career-ending injury in horses, with well-predictable etiologies including trauma, chronic sepsis, and exercise-induced degeneration. Traumatic lacerations, whether partial or complete, can lead to acute tendon failure and have been described in cases were sharp trauma or penetrating wounds compromise tendon integrity [11]. In cases of chronic sepsis within the digital sheath, persistent infection and subsequent tendon necrosis may result in spontaneous rupture, particularly if treatment is delayed or ineffective [12]. Additionally, high-intensity exercise remains a major risk factor, as repetitive strain and micro damage can lead to progressive tendon degeneration and eventual rupture, particularly in racehorses and elite sport horses [13].

Traditional methods of tendon repair often yield suboptimal outcomes, as healed tendons frequently develop scar tissue with inferior biomechanical properties compared to healthy tendon tissue, increasing the risk of reinjury [8]. Even with recent advancements in orthopedic surgery, chronic, non-healing tendon injuries often require surgical treatment. Moreover, there is a considerable risk of re-rupture since many common surgical instruments, such as suture anchors, cannot regenerate the enthesitis at the tendon–bone interface [14]. Various surgical strategies have been explored to improve tendon repair, including the use of prosthetic materials, bioabsorbable scaffolds, tendon autografts/allografts, and specialized suture patterns [15].

While these methods can provide initial stabilization, they often do not replicate the native tendon's structural integrity or functional elasticity. Additionally, synthetic materials may provoke inflammatory reactions, whereas bioabsorbable options may degrade before sufficient tissue regeneration occurs [8].

Emerging regenerative therapies, such as stem cell therapy, platelet-rich plasma (PRP), and extracellular matrix (ECM) scaffolds, aim to enhance tendon healing by promoting organized collagen deposition and reducing fibrosis [16]. However, challenges remain in achieving full enthesis regeneration, particularly in high-motion, high-load environments like the equine limb [17].

Given these limitations, future research focuses on bioengineered tendon constructs, growth factor delivery systems, and biomimetic materials that better replicate native tendon mechanics and biology [16]. Until such innovations become clinically viable, a combination of surgical stabilization, controlled rehabilitation, and adjunctive regenerative therapies remains the best approach to optimize outcomes in tendon repair.

The amniotic membrane (AM) has emerged as a promising regenerative therapy due to its anti-inflammatory, anti-fibrotic, and pro-healing properties. It is particularly valuable in managing tendonitis, desmitis, and suspensory ligament injuries in equine. Tendon injuries trigger an excessive inflammatory response, leading to fibrosis and scar tissue formation, which compromises tendon elasticity and strength. The amniotic membrane acts as a physical barrier, reducing inflammation by trapping immune cells (e.g., T cells and macrophages) and suppressing pro-inflammatory cytokines [18]. Additionally, amniotic epithelial cells (AECs) secrete transforming growth factor beta (TGF-β) inhibitors, Interleukin-10 (IL-10), and prostaglandin E2 (PGE2), which modulate the immune response and prevent excessive collagen deposition [19]. This helps maintain better tendon architecture and reduces the risk of adhesion and reinjury.

Furthermore, the extracellular matrix (ECM) of the AM is rich in collagen type I and III, fibronectin, and elastin, which closely mimic the natural tendon structure, providing a scaffold for tenocyte migration and proliferation [20]. Growth factors such as platelet-derived growth factors (PDGFs), vascular endothelial growth factor (VEGF), present in the AM enhance angiogenesis and tissue remodeling, accelerating functional recovery. Studies in human and veterinary medicine suggest that AM-derived stem cells can differentiate into tendon-like cells, further supporting tissue regeneration [21].

The amniotic membrane meets all five criteria for an ideal tendon scaffold, offering: 1. Excellent mechanical properties to provide sufficient strength and stiffness of the tendon to withstand the stress and tensile forces generated by the surrounding environment; 2. Favorable biological functionality to support cell adhesion, growth, proliferation, and differentiation to facilitate matrix secretion and tendon tissue development; 3. Strong biodegradability and resorption rate to match the cell growth rate of the repaired tissue; 4. Low immunogenicity and satisfying biocompatibility with the host both pre- and post-degradation; and 5.

Excellent processability that enables fabrication into intricate structures and shapes, such as knitting, weaving, and electrospinning. Its natural composition, processability, and clinical efficacy position it as a superior alternative to synthetic grafts or standalone cell therapies [22].

The bovine amniotic membrane (BAM) has gained attention in regenerative medicine due to its unique biological properties. Bovine amniotic membrane as a xenograft is a versatile, cost-effective biomaterial with broad applications in wound healing, ophthalmology, dentistry, and surgery. While it shares many benefits with human amniotic membrane, its greater availability, larger surface area, fewer legal or ethical concerns, and lower cost make it a promising alternative. Ongoing research aims to expand its use in advanced tissue engineering and personalized medicine [23,24]. Despite the therapeutic potential of bovine amniotic membrane-derived mesenchymal stem cells (BAMSCs), research on buffalo (Bubalus bubalis) AMSCs remains limited. To date, only a few articles have studied the characteristic features of buffalo amniotic membrane- and amniotic fluid-derived AMSCs [25-31], highlighting a gap in translational research.

Ultrasonography is the gold standard for non-invasive assessment of equine soft tissue injuries, offering unparalleled visualization of musculoskeletal structures. This imaging modality proves particularly valuable for evaluating tendons and ligaments in the metacarpal and pastern regions, with specialized application in examining the suspensory apparatus [32]. Recent studies have demonstrated its efficacy in establishing baseline measurements, including the normal cross-sectional area of tarsal ligaments in Standardbreds [33], while also detecting pathological changes in the palmar/plantar structures of gaited horses' metacarpal and metatarsal regions [34]. The technique's diagnostic precision and repeatability make it indispensable for both clinical evaluation and research applications in equine sports medicine [35].

Ultrasonography remains the first-line diagnostic tool for routine tendon screening, and MRI provides definitive, high-precision imaging for complex or ambiguous cases. Its ability to reveal occult lesions, assess healing progression, and guide treatment decisions makes it indispensable in advanced equine sports medicine, particularly for high-value performance horses where early, accurate diagnosis is critical to preserving athletic function [36].

This study aimed to evaluate the healing quality of experimentally induced full-thickness tenotomy of the Superficial digital flexor tendon (SDFT) in donkeys (*Equus africanus asinus*), as a model of equine SDFT rupture, treated using tenorrhaphy assisted with xenograft Buffalo (*Bubalis bubalis*) fresh amniotic membrane scaffold using multimodality diagnostic imaging (Ultrasound and MRI).

Material and Methods

Experimental animals:

The study comprised twelve clinically healthy male donkeys. They were kept in the Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Cairo University. Food and water were introduced to animals ad libitum. The animals were randomly divided into two equal groups (positive control and AM groups). The contralateral limb in each donkey was used as the negative control group, where no surgical intervention was performed. This study was carried out in compliance with the ARRIVE guidelines [37] and the Studies of Diagnostic Accuracy STARD guidelines [38].

Preparation of amniotic membrane:

Amniotic membranes were collected from fresh placenta delivered by normal birth, with an intact membrane obtained from three buffalo. After the chorion was peeled out, it was washed with sterile saline solution to get rid of any debris. The membrane was completely cleaned under aseptic conditions using sterile normal saline that contained 0.025 mg/ml amphotericin B, 100 U/ml penicillin, and 0.2 mg/ml streptomycin (Pen & Strept; Norbrook, the Netherlands). In a sterile plastic Petri dish, the membrane was repeatedly washed with a standard saline solution (Figure 1). The epithelial side was marked with Mer-silk 4/0 suturing material. After that, AM was put in a Petri dish with the same mixture. Specimens of amniotic membranes were preserved for use within one week and kept in a refrigerator at 4 °C [39].

Surgical technique:

Animal preparation

All donkeys underwent physiological assessment (temperature, heart rate [HR], respiratory rate [RR], capillary refill time [CRT]) and gait analysis to confirm normal musculoskeletal function and absence of lesions. Eligible animals were dewormed with piperazine citrate (40 g/100 kg body weight; Piperazine Citrate 100%®, UCCMA, Egypt) to eliminate parasitic confounders [40]. Food was withheld for eight hours before the operation to minimize the risk of regurgitation and aspiration pneumonia, consistent with standard preoperative fasting guidelines for equines [41]. A prophylactic intramuscular dose of Pen & Strep® (procaine penicillin dihydrostreptomycin sulfate, 10 ml/donkey) was administered 30 minutes before

surgery to reduce the risk of postoperative infections, following recommendations for perioperative antimicrobial use in equine surgery [42]. The mid-metacarpal region of the left forelimb was clipped, shaved, and aseptically prepared using BETADINE® antiseptic Solution 10% (povidone-iodine, Mundipharma, Egypt), following a standardized surgical scrub protocol to minimize bacterial load [43]

Anaesthesia Protocol

Anaesthesia was induced using intravenous administration of 1.1 mg/kg B.W. of xylazine Hcl (XYLAJECT 2%, Adwia Pharmaceuticals Co., Egypt) and 2.2 mg/kg B.W. of Ketamine Hcl (Ketamine, Rotexmedica 50 mg/ml, Germany). Maintenance of anaesthesia during the surgical procedure was achieved using a triple-drip combination consisting of Guaifenesin (25 g), Ketamine (500 mg), and Xylazine (250 mg), in 500 mL normal saline solution given by intravenous route [44, 45].

Surgical full-thickness tenotomy of the Superficial Digital Flexor Tendon

Following the induction of general anaesthesia, proper positioning in lateral recumbency and tourniquet application below the carpal joint to minimize haemorrhage, a complete transverse full-thickness tenotomy of the superficial digital flexor tendon (SDFT) was performed in the left forelimb at the mid-metacarpal region (Figure 2) [40]. Donkeys in the AM group were subjected to superficial digital flexor full-thickness tenotomy and then sutured using the modified Kessler technique (Fig.2. E). AM was wrapped around the sutured tendon and fixed with Vicryl size 2 using a simple interrupted suture pattern (epithelial surface of AM outside and endothelial surface facing the tendon) (Fig.2. D). The donkeys in the positive control group were subjected to superficial digital flexor full-thickness tenotomy and then treated with suturing only (Fig.2. C). The operated limb was kept in a soft bandage in combination with a splint from the hoof to above the carpal joint for fixation, in addition to application of a high-heel caulkin shoe as a supporting shoe to reduce the weight-bearing on the SDFT.

Post-operative care:

Following surgery, all donkeys received a 5-day course of intramuscular antibiotics using Pen & Strep® (Procaine Penicillin 200 mg and Dihydrostreptomycin Sulphate 250 mg, Norbrook, UK) at a dose of 10 ml daily. Skin sutures were removed 10 days postoperatively. All animals received 1500 IU of antitetanic serum administered subcutaneously during recovery [43].

Method of evaluation:

Clinical index score

All the animals were maintained under continuous clinical observation post-surgery, with subsequent twice-daily monitoring throughout the recovery period. Donkeys underwent systematic clinical evaluations at 2, 4, 6, 8, 10, and 12 weeks postoperatively. This comprehensive surveillance protocol included: subjective evaluation of clinical signs (including lameness grade, discomfort, and pain); and objective circumferential limb measurements at the repair site (Table 1).

Lameness was evaluated at each experimental time point using a 0–3 grading scale, where 0 indicated no lameness, 1 represented mild lameness (subtle gait irregularity), 2 denoted moderate lameness (obvious limping with partial weight-bearing), and 3 reflected severe lameness (inability to bear weight) [46]. Pain was monitored postoperatively through clinical and behavioral indicators, including reluctant or difficult ambulation, prolonged recumbency, and elevations in pulse rate, respiratory rate, or rectal temperature. Discomfort was assessed based on deviations from normal behavior, such as reduced activity, decreased appetite, and alterations in alertness or attitude. The frequency of limb hanging (spontaneous lifting of the affected limb) and extended periods of recumbency were also recorded as signs of discomfort [15].

To objectively evaluate swelling, limb circumference was measured at the mid-metacarpal region in three levels (IIA, IIB, and IIIA) (Figure 2) using a standardized tape measure. Baseline measurements were taken in the contralateral (right) limb of each donkey as the negative control group, and both positive control and AM-treated donkeys directly after the operation (week 0), with follow-up assessments conducted postoperatively at 2, 4, 6, 8, 10, and 12 weeks for negative & positive control and AM-treated groups [15].

Ultrasound evaluation:

Careful preparation of the skin was done for optimal ultrasound imaging, including clipping of the palmar metacarpal region from the level just distal to the accessory carpal bone down to the ergot, The skin was thoroughly scrubbed, degreased using surgical spirit (alcohol) before applying a liberal amount of coupling gel [47]. A high-frequency (8 MHz) linear array transducer, Hitachi Aloka F31 ultrasound machine (Japan), was used for evaluation. The examination was performed in both weight-bearing and non-weight-bearing positions, transverse and longitudinal planes, and sagittal and transverse scans. Regarding standardized anatomical reference points, the metacarpal region was divided into three

equal tiers (proximal, middle, and distal), designated as Zones I to III from the carpometacarpal joint to the proximal edge of the palmar annular ligament at the fetlock [48,32]. (Figure 2).

Quantitative evaluation of tendon echogenicity was performed using grayscale brightness analysis, where pixel intensity values ranged from 0 (black) to 255 (white). This was accomplished using specialized image analysis software (Image J, NACL Co. Ltd., Tokyo, Japan) to calculate the mean grayscale level within a defined region of interest (ROI) on cross-sectional ultrasonographic images [49].

MRI evaluation:

Magnetic resonance imaging (MRI) evaluations of the operated limbs were performed post-euthanasia at 6 and 12 weeks following superficial digital flexor tendon (SDFT) tenorrhaphy in each experimental group. All imaging was conducted at Police Heights Equine Hospital using the O-Scan Equine MRI system (ESAOTE S.P.A., Genova, Italy), a specialized low-field MRI unit designed for equine musculoskeletal assessment. The system specifications included an input voltage of 100-120V/220-240V, operating frequency of 50/60 Hz, and power consumption of 1000 VA [10].

A comprehensive imaging protocol was implemented utilizing three standard sequences: gradient echo T1-weighted for anatomical detail, fast spin echo T2-weighted for evaluation of fluid accumulation and tissue integrity, and short-time inversion recovery (STIR) sequences for enhanced detection of edema and inflammation. All sequences were acquired in sagittal, transverse, and frontal planes with a consistent 4-mm slice thickness to ensure thorough evaluation of the surgical site [50].

Statistical analysis

Kolmogorov–Smirnov test was conducted to confirm that the data were within normal distribution. The descriptive statistics (Mean ± SD) for "transverse dimension, Longitudinal dimension, CSA, Echogenicity of SDFT" in each AM, Control, and Normal groups were calculated then the differences between the three groups were evaluated by one-way ANOVA (Table 3) and post hoc test (Scheffe test) was conducted to detect which groups differ (Table 4).

Moreover, the descriptive statistics (Mean ± SD) for "Lameness, Discomfort, Pain and Limb Circumference" scores in each AM and Control group were calculated, and the Pearson Chi-Square test was used to evaluate the differences in scores between the two groups (Table 2).

All statistical tests were conducted using SPSS (IBM SPSS version 25 × 64-bit edition), and the data were considered statistically significant when the P Value < 0.05.

Results

Clinical index score

The results of the subjective evaluation of clinical signs, including lameness, discomfort, and pain scores, were recorded (Table 2) (Fig. 3).

The objective evaluation of limb circumference was measured at the mid-metacarbal region in three levels (the tenorrhaphy site (IIB) and its proximal (IIA) and distal (IIIA) levels) of AM, positive, and negative control groups at 0, 2, 4, 6, 8, 10, and 12 weeks postoperatively (Table 2) (Fig. 4).

Ultrasound evaluation:

The ultrasound evaluation of AM, positive, and negative control groups was performed to measure the size of SDFT, including Transverse (T) and Longitudinal (L) dimensions, and Cross-sectional area (CSA); and quantitative evaluation of tendon echogenicity was performed using grayscale brightness analysis to measure the Echogenicity at 4, 8, and 12 weeks postoperatively (Table 3) (Figs. 5, 6, and 7). The sagittal and transverse ultrasonographic scans of the SDFT at the palmar aspect of the mid-metacarpal region (zone IIB) revealed, in the amniotic membrane group, thickening of the SDFT with heterogenous echogenicity (Figs. 5A & 6A), presence of hypoechoic gap within the tendon fiber due to reaction of amniotic membrane during healing of SDFT (Fig. 5B & 6B), and increase in echogenicity near to normal with normal size and without adhesion with deep digital flexor tendon (DDFT) (Fig. 5C & 6C) at 4, 8, 12 weeks post-tenorrhaphy respectively. In the control positive group, showing decreased SDFT echogenicity (Figs. 5A & 6A), showing increased SDFT echogenicity and an increase in its size (Fig.5B & 6B), and showing loss of SDFT shape with severe adhesion with DDFT with heterogenous echogenicity (Fig. 5C & 6C) at 4, 8, 12 weeks post-tenorrhaphy, respectively.

MRI evaluation:

Magnetic resonance imaging (MRI) evaluations of the SDFT-operated limbs were recorded in the amniotic membrane group of transverse and sagittal planes at 6- and 12-week post-tenorrhaphy (Fig. 8). The results showed that the graft bed (AM Wrapped the SDFT) was a highly intense area with an increase in size at 6 weeks post-tenorrhaphy. SDFT returned to normal crescent shape and size with low signal intensity of the grafted tendon with normal fiber alignment at 12 weeks post-tenorrhaphy.

MRI assessments of the SDFT-operated limbs of the positive control group were noted in the same planes and times (Fig. 8). The results illustrated a moderate increase in signal intensity at 6 weeks post-tenorrhaphy. SDFT showed high signal intensity with tendon thickening, an incomplete tendon fiber alignment pattern, homogeneity, and severe adhesion at 12 weeks post-tenorrhaphy.

Discussion

The superficial digital flexor tendon (SDFT) plays a crucial role in the stay apparatus of the equine, with special emphasis on the donkey limb, which significantly contributes to the support of the fetlock joint and the dissipation of forces during locomotion [51].

In the present study, the experiment depended on the hypothesis of regenerative power of amniotic membrane as a biological scaffold and source of stem cells that can enhance the healing steps of equine tendon rupture via restoring tendon elasticity and limiting/preventing the adhesion with neighboring tissues [52].

Donkeys were a good, reliable, ethical, and cost-effective alternative model of equine SDFT rupture as it has the same anatomical characteristics of the horse metacarpal region, and alignment of flexor tendons and ligaments, in addition to the symmetrical pathophysiology of the tendon healing [53]. On the other hand, horses are heavier, taller, faster, more svelte, and sensitive than donkeys [54].

In the current study, amniotic membrane implant of induced full-thickness SDFT tenotomy was assisted with fresh xenograft Buffalo (Bubalis bubalis) amniotic membrane. Bovine fetal membranes, including the bovine amniotic membrane (BAM) and allantoic membrane, have been proposed as effective biological dressings for wound healing [55]. Early experimental work by Silvetti et al. (1957), as cited by Rao, first introduced the concept of using bovine amnion xenografts in medical applications [56]. Since this pioneering study, extensive basic and clinical research has explored the therapeutic potential of bovine amniotic membranes in regenerative medicine, demonstrating their utility in tissue repair and regeneration [23]. According to the authors' knowledge, no available literature exists about using buffalo amniotic membrane in medical applications. While the availability of buffalo amniotic membrane is more than that of cattle one in the Egyptian large animal clinics and farms, in addition to a wider surface area, and is worth nothing for human consumption, it emerges as a highly promising source for regenerative therapies, and it seems like a discovery of a lost treasure in the waste.

From the obtained results, the clinical evaluation of lameness, discomfort, pain, and limb circumference revealed progressive improvement in both the amniotic membrane (AM)-treated and control groups over the 12-week postoperative period. However, the AM-treated group demonstrated earlier resolution of clinical signs, particularly in pain reduction and swelling. By 8–10 weeks postoperatively, the AM group exhibited significantly lower pain scores when compared to the control group ($p < 0.05$), suggesting an accelerated recovery phase. These findings align with previous studies indicating that AM possesses potent anti-inflammatory and analgesic properties [analgesic products such as anandamide (AEA), oleoylethanolamide (OEA), palmitoyl ethanolamine (PEA)], likely due to its rich composition of growth factors and immunomodulatory cytokines [57, 58].

Limb circumference measurements, an objective indicator of postoperative inflammation, further supported these observations. The AM-treated group showed significantly reduced swelling at the tenorrhaphy site (IIB) and adjacent regions (IIA, IIIA) by 6–8 weeks ($p < 0.05$), whereas the control group maintained persistent edema. This reduction in swelling may be attributed to AM's ability to suppress pro-inflammatory cytokines such as TNF-α and IL-6, as demonstrated in previous tendon healing models [59]. By 12 weeks, both groups exhibited complete resolution of lameness and discomfort, indicating functional recovery, but the earlier improvements in the AM group suggest a more efficient healing trajectory.

In the present experiment, the AM-treated tendons exhibited initial thickening (4–8 weeks) followed by progressive normalization in transverse (T) and longitudinal (L) dimensions, whereas the control group displayed delayed and irregular remodeling Fig (5,6). Notably, the cross-sectional area (CSA) of AM-treated tendons stabilized by 12 weeks, while the control group showed persistent enlargement, indicative of fibrotic scarring. These findings are consistent with studies demonstrating that AM enhances extracellular matrix organization by promoting collagen type I synthesis over collagen type III, thereby reducing fibrosis [60].

Echogenicity analysis further highlighted the superior tissue quality in AM-treated tendons. At 4 weeks, heterogeneous echogenicity in the AM group suggested active remodeling, whereas the control group exhibited hypoechoic regions, reflecting disorganized fiber structure [49]. By 12 weeks, the AM-treated tendons achieved near-normal echogenicity without adhesions, while the control group developed severe adhesions and heterogeneous echotexture. This aligns with prior research

indicating that AM prevents peritendinous adhesions by modulating Transforming Growth Factor Beta 1 TGF-β1-mediated fibrosis [50].

Magnetic resonance imaging (MRI) corroborated these findings, demonstrating that AM-treated tendons exhibited high signal intensity (edema) at 6 weeks but transitioned to low signal intensity (normal tendon) with well-aligned fibers by 12 weeks. In contrast, the control group displayed persistent high signal intensity, tendon thickening, and adhesions, consistent with failed remodeling. These MRI findings support the research work that AM promotes organized collagen realignment, as previously observed in equine tendon repair models [61,62].

Conclusion

The results of this study demonstrated that fresh buffalo amniotic membrane xenograft-derived scaffold enhanced tendon healing of donkey SDFT, after induced full-thickness tenotomy, by accelerating functional recovery, reducing inflammation, and promoting structured remodelling. The AM-treated group exhibited earlier resolution of pain and swelling, superior tendon architecture on ultrasound, and near-physiological tendon morphology on MRI compared to controls. These benefits were likely mediated by AM's anti-inflammatory, antifibrotic, and pro-regenerative properties. Further studies should investigate long-term biomechanical strength, histopathological, and biomolecular studies to further validate these findings. Nevertheless, this study supported using AM as a promising adjunct in tenorrhaphy, offering improved functional and structural outcomes in tendon repair.

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Declaration of Conflict of Interest

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethical of approval

The study protocol was approved by the Institutional Animal Care and Use Committee (Faculty of Veterinary Medicine, Cairo University) (Vet CU131020241062).

TABLE 1. The clinical index scores for subjective assessment of clinical parameters in donkeys subjected to digital flexor tendon tenorrhaphy

Clinical index	Description and level
Lameness	0 = negative; 1 = mild; 2 = moderate; 3 = severe
Discomfort	0 = comfort; 1 = discomfort
Pain	0 = negative; 1 = mild; 2 = moderate; 3 = severe
Limb circumference	0 = 15cm; 1 = 16cm; 2= 18cm; 3= 20cm

TABLE 2. Results of the clinical index scores of AM and the positive control groups just after tenorrhaphy (0W) and at 2, 4, 6, 8, 10, and 12 weeks postoperatively (Pearson Chi-Square)

Parameters		AM **Treated**			Positive **Control**			
		Mean ± Sd	**Min.**	**Max.**	**Mean ± Sd**	**Min.**	**Max.**	**Pearson Chi-Square**
Lameness Score	**0W**	2.67 ± 0.58	2	3	3 ± 0	3	3	0.273
	2W	2.33 ± 0.58	2	3	2.67 ± 0.58	2	3	0.414
	4W	2.33 ± 0.58	2	3	2.33 ± 0.58	2	3	1.000
	6W	1.67 ± 0.58	1	2	2 ± 0	2	2	0.273
	8W	1.67 ± 0.58	1	2	1.67 ± 0.58	1	2	1.000
	10W	1.33 ± 0.58	1	2	1.33 ± 0.58	1	2	1.000
	12W	0	0	0	0	0	0	------
Discomfort	**0W**	1 ± 0	1	1	1 ± 0	1	1	------
	2W	1 ± 0	1	1	1 ± 0	1	1	------
	4W	0.67 ± 0.58	0	1	1 ± 0	1	1	0.273
	6W	0.67 ± 0.58	0	1	0.67 ± 0.58	0	1	1.000
	8W	0.33 ± 0.58	0	1	0.67 ± 0.58	0	1	0.414
	10W	0.33 ± 0.58	0	1	0.33 ± 0.58	0	1	1.000
	12W	0	0	0	0	0	0	------
Pain	**0W**	3 ± 0	3	3	2.67 ± 0.58	2	3	0.273

	2W	2 ± 1	1	3	2.67 ± 0.58	2	3	0.513
	4W	1.67 ± 0.58	1	2	2.33 ± 0.58	2	3	0.368
	6W	1.33 ± 0.58	1	2	1.67 ± 0.58	1	2	0.414
	8W	1 ± 0	1	1	1.67 ± 0.58	1	2	0.083
	10W	0.33 ± 0.58	0	1	1.33 ± 0.58	1	2	0.189
	12W	0	0	0	0	0	0	------
Limb Circumference (IIA)	**0W**	0	0	0	0	0	0	------
	2W	0	0	0	0.33 ± 0.58	0	1	0.273
	4W	0.33 ± 0.58	0	1	0.67 ± 0.58	0	1	0.414
	6W	1.33 ± 0.58	0	1	1.67 ± 0.58	1	2	0.189
	8W	0.67 ± 0.58	1	2	1.67 ± 0.58	1	2	1.000
	10W	0.67 ± 0.58	0	1	1.33 ± 0.58	1	2	0.189
	12W	0	0	0	0.33 ± 0.58	0	1	0.273
Limb Circumference (IIB)	**0W**	0	0	0	0	0	0	------
	2W	1.33 ± 0.58	1	2	1.33 ± 0.58	1	2	1.000
	4W	1.67 ± 0.58	1	2	2.33 ± 0.58	2	3	0.368
	6W	2 ± 1	1	3	2.33 ± 0.58	2	3	0.513
	8W	1.33 ± 0.58	1	2	2 ± 1	1	3	0.513
	10W	0.33 ± 0.58	0	1	0.67 ± 0.58	0	1	0.414
	12W	0	0	0	0.33 ± 0.58	0	1	0.273
Limb Circumference (IIIA)	**0W**	0	0	0	0	0	0	------
	2W	0.33 ± 0.58	0	1	0.33 ± 0.58	0	1	1.000
	4W	0.67 ± 0.58	0	1	1 ±0	1	1	0.273
	6W	1.33 ± 0.58	1	2	1.67 ± 0.58	1	2	0.414
	8W	0.67 ± 0.58	0	1	1.33 ± 0.58	1	2	1.000
	10W	0.33 ± 0.58	0	1	0.33 ± 0.58	0	1	1.000
	12W	0	0	0	0.33 ± 0.58	0	1	0.273

The contralateral limb in each donkey was used as the negative control group with a score of 0 for Lameness, Discomfort, Pain, and Limb circumference.

TABLE 3. Results of the ultrasound evaluation of AM, positive, and negative control groups, including Transverse (T) and Longitudinal (L) dimensions, Cross-sectional area (CSA), and Echogenicity (Echo) of SDFT at 4, 8, and 12 weeks postoperatively (One-way ANOVA test).

Parameters		**AM Treated**			**Positive Control**			**Negative Control**			
		Mean ± SD	**Min.**	**Max.**	**Mean ± SD**	**Min.**	**Max.**	**Mean ± SD**	**Min.**	**Max.**	
(T)	**4W**	3.38 ± 0.2	3	3.6	2.35 ± 0.13	2.2	2.5	1.9 ± 0.66	0.9	2.7	≤ 0.0001
	8W	3.07 ± 0.34	2.8	3.7	2.83 ± 0.31	2.4	3.1				0.003
	12W	2.37 ± 0.16	2.2	2.6	3.03 ± 0.26	2.8	3.4				0.006
(L)	**4W**	4.57 ± 0 .3	4	4.8	3.08 ± 0.22	2.8	3.3	1.62 ± 0.39	1.2	2.2	≤ 0.0001
	8W	3.42 ± 0.57	2.7	4.1	3.13 ± 0.26	2.9	3.5				≤ 0.0001
	12W	2.18 ± 0.56	1.5	2.8	3.23 ± 0.22	3	3.5				0.001
CSA	**4W**	5.03 ± 0.5	4.5	5.5	3.5 ± 0.8	2.7	4.3	2.55 ± 0.58	2	3.1	0.004
	8W	2.9 ± 0.26	2.6	3.1	4.6 ± 0.76	4.1	5.5				0.008
	12W	2.63 ± 0.23	2.5	2.9	4.83 ± 1.17	3.5	5.7				0.010
ECHO	**4W**	41.55 ± 3.14	37	44	34.03 ± 5.85	28.1	42	47.43± 3.96	42	53.3	0.001
	8W	40.26 ± 13.21	24	58	40.75 ± 6.6	32	48				0.295
	12W	46.4 ± 4.08	41.5	50	50.93 ± 2.3	48.3	53.1				0.215

TABLE 4. Results of the ultrasound evaluation of AM, positive, and negative control groups, including Transverse (T) and Longitudinal (L) dimensions, Cross-sectional area (CSA), and Echogenicity (Echo) of SDFT at 4, 8, and 12 weeks postoperatively using Post hoc test (Scheffe test).

Parameters		P Value					
		AM		Control		Normal	
		Control	Normal	Treated	Normal	Treated	Control
(T)	AM4	0.002	≤ 0.0001	0.002	0.247	≤ 0.0001	0.247
	AM8	0.747	0.004	0.747	0.036	0.004	0.036
	AM12	0.106	0.224	0.106	0.006	0.224	0.006
(L)	AM4	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001
	AM8	0.618	≤ 0.0001	0.618	0.001	≤ 0.0001	0.001
	AM12	0.015	0.182	0.015	0.001	0.182	0.001
CSA	4W	0.058	0.004	0.058	0.214	0.004	0.214
	8W	0.046	0.595	0.046	0.009	0.595	0.009
	12W	0.025	0.989	0.025	0.015	0.989	0.015
ECHO	4W	0.088	0.140	0.088	0.001	0.140	0.001
	8W	0.996	0.376	0.996	0.470	0.376	0.470
	12W	0.254	0.905	0.254	0.344	0.905	0.344

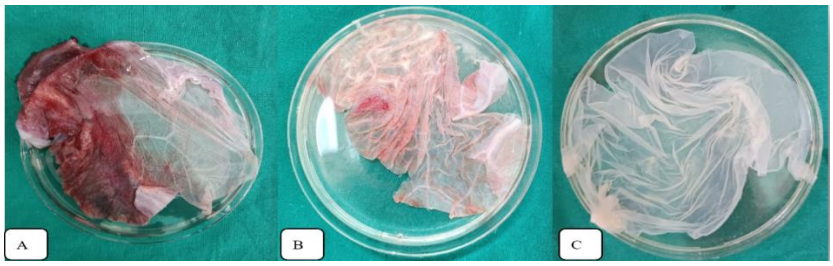

Fig. 1. A) AM after being peeled out of the chorion.
B) AM was washed with sterile saline solution that contained 0.025 mg/ml amphotericin B, 100 U/ml penicillin, and 0.2 mg/ml streptomycin (Pen & Strept; Norbrook, the Netherlands) to get rid of any debris. C) In a sterile plastic Petri dish, the membrane was repeatedly washed with a standard saline solution and stored in a refrigerator at 4 °C.

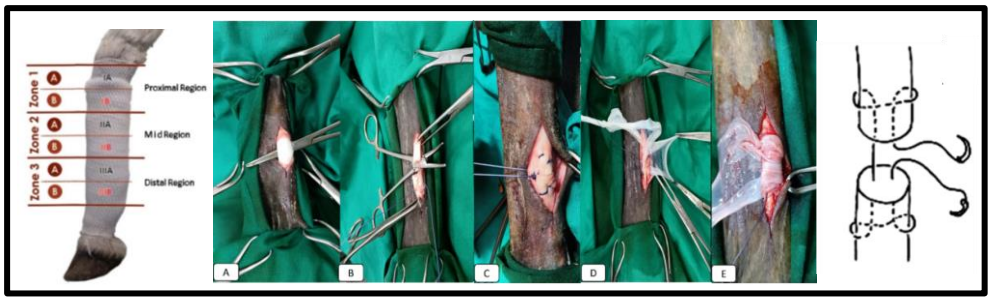

Fig.2. The Full-thickness tenotomy in donkeys was induced through incision in the skin and dissection of the subcutaneous tissue and paratenon to isolate the superficial flexor tendon (A) which was tenotomized (B) then sutured using Ethicon Coated VICRYL™ (polyglactin 910) suture material, size 2 (Ethicon Johnson & Johnson MedTech, USA) using modified Kessler technique in positive control group (c) and sutured with wrapping of amniotic membrane which act as scaffold on tenotomized area in AM group (D) & (E). Notes: The Zones of the donkey metacarpal region (the intended tenotomy site was IIB) and Schematic of Modified Kessler suture tendon repair technique.

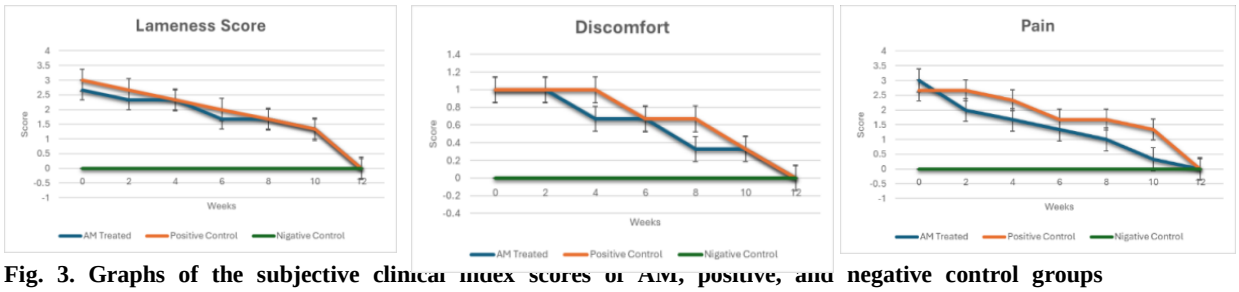

Fig. 3. Graphs of the subjective clinical index scores of AM, positive, and negative control groups (including lameness score, discomfort, and pain); at 0, 2, 4, 6, 8, 10, and 12 weeks postoperatively.

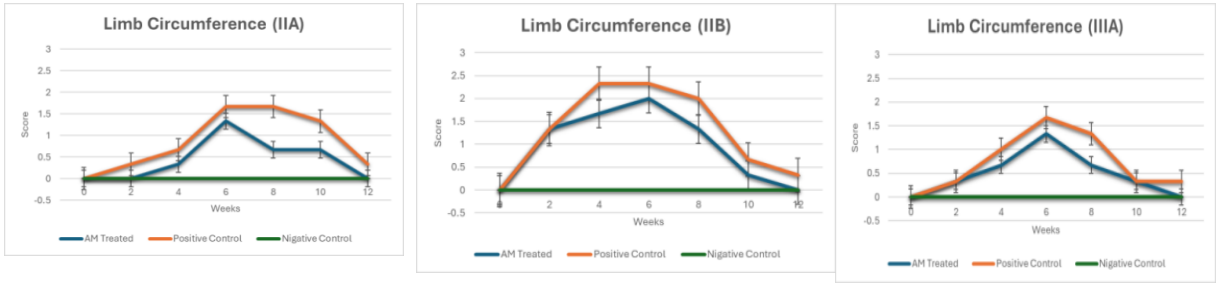

Fig. 4. Graphs of the objective circumferential limb measurements at three levels (IIA, IIB, and IIIA) of the metacarpal region of AM, positive, and negative control groups at 0, 2, 4, 6, 8, 10, and 12 weeks postoperatively.

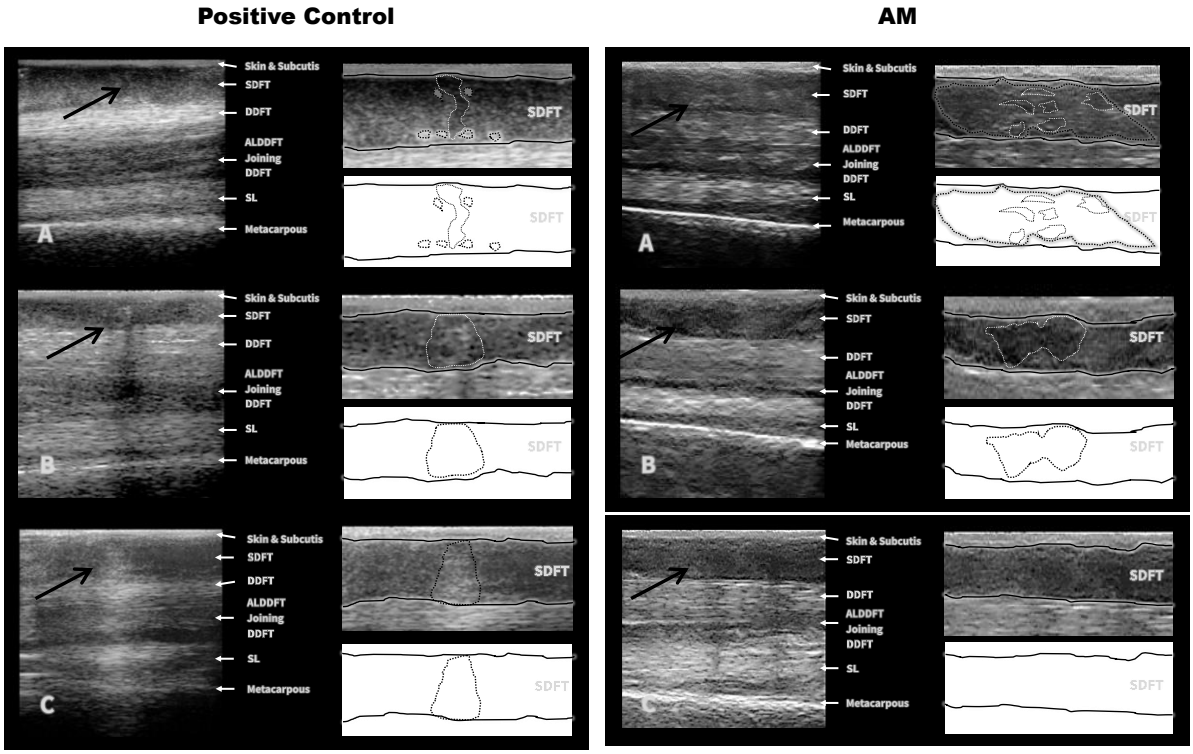

Fig. 5. A sagittal ultrasonographic scan of the SDFT at the palmar aspect of the mid-metacarpal region (zone IIB) revealed, in the amniotic membrane group, (A) thickening of the SDFT with heterogenous echogenicity (black arrow), (B) presence of hypoechoic gap within the tendon fiber due to reaction of amniotic membrane during healing of SDFT (black arrow), and (C) increase in echogenicity near to normal with normal size and without adhesion with deep digital flexor tendon (DDFT) (black arrow) at 4, 8, 12 weeks post-tenorrhaphy respectively, In the control positive group, (A) showing decreased of SDFT echogenicity (black arrow), (B) showing increase SDFT echogenicity and increase in its size (black arrow), and (C) showing loss of SDFT shape with sever adhesion with
DDFT with heterogenous echogenicity (black arrow) at 4, 8, 12 weeks post-tenorrhaphy, respectively.

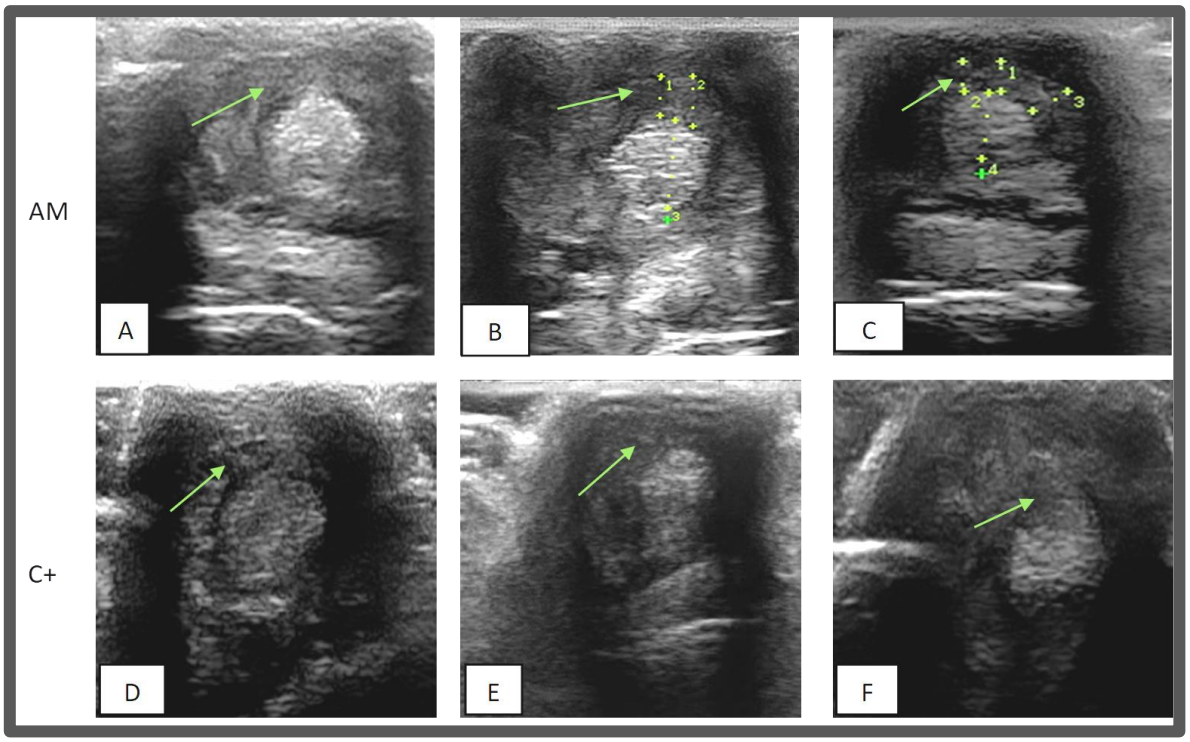

Fig. 6. Transverse sonogram of the SDFT in palmar aspect of the mid-metacarpal region obtained thickening of the SDFT with heterogenous echogenicity at 4 weeks after operation in amniotic membrane group (A). at 8 weeks after operation SDFT characterized by presence of hypoechoic gap within the tendon fiber due to reaction of amniotic membrane during healing of SDFT (B) and at 12 weeks after operation SDFT showing increase in echogenicity near to normal with normal size and without adhesion with deep digital flexor tendon (DDFT) (C).In the control positive group SDFT showing decreased echogenicity at 4 weeks after operation (D) while at 8 weeks SDFT showing increase echogenicity and increase in the size (E) but at 12 weeks SDFT loss its shape and showing sever adhesion with DDFT with heterogenous echogenicity (F).

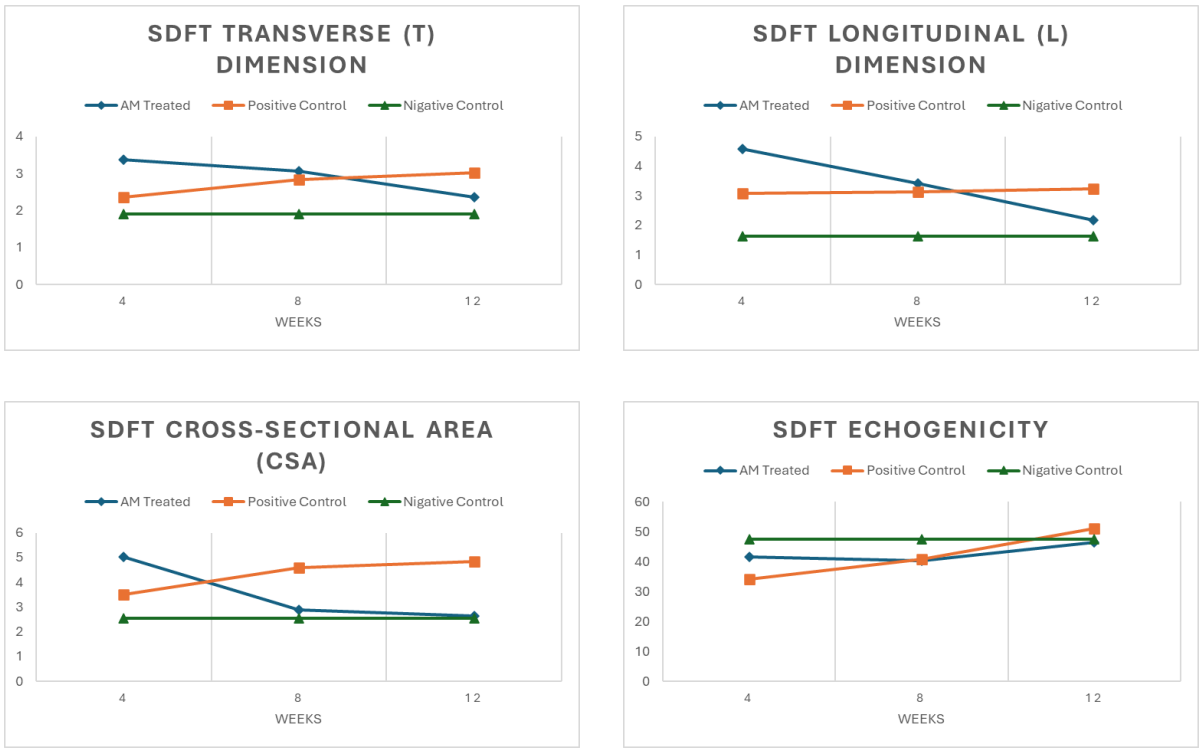

Fig. 7. Graphs of the ultrasound evaluation of AM, positive, and negative control groups, showing Transverse (T) and Longitudinal (L) dimensions, Cross-sectional area (CSA), and Echogenicity (Echo) of SDFT at 4, 8, and 12 weeks postoperatively.

Positive Control **AM**

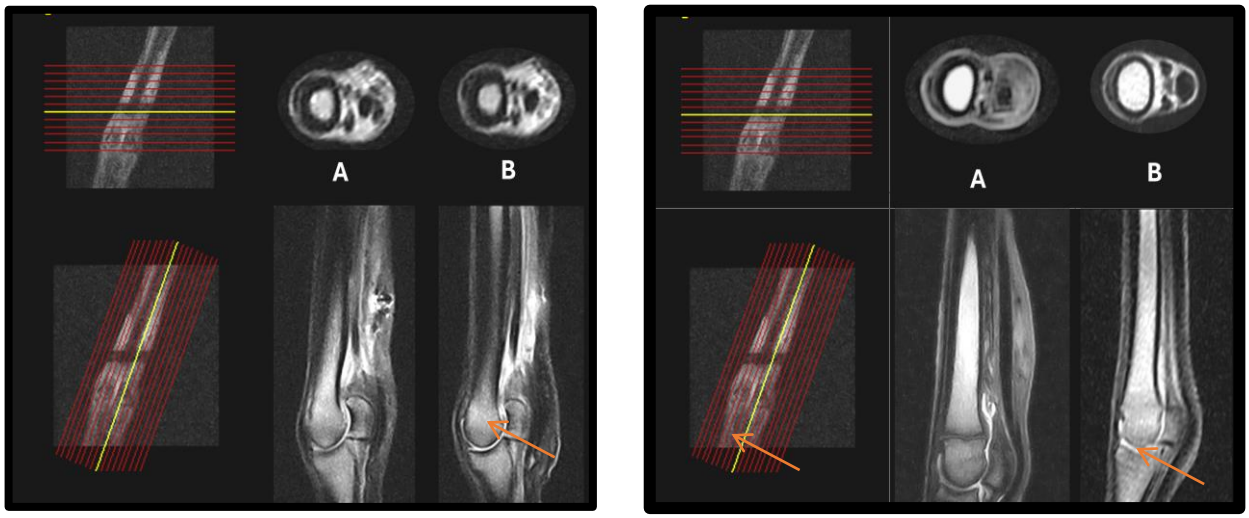

Fig. 8. MRI of SDFT at the palmar aspect of the mid-metacarpal region (zone IIB) revealed, in the control positive group, (A) transverse and sagittal planes at 6 weeks post-operation showing a moderate increase of signal intensity with thickening of the tendon (orange arrow); (B) SDFT showing high signal intensity of the grafted tendon with incomplete pattern of tendon fibers alignment, homogenicity, and severe adhesion at 12 weeks post-operation (orange arrow). In the amniotic membrane treated group, (A) transverse and sagittal planes at 6 weeks post-operation showing the graft bed (AM Wrapped the SDFT) appears as a high intense area with increase in size (orange arrow), (B) grafted SDFT returned to normal crescent shape and size with low signal intensity and normal fiber alignment at 12 weeks post-operation.

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تقييم زراعة الغشاء الأمنيوسي (السَّلَى) من الجاموس لإعادة بناء قطع كامل السُمك وتر المثنية الرقمية السطحي في الحمير: تقييمات باستخدام الموجات فوق الصوتية/التصوير بالرنين المغناطيسي

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الملخص

يُعد وتر المثني السطحي للأصابع أكثر الأوتار تعرضًا للإصابة لدى الخيول. تم تصميم الدراسة الحالية لتقيّم فعالية استخدام الغشاء الأمنيوسي (السَّلَى) المعد من الجاموس كطعم بيولوجي لإصلاح قطع كامل السُمك ومُستحدث تجريبيًا لوتر المثني السطحي للأصابع في الحمير، وذلك كنموذج لتمزق وتر المثني السطحي للأصابع لدى الخيول. تم تقُسّيم الحيوانات تحت التجربة (اثنا عشر حمارًا ذكرًا سليمًا) عشوائيًا إلى مجموعتين: المجموعة المعالجة، حيث خُيط وتر المثني السطحي للأصابع باستخدام خيط فيكريل مقاس 2 ولُفّ بالغشاء الأمنيوسي (السَّلَى). أما المجموعة الثانية، فكانت المجموعة الضابطة الإيجابية، حيث خُيط الوتر باستخدام خيط فيكريل مقاس 2 دون استخدام الغشاء الأمنيوسي (السَّلَى). أما الأطراف المقابلة، فكانت بمثابة مجموعات ضابطة سلبية في جميع الحمير. تم تقييم شفاء الأوتار باستخدام الموجات فوق الصوتية والتصوير بالرنين المغناطيسي. أظهرت المجموعة المعالجة بـالغشاء الأمنيوسي (السَّلَى) تحسنًا أسرع في العرج والألم والتورم مقارنةً بالمجموعة الضابطة الإيجابية. كشف التصوير بالموجات فوق الصوتية عن صدى ومحاذاة ألياف شبه طبيعية في الأوتار المعالجة بـالغشاء الأمنيوسي (السَّلَى)، بينما أظهرت المجموعة الضابطة صدى غير متجانس مع الالتصاقات. أوضحت نتائج التصوير بالرنين المغناطيسي انخفاض شدة الإشارة، ومحاذاة الألياف بشكل طبيعي، واستعادة الشكل الهلالي في مجموعة الغشاء الأمنيوسي (السَّلَى)، مما يشير إلى إعادة تشكيل هيكلية. في المقابل، أظهرت المجموعة الضابطة إشارة عالية، وسماكة في الأوتار، وأليافًا غير منظمة. تشير هذه النتائج إلى أن استخدام الغشاء الأمنيوسي (السَّلَى) المعد من الجاموس كطعم بيولوجي لإصلاح قطع كامل السُمك قد أدي إلى تُحسّن ملحوظ في التئام الأوتار من خلال تسريع التعافي الوظيفي، وتقليل الالتهاب، وتعزيز تجديد الأنسجة بشكل منظم. تدعم نتائج الدراسة استخدام الغشاء الأمنيوسي (السَّلَى) كعلاج مساعد واعد في إعادة بناء الأوتار، مما يُحسّن النتائج الهيكلية والوظيفية في إصلاح الأوتار. قد يُقدم هذا النهج استراتيجية علاجية فعّالة للتعامل مع حالات تمزق وتر المثني السطحي للأصابع في الخيول.

الكلمات الدالة: الغِشاءُ الأمنيوسي، الجاموس، الحمار، التصوير بالرنين المغناطيسي، وَتَرُ المُثْنِيَ السَّطْحِيّ للإِصْبَع، تقويم الوتر، الموجات فوق الصوتية، طُعْمٌ أَجْنَبِيّ.