



MONITORING OF THE REPAIR PROCESS OF SURGICALLY CREATED LESIONS IN BUCKS SUPERFICIAL DIGITAL FLEXOR TENDONS BY THE USE OF AUTOLOGOUS CONDITIONED SERUM CLINICAL AND ULTRASONOGRAPHICAL STUDY

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Abstract

The aim of this study was to describe the effect of single intra-tendonous injection of autologous conditioned plasma (ACS) on the repair of surgically induced acute superficial digital flexor (SDF) tendonitis in bucks on clinical follow-up and ultrasonographic parameters. For this purpose 24 adult local bucks were used. All subjected to splitting of left superficial digital flexor tendon (SDFT) of forelimb. The left forelimb of each buck was prepared aseptically, anesthetized locally then 5-cm., lateral incision was made in the skin above the SDFT. Blunt dissection was performed to separate SDFT from the deep digital flexor tendon (DDFT). The tendon was splitted longitudinally in its mid portion. The subcutaneous layer and skin were closed routinely and the operative limbs were left for 48hrs., after 48hrs; post-splitting. The bucks were allocated randomly and equally into two groups, (12 bucks/group). The first (control group) was injected with 2ml phosphate buffer saline (PBS). The second (treatment group) injected with 2ml ACS. The injection into the tendon defect was performed under ultrasonographic guidance. The ACS was obtained from autologous blood without anticoagulant which was centrifuged at 3000 rpm for five minutes. Clinical results reflected two main secondary health problems represented by lameness and swelling of the operative site in two buck groups. The lameness was severe during the 1st week in both groups with significant differences $P \leq 0.05$ between them. With the advancement of time, lameness subsided gradually and absent earlier in ACS group. Furthermore mild degree of lameness (0.45 ± 0.22) was noticed in PBS group until the end of 7th week. Swelling was noticed few hours post-surgery in the both animals groups and reached its peak at 1st week with significant differences $P \leq 0.05$ between them, then started to subside gradually and the diameter of operative limbs retrained nearly to its normal size as compared with zero time. The ultrasonographical results of normal tendon (zero time) revealed homogenous normoechoic pattern of the skin, SDFT and DDFT (parallel collagen fibers). Forty-eight hours post-splitting. The some area of SDFT had a hypoechoic area with loss of parallel collagen fibers formation and mostly anechoic central lesional. At 4 weeks post-treatment, the SDFT echogenicity of PBS group showed severely enlarged hypoechoic area (score 4 or 100%) with irregular orientation of collagen fibers, in addition to large anechoic central lesion The ACS group displayed hypoechoic pattern (score 3 or 75%). At the end of experiment, the hypoechoic lesion was continued until 16 week in PBS group (score 1 or 25%) whereas the sonogram of ACS group was reflected closely similar pattern to that noticed in healthy tendinous structure (score zero). In conclusion, this clinical trial in bucks with created acute SDFT tendinitis, showed that a single intra-lesional injection of ACS contributes to significant reduction of secondary health problems and improvement of ultrasonographical findings in comparing with PBS group.

Key words: SDFT, Tendinitis, PBS, ACS, Bucks.

Introduction

Tendon injuries especially of the superficial digital flexor tendons (SDFTs) are one of the most common forms of musculoskeletal injuries injury in Thoroughbred

racehorses and other horse breeds and is regarded as a career-limiting disease with a high recurrence rate and considered as a significant clinical problem for orthopedic surgeons and investigators (Patterson-Kane and Firth 2009). Numerous treatment modalities have shown limited

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success in improving tendon repair and mostly result in scar formation which is inferior to original tendon strength (Smith and his team 2013). Recently, Regenerative therapy aims to restore structure and function after application of biocompatible materials, cells and bioactive molecules (Frisbie *et al.*, 2007). There is growing knowledge about the clinical effects of potentially regenerative substrates, e.g. mesenchymal stem cells (MSCs) (Heisterbach *et al.*, 2012) and autologous blood products such as platelet rich plasma (Carvalho and co-worker 2013). Autologous blood-derived biological, including autologous conditioned serum (ACS), are frequently used to treat tendinopathies in horses (Ionita and Brehm 2008).

Autologous conditioned serum (ACS; synonyms irap®, Orthokine®, Orthogen,) is used for intra-lesional treatment of tendinopathy in various animal species (Baltzer *et al.*, 2009). The ACS is prepared by exposing whole blood samples to glass beads and using of special kits which has been shown to stimulate the secretion of anti-inflammatory cytokines, including interleukin (IL)-4 and IL-10 and IL-1 receptor antagonist (IL-1Ra) (Meijer *et al.*, 2003 and Hraha *et al.*, 2011). A recent investigation has shown that ACS contains high levels of IL-1Ra and IL- interleukin (IL)-4 and IL-10. Equine studies have focused on the IL-1Ra-mediated anti-inflammatory effects of ACS; however, in tendon healing, the high concentrations of growth factors such as insulin-like growth factor-1 (IGF-1) and transforming growth factor-beta (TGF-β) may be equally or more important (Dahlgren *et al.*, 2002 and Molloy *et al.*, 2003).

The uses of ACS have not been investigated widely in large animals especially in ruminant tendonitis therefore it is poorly understood in these species, thus the present research was designed to prospectively evaluate the influence of a single intra-tendinous injection of ACS on the restoration of surgically created acute SDF tendonitis in bucks based on clinical and ultrasonographic parameters.

Materials and Methods

Experimental animals: Twenty-four adult local breed bucks were used for the present clinical experiment. They were weighing 28-32 Kg. and their ages were (2-3) years. All bucks were maintained hygienically in animal shed and approved by the animal welfare of the Veterinary Medicine, University of Baghdad. The animals were housed in pens and acclimatized randomly for three weeks prior to commencement of the experiment. The experimental animals were kept on tethered grazing (alfalfa hay) and fed with concentrate in three meals

daily and water ad libitum. All animals were kept under same circumstances and dewormed with Ivermectin (Ivomic 1% Spain) at a dose rate of 0.2 mg/kg B.W., administered subcutaneously in hairless area. During the acclimatization period, bucks were given identification by ear-tagging. Bucks were examined clinically and ultrasonographically to check any abnormalities of the SDFT of the left forelimb prior to surgery which deemed a normal echogenic aspect of the tendon tissue and no any tendinous lesion was observed. Bucks were allocated randomly and equally into two groups (12bucks/group), first control group and second treatment group.

Preparation and application of autologous conditioned serum: This was done according to the method mentioned by Darabos and co-workers (2011) as follow: Ten milliliters of autologous blood were collected from each buck by single venipuncture of jugular vein and put in sterile test tubes Fig. 1, then samples were incubated at 37°C for 6 hrs., Fig. 2. Subsequently, the blood was centrifuged at 3000 rpm for 10 minutes to separate serum from blood Fig. 3. The supernatant conditioned serum is aseptically aspirated and collected

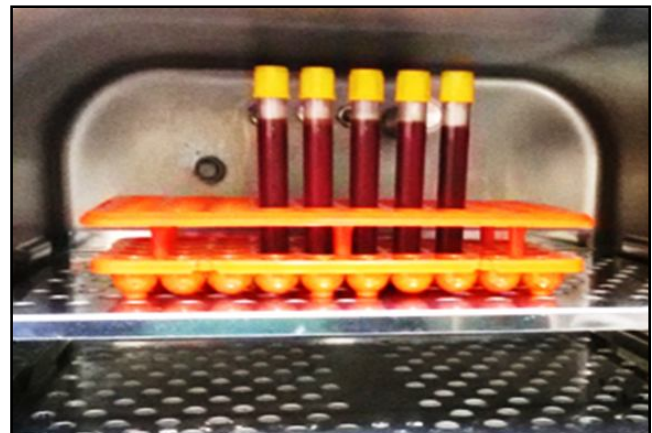


Fig. 1. The blood is collected in sterile test tubes.

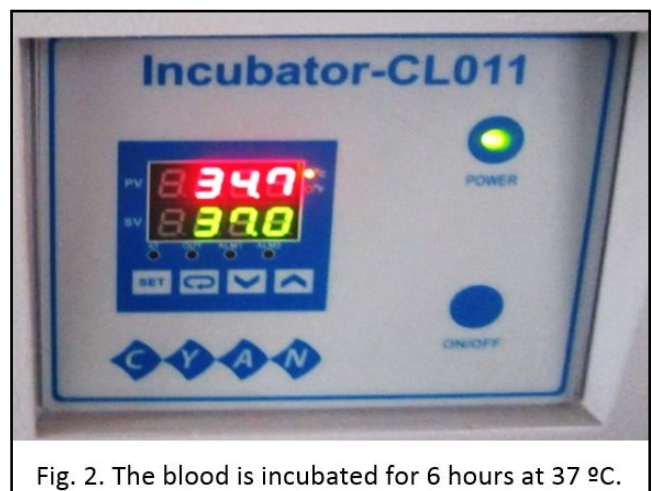


Fig. 2. The blood is incubated for 6 hours at 37 °C.



Fig. 3. The blood is centrifuged to separate the therapeutic serum.



Fig. 4. After aseptic harvesting, the serum is ready to use.

in a tube. The resulting conditioned serum was injected intra-lesional into the SDFT defect Fig. 4 or frozen at -20°C until use.

Technique of inducing tendinitis (surgical procedure)

Food was withheld for overnight before tendinitis induction in all bucks. The animals were sedated with Xylazine hydrochloride 2% (Xylapan®, Switzerland) in a dose rate of 0.25 mg/kg via intramuscular injection. The left superficial digital flexor tendon (SDFT) of forelimb of each buck was prepared surgically from carpal joint to the end of the limb. The animal placed on right recumbency with operated limb up which was anesthetized locally with lidocaine hydrochloride 2% (Xylocaine, Fatro, Italy 20 mg/ml) in a dose rate of 3 mg/kg B.W., according to Gabel, (1996) infiltrated subcutaneously above the SDFT. Animal was placed in a sterile field on a surgical table and covered with a sterile surgical drape with the exception of the SDFT of the left

forelimb which remained exposed. a straight Approximately 5 cm straight incision was made at the mid metacarpal region, including the skin, subcutaneous fascia and tendon paratenon, to expose the dorsal surface of the tendon and bleeding was carefully arrested by routine method. Blunt dissection was made to expose the SDFT which was separated from the deep digital flexor tendon (DDFT). A small curved forceps was introduced under the isolated tendon to prepare it for splitting The tendon was splitted longitudinally with the direction of collagen fibers in its mid portion with a surgical scalpel (full-thickness) then incision is enlarged with scissor. The subcutaneous layer and skin were closed by horizontal interrupted mattress suture with silk No. 0 (Ethicon) and the operative limbs were left for 48hrs., without any therapy to evocate (acute tendonitis). After that time the animals were randomly assigned into two equal groups (12bucks /group). The first group served as control group which was treated by intra-lesional injection of sterile (PBS). The second group (treatment group), their animals were treated by intra-lesional injection of ACP in the SDFT lesion. The doses were (2ml) for each agent. The injections of PBS and ACP into the tendon defect were performed through a 21 gauge needle under ultrasonographic guidance. Following treatment, the operated animals were kept in a single cages and broad spectrum antibiotic represented by pencillin-streptomycine (Norbrook Company. N. Ireland) in a dose of 10000 IU and 5 mg/Kg B.W., respectively were injected IM for five successive days to prevent infections. No forelimb immobilization (cast) was adopted in order to allow tendon movements along the postoperative period.

Assessment of study parameters: Bucks were re-examined clinically and ultrasonographically at regular intervals.

Clinical evaluations: these were consisted monitoring of the followings.

Degree (score) of lameness: The lameness was evaluated at zero time (data base) and then weekly for seven consecutive weeks. Lameness scores (0 to 4) were assigned based on the following scale as mentioned by Stashak, (2002) table 1.

Table 1: Show the lameness scores (Stashak, 2002).

Grade	Description
0	No lameness (normal gait).
1	Mild weight-bearing lameness
2	Moderate weight-bearing lameness
3	Severe weight-bearing lameness
4	Non-weight-bearing lameness (animal unable to bear weight on the affected limb).

Local swelling of the operative area:

Postoperative swelling of the SDFT was determined by palpation as an increase in diameter relative to normal tendon. In addition external width (distance from skin to skin, medial to lateral) of the region was measured at the level of the surgical site by using a digital caliper to determine the presence and degree of swelling of the operative tendons (Kavaguchi De Grandis *et al.*, 2012). The external width was evaluated at zero time and then weekly for seven weeks. The same person performed all the measurements with a digital caliper (Castorama, 59175 Templemars, France).

Ultrasonographical evaluation: The palmar aspect of metacarpal region of the left forelimb was prepared by shaving. With the help of a half dose of Xylazine hydrochloride 2%, ultrasound gel had been applied on the site to ensure good contact between the skin and the transducer. The ultrasonography was performed by WellD Ultrasonographic apparatus with 7.5 MHz linear transducer (Logiqe, GE Healthcare, Wauwatosa, WI, USA). Longitudinal ultrasonographic images were performed before surgery for each animal to serve as a control normal images then 48 hrs., post induced tendonitis as well as at (4, 8, 12 and 16 weeks post injection of the PBS and ACS to evaluate the echogenicity of the operative site of tendons. The echogenicity was determined and scored, as tendon echogenicity score (TES), between (0-4) suggested by Reef, (2001). Echogenicity was assigned as listed in table 2. Analyses of ultra-sonograms were carried out by the same operator to avoid inter-observer error. The lesion was tagged on the images to help for the next examination and to assure repeatability.

Statistical Analysis: Statistical analysis of the data was performed using SAS (Statistical Analysis System-version 9.1). Mean and standard error (M ±SE) were calculated for all variables and differences between the two animal groups. One-way Analysis of Variance ANOVA and Least Significant Differences (LSD) were used to assess the variables. The P ≤ 0.05 values were defined as level of significance. (SAS, 2010).

Results

Lameness: Severe lameness was noticed in both

Table 2: Show the echogenicity scores (Reef, 2001).

Score	Description
0	Normoechoic
1	Slightly less echogenic (25%) than normal.
2	Half echogenic and half anechoic (50%).
3	Mostly anechoic (75%).
4	Completely anechoic (100%).

buck groups during the first week (4.0±0.00) and (3.5±0.40) in PBS and ACS respectively with significant differences P ≤ 0.05 between them and the animal cannot bear weight on the operative limb. Moreover, lameness was prominent at 2nd and 3rd weeks post-surgery in the two groups, then gradually decreased from 5th month in PBS and ACS groups (bucks became sound) whereas, lameness was disappeared at 6th week in ACS groups (bucks became sound). Furthermore, mild degree of lameness (0.45±0.22) was noticed in PBS group until the end of 7th week table 3 and Fig. 5. Throughout the remainder of the study, all tendons exhibited normal tension comparable to the contralateral limb with none of the fetlock joints exhibiting hyper-flexion (normal stance position) regardless of treatment modality.

Local swelling: Data fixed in table 4 and Fig. 6 reflected the degrees of swelling; this sign was noticed few hours post-surgery in all animals groups. There were no significant differences P>0.05 in zero time between the two groups. Swelling reached its peak at 1st week post-treatment with significant differences Pd*0.05 between the groups, the values were (14.0±0.37) and (13.5±0.15) cm in PBS and ACS groups respectively. In the beginning of the 5th week until the 7th week, there were no significant differences between groups and

Table 3: Show the mean values of lameness of the two groups.

Group M±SE			
LSD	ACS	PBS	Time (weeks)
---	Zero	Zero	Zero
0.894	3.55±0.40 b	4.00±0.00 a	One
1.361	3.25±0.30 b	3.80±0.33 a	Two
1.502	2.70±0.50 b	3.20±0.60 a	Three
1.173	1.25±0.22 a	2.50±0.20 a	Four
1.225	0.5±0.20 a	1.25±0.12 a	Five
1.414	0.00±0.00 b	1.00±0.66 a	Six
0.576	0.00±0.00 b	0.45±0.22 a	Seven

(Zero): Mean value prior to treatment.
Horizontal small letters denote significant differences P ≤ 0.05 between groups.

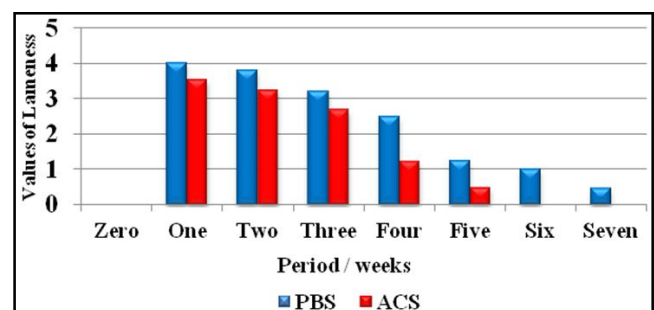


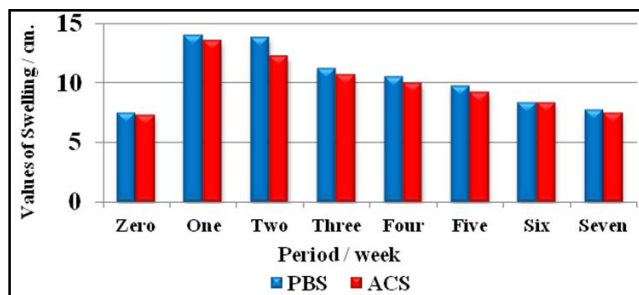
Fig. 5: Photogram show the mean values of lameness of the two groups.

Table 4: Show the mean values of swelling (cm) of the two groups.

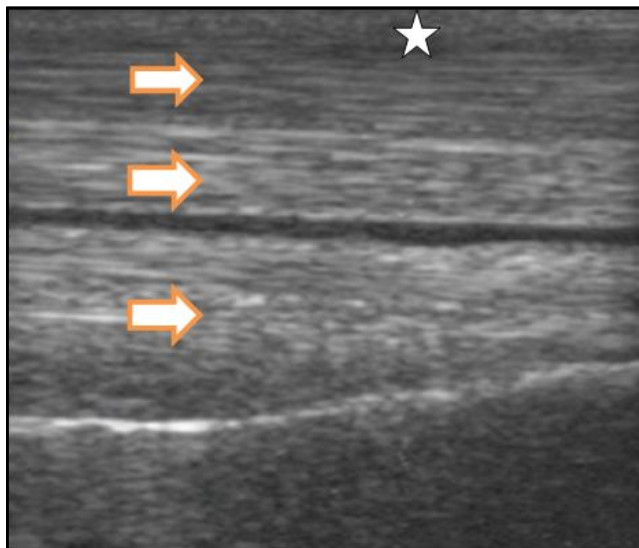
Group M±SE			
LSD	ACS	PBS	Time (weeks)
0.114	7.25±0.25 a	7.50±0.30 a	Zero
1.733	13.5±0.15 b	14.0±0.37 a	One
1.356	12.25±0.20 b	13.8±0.40 a	Two
1.227	10.70±0.10 b	11.2±0.14 a	Three
1.148	9.95±0.26 b	10.5±0.33 a	Four
0.151	9.20±0.22 a	9.75±0.12 a	Five
0.122	8.29±0.33 a	8.35±0.36 a	Six
0.104	7.47±0.31 a	7.60±0.60 a	Seven

(Zero): Mean value prior to treatment.

Horizontal small letters denote significant differences $P \leq 0.05$ between groups.

**Fig. 6:** Photograph show the mean values of swelling (cm) of the two groups.

swelling started to subside gradually and the diameter of operative limbs retrained nearly to its normal size as compared with zero time.

**Fig. 7:** Normal longitudinal ultra-sonogram of buck palmar tendon, show homogenous normoechoic pattern of the skin (star), and parallel collagen fibers) of SDFT (upper arrow), and DDFT (middle arrow) and homogenous hyperechoic parallel collagen fibers of SL (lower arrow).

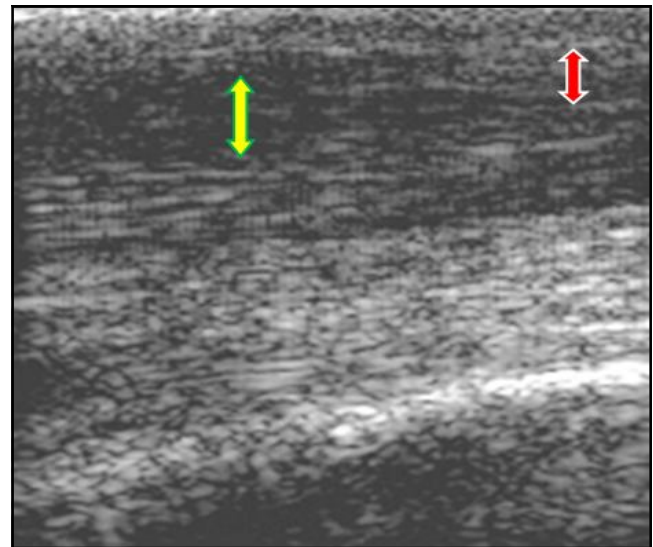
Ultrasonographical findings

Ultrasonographical descriptions of the metacarpal region: The normal longitudinal ultrasound image of metacarpal area showed a homogenous normoechoic pattern of the skin, SDFT and DDFT (parallel collagen fibers) at the upper side. The SL located closely under DDFT as a homogenous hyperechoic parallel collagen fibers Fig. 7.

Ultrasonographical descriptions of the metacarpal region post-splitting: The longitudinal ultrasound image of metacarpal area has been done after 48hrs., post-splitting. The some area of SDFT had a hypoechoic area with loss of parallel collagen fibers formation and mostly anechoic central lesional. The DDFT was suffered from hypoechoic pattern Fig. 8.

Ultrasonographical descriptions of the SDFT at 4 weeks post-treatment: The SDFT echogenicity of PBS group in this period showed severely enlarged hypoechoic area, (score 4 or 100%) with irregular orientation of collagen fibers, in addition to large anechoic central lesion as well as missing of the metacarpal structural landmarks Fig. 9A. The ACS group displayed hypoechoic pattern (score 3 or 75%) with few parallel collagen fibers and mostly anechoic central zone Fig. 9B.

Ultrasonographical descriptions of the SDFT at 8 weeks post-treatment: The PBS group echogenicity in this period was similar to the previous period as a hypoechoic pattern and anechoic central zone (score 3 or 75%), as well as the alignment of collagen fibers was lost Fig. 10A. The ACS group revealed mild anechoic

**Fig. 8:** The ultra-sonogram of the splitting lesion of SDFT after 48 hrs., show hypoechoic tendinous region (red arrow) with completely anechoic central lesion (yellow arrow).

central lesion surrounded by hypoechoic area (score 2 or 50%) Fig. 10B.

Ultrasonographical descriptions of the SDFT at 12 weeks post-treatment: The majority was hypoechoic central area with irregular orientation of collagen fibers surround by normoechoic tendinous tissue in PBS group (score 2 or 50%) Fig. 11A. In contrast the ACS group reflected only slightly hypoechoic central area (score 1 or 25%) with parallel collagen fibers with normal tendinous pattern Fig. 11B.

Ultrasonographical descriptions of the SDFT at

16 weeks post-treatment: The hypoechoic lesion was continued until 16 week in PBS group (score 1 or 25%) Fig. 12A whereas the sonogram of both ACS groups was revealed closely similar pattern to that noticed in healthy tendinous structure (score zero) Fig. 12B.

Discussion

Clinical follow-up: During a clinical follow-up of treatment bucks there were two main secondary complications include lameness and swelling.

Lameness: All operated limbs showed a unilateral left forelimb lameness which was pronounced in 1st, 2nd

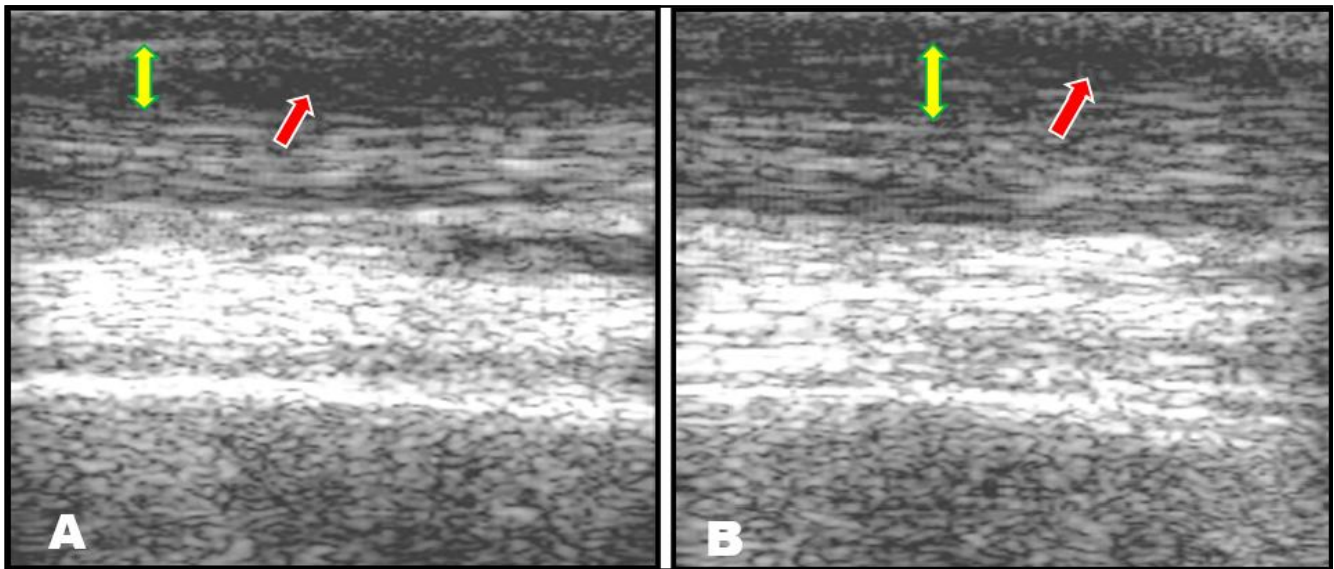


Fig. 9: Show the echogenicity of SDFT at 4 weeks post-treatment; (A) The PBS group reflected an enlarged hypoechoic tendinous region (yellow arrow) with huge anechoic central area (red arrow). (B) The ACS group had a hypoechoic tendinous region (yellow arrow) with mostly anechoic central area (red arrow).

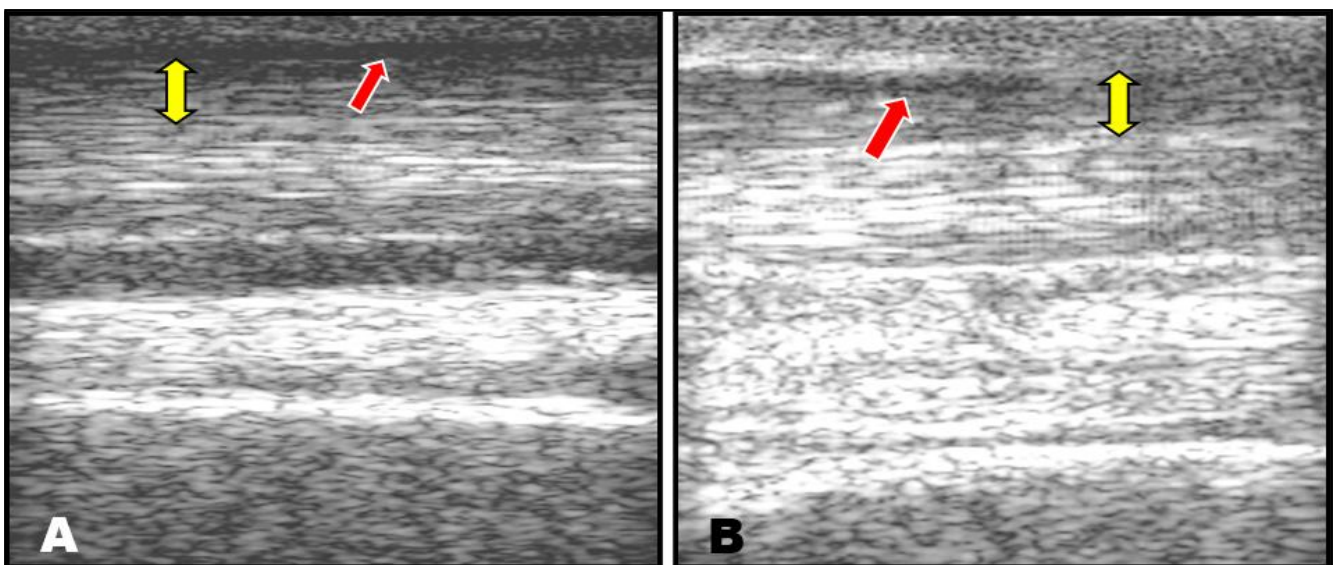


Fig. 10: Show the echogenicity of SDFT at 8 weeks post-treatment; (A) The PBS group displayed a hypoechoic tendinous region (yellow arrow) around anechoic central lesional zone (red arrow). (B) The ACS group had a hypoechoic tendinous region (yellow arrow) surrounding by anechoic central zone (red arrow).

and 3rd week post-injection of PBS and ACS. Lameness noticed in the current study may be ascribed firstly; to pain evocated from cutting of the nerves at the operative site during surgery which finally leads to inflammation, secondly to swelling which cause pressure on nerves ending in the operative site. There were high concentrations of the neurotransmitter with elevation of the pro-inflammatory prostaglandin PGE₂, this interpretation was agreed with Zubrzycka and Janecka, (2000). Chemical irritants and neurotransmitters may generate pain in tendinopathy and increase in lactate levels (Alfredson *et al.*, 2002).

Khan and colleagues (1999) indicated that pain may originate from a combination of mechanical and biochemical causes. Tendon degeneration with mechanical breakdown of collagen could theoretically explain the pain.

A study in horse that received a single PRP treatment, Bosch *et al.*, (2011) were recorded lameness scores, ranging from 2 to 3, in all horses 24 hours after induction of injury, decreasing gradually after this time. On day 30, only one horse of each group was lame (grade 1), which lasted until day 45. After that time, all horses were sound. Furthermore, Senzel and co-worker (2009) were noticed

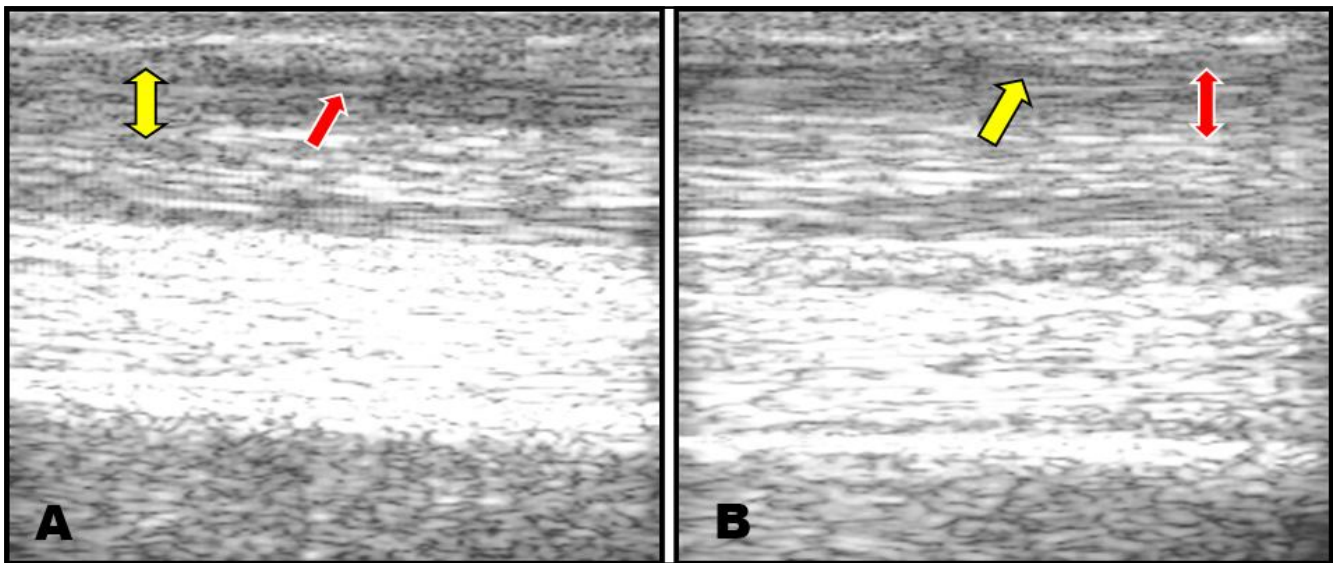


Fig. 11: Show the echogenicity of SDFT at 12 weeks post-treatment; (A) The PBS group revealed a normoechogenic tendinous region (yellow arrow) with major hypoechoic central area (red arrow). (B) The ACS group had a hypoechoic central area with parallel collagen fibers (yellow arrow) and returned of other portions to the normal tendinous pattern (red arrow).

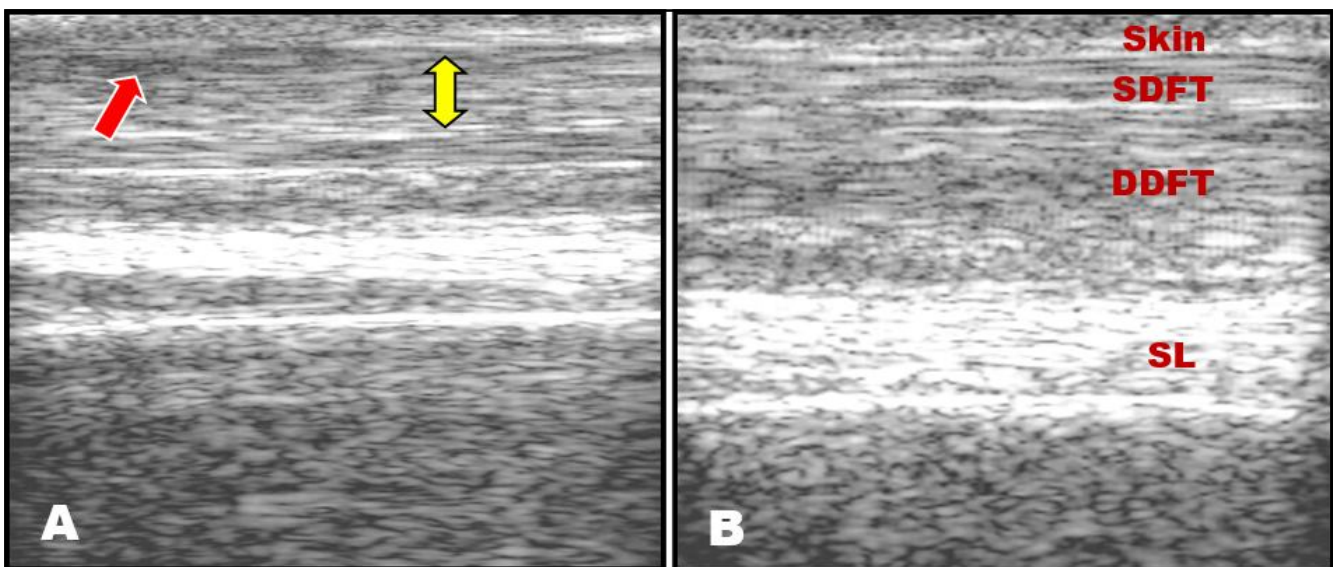


Fig. 12: Show the echogenicity of SDFT at 16 weeks post-treatment; (A) the PBS reflected mild hypoechoic central area surrounding by the normoechogenic structure. (B) in ACS group, the tendon was rebounded to the normoechogenic tendinous fashion with normal parallel collagen fibers.

that horses injected with a single PRP treatment showed lameness which was severe at the 1st week then started to disappear gradually with the time then diminished totally after 62 days.

Results of the present controlled clinical trial concerning the lameness, demonstrate persistent of lameness at a lower level until 7th week in PBS group. On other hand single intra-lesional injection of ACS leads to an earlier reduction of the lameness score of the SDFT region. This outcome may be attributed to anti-inflammatory and analgesic properties of ACS and activation of GFs and cytokine release from these agents which continues their effect starting from 7 days post-injection and play an important role in enhancing tendon repair, this interpretation was parallel to that mentioned by Frizziero and his team, (2013).

Swelling: Localized swelling was noticed few hours post-surgery in all animals groups. Swelling reached its peak at 1st week post-treatment with significant differences between the two groups. Swelling could be attributed to the formation of intratendinous hematoma and postoperative edema that develops after injury, which considered as part of the inflammatory process, remaining visible in all animal groups until the 4th week. Persistent vascularization was suggested to be the cause of lasting swelling in tendon disorders. Reports of clinical observations by Keg *et al.*, (1992); Alves *et al.*, (2001); and Bosch, (2009) corroborate these explanations.

Geburek *et al.*, (2015) demonstrated the swelling of tendinopathy of forelimb SDFT in the equine occurred by the accumulation of the inflammatory cells and exudate fluid as well as the proliferated cells into the skin, subcutaneous and peritendinous tissue.

Sharma and Maffulli (2006) recorded swelling after using Matrix Metalloproteinase (MMP) due to degradation of collagen fibers of the ECM at the proliferative stage and continue until 28 day which was the same period noticed in our study.

Han *et al.*, (2017) referred that edema of both peritendinous tissues and tendons inevitably persist in the early period of tendon healing. Subcutaneous tissue edema was found to increase energy and force required to move the tendons and it is an inevitable biologic process, contributing substantially to increase the resistance to tendon gliding but changes in the length of the edematous tissue increased the resistance by only 10%, 20% and 30% in the presence of mild, moderate or severe edema respectively. Pingel *et al.*, (2013) were indicated that the resistance to tendon gliding was affected more by the severity of tissue edema rather than by the extension of edema in the digits, which suggests that edema severity is a concern in determining the timing of commencement and methods of tendon motion exercise. Edema of the

tendon is a common finding during delayed primary repair and is especially detrimental to tendon gliding in the flexor sheath region.

In the current study, the palpable swelling declined noticeably with the experimental time and totally disappeared at the end of 4th week in the two groups and the diameter of operative limbs retrained nearly to its normal size as compared with zero time. This may be due to a therapeutic effect of inadvertent reflux of ACS doses which has anti-inflammatory and anabolic effect of ACS which represent by high level of GFs it contain that decreased the inflammatory processes faster than the PBS. Sarrafian *et al.*, (2010) corroborated these documents, in evaluating Achilles tendinitis in sheep for the inflammatory processes or swelling grade which was rebounded at 8th week after the splinted tendon while earlier disappearance of swelling in the present study were noticed at the end of 4th week.

The abnormalities which were happened in this study including (pain, locally increased temperature, lameness and swelling) gradually started to relieve so that the physical activity of the animals returned back to an almost normal level and animals were grazing freely.

Ultrasonographical findings: Ultrasonography is widely used to evaluate the tendon lesions severity and progression, this technique is considered one of the most accurate diagnostic tools in the early stage, advanced healing lesions tend to present normal echogenicity although the tendon has not yet completely recovered (Whitcomb, 2009). In particular, the mechanical properties of tendon play a determinant role in its ability to resume work (Wood *et al.*, (1994). Vergari *et al.*, (2012) were mentioned that, the application of ultrasonographic scan for the diagnosis of flexor tendon injuries in horses has provided a safe, non-invasive objective means for assessments of tendon shape, echogenicity, edema and fibers alignment as indicators for the stage of tendon healing and comparisons of ultrasonographic and pathological findings that accurately reflect the extent of lesions in horse. Furthermore, allow the patient rehabilitation program to be tailored to tendon healing as evidenced by direct visualization of tendon architecture.

The normal ultrasound horizontal images of normal SDFT of bucks forelimb in the current study showing a fine arrangement of dots, homogeneous textures and linear fiber patterns, these finding were in accordance to normal tendon tissue US presentation. Mannion (2006) referred that the ultrasound images of normal tendons revealed hyperechoic appearance due to its highly fibrous composition and if the transducer is positioned parallel to the tendon long axis, it appears rectangular in shape and the tendon fibers appear as a linear arrangement

surrounded by hyperechoic peritendon.

Forty-eight hours post-splitting of SDFT, the US images revealed hypoechoic area and mostly anechoic central lesional resulting from splitting which lead to hemorrhage or edema. Ali *et al.*, (1992) indicated that the interval between tendon fragments in partial and complete tendon rupture might be filled by hemorrhage, serous effusion or edema yielding an echo pattern of increased and decreased echogenicity. Serous fluid and edema are hypo- to anechoic. Nicoll *et al.*, (1992) found that the resolution of the initial peritendinous and intratendinous edema, are an accurate assessment of the lesion that may be often made. Marked ultrasonographic improvement will be recognized in the initial 4-8 weeks, with some degree of echogenicity corresponding to granulation tissue. As healing progress, echogenicity gradually increases possibly corresponding to increasing collagen and fluid content.

Rantanen (1993) induced tendonitis which was observed through US view as anechoic and hypoechoic areas matching the points of hemorrhage, edema and tendon fibers rupture. These findings may be explained by the inflammatory exudate, macrophages that continuously release protease and collagenase, which exert enzymatic activity.

The improvement of ultrasonographical examination was observed at 12 weeks in ACS group. However in PBS treated tendons required 16 weeks to recover or resumed their normal shape and structure. A study by Georg (2010) presented an evidence that the ultrasonographic enhancement initiated within two months after intra-lesional injection of ACS therapy for tendinitis in equine. Furthermore, González *et al.*, (2016) induced Achilles tendinitis by collagenase injection and they noticed an increased in cross sectional area at 2nd week. At 2nd month post-treatment with PRP, the lesion was decreased saliently due to the effect of GFs found in PRP.

A recent study by Kuffler, (2019) who noticed that the use of autohemotherapy resulted in reduction of the inflammatory processes especially when applied through ultrasound guided. These therapies have anti-inflammatory and analgesic effect because of it contains high levels of anabolic growth factors (GFs) mainly (IGF-1, bFGF and VEGF) and anti-inflammatory cytokine (IRAP1 and IRAP II) which accelerated tendon healing.

In the current study the ultrasound evaluation carried out for 16 weeks after induction of tendinitis revealed a gain longitudinal orientation of collagen fibers of the tendons in the two groups and it was faster in ACS group (earlier maturation phase) which showed normal tendon echogenicity with the progress of healing process.

Conclusions

This clinical trial in bucks with acute tendinopathies of the SDFT clearly demonstrated that a single intra-lesional ACS injection contributes to significant reduction of lameness and to improvement of ultrasonographic parameters of repair tissue within the experimental time.

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