

EVALUATION ROLE OF THE AUTOGENOUS PERITONEUM AS A SCAFFOLDS ON THE HEALING OF ACHILLES TENDONS IN DOGS

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ABSTRACT

The objective of the current study was to evaluate the healing of experimentally severed Achilles tendons of the dogs repaired by Kessler suture pattern alone or with autologous peritoneal scaffold. A total of twelve healthy, neurologically and orthopedically local breed dogs were used in this study, which were randomly allocated into two equal groups (6 of each). The 1st group as a control, while the 2nd as a treated group. In both groups the Achilles tendon of one hind limb was completely severed transversely, in 1st group the tendon was repaired by Kessler suture pattern using silk No.3, while in the 2nd group a combination of Kessler suture and autogenous peritoneal scaffold was applied to reinforce the tendon repair. The site of surgery was evaluated clinically, while gross and histological study was performed at 15, 30 and 60 days post-operative days. Results was revealed that autologous peritoneum graft has a remarkable effects on Achilles tendon healing represented by improving fibroblastic proliferation, reduce adhesion, increase tendon strength and improve tendon function when compared with the control group. We concluded that peritoneal graft application has valuable and beneficial effects on treatment of ruptured Achilles tendon in dogs when utilizing it in conjunction with the traditional procedures.

INTRODUCTION

In dogs is the strongest tendon in structure of the musculoskeletal system is the Achilles tendon (1), it is essential for normal movement of hock joint especially for activities like stair climbing and running (2).Achilles tendon consists of the connection

of three distinct structures: the tendons of the superficial digital flexor muscle, gastrocnemius muscle and the common (conjoined) tendons of the gracilis, semitendinosus and biceps femoris muscles (3,4). Tendon and muscle injuries are recognized as an important cause of lameness in domestic animals (5,6). Partial and total rupture of Achilles tendon is common traumatic lesion occurring mostly in athletes. Classification of lesions is based upon their location, severity and chronicity. Clinical signs include lameness, plantigrade stance and various degrees of hock joint hyper flexion according to the severity of injury, and the most common site for injury is 2-6 cm from the heel (7,5). Treatment of Achilles tendon rupture is remain controversial, and the most frequently applied methods are conservative, percutaneous and surgical repair (2,7), and the surgical repair is usually advisable by many of surgeon to be the best way to restore tendon integrity, strength and functional performance (7,2,8). The blood supply of tendon is relatively poor, therefore, the healing process is usually difficult, progresses slowly and characterized by the potential in evitable formation of adhesion with surrounding tissue. As result the treatment process is slow and re rupture may be occur (9, 10). Therefore, several tissue engineering either synthetic or natural have been described in animals and human being to improve the healing process and directed it toward complete tendon regeneration without development of fibrotic tissue formation such as polypropylene mesh, collagen fiber implantation, pericardium, small intestine submucosa, dermal patch, mesenchymal stem cells, vein graft, platelet rich plasma and omentum (2, 3,9,11,12,13). Peritoneum is a serous membrane covered by mesothelial cells supported by stromal layer composed of elastic and collage fiber, mast cells, macrophage, lymphocyte, adipose cells and glycosaminoglycans (14,15). It has been used in surgery as bioscaffold for reconstructing of corneal surface in dogs (16). It used based on the capacity of cells that it is contain to release growth factor such as transforming growth factor, fibroblast growth factor and epidermal growth factor which demonstrated positive effect on healing process (17,29). Therefore, in this study the role of peritoneum in healing of experimental Achilles tendon rupture was evaluated in dogs.

MATERIALA AND METHODS

A total of twelve local breed dogs from both sex with mean weight of (12.2 ± 0.5)kg and mean age of (16.2 ± 0.7)months were used. Animals were clinically healthy, had no history or clinical signs suggestive of orthopedic or neurological diseases. Animals were housed in animal housing facility of veterinary teaching hospital, college of veterinary

medicine university of Mosul. All animals were housed for 3 week at the facility for acclimatization .Animals were anesthetized through intra muscular injection a mixture of 10 % Ketamine (10 mg /kg) and 2% Xylazin (3 mg /kg) ,anesthesia was maintained with this mixture . The surgical procedure was performed under aseptic conditions. To obtain the peritoneum grafts, the animal positioned in dorsal recumbency, an a ventral midline abdominal incision was made caudal to umbilicus (3-4 cm in length). After opening of the abdomen a flap of the parietal peritoneum membrane was then isolated and harvested .The harvested peritoneum segment (approximately 3×3 cm) was preserved in normal saline solution containing 10% gentamicin. The recipient site was aseptically prepared. A longitudinal skin incision (5 cm length) was made over the Achilles tendon starting just 5 cm proximal to the calcaneus on the lateral aspect of the limb, and the paratenon was identified and incised longitudinally as a separate layer. The Achilles tendon was identified and transversally severed by scalpel. In the control group, the tendon was only sutured using Kessler technique, without grafting, using silk NO3. In treatment group, the tendon was sutured as in control group, the harvested peritoneum segment was inserted through the sutured ends of the tendon with the peritoneum mesothelium layer facing the paratenon layer of the tendon, then the inserted graft was gently pulled over the sutured tendon, the free edges, proximally and distally of the grafted peritoneal scaffold were sutured to the tendon using 2-0 silk suture. The surgical site was then closed routinely and covered with a non-adhesive dressing bandage. The operated limb immobilized using Gipson (with window) for 2 week post operatively. All dogs received penicillin streptomycin (10000 IU and 10 mg /kg respectively) for 5 days as a post-operative antibiotic therapy. The site of surgery was evaluated clinically, while gross pathological study and biopsy collection was performed at 15, 30 and 60 days post-operative days. The tissue section stained by Harri's hematoxylin and alcoholic eosin (18). Later the stained slides examined at 100 Xs powers.

RESULTS

Clinically all experimental animals were recovered well from surgery. Operated animals of all groups were seem in a good healthy condition, normal activity and appetite, and there was no evidence of clinical complications such as local infection or wound dehiscence at the site of operation. All animals of two groups were showed lameness after operation, and this lameness was classified according to lameness score system as

following grade 1 lameness was noted and persisted for 15 days in control group and for 8 days in treated group. Less severe lameness classified as grade 2 was observed at 30 days post operation in control group and 20-22 days in group treated with peritoneum. While at days 55 in control group and day 42 in treated group no signs of lameness were observed. Thickening at site of operation was palpated in the two groups, and was subsided gradually, and at 20 days in control group and at 27 days in treatment group the thickening had been vanished.

Gross pathological evaluation. The gross examination in control group before specimen collection at 15 Post-operative day was revealed presence of severe adhesion between the Achilles tendon and neighboring tissue with partial healing of two tendon ends, presence of suture material (Fig 1: A). While at day 30 postoperatively moderate adhesion between the Achilles tendon and neighboring tissue with thickening of tendon at the site of operation was observed (Fig 2:A). After 60 days postoperatively presence of mild adhesion and fibrous tissue around the site of operation with complete healing of two incisional tendon ends (Fig 3: A). In treatment group gross evaluation at day 15 after operation was revealed presence of moderate adhesion between the Achilles tendon and surrounding tissue with partial healing of two incisional tendon ends rather than presence of surgical suture material and very thin layer of the graft around the site of operation (Fig. 1 :B). At 30th days mild adhesion with complete tendon ends healing was observed, absence of graft material was seen (Fig 2 : B). While at day 60 postoperatively lesser adhesion between the Achilles tendon and surrounding tissue was observed comparison with control group at the same period and absence of peritoneal graft tissue around the site of operation was seen with presence of complete healing of two tendon ends (Fig 3: B). By visual inspection, no change in the total tendon length or the length of the repair site was observed in all experimental animals.

Histopathological examination.

Injured tendons in control groups at 15 days post operation were showed formation of granulation tissue with hypercellularity of fibroblast characterized by large spindle nucleus with low density of collagen fiber. Formation of new blood vessels and severe inflammatory response characterized by infiltration of mononuclear cells represented by macrophage, lymphocyte and plasma cells with only a few scattered neutrophils being present were seen (Fig 4 :A). At 30 days PO the histological examination was revealed

presence of maturation for granulation tissue characterized by dense collagen fiber with moderate proliferation of active fibroblast. Also a mild inflammatory response of mononuclear cells between the collagen fiber was seen (Fig 5 :A), while at day 60 post operation mature connective tissue was present that characterized by organization of collagen fiber and presence of a little number of fibroblast and the inflammatory reaction was less strength compression to 15 and 30 days after operation (Fig 6 :A). In treatment group at 15 days after operation severe proliferation of active fibroblast with little number of blood vessels were seen, furthermore decrease in the density of collagen fiber with mild inflammatory response were seen. In addition to that. Foreign body granuloma reaction around the suture materials represented by foreign body giant cell, mononuclear macrophages and lymphocyte was present next to the suture lines. Graft degradation was characterized by loss of a distinct boundary between newly deposited host tissue and the original graft material (Fig 4 :B). At day 30th post operation maturation of granulation tissue was present characterized by have more eosinophilic organized collagen fiber with little number of fibroblast than which was seen at 15 days, also. There was loss of all morphologic evidence of the graft material, which was replaced by a relatively homogeneous deposition of new host-derived extracellular matrix material, the inflammatory reaction was not seen. Furthermore dilation and congestion of blood vessels with presence of edema between the collagen fiber were seen (Fig 5: B). At day 60 histological examinations showed decrease in the number of spindle fibroblast cells. In addition collagen fiber organization and vascularity were qualitatively similar to those of normal tendon tissue. Histological examination showed no signs of cartilage or bone formation within the tendon in control and treatment groups (Fig 6: B).



Fig 1(A) Control group show sever adhesion with partial healing of the Achilles at 15 days



Fig 1(B) treatment group show moderate adhesion and faster healing than control group. At 15 days

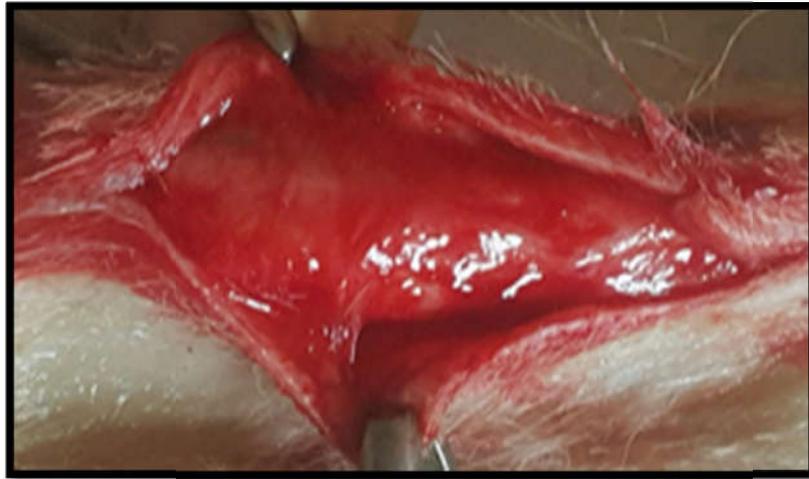


Fig2 : (A) : Control group show moderate adhesion at 30 days



Fig 2 (B): Treatment group show mild adhesion with complete absorption of peritoneum graft with faster healing than control group

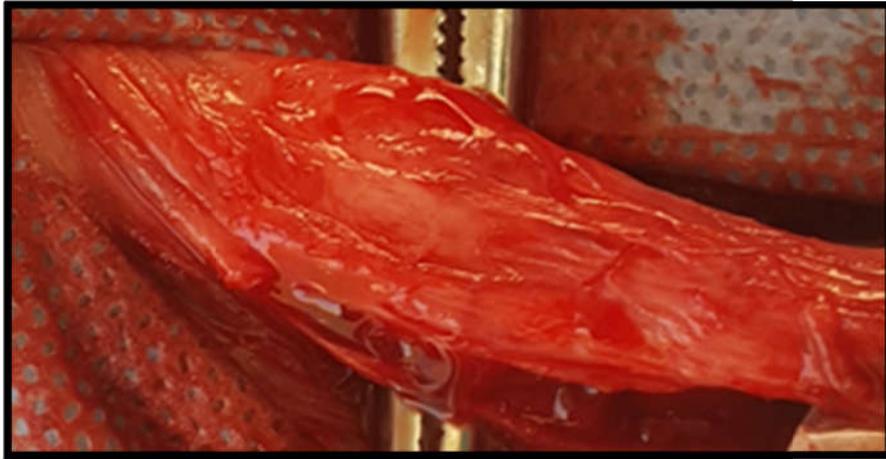


Fig 3 : (A): Control group show mild adhesion compression with day 60



Fig. 3(B): Treatment group show very minor adhesion with complete healing at 60 days

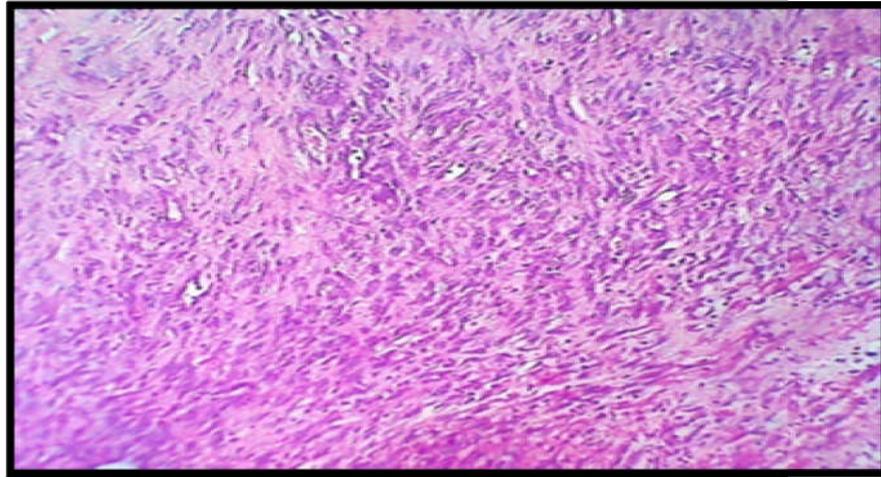


Fig.4: (A): control groups at 15 days post operation were showed formation of granulation tissue with hypercellularity of fibroblast with sever inflammatory response. H&E, 100x.

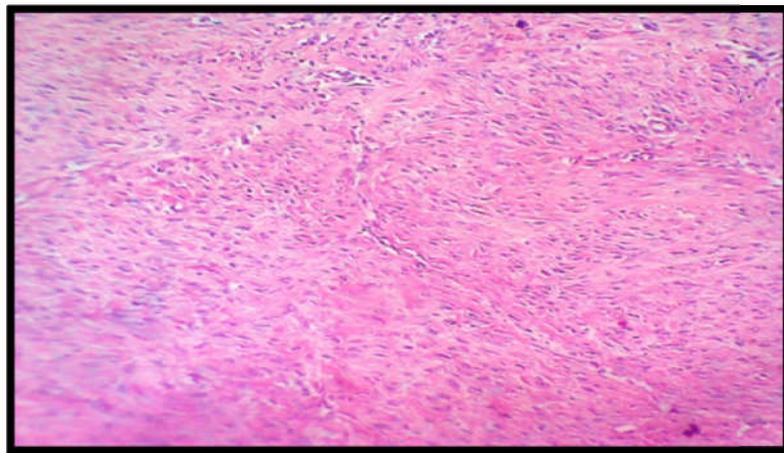


Fig.4: (B): In treatment group 15 days post operation showed sever proliferation of active fibroblast with little number of blood vessels with mild inflammatory reaction. H&E, 100x.

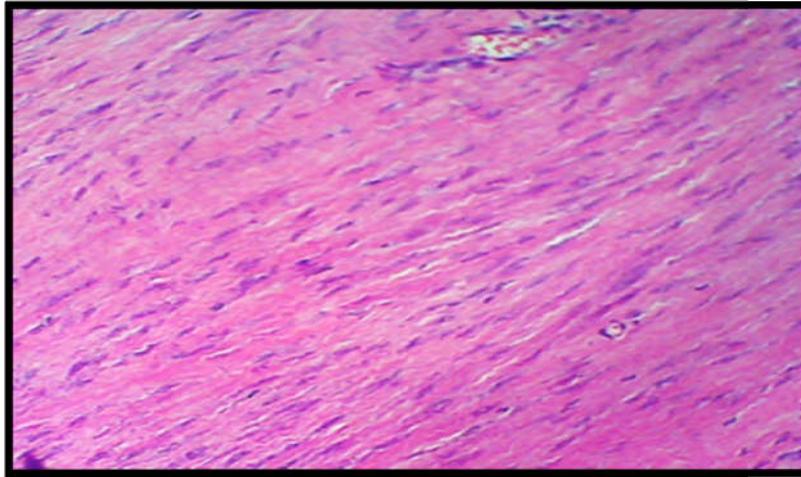


Fig.5: (A): control groups at 30 days post operation showed maturation of granulation tissue.with mild inflammatory response between the collagen fibers. H&E, 100x

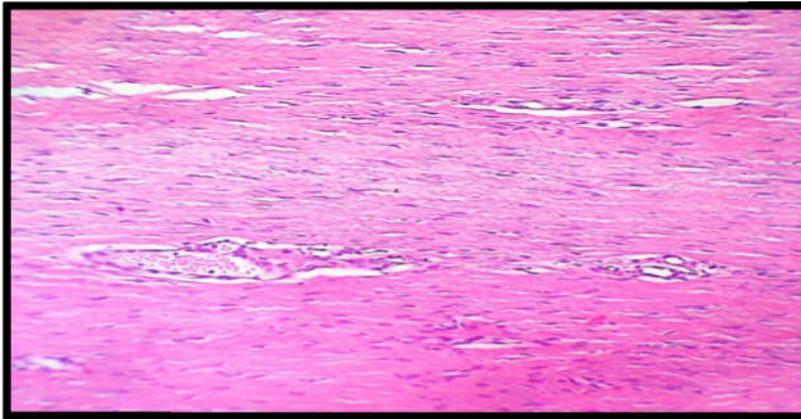


Fig.5(B): Treatment group at 30 days show maturation of granulation tissue characterized by have more oesinophilic organized collagen fiber with deposition of new host-derived extracellular matrix material. H&E, 100x.

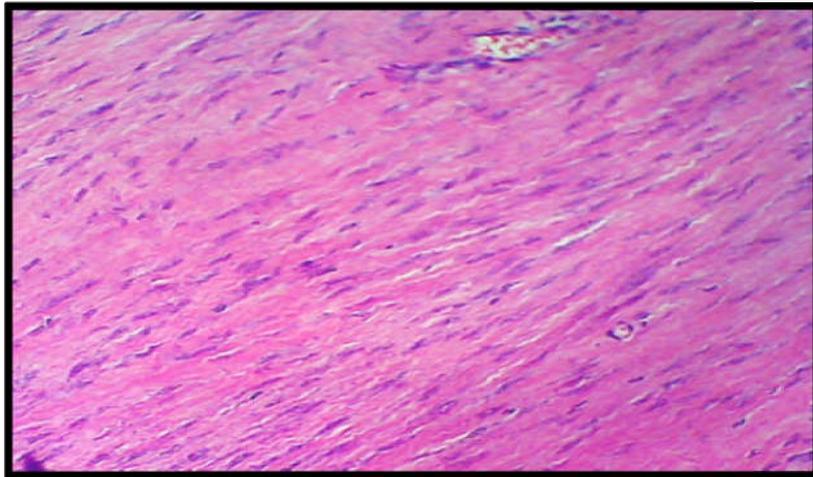


Fig.6 : (A): Control groups at day 60 post operation show mature connective tissue . H&E, 100x.

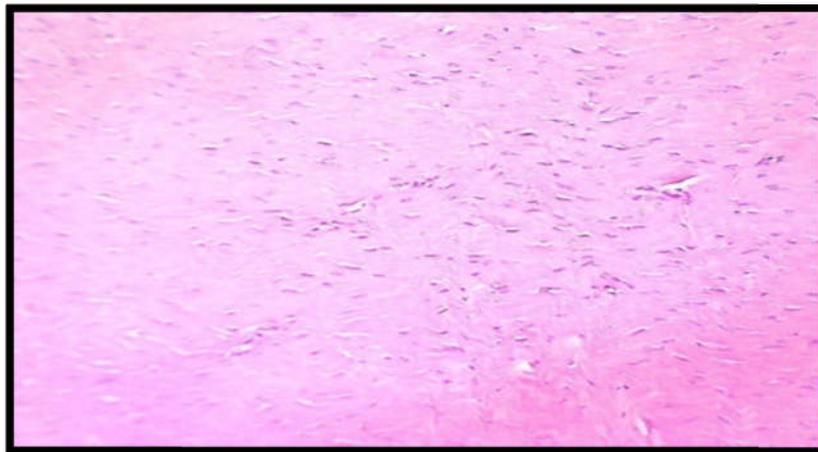


Fig 6.(B) :Treatment group at 60 days show decrease in the number of spindle fibroblast cells. In addition collagen fiber organization H&E, 100x.

DISCUSSION

Degenerative and Injuries situation of tendons represent about 50% of the musculoskeletal damage treated in orthopedic clinics (10, 19). For many years healing processes of tendon like other connective tissue has been an attractive matter for researchers. The capacity to restore functional healing in the treatment of tendon injuries is significantly improving with the development of advance surgical techniques (20, 4, 21, and 22). A successful restoration should have the suitable durability, tension and strength to meet the dynamic work load (9). The present study showed that treatment

with a single layer of autologous peritoneum scaffold resulted in apparently faster tendon healing. Results of this study suggest that, in a dogs model, peritoneum graft can be used as a tissue-engineered. The peritoneum graft maintained sufficient strength, while serving as a temporary scaffold for host tissue in growth and remodeling. Furthermore, peritoneum graft was not associated with presence infection, wound and graft dehiscence and showed a good incorporation of two transected tendon ends. Compared to operated tendons in control group (9). In this study, the gross and histological examination of repaired tendon healing by means of conventional surgical techniques improved by an autologous peritoneal graft suggested a clinically favorable outcome when compared to healing of non-grafted tendons. The healing of grafted tendons was more complete with considerably less adhesion formation that occurs with conventionally performed surgical techniques by using Kessler methodes. Adhesion formation after tendon repair is one of the major clinical concerns because it may contribute to poor functional outcome (2,9,24,25,23). The gross examination revealed considerably less adhesion formation in treatment group comparison with control group it was proposed that autologous peritoneum grafts may act as a protective shield to aid in decrease adhesion formation with the surrounding tissue and thus maintaining the gliding movement of the tendon. In this study, the groups that received the peritoneum grafts had a more production of collagen, higher extent of vascularization, more fibroblastic reactions and earlier granulation tissue maturation. These results may owing to the using of peritoneum graft which thought to be act as a source of cellular elements that accelerate healing. Peritoneum is a serous membrane consist of collagen, glycosaminoglycan and different cells (mast cells ,macrophage ,lymphocyte ,adipose cells) act as a source for growth factor (14,15). The collagenous resorbable material has been successfully used as an autograft for repairing different tissue such as tendon (26,27). The growth factors released from cell that's present in peritoneum play a critical role for tendon repair (17).TG Factor present in fibroblasts, macrophages, platelets and other cell types, it is exerts various effects such as cell proliferation, fibroblast stimulation, macrophage recruitment, , and collagen production. It enhances tendon healing. FGF stimulates angiogenesis, endothelial cell proliferation, collagen synthesis, wound contraction, matrix synthesis, and epithelialization. Insulin-like growth factor (IGF-I) have curative effects on tendon healing via numerous mechanisms, including stimulation , proliferation and migration fibroblast, collagen gene representation and protein synthesis, in addition to extracellular matrix synthesis and

improves the ultimate load of tendon (28,13) . There are several synthetic and natural materials have been used to supported healing process and the ideal graft material must be inert ,elastic, easy to collect and apply, have a suitable size and thickness , not adding unwarranted bulk, strong and should stimulate collagen formation (5,10,22). The peritoneum graft in this report was technically not difficult to collect or apply to the injured tendon. Additionally the grafted peritoneum was incorporated into the recipient site without added any undue bulk, it is rapidly degraded after implantation. The healing process of tendon is prolonged and characterized by formation of inevitable adhesion with the surrounding tissues which can compromise the limb function. The most significant finding of this study was that the use of peritoneum graft results in less adhesion formation and more clinically favorable outcome conduct compared to a non-treated control. Based on these results we recommend the use of an autologous peritoneum graft to support the tendon repair using the traditional Kessler techniques in ruptured Achilles tendon in dogs (29).

CONCLUSION

The peritoneal graft application has valuable and beneficial effects on treatment of ruptured Achilles tendon in dogs when utilizing it in conjunction with the traditional procedures.

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تقييم دور سقالة البريتون الذاتية على التئام وتر اكيلس في الكلاب اسماء حسين علاوي ليث محمود القطان

هدفت الدراسة الحالية لتقييم التئام وتر اكيلس الممزق تجريبيا والمعالج بالخياطه بتقنيه كسلر لوحدها او مع استخدام سقالة البريتون الذاتية في الكلاب
استخدم ١٢ حيوانا محليا خاليا من الاصابات العصبية والعظمية .قسمت الحيوانات عشوائيا الى مجموعتين متساويتين في كل مجموعة ٦ حيوانات .المجموعة الاولى مجموعته السيطره والمجموعه الثانيه مجموعته المعالجه . في كلتا المجموعتين تم قطع الوتر بشكل تام ثم خياطته بطريقه كسلر مع تثبيت سقالة البريتون الذاتية لتدعيم الوتر في المجموعه الثانيه .تم تقييم موضع العمليه سريريا اما الفحص النسجي المرضي فقد تم اخذ خزعات لفترات ١٥ و ٣٠ و ٦٠ يوما بعد العمليه . اظهرت النتائج ان استخدام رقع البريتون الذاتية لها تاثير واضح على التئام وتر اكيلس وكان جليا من خلال تكوين الارومات الليفيه وتقليل الالتصاق وزيادة شد الوتر وقوته وتحسين وظيفته مقارنة

بمجموعة السيطرة نستنتج من ذلك ان استخدام رقع البريتون الذاتية ذات اهميه وتأثير فاعل في علاج وتر اكليس الممزق تجريبيا في الكلاب اذا استخدم مقترنا مع الخياطه التقليديه.

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