Development of a Clinically Relevant Chronic Rotator Cuff Model in Sheep

Robert Hendrickx[1]

Master student Veterinary Medicine, Companion Animal Health, 3922715

Supervisors: C.F. Wolschrijn[1] **& J.E. Easley**[2]

[1]**Faculty of Veterinary Medicine, Utrecht University, The Netherlands**

[2]**Preclinical Surgical Research Laboratory (PSRL), Colorado State University, Fort Collins, United States of America**

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Abstract

Introduction: Rotator cuff tendon tears (RCT) are the most common soft tissue injuries in the shoulder joint. Various animal models have been described to investigate the rotator cuff, but all translational animal models have inherent weaknesses to examine the physiology of injury and repair of the chronically degenerated rotator cuff. The objective of this study was to evaluate a partial infraspinatus (IF) tendon transection model for chronic RCT and compare the injury characteristics of this model shown in sheep to those characteristics shown in people with severe chronic RCT injuries. It was hypothesized that a partial RCT of the sheep IF tendon would be repairable following a specified healing period and that characteristics of chronic RCT would closely mimic chronic rotator cuff tendinopathy characteristics noted in people.

Methods: The right side IF tendons of six sheep were partially detached followed by capping of the detached medial section of the tendon with Gore-Tex^{TM}. Sheep were sacrificed at a 6-week (n=3) and 12-week (n=3) time point. At both time-points, the reattachment of the IF tendon to the humeral footprint was simulated. Tissues were collected from both the detached and attached portions of the IF tendon. Human tissue samples of the supraspinatus tendon were harvested from patients undergoing primary reverse shoulder arthroplasty and served as positive controls of chronic rotator cuff tendinopathy for comparison.

Results: Sheep tendons were characterized by a more acute reactive and reparative pathological process when compared to the human tissues, which were more evident within the medial (detached) portion of the tendon. The lateral (attached) portion of the tendon also showed mild changes, which appeared to be more chronic and degenerative in nature when compared to the medial (detached) tendon. These changes in the lateral (attached) tendon were more similar to those observed in the chronic degenerated human tendon.

Conclusions: This translational model demonstrated similar histological changes within the tendinous and muscular portions of the sheep IF muscle that were noted in people with chronic rotator cuff tendinopathy. Six weeks post-surgery in sheep was an appropriate time to result in comparable histological findings of chronic rotator cuff tendinopathy to people, and the limited retraction of the detached tendon at that time point allows for anatomically correct reattachment to the humeral footprint.

Clinical relevance: This model is translational to the human condition and has potential to have positive impacts on basic science research as well as advanced development of rotator cuff devices or biologics.

Introduction

Rotator cuff tendon (RCT) tears (i.e., tears to the subscapularis, supraspinatus, infraspinatus, and teres minor tendons) are the most common soft tissue injuries in the human shoulder causing pain and dysfunction[1–4]. Around 250,000 repairs are performed annually in the USA, with a $3.44B annual societal impact[5,6]. The original insult causing the RCT tears can be multifaceted, but generally stems from an acute injury resulting in chronic degeneration or emanates from a combination of overloading and degeneration[7,8]. The tendon itself is not capable of healing naturally from a full-thickness tear[9], and degeneration is aggravated by several factors including, but not limited to, low blood supply within the tendon, the lack of acute treatment in most patients, and the thinning and disorientation of collagen fibers[8,10,11]. Therefore, it is oftentimes necessary to pursue surgical treatment if patients have functional impairment and persistent symptoms after conservative treatment[12]. Despite progressive approaches for rotator cuff repair, most surgical repairs experience a wide spectrum of failure rated (20 – 94%)[13,14]. For surgical repairs that fail to heal, the failure rate has been shown to correlate with the size of the tear, the time from the initial injury, the tendon quality, presence fatty atrophy of within both the tendon and adjacent muscle, and the surgical repair technique[12,14–20]. To investigate the tendinopathy of rotator cuff injuries, it is necessary to perform controlled experiments. As it is not possible to control extent of injury or to simulate the degeneration cascade with human cadavers, a translational animal model is the most appropriate; allowing the simulation of physiology of injury and repair.

There is a critical need to refine / re-develop a large animal model of the chronically degenerated rotator cuff injury. The ideal large animal model of RCT tears should be easy to produce and repeatable. Furthermore, a model with a similar tendon size to the human tendon should be used as it would allow application of devices intended for human use, and thus allow researchers to determine device effects on rotator cuff tendinopathy. Ideally, the biomechanical, structural, material, cellular, genetic, and histopathological observed in the animal model should be translatable and correlated with those changes observed within the human population.

The murine, leporine, canine, ovine, and non-human primates have been described as animal models for investigating the rotator cuff[21–25]. However, it still is unclear which animal model best represents the physiology of injury and repair of the chronically degenerated rotator cuff[26]. There are several inherent limitations to many of the currently used animal models. First, most of the tendons of the rotator cuff in animals are different in shape and size. Due to this difference to the human condition, it is not possible to test devices intended for human use, and the small size increases the difficulty performing the surgical techniques. Another shared limitation of these models is that most are quadrupeds (except for the non-human primate) and are considered to be more weight bearing on the

front limbs than humans. The human shoulder is often described as nonweight-bearing, but it has been shown that human shoulders do bear loads during normal everyday activities[24,27]. The daily activity is limited during the recovery phase following rotator cuff surgeries, which is not possible for quadrupeds. Conversely, non-human primates are more like humans because they are bipeds; however, the ethical burden and the increased expenses in their use make them unattractive as a model.

Previous research by our group and others have shown that the sheep is a well proven animal model for evaluation of basic science questions, as well as, a model to develop novel surgical devices or techniques[4,26,28–32]. Comparing anatomy of the shoulder regions of the different animal models, the infraspinatus tendon of the ovine model is similar in size and shape to the supraspinatus tendon in human[32,33]. Because human rotator cuff tears are often chronic and large in nature, it is possible to perform surgical techniques in sheep and studying the *in vivo* effects of chronic rotator cuff tears[13]. Furthermore, sheep are a convenient large-animal model because of availability, ease of handling and housing, animal cost and acceptance to society as a research model[32]. An earlier study performed by our group evaluated how a sheep model of acute repair responded following recovery. It showed that fatty infiltration and fibrosis are significantly increased after an acute rotator cuff and that an immediate repair following a rotator cuff tear is not sufficient[34]. In this study, the amount of fibrosis and fatty infiltration were not quantified in the histological sections, but was rather used as a qualitative review. This study evaluated the acute tear model. However, the acute tear model aims to test devices and biologics prior to the long-term signs of muscle and tendon injury take place, that is why the acute tear model does not translate well to the chronic rotator cuff tear condition in people. Research investigating the chronic ovine RCT tear model where the infraspinatus tendon was fully transected has some limitations, including significant tendon changes during the degeneration cascade, and increased magnitudes of tendon and muscle degeneration as compared to human tissues[22,26,32].

The purpose of this study was to develop and evaluate a novel ovine infraspinatus tendon transection model (i.e., partial transection), to improve the translatability of the current ovine chronic rotator cuff model (i.e., full transection). Specifically, the histopathologic injury characteristics of a partially transected infraspinatus tendon model in sheep were compared to the histopathologic injury characteristics of a chronically injured rotator cuff in humans. Human tissue collected during primary reverse shoulder arthroplasty procedures served as a control to compare against.

It has been shown that muscular and tendinous tissues will undergo degenerative changes if they are not loaded for an extended period. It was hypothesized that by transecting half of the tendon, the sheep would still be able to ambulate normally, yet degenerative changes would be induced in the transected half of the tendon. This would allow the simulation of reattachment of degenerated tendon tissue that

more closely mimics the mild histological degeneration seen in human chronic RCT's. It was hypothesized that a partial RCT of the sheep infraspinatus tendon would be more easily repairable than a full transection model following the degeneration period and that histopathologic changes of chronic RCT would more closely mimic chronic rotator cuff tendinopathy characteristics noted in people. To accomplish these goals, the histopathologic characteristic of ovine and human tissues were determined and compared.

Materials and Methods

The surgeries were performed at the Preclinical Surgery Research Laboratory (PSRL) at Colorado State University (CSU, Fort Collins, Colorado, CO) under IACUC (#15-6202A) approval. Human tissue was collected from Rush Medical Hospital (Chicago, IL) under IRB (IRB ID #16110707-IRB01) approval.

Human 'Model' - Tissue Collection –

Human tissue samples (20 mm x 10 mm) of the supraspinatus tendon were harvested for this study from seventeen (n =17) patients undergoing primary reverse shoulder joint replacement because of chronic rotator cuff injuries. Primary reverse shoulder arthroplasty is a surgical procedure in which the humeral head and glenoid are replaced with prostheses, allowing the joint to be stabilized. These served as positive controls of chronic rotator cuff tendinopathy. Tissue was placed in 10% neutral buffered formalin for fixation at the time of collection.

Ovine Model - Surgical Procedure –

Six (n = 6) skeletally mature female sheep (≥ 3.5ys of age) were utilized in this study. The sheep were divided between two sacrificial time points; three (n = 3) sheep were euthanized at 6 weeks and three (n = 3) at 12 weeks post-surgical creation of the tear.

All ewes were fasted 12 hours prior to surgery and were given procaine G penicillin preemptively (3 million units/kg, SQ – PenOne Pro™, Norbrook Laboratories Limited, Newry, Ireland), 1 gram of oral phenylbutazone (Bute Buloses, VEDCO, Inc., St. Joseph, MO, USA) and transdermal fentanyl patches (100 mcg, 50 mcg – Fentanyl Transdermal System, Watson Pharma, Inc., Corona, CA, USA) 24 hours before surgery.

The sheep were placed in left lateral recumbency under general anesthesia. The skin over the right shoulder joint was shaved and prepared for aseptic surgery using alternating scrubs of povidone-iodine (Betadine) and alcohol. The shoulder joint was draped for aseptic surgery. A skin incision approximately 12 cm in length was made in the shoulder joint. The *m. subcutaneous coli* was divided in line with the incision and the *m. deltoideus* was split along the tendinous division between the heads

of the acromion and scapula. The superficial head and insertion of the infraspinatus tendon was isolated, followed by sharp transection/detachment of the cranial 50% of the infraspinatus tendon at its insertion to the greater tubercle of the humerus (**Figure 1, A, B)**. The released portion of infraspinatus tendon was then capped/wrapped with an approximately 3 cm x 3 cm patch of Gore-Tex (**Figure 1, C**).

The capped end was not re-attached to the footprint of the humerus (**Figure 1, C**). Two 1.2 mm k-wire markers were inserted along the lateral edge of the humeral footprint to serve as anatomical landmarks indicating the site of partial transection. After k-wire insertion, the brachial fascia and subcutaneous tissues were closed as separate layers using 2-0 absorbable suture material (Polysorb), followed by skin closure with stainless steel staples. Following complete recovery, the sheep were allowed to eat and move *ad libitum*. The sheep were monitored daily throughout the study period for any signs of adverse events or complications and to evaluate pain, lameness/ambulatory function and incisional site healing.

Surgical assessment after 6 or 12 weeks of healing

In anesthesia both the 6 and 12-week time point animals, surgical assessment of scar tissue formation (i.e., was the scar tissue removable or affixed) and tendon retraction (measured in cm, by simulating re-attachment) was performed. After the *m. deltoideus* was split along the tendinous division between its acromial and scapular head, the acromial head of the *m. deltoideus* was reflected with malleable retractors. Overlying fibrous scar tissue was dissected away, and the Gore-Tex patch capping the transected portion of the infraspinatus tendon was localized and removed. The capped portion of tendon was assessed, and the retraction distance was measured. Surgical reattachment of transected portion was simulated by three board certified veterinary surgeons familiar with the acute repair technique.

Specimen collection

Sheep were euthanized humanely by intravenous barbiturate overdose (Pentobarbitone sodium, -88mg/kg). Following euthanasia, a complete gross necropsy was performed. The surgically treated shoulder was removed, and via dissection, the infraspinatus muscle and tendon attached to the humeral attachment were harvested. Following dissection, biopsies were taken from regions within both the attached and detached halves of the tendon and muscle. The regions of interest included the proximo-lateral and proximo-medial infraspinatus muscle, disto-medial and disto-lateral infraspinatus musculotendinous junction, the capped medial infraspinatus tendon, and the uncapped lateral infraspinatus tendon (**Figure 2**). Biopsies obtained from these regions allowed for the quantification of degeneration and comparison to data from the human chronic RCT tendon samples.

Histological Analysis

Following the tissue collection and fixation for both ovine and human samples, standard paraffin techniques were used, and one section was cut from each formalin-fixed sample and stained with Hematoxylin & Eosin (H&E). H&E stain was used to highlight and quantify the amount of muscle and fat within the samples. Sections were evaluated using a semi-quantitative scoring system based on an adaptation of the scheme put forth by Longo et al.[35]. Briefly, tenocyte reactivity, stromal cell proliferation, inflammation, neo-vascularization, discreteness of muscle-tendon junction, and myocyte degeneration/atrophy were quantified. Slides were scored on a five-point scale where zero corresponds to normal, 1 with minimal change, 2 with mild change, 3 with moderate change, and 4 with severe change. A board-certified veterinary pathologist (D.P. Regan) unblinded to species but blinded to tendon/muscle section performed the histopathological analysis on all samples.

Results

All six animals survived to the 6 and 12-week time points. No intra- or postoperative complications were noted. At both time-points the capped portion of the tendon was covered with a moderate amount of scar tissue that was easily removed via surgical dissection to expose the Gore-Tex for removal.

Reattachment of Medial Half of Tendon

To decide if the medial half of the tendon could still be re-attached, the distance of the medial half of the tendon was measured from the end of the detached portion to the original insertion of the greater tubercle of the humerus. A simulation of the re-attachment was performed to confirm if the tendon could still be re-attached. At the six-week time point, the detached portion of the infraspinatus tendon was able to be re-attached to the humeral footprint to create an anatomically correct repair (3 out of 3); the average retraction at this time point was 1.6 ± 0.2 cm (mean ± standard deviation). It was not possible to re-attach the infraspinatus tendon to the humeral footprint at the twelve-week time point due to significant retraction (2.2 cm ± 0.6 cm) and fibrosis of the detached tendon (0 out of 3).

Histology Results

Tendon Region

The medial infraspinatus tendon within the ovine model that underwent transection (**Figure 1, B**) was characterized by plump, reactive tenocyte nuclei and marked proliferations of stromal cells, which replaced normal tendon collagen (**Figure 3, B and C**). The tendon histologic scoring for inflammation, neo-vascularization and tenocyte reactivity & stromal cell proliferation are shown in Table 1. The ovine 12-week, transected, post-surgery sample scores had an average score of 2.67 out of 4 (mild-moderate changes) in the tenocyte reactivity and stromal cell proliferation category; human samples were scored at an average of 2.44 in the same category . The remaining ovine tendon collagen

frequently appeared degenerately, characterized by loss of the tightly-packed parallel fiber arrangement, with increased waviness and separation of palestaining collagen fibers (**Figure 3, B and C**). The 12-week transected ovine tendons scored closest to human tendons in the inflammation category, scoring 1.00 and 0.59 respectively (**Table 1**). The increased proliferation in all samples of capillary-sized new blood vessels showed that there is neo-vascularization (**Figure 3, C**). Infiltrates of mononuclear inflammatory cells, primarily composed of lymphocytes, with fewer plasma cells and rare macrophages, were often present between collagen bundles and surrounding neo-vessels (**Figure 3, B and C**).

The lateral (non-resected) infraspinatus tendon (**Figure 3, D and E**) was typified by a less inflamed yet more degenerative process characterized by pallor and marked separation and haphazard arrangement of collagen bundles, with loss of collagen fiber structure. Tenocyte nuclei in these tendons often appeared plump, reactive and occasionally resembled chondrocytes and were forming clusters of chondrocytes with multiple nuclei per lacunae.

Most evident within the human tendons was the loss of the tight, parallel organization of collagen bundles, with haphazard arrangement of collagen bundles, fibrillation and separation of collagen bundles. Additionally, multifocal regions of increased tenocyte reactivity and stromal cell proliferations were also present, again characterized by increased overall tendon cellularity, enhanced prominence of tenocytes between collagen bundles, and occasional streaming bundles of plump, reactive mesenchymal cells. In addition, the overall tinctorial properties of the tendon collagen was markedly different from normal tendons, characterized by diffuse pallor (pale pink staining) which coincided with marked loss of normal collagen bundle architecture and presence of thin haphazardly arranged collagen fibers, and occasional hyalinization and/or mucinous degeneration. Mild neovascularization was present in the majority of samples, while inflammation was minimal to essentially non-existent (**Figure 3, A**). Similar histological findings were noted across all animals at both 6- and 12-weeks and severity of histological changes did not seem to progress beyond the 6-week (**Figure 3, B, C, D, and E**).

Musculotendinous Junction Region

The infraspinatus musculotendinous junction in sheep was characterized by varying degrees of infiltration and separation of myocyte bundles by adipocytes (fatty infiltration). There is also presence of multifocal areas of myofiber atrophy and fibrosis. Relatively significant pathological changes were observed within the proximal infraspinatus muscle and musculotendinous junction, somewhat similar to those described for “fatty atrophy” of human rotator cuff muscles (**Figure 3, F**). No human samples of musculotendinous junction were available for analysis. There was an increased degree of fatty infiltration within the medial (transected) portion, as compared to the lateral (non-transected) portion

of the infraspinatus musculotendinous junction (2.17 vs. 0.80, **Table 2**). Additionally, the medial portion demonstrated rare myofiber atrophy and associated fibrosis, which was predominately absent from the lateral (non-transected) portion.

Infraspinatus Muscle Region

In the infraspinatus muscle biopsies, there is a clear difference in the level of inflammation between the transected and non-transected sides at both 6 and 12 weeks, with the non-transected side showing minimal to no signs of inflammation (scores of 1.08 and 0.20 for 6-week transected and non-transected, respectively, **Table 2**). The level of atrophy seen in the transected side of the tendon was much greater than in the non-transected side at the 6-week time point (2.17 and 0.80 respectively, **Table 2**). At 12 weeks, the level of atrophy was similar between both sides (1.83 and 1.42 for transected and non-transected sides, **Table 2**). The muscle tissue from the transected half had a noticeably larger level of neo-vascularization than did the non-transected portion (0.92 and 0.50 vs. 0.00 and 0.17 for 6 and 12-week transected vs. 6 and 12-week non-transected, **Table 2**). No human samples of infraspinatus muscle were available for analysis.

Discussion

This translational model development study describes an ovine model of chronic rotator cuff repair. It was hypothesized that the degeneration resulting from the partial transection model would mimic the degeneration seen in chronic rotator cuff injuries in humans. For this to be an effective model, it was necessary that the transected portion of the ovine infraspinatus tendon could be easily reattached in an anatomically correct position. In previous work performed by our group, Coleman et al[26] transected the entire infraspinatus tendon followed by capping. While reattachment of the infraspinatus tendon at 6 weeks following release was possible, it was not at 6-weeks and impossible at any time point after. In this study, 6-weeks appears to be a sufficient amount of time for signs of tendon and muscle degeneration to occur, while minimizing retraction of the tendon from the humeral head.

This research showed that sheep tendons in general were characterized by a reactive and reparative pathological process, as evidenced by increased inflammation, neovascularization, and markedly increased tenocyte reactivity and stromal cell proliferation. Histological changes within the human tendons were characterized by more diffuse, chronic, degenerative changes. The lateral (non-transected) infraspinatus tendon showed mild histological changes, which appeared more chronic and degenerative in nature as opposed to the reactive/reparative changes observed for the medial tendons. These changes in the lateral tendon, although less severe in terms of collagen degeneration, were more similar to those observed for the human tendon samples.

When comparing the transected infraspinatus muscle to the non-transected infraspinatus muscle, the non-transected portion showed minimal to no signs of inflammation; while the transected portion showed minimal signs at both 6 and 12-weeks. In a previous study, Gerber et al.[36] released the entire infraspinatus tendon, encased the tendon in a silicone tube, and carried out a tendon repair at forty weeks. Using electron microscopy, they found that the vascularity after tenotomy was not significantly affected. Similar to Gerber et al.[36], our study showed little to no changes in neo-vascularization at the 6-week and 12-week time points in both sides of the infraspinatus muscle. It can then be inferred that the level of neo-vascularization seen in the infraspinatus muscle is not sensitive to size of tear in the tendon.

The loss of the attachment from tendon to bone changes the physiology, structure and function of the muscle[37]. Tears in the rotator cuff lead to degeneration of the muscle with the amount of fatty infiltration and muscular degeneration increasing with tear size[38]. This study revealed that a larger level of degeneration/atrophy occurred on the transected side than the intact side at the 6-week time point. By the 12-week time point, the level of atrophy had evened out between sides, with both sides having mild atrophy levels. Steinbacher et al.[39] found that human patients with a massive rupture (> 5 cm) of the supraspinatus muscle contained a significantly higher amount of fatty tissue and almost double the amount of intracellular lipids than those in the control patient group. Gerber et al.[36] found a development of muscular atrophy and an increase of fatty infiltration at both 16 and 40 weeks after tendon release. Similar to Gerber and Steinbacher's findings in sheep and humans, both fatty infiltration and atrophy were present in both sides of the muscle samples at both time points.

As with all translational animal models, there are limitations correlating results to clinical human cases due to differing anatomy and mechanics. Rat models are unique in that their shoulders have a supraspinatus tendon that passes under an enclosed arch, similar to humans[13,24]. Due to sizing difference to the human condition, it is not possible to test devices intended for human use, and the small size increases the difficulty of performing the surgical techniques.

A limitation of this research is that the histological sections were scored semi-quantitatively following a grading scheme, which does not calculate exact levels of features of interest such as fatty infiltration or cellularity. This study compared the medial and lateral sides of the muscle, musculotendinous junction, and tendon; however, only part of one half of the tendon was transected. Since the rest of the muscle and tendon were still attached, the degenerative effects may be seen in both sides of the muscle and musculotendinous junction. Human tissue samples were limited to only tendon; no muscle was available for analysis. Within the human samples, there was a large amount of variation. This is likely due to the uncontrolled nature of the damage in the human tendons (samples were taken at various time points after differing levels of injury).

The low sample size in this study (n = 3 at both time points) resulted in low statistical powers, making it difficult to prove statistical differences. While sample size was small, a direct comparison within sheep was possible and results were similar across all animals at each time point. Comparisons within the same animal and across time points and species was still possible with a small sample size. A statistical power analysis was performed for sample size estimation. Running a GPower 3.1 software for power analysis with an alpha = 0.05, a size effect of 0.80 and a power of 0.80, the projected sample size needed would be approximately N=26 for each group (human and sheep). A recommendation for future work is to propose a sample size of n=26 for each group, this sample size would be adequate for this study to prove statistical differences.

Future work in the development of a more translatable large animal model for chronic rotator cuff degeneration will investigate the RNA changes associated with the degeneration of both human and ovine rotator cuffs; adding a level of validation in the similarity between the model and human condition. Furthermore, non-destructive biomechanical testing will be utilized to quantify intact ovine tissue and degenerated ovine tissue at several different time points. This testing will allow for histologic biopsies to be taken after mechanical testing, providing a way to correlate mechanical properties, histologic changes, and RNA expressions with levels of degeneration of the tissue.

Development of a more clinically translatable chronic rotator cuff injury model is an important step in furthering the state of human medicinal treatments of RCT's. An accurate model will help improve basic science research and enable researchers to investigate implantable devices, cellular therapies, prevention of degenerative changes, rehabilitation options, or new surgical techniques. These improvements will have a positive impact on the many people who suffer from RCT's globally.

Conclusions

In summary, this translational model of chronic rotator cuff injury demonstrated similar histological changes within the tendinous and muscular portions of the sheep infraspinatus muscle that were noted in people with chronic rotator cuff tendinopathy. Six weeks post-surgery in sheep was an appropriate time to result in comparable histological findings of chronic rotator cuff tendinopathy to the human tissue we examined, and the limited retraction of the detached tendon at that time point allows for anatomically-correct reattachment to the humeral footprint.

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Appendix I: Figures

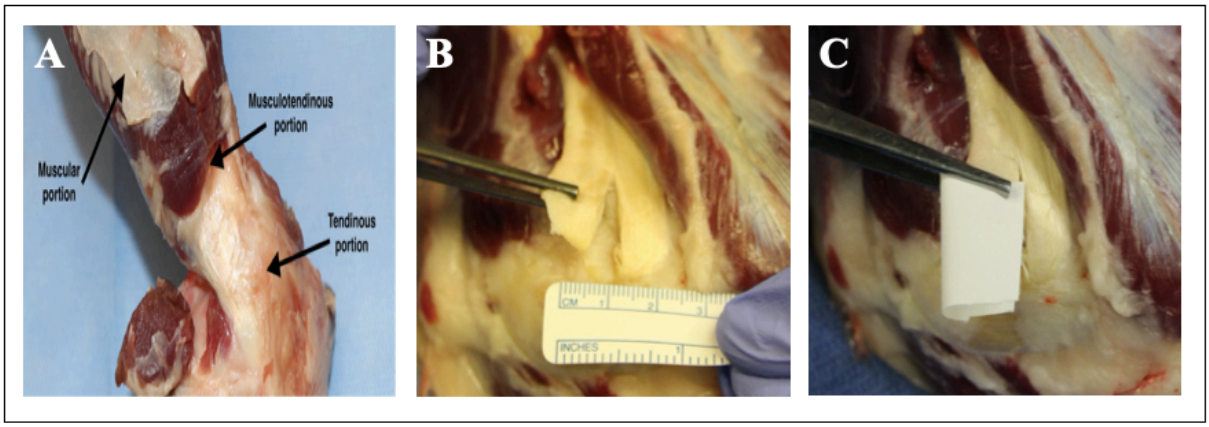

Figure 1. Surgical photographs; **(A)** Full non-transected tendon attached to the humerus **(B)** Uncapped transected IF tendon, **(C)** Transected IF tendon capped with Gore-tex™.

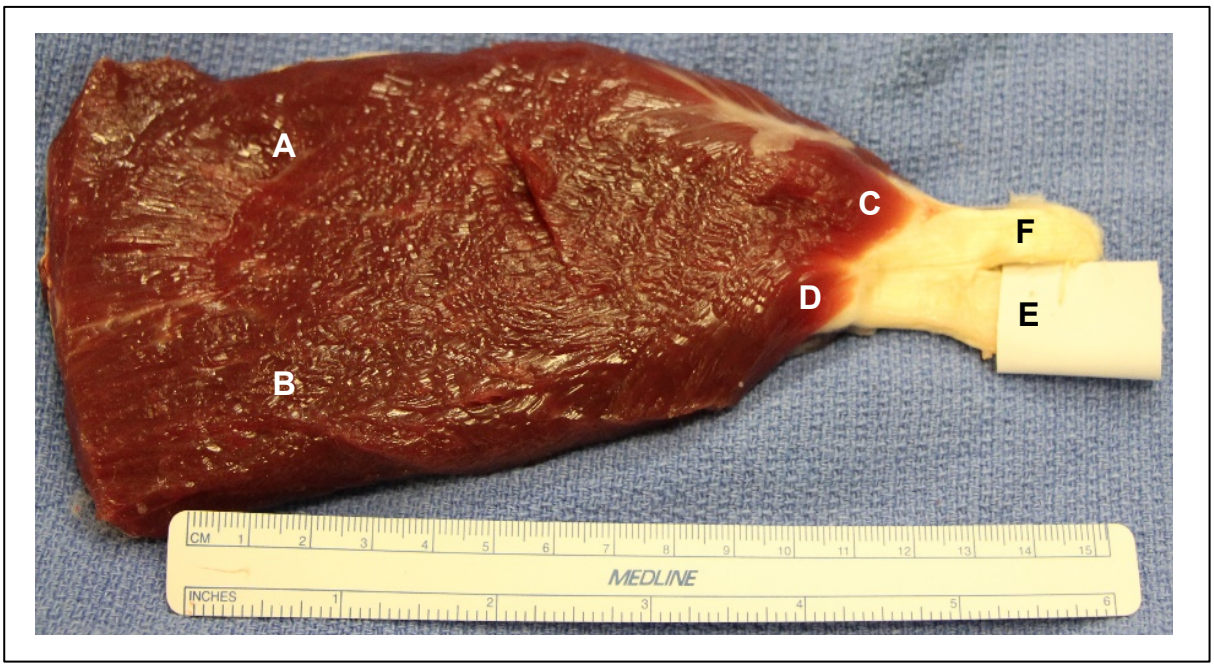

Figure 2. Histologic biopsy locations: **(A)** Proximo-lateral IF muscle, **(B)** Proximo-medial IF muscle, **(C)** Disto-lateral IF musculotendinous junction, **(D)** Disto-medial IF musculotendinous junction, **(E)** Transected medial IF (shown capped with Gore-Tex™), **(F)** Non-transected lateral IF.

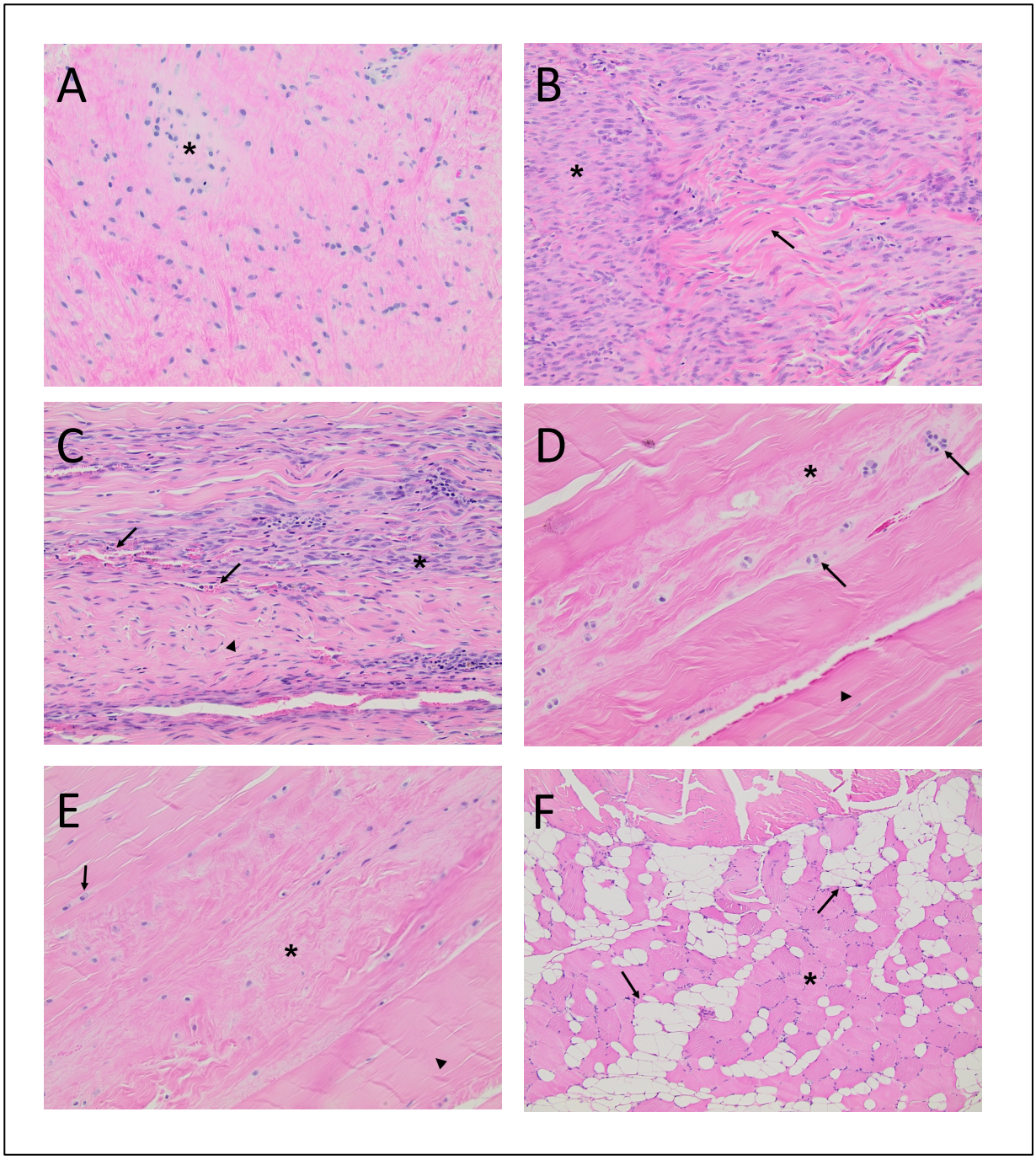

Figure 3. IF specimens stained with Hematoxylin and Eosin: (**A**) Human RC supraspinatus tendon, 10x. Asterisk = cartilaginous metaplasia. (**B**) and (**C**) Transected portions of the sheep IF tendon, 10x. Asterisk = proliferations of stromal cells. Arrowhead = palestaining collagen fibers, arrows = neo-vascularization. (**D**) and (**E**) Non-transection portions of the sheep IF tendon, 10x. Asterisk = collagen fiber structure, arrows = nuclei per lacunae. (**F**) Proximo-medial aspect of the sheep IF musculotendinous junction, 10x. Asterisk = myocyte bundels, arrows = adipocytes.

Appendix II: Tables

		Human Mean / SD	Ovine: 6-Week Mean / SD	Ovine: 12-Week Mean / SD	
Tendon Histologic Scoring	Inflammation	0.59 / 0.87	1.83 / 0.85	1.00 / 0.00	Transected
			1.50 / 0.71	1.33 / 0.47	Non-Transected
	Neo-Vascularization	2.24 / 1.03	3.67 / 0.47	3.33 / 0.94	Transected
			3.00 / 0.00	3.00 / 1.41	Non-Transected
	Tenocyte Reactivity & Stromal Cell Proliferation	2.44 / 0.79	3.67 / 0.47	2.67 / 0.94	Transected
			3.33 / 0.47	2.00 / 0.82	Non-Transected

Table 1: Histologic scoring of tendon biopsies. Human samples N=17. Ovine 6 and 12-week, transected and non-transected samples N=3.

		Ovine: 6-Week Mean / SD	Ovine: 12-Week Mean / SD	
Musculotendinous Junction	Discreteness of Muscle-Tendon Junction	2.50 / 0.87	1.67 / 0.47	Transected
		1.00 / 0.82	1.50 / 0.50	Non-Transected
	Neo-Vascularization	1.25 / 0.43	1.00 / 0.00	Transected
		0.33 / 0.47	0.50 / 0.50	Non-Transected
	Tenocyte Reactivity & Stromal Cell Proliferation	2.75 / 0.83	1.00 / 0.00	Transected
		1.00 / 0.82	0.50 / 0.50	Non-Transected
Muscle	Inflammation & Cellular Composition	1.08 / 0.84	0.83 / 0.69	Transected
		0.20 / 0.40	0.17 / 0.37	Non-Transected
	Neo-Vascularization	0.92 / 0.45	0.50 / 0.50	Transected
		0.00 / 0.00	0.17 / 0.37	Non-Transected
	Myocyte Degeneration / Atrophy	2.17 / 0.69	1.83 / 0.69	Transected
		0.80 / 0.75	1.42 / 0.61	Non-Transected

Table 2: Histologic scoring of musculotendinous junction and muscle biopsies. Ovine 6 and 12-week, transected and non-transected samples N=3.