Author's response to reviews

Title: Short and long terms study of the experimentally ruptured tendon healing in rabbits

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Version: 2 Date: 1 July 2011

Author's response to reviews: see over
Dear Chief Editor

Ref.: MS: 1268797133535166

In response to the comments and suggestions in your letter of 9th May 2011 we have followed the referee’s suggestions and made the following amendments to our MS. I would like to thank you and the referees for your valuable point of views and suggestions. Our changes in the text are shown pink. Q is the question or suggestion raised by the respected referees and Answer is our explanation in response to the respected referees’ suggestions.

With the best wishes and good luck

A. Moshiri, DVM, DVSc, Assistant Professor of Veterinary Surgery

ANSWER TO THE REVIEWERS COMMENTS

Reviewer number: 1

Q 1: This is a well designed and executed animal study on the naturally healing course of a surgically cut and repaired flexor tendon. As such it provides support for previous evidence of the long term healing conditions for tendon.

Answer 1: We would like to thank the respected referee for his/her scientific attention and concerns.

Q 2: From an academic clinical viewpoint the validity of animal studies must however always be kept in mind and stressed. Firstly a healthy human tendon does not rupture
and it does not rupture as a surgical cut. Further it does not rupture isolated, as can be seen from enclosed excellent photos. The importance of the surrounding hematoma and disrupted peritendon structures is thus not studied. Such factors are outlined well in the recent review article "The Validity of animal studies by Liu et al 2010".

**Answer 2:** The respected referee's statement is right that a healthy human tendon does not normally rupture and it does not usually rupture as a surgical cut, however, in the cases of trauma such as, accident, transaction by a sharp instrument and war traumas this type of lesion is common both in Human and Veterinary Medicine. In addition, tendon rupture may happen during or after orthopedic surgery and application of internal fixation techniques by fixative materials and screws. Importance of the surrounding hematoma and disrupted peritendon structures at earlier stages of healing are properly studied by many investigators, while studies on the later stages of healing are very scanty. The present experiment was designed to compare the structural and physical properties of ruptured tendon on 28 and 84 days post injuries. At both stages, the hematoma and fibrin clots are disappeared and the injured areas are in the fibroplasias and remodeling stages of healing. Please see the review by Sharma and Maffulli, 2005.


I searched PubMed, SCOPUS and ISI Web of Knowledge to find the review “The Validity of animal studies by Liu et al 2010” that was suggested by the respected referee but unfortunately I was not able to find this review.

**Q 3:** Secondly the validity of using a model with a flexor tendon in an animal in relation to clinical human problems such as Achilles tendon and Rotator cuff ruptures must also
be considered so that too wide implications are not drawn. However, subject to such
limitations highlighted this article is well worth of publication.

**Answer 3:** This is basically true that the validity of using an animal model in relation to
clinical human problems such as Achilles tendon and Rotator cuff ruptures must be
considered so that too wide implications are not drawn, but animal studies in such
experiments is inevitable and many investigators including ourselves performed similar
experiments using animal models to study different aspects of tendon healing. This study was
designed to simulate the sharp tendon rupture in human and animals. This experimental
model is suitable for hand flexor tendon surgery in human practice and SDFT injury in
equine practice. Some of our previous published papers in this respect are as follows:

injury to treatment with a polysulphated glycosaminoglycan (ADEQUAN). Connective Tissue
Research, 49(5), 351-360.


remodeling in the early stages of healing of tendon injury in rabbit. Archives of Orthopaedic

Q4: Level of interest: An article of importance in its field.

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

**Answer 4:** We would like to thank the respected referee for his/her encouraging point of
views.

**Reviewer number:** 2
Q1: This study was designed to compare the outcome of healing of the ruptured superficial digital flexor tendon (SDFT) on 28 and 84 days post injury in rabbits. They concluded that tendon healing is very slow and that the morphological and biomechanical parameters were still inferior to the normal tendons at 84 days post-injury. Although the study is interesting, however, the manuscript includes many careless mistakes such as lack of explanation and findings. Also it was very difficult to understand the hypothesis because of too many meaningless figures and too less explanation. The authors should address the hypothesis. The authors should address the points to really prove their hypothesis.

Answer 1: We tried to explain some of the terminologies and findings in the revised version. However, most of these terminologies and findings are usual and well known for the orthopedic surgeons and pathologies and defining them increases the volume of the paper. However, if the respected referee still believes to expand or clarify some of the definitions I will request him (her) to specify those terms or findings; I will introduce them in a second revised form before the paper goes for production. However, the respected referee can see most of these terminologies in some of our previous papers too. I request the respected referee to see the following papers:


As the respected referee suggested the hypothesis was addressed at the Abstract (please see page 1, lines 17-20) and also at the end of the Introduction (please see pages 3 and, lines 68-76) and we addressed the points to prove the hypothesis in the Discussion and Conclusion (please see pages 17,18, lines 414-437).

Q 2: Page 1, line 15: in abstract part, there is no hypothesis. The authors should address the hypothesis.

Answer 2: As the respected referee suggested, the hypothesis was added at abstract (please see page 1, lines 17-20)

Q 3: Page 2, line 25: not need “but”

Answer 3: It was corrected as the respected referee suggested.

Q4: Page 5, line 123: How did authors analyze walking activity quantitatively?

Answer 4: Lameness and comfortable/uncomfortable physical activities were defined as below:

1-tarsal flexion degree of the animal in the cage (the animals were observed for 30 second in the cage) and in the room (the animals were observed in the room for 30 seconds): a) below 30 degree, b) between 30-60 degree, c) between 60-90 degree.

2-weight distribution on the hind limbs in the cage and in the room: a) most of the weight bearing was on the thoracic limbs, b) most of the weight bearing was on the normal hind limb (the right leg), c) most of the weight bearing was distributed equally on the bind limbs.
3-pain in palpation of the injured area: a) the animals show reaction, b) the animals show no reaction

4-pain in the complete extension of the hind paw: a) the animals had pain and flexed the extended limb, b) the animals had no pain and did not flex the extended limb.

5-toe and heel position of the injured leg (the left leg): a) heel was up and toe was on the ground, b) heel and toe were on the ground, c) heel was on the ground and toe was up.

The authors added this part of the answer in a brief paragraph at pre euthanasia section of the materials and methods (Please see Page 5 lines 119-123). However, the assessments were qualitative but not quantitative.


Q 5: Page 7, line 155: “based on their diameter, cytoplasmic granules and cell staining capacity” This is not enough to detect mesenchymal cells. If authors said so, you need to add previous reports regarding to the methodology.

Answer 5: This method for tenocytes detection has been previously been described by different researchers. Please see:


Q6: Page 8, methods section: authors need to describe ultrasonography methods.

Answer 6: As the respected referee suggested the ultrasonography method was described in the revised version (Please see page 6, lines 131-142).

Q7: Page 9, line 212: “The diameter of the injured tendons was significantly larger than those of their uninjured normal contralateral at days 14, 21 and 28 day post-injury” This is not gross morphology.

Answers 7: All the clinical symptoms, necropsy findings and tendon diameter are explained in the subtitle “Clinical findings and gross morphology”. However, if the respected reviewer has a better suggestion please informs me to do look after it.
Q 8: Page 9, line 219: How did you recognize? How did you analyze? Authors should mention the quantification method.

**Answers 8:** As the respected reviewer suggested, the quantification method has been mentioned in the Materials and Methods section.

Q9: Page 10, line 223: Fig. 3 does not make sense. Authors cannot say by showing only one sample.

**Answer:** As the respected referee suggested Fig. 3D (Radiography) was deleted in the revised version

Q 10: Page 10, line 227: authors need to explain measurement method of ultrasonography. Which time point the diameter of tendon and weight are measured?

**Answer 10:** As the respected referee suggested this assessment was described in the Materials and Methods (Please see page 6, lines 131-142). As it was mentioned in the earlier version of the manuscript too, weight of the animals and tendon diameter over the skin were weekly measured until euthanasia (Please see page 5, lines 109-113).

Q11: Page 10, line 243: in Fig. 4, B and D are both intact. The thicknesses look quite different between intact at day 28 and day 84. Please explain.

**Answer 11:** The figure 4 D was corrected as the respected referee suggested.
Q 12: Page 11, line 258: how did authors detect mature cells? What is the difference between mature and pre-mature cells?

Answer 12: Please see my answer to question #5.

Q 13: Page 13, line 316: there are many suture techniques for tendon rupture. Is this suture procedure is enough? Did authors compare other suture techniques? How did you decide this suture procedure?

Answer 13: The modified Kessler core technique following running pattern is an accepted method for early biomechanical performance of the ruptured tendons. The purpose of the present study was not to evaluate the effects of suture techniques on tendon healing. However, there are many studies on the effects of the suture techniques on the biomechanics and performance of the tendons. The concept of sharp ruptured tendon healing related to time was the real purpose of this study.

Please see the following references for Kessler pattern in primary repair of tendon rupture.


Q14: Page 14: Sham operation is needed in comparative in vivo study. Did authors have sham group?

Answer 14: The purpose of the present study was to evaluate the healing process of the standard sharp rupture induced in experimental practice in rabbits in two different times. It seems there is no need for sham operation in such an experiment. The 28 day post injury group could be determining as a sham group for day 84 post injury.

Q 15: Did authors analyze repaired tendon such as immunohistochemistry, PCR, or western blot?

Answer 15: No we studied the structural hierarchy of the injured tendon using radiography, ultrasonography, gross morphology, histopathology and electron microscopy methods together with their biomechanical properties. Biochemical and molecular analysis are more applicable for early inflammatory and fibroplasia stages of healing and we are going to concentrate on molecular and biochemical assessment in the early stages of tendon and ligament healing in near future. Many investigators did similar studies to our experiment. Please see the following references:


Q16: Page 21, figure legends

Fig. 2: Which time points the diameters were measured? No mention in methods section.

The time of measurement is mentioned in the methods section. Please see page 5, lines 112-114.

As it is mentioned in the Materials and Methods, “the tendon and the covering skin diameter around the injury site and in a comparable area of the uninjured contra-lateral tendon were measured, using a micrometer measurement device (Samsung, Seocho-gu, Seoul, Korea), before injury, and then weekly, until the animals were euthanized.”

Q 17: It is better to put together, Fig. 2B and Fig 3

Fig 3D: no need

Answer 17: As the respected referee suggested Fig. 2B and Fig.3 were put together and Fig 3D (Radiography) was deleted. It was corrected
Q 18: Fig.5: authors should show border between injured site and normal area in lower magnification section.

Answer 18: The tissue sections are collected from the central areas of the lesions. However, after full thickness failure of the tendon, the injury will expand throughout a long distance of the tendon. This is because the haematoma, edema and inflammatory cells migrate from the injury site to the surrounding tendon proper and peritendinous fascia.

Q 19: Fig. 6C: which time point of this normal contralateral tendon?

Answer 19: At euthanasia (at the end of the experiment)

Reviewer number: 3

Editors comment: The topic is interesting and the paper is well written and well organized in almost all its sections. However, some clarifications are needed before to be considered for acceptance.

Answer: We would like to thank the respected referee for his/her scientific attention and concerns.

Q1. The Introduction is too long and long winded. It should be shortened, mostly focusing on the sense of the study. The authors should also clarify the hypothesis of the study.
**Answer 1**: As the respected referee suggested the introduction has been shorten in the revised version and focused on the concept of the study. In addition, the authors tried to clarify the hypothesis of the study at the end of introduction section.

**Q2**: Materials and methods are reported in detail in each section. It is unclear whether radiographs were performed every week? In this case, the authors should explain the reason why.

**Answer 2**: The radiographs were performed every week to find out if the tendon injury could alter the joint space or induce other abnormalities in the surrounding musculoskeletal system. In addition, the authors took the radiographs to show that the animals were mature.

**Q3**: Results: The authors do not specify how they measure the tendon (ultrasonography, micrometry). As reported in Material, they assessed the covering skin diameter and compared it to the uninjured side. In results there are no data on this. Is this a validated way to measure this variable? It appears very indirect

**answer 3:**
Measurement of the tendon diameter from the skin and fascia over the injury site is an accepted method. Please see the following references in this respect:


This method is an index of tendon inflammation and edema following a tendon injury. The authors controlled the skin and fascia over the lesion by ultrasonography observations. This could well be responsible to describe the difference between the injured treated and control tendons. Moreover the relation between the diameter of the inflamed tendon, skin and fascia is not important for the authors to investigate the tendon healing process. The purpose of the present study was to measure the total swelling of the injured area as an index of tendon inflammation and edema beside other investigations.

The ultrasonography and micrometry methods were added as the respected referee suggested. Please see page 5, lines 108-117 and page 6, lines 132-143.

Q4: The US patterns should be better described, reporting findings in detail in table.

Answer 4: It was corrected as suggested by the respected reviewer.

Q5: The discussion is coherent with study findings. The Authors should mention the main weaknesses of the study. Firstly, the short follow up. We believe this follow up is too short to allow to draw definitive conclusions.
Answer 5: As the respected referee suggested we mentioned the main weaknesses of the study including the short follow up at the end of discussion (please see pages 17 and 18, lines 414-428).

However, when we refer to long term study, it means that this time is long for tendon healing in rabbits, but not for the humans. It means that if we accept that the mean of human's age is 70 years, and for rabbits is 7 years, it means that the evolutionary period of rabbits is 10 times faster than humans. Thus 84 days post injury in an experimentally induced tendon rupture is approximately equal to 840 days post injury in human cases of tendon rupture. 840 days means 2.3 year. To the knowledge of the authors, more than one year after tendon injury, spotted as a long term in human patients by Sharma and Maffuli, 2005.


Q 6: As the rupture was iatrogenic, and the tendon was transected, the healing process would be different compared to the process after rupture occurring in diseased tendons with the classical features of the condition.

Answer 6: The healthy human tendon does not rupture accidentally, however in the cases of trauma, during the surgery and in the similar conditions, it can be transected by a sharp instrument. There are many traumatic cases in human and veterinary medicine such as car accident that results to tendon transection because of sharp and hard metal materials that penetrate the skin and cut the intact tendons. In another approach, in the cases of orthopedic surgery, for example the internal fixation techniques to reduce movement of the tibia or metatarsal bones, the surgeon should expose the fractured site in a manner to implant the fixation plate or other fixation materials, in such cases it is very common to cut the intact tendons during these procedures. The present study pays attention to such happenings and
investigates the healing process of injured transected tendons, in such situations that surgeon decides to repair the transected tendons, during surgery or in which cases of sharp traumatic tendon rupture.