



## Structural Appearance of Degenerative Changes in Deep Digital Flexor Horse Tendon using Image Analysis Microscopy

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### Abstract

Tendon injury is one of the most common causes of wastage in the performance horse; the majority of tendon injuries occur to the superficial digital flexor tendon (SDFT) deep digital flexor tendon (DDFT) and suspensory Ligament of the forelimbs. The selected structure of this sample is the deep digital tendon (DDFT) which measured to the mid-metacarpal region of the forelimb, out of a count population by thirty adult horses examined using Image analysis microscopy (IAM) sex distribution was not included into account but ages including. A change in its structural organization due to aging following tendon degeneration was unknown. The tendon structure under (IAM) showed a dense regular connective tissue arrangement that reflects the mechanical requirement of this tissue. It is defined by thick regular bundles of longitudinal collagen fibres arranged in a zig-zag conformation. All tendons examined from these horses had no history of deep digital flexure tendinitis and were all macroscopically normal.

In conclusion, the study showed that the normal structure of equine DDFT can be clearly assessed using Novel technique of Image Laser analysis Microscopy.

**Keywords:** Horses; DDFT; Degeneration; IAM; Tendon

### Introduction

Tendon pathologies are among the most common musculo-skeletal disorders in equine [21]. Injuries to the tendon are major causes of wastage throughout the equine industry [8,28] and also highly significant because of its prevalence and effects [8]. Tendons may be injured through excessive strain or acute percutaneous trauma, such as lacerations or kicks. Overstrain injuries may occur as a result of a sudden overload of the tendon which exceeds its capacity for resistance, or as a result of degenerative changes within the tendon that weaken it. Lack of fitness, fatigue, and poor conformation may promote excessive biomechanical forces on a tendon in the equine athlete [6,12,16,18,20,23,25]. In these cases, the tendon can lose its structural organization and eventually ruptures as a result of excessive use.

Tendons are elastic structures that transmit forces generated by muscle to bones to allow movement of the skeleton such as the superficial and deep digital flexor tendons [14]. These energy storing tendons act as springs with associated muscle bodies serving to dampen vibrations [31].

Some tendons are more prone to injury than others. Structures and the superficial digital flexor tendon (SDFT) and the deep digital flexor tendon DDFT were the most commonly affected structures especially in the forelimbs [10,16,21,26]. The main causes of horse tendon injuries are sudden tendon extension with a sharp contraction of muscles, an early high level of physical activities, and tendon hyperthermia [9,17]. The death of tenocytes in the tendon injury may have a significant impact on the onset of tendinitis which characterized by microtrauma lead to rupture of tendon fibers [28].

## Material and Method

### Tendon samples

The structure of the equine DDFT was sampling collection of thirty horses divided in 3 category groups of different ages adults (n = 15) horses, middle aged (n = 13) horses and (n = 2) old horses. The samples collected from the mid-metacarpal region of the fore-limb using Image analysis Microscopy (IAM). (Table 1) All horses were clinically normal without any Signs of injury or lameness.

### Image Analysis Microscope (IAM)

#### Techniques

Fresh samples of the DDFT from the left limb were isolated from young, middle-aged and old horses at the level of the mid-metacarpal region. The tendon specimens were collected by cutting the tissue into 1 cm cubes and preserved in Karnovsky's fixative for 12 to 24 hours at 4°C. The samples were then washed with 3 changes of 0.1M sodium cacodylate buffers for 10 minutes each. The samples were post fixed in 1% buffered osmium tetroxide for two hours at 4°C again washed with another 3 changes 0.1M sodium cacodylate for 10 minutes each. The samples were dehydrated in a series of acetone of different concentrations (35%, 50%, 75% and 95%) for 10 minutes each and lastly washed in 3 changes absolute acetone for 15 minutes each [13,15,24]. The samples were then transferred into specimen baskets, put into critical point drying for approximately 180 minutes and mounted onto the stub using double-sided tape. Lastly the specimens were gold coated in the sputter and examined under scanning electron microscope (Joel, Japan).

Number of Horses	Age of Horses
15	8 -10 years
13	12- 17 years
2	18 -25 years
Total 30 Horses	

**Table 1:** The number of Horses and their ages using in this study.

An image analysis was used to determine the degree of degenerative changes in SDFT and DDFT and suspensory ligament. the system used was built around a sun workstation connected to modified Leica DMRA2 installed with the Q-win and Q-fluro software the recognition function was performed on digitalized microscope image of conventionally H and E- stained histological sample.

## Results and Discussion

### Overall study population

The inclusions to the study population for 30 cases were predominantly racing horses. For the overall study population the most commonly affected structure was the DDFT at the cross-section area (CSA). The morphology of DDFT incidence of degenerating lesions of (n = 15) adult horses aged (8-10) years old was 50%, (n = 13) middle-aged horses aged (12-17) years old was 43.3% and (n = 2) old horses aged (20-28) years old was 6.7%. As a result of all these physiologically age related changes, an aged tendon is weaker with a likely hood to tear or suffer injury from overuse.

All samples of tendon was observed under (IAM) which showed that the small and large bundles were of different lengths and surrounded by a reticulum of connective tissue. The wave pattern of the DDFT appeared as zigzag formation on the surface under the (IAM). The changes due to degeneration at the mid-metacarpal region of the DDFT appeared as homogenous areas indicating a loss of the uniform wave pattern .The results of this study suggest that (IAM) could play an important role in investigating normal dynamic tendon structure.

In general, degeneration leads to a decrease in function from the cellular level to the organ level [5] Although the exact mechanism of tendon degeneration is not properly understood, it was suggested that the mechanism of tendon degeneration is due to hyperthermia of the tendon [30]. Several other theories have been forwarded including passive mechanical degeneration, active degradation through the action of vascular reduction and neural over stimulation. Degenerative changes, if predisposing the tendon to injury, must be associated with a reduction in the mechanical integrity of the tendon [4]. These degenerative changes have been studied [1,2,29] Overstress of tendon may also lead to injury and increases with increasing age [10,11,16,23]. The results of this study were similar to their findings.

Degenerative change has been reported in the homologous tendon in humans [3] where the most common site of injury is between the central carpal and of this study. This area is particularly poorly vascularised compared to the rest of the tendon. As a consequence of prolonged exercise and restriction of blood supply, ischemia may develop which lead to tendon damage. These observa-

tions are similar to those in thoroughbreds [19] and standardbreds [22] Tendons have lower oxygen consumption compared to other tissues allowing tendons to function for longer periods of time without fatigue [32] Some research had suggested that the raising of a horse's heel will decrease the load on the DDFT, increasing fetlock joint extension and subsequently the load on SDFT and SL [7] At this moment no references have been found to describe the degenerative change in the DDFT at the level of CSA.

## Conclusion

In conclusion, the study showed that the normal structure of equine DDFT can be clearly assessed using Novel technique of Image Analysis Microscopy (IAM). This new technique for equine tendon and can be a useful instrument for the characterization of thick tendon sections. It is anticipated that the technique can be useful tool in the diagnosis of tendon injury.

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