

## **Morphological and morphometric analyses of the suspensory ligament in Standardbreds**

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## **Abstract**

Ultrasound techniques allow examination of some parts of the suspensory ligament (SL) but "anomalies" are regularly observed. Their significance is not known. Few studies have described the relationship between ultrasonographic appearance and the exact morphology in histological sections. The aim of this study is to develop good techniques for cutting, staining, and showing the variation in the tissue composition within the SL. The SLs from the right limbs of 11 horses were collected. Samples were taken from cross-sections at six levels of the SL and they were embedded in paraffin or in Tissue-Tek®. Most of the paraffin sections were shredded. By using the cryosection technique, some freezing artifacts (holes) appeared. Therefore, a technique of freezing with cryoprotection was carried out, which produced the best results. Hematoxylin-phloxine-saffron gives a good contrast of colors between the tissues observed allowing the use of an image analysis program. The percentage of each tissue within the SL for each section and for six levels of the ligament was calculated. Results were analyzed by SAS software. The muscle tissue (PMT) and adipose tissue (PAT) decreased significantly ( $p < 0.0001$ ), whereas the connective tissue (PCT) increased significantly ( $p < 0.0001$ ) with age and when descending from the proximal to the distal level of the SL. The PMT was significantly higher ( $p < 0.0001$ ) in females than males, while the PCT was significantly higher ( $p < 0.0001$ ) in males than females. The PAT was significantly higher ( $p = 0.0278$ ) in hindlimbs than in forelimbs.

**Keywords:** suspensory ligament, cryosection, Hematoxylin-phloxine-saffron, horse, anatomy