

Characterization of collagen fibrils after equine suspensory ligament injury: an ultrastructural and biochemical approach

M. K. Shikh Alsook¹, A. Gabriel¹, M. Salouci¹, J. Piret², N. Moula³, J.-M. Denoix⁴, N. Antoine² and E. Baise⁵

¹*Anatomy Unit, FARAH Research Center & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.*

²*Histology Unit, FARAH Research Center & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.*

³*Sustainable Animal Production Unit, FARAH Research Center & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium*

⁴*CIRALE -14430 Goustranville, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Paris, France*

⁵*FARAH Research Center & Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.*

Corresponding author: mkal.sook@doct.ulg.ac.be (M. K. Shikh Alsook).

Abstract

Suspensory ligament (SL) injuries are an important cause of lameness in horses. The mechanical properties of connective tissue in normal and pathological ligaments are mainly related to the fibril morphology, as well as the collagen content and types. The purpose of this study was to evaluate, using biochemical and ultrastructural approaches, the alterations in collagen fibrils after injury. Eight Warmblood horses with visible signs of injury in only one forelimb SL were selected and specimens were examined by transmission electron microscope (TEM). Collagen types I, III and V were purified by differential salt precipitation after collagen extraction with acetic acid containing pepsin.

TEM revealed abnormal organization as well as alterations in the diameter and shape of fibrils after SL injury. The bands corresponding to types I, III and V collagen were assessed by densitometry after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Densitometric analysis indicated that the proportions of type III and type V

collagen were significantly higher ($P < 0.001$) in damaged tissues compared to normal tissues with a mean increase of 20.9 and 17.3% respectively. Concurrently, a significant decrease ($P < 0.001$) in type I collagen within damaged tissues was recorded with a mean decrease of 15.2%. These alterations could be the hallmark of a decrease in the tissue quality and mechanical properties of the ligament. This provides new insight for subsequent research on tissue regeneration that may lead to the development of future treatment strategies for SL injury.