

# MULTIPOTENT EQUINE ADIPOSE TISSUE-DERIVED PROGENITOR CELLS. CLINICAL CASE REPORTS OF ALLOGENEIC CELL-THERAPY IN HORSES.

**Mambelli Lisley I. BS.** Butantan Institute; Laboratory of Genetics; Avenue Vital Brasil, 1500; Sao Paulo; SP; Brazil; 05503-900; +55 11 71696204; +55 11 58422135; lisley@usp.br

**Mambelli Lisley I.<sup>1</sup> BS, Santos Enrico J. C.<sup>2</sup> PhD, Frazão Paulo J. R.<sup>3</sup> MS, Chaparro Mariana B.<sup>3</sup> MS, Zoppa André L. V.<sup>3</sup> PhD, Kerkis Irina<sup>1</sup> PhD, Kerkis Alexandre<sup>2</sup> PhD.**

<sup>1</sup>Laboratory of Genetics, Butantan Institute; <sup>2</sup>CELLTROVET – Applied Genetics, Veterinary Activities Ltda.; <sup>3</sup>Department of Surgery, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, SP, Brazil.

**Introduction** In horses, stem cell therapies are a promising tool to the treatment of many injuries, which are common consequences of athletic endeavor, resulting in high morbidity and often compromising the performance. Previously, we reported the isolation and differentiation of equine adipose tissue-derived progenitor cells (eAT-PC) into mesodermal derivatives and also showed the potential of these cells to maintain their stemness even after cryopreservation. The aim of this study was further characterization of eAT-PC differentiation potential and application of allogenic eAT-PC for the treatment of tendonitis in horses.

**Methods** eAT-PC were maintained under conditions previously described (Mambelli *et al.*, 2009). Differentiation towards smooth and skeletal muscles and also neuronal cells was performed after thawing following routine protocols, slightly modified. Mouse anti-human antibodies: anti-myosin, anti- $\alpha$ -actinin, anti-MyoD1, anti-beta-tubulin-III; as well as rabbit anti-human anti-nestin and anti-glial fibrillary acidic protein (GFAP) were used after cell fixation in 4% paraformaldehyde. The expression of cell specific proteins was analyzed under confocal microscopy. Twelve animals with tendonitis received  $10^7$  of eAT-PC into the injured tissue under local anesthetic and ultrasonographic control. After one month, ultrasonographic control was performed again. All procedures were approved by horse owners under signature of a veterinary service contract.

**Results** After the induction of myogenic differentiation, the cells presented first signs of morphological changes similar to muscle cells, at day 10. Myosin,  $\alpha$ -actinin and MyoD1 antibodies showed positive immunostaining with progenitor cells confirming muscle cells differentiation. Neuronal differentiation was evidenced by morphological changes, which lead to outgrowth formation and nucleus dislocation. Prior differentiation into neuronal lineages, eAT-PC already presented strong nestin positive immunolabelling. After differentiation neuron-like cells derived from eAT-PC reacted positively with such markers as beta-III-tubulin and GFAP. Functional tests are being provided. Respective controls used in both studies did not present any specific labeling with the same antibodies. Since our study was based on clinical cases, the animals were heterogeneous for age, weight and sex, but all of them were athletic horses. One month after eAT-PC application into the lesion, the formation of healthy tissue has been observed. All treated horses showed a functional recovery and were able to return to their normal activity, without lesion recurred.

**Conclusion** Extending our previous findings, we showed that eAT-PC possess all characteristics of multipotent adult stem cells. Besides differentiation into bone, cartilage and adipose cells (Mambelli *et al.*, 2009) additionally, they were able to produce smooth, skeletal muscles and neuron-like cells. Their application in horses provided functional recovery of damaged tendons and treated animals were capable to return to their normal activity. Our findings classify eAT-PC isolated and cultured *in vitro*, as a promising tool for cell-therapy, which maintain their potential even after cryopreservation. Further studies are needed in order to understand the mechanism of their action on damaged tissues recuperation.