Isolation and Characterization of Adult Progenitor Cells From Healthy and Laminitic Hoof Tissue

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Abnormal progenitor cell differentiation and maturation may contribute to hoof abnormalities during and after laminitis. Authors' address: Louisiana State University School of Veterinary Medicine, 1909 Skip Bertman Drive, Baton Rouge, LA 70803; e-mail: vpinto@tigers.lsu.edu. *Corresponding and presenting author. © 2012 AAEP.

1. Introduction
Treatments for laminitis are limited and minimally effective. The hypothesis tested was that equine adult laminar progenitor cells are irreversibly altered in laminitis.

2. Materials and Methods
Progenitor cells from unaffected (UC) and laminitic (LC) hooves were evaluated with a novel in vitro model of equine lamina. Cell doublings (CD) and doubling times (DT) were quantified for passage (P) 0–5 cells. For P0, P2, and P5, fibroblastic colony-forming units (CFU-F), progenitor (OCT4, SOX-2, CD29, CD44, and CD105), and keratin (K14, K15, and K19) target gene mRNA levels (qRT-PCR), protein expression (flow cytometry), and multipotentiality were assessed. Keratins were localized with immunohistochemistry.

3. Results
Overall, LC CD was significantly higher than UC. Between groups, progenitor gene mRNA levels were significantly higher in P0 LC versus UC. Within groups, P0 UC had significantly lower progenitor mRNA levels than P5. Levels of K15 and K19 mRNA were significantly lower in P0 versus P5 in LC. CD44 mRNA levels were significantly higher in P5 versus P0 in LC and UC. Flow cytometry showed significantly higher percentages of CD105+ and CD44+ cells in P0 versus P5. Keratins were localized to secondary epidermal lamina. Osteogenic, adipogenic, and chondrogenic differentiation was confirmed in both cell types.

4. Discussion
Results suggest that progenitor cells in laminitic hooves are in a constant state of hyperproliferation without maturation. These findings may provide a target for future treatment and prevention strategies.