

Myostatin mRNA expression in cultured equine satellite cells

Abigail Hansen, Marcia Hathaway, Christie Ward and Mary Pampusch

Department of Animal Science, Univ. of Minnesota, St. Paul 55108

Introduction

Satellite cells, mono-nucleated myogenic cells found in all skeletal muscle, were used as a model for muscle growth in horses.¹ The expression of specific myogenic regulatory factors (MRFs), such as MyoD, Myf-5 and myogenin, control the proliferation and differentiation process of satellite cells.² Myostatin is a MRF that inhibits muscle cell differentiation and growth.³ In thoroughbred horses, the myostatin gene has been linked to different muscle phenotypes and ideal racing distances.³

Objectives

- To assess myostatin mRNA levels over time in cultured equine satellite cells.
- To correlate myostatin mRNA levels to cell growth and differentiation as indicated by MyoD, Myf-5, and myogenin mRNA levels, along with visual assessment of percent fusion of satellite cells.

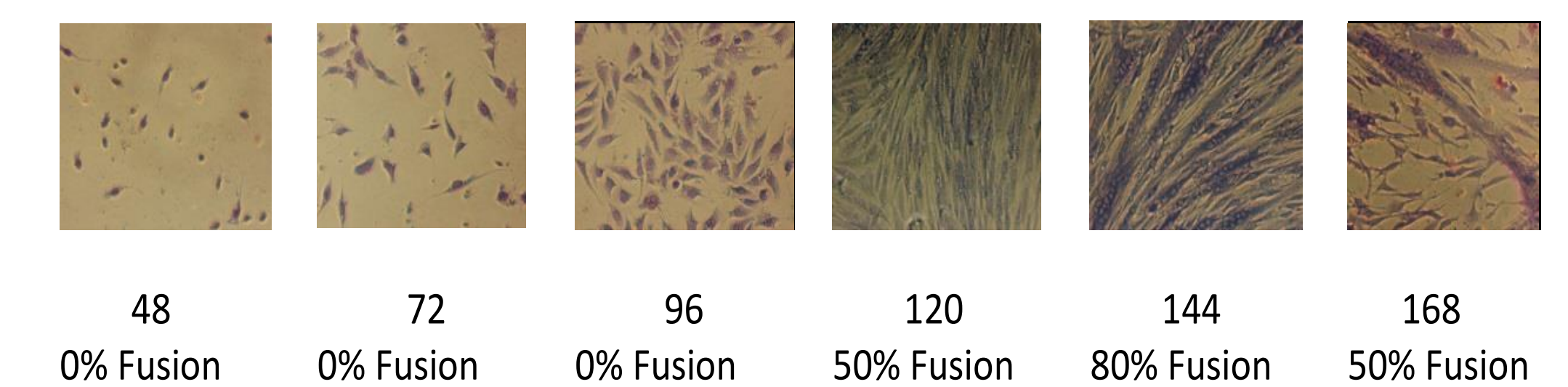
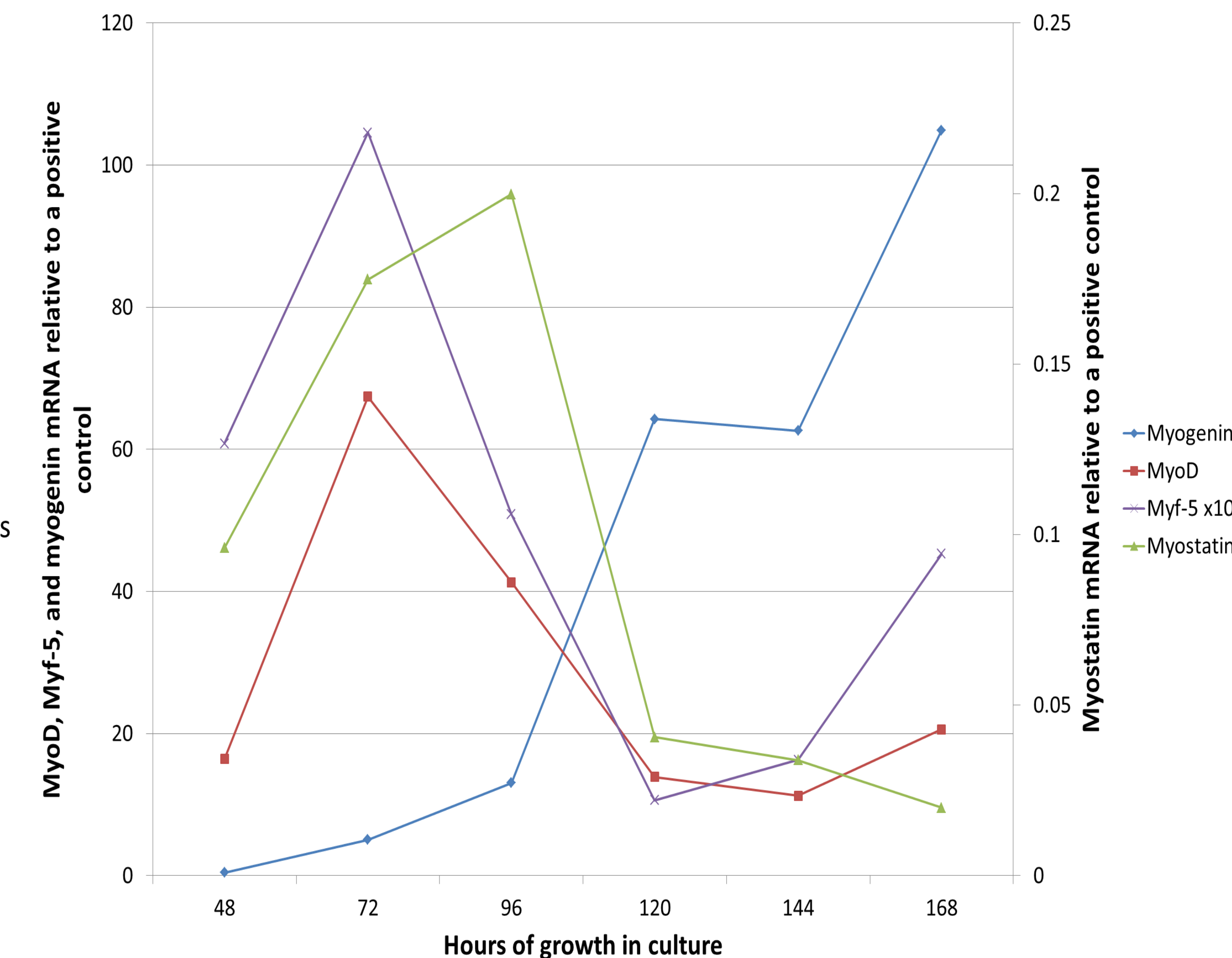
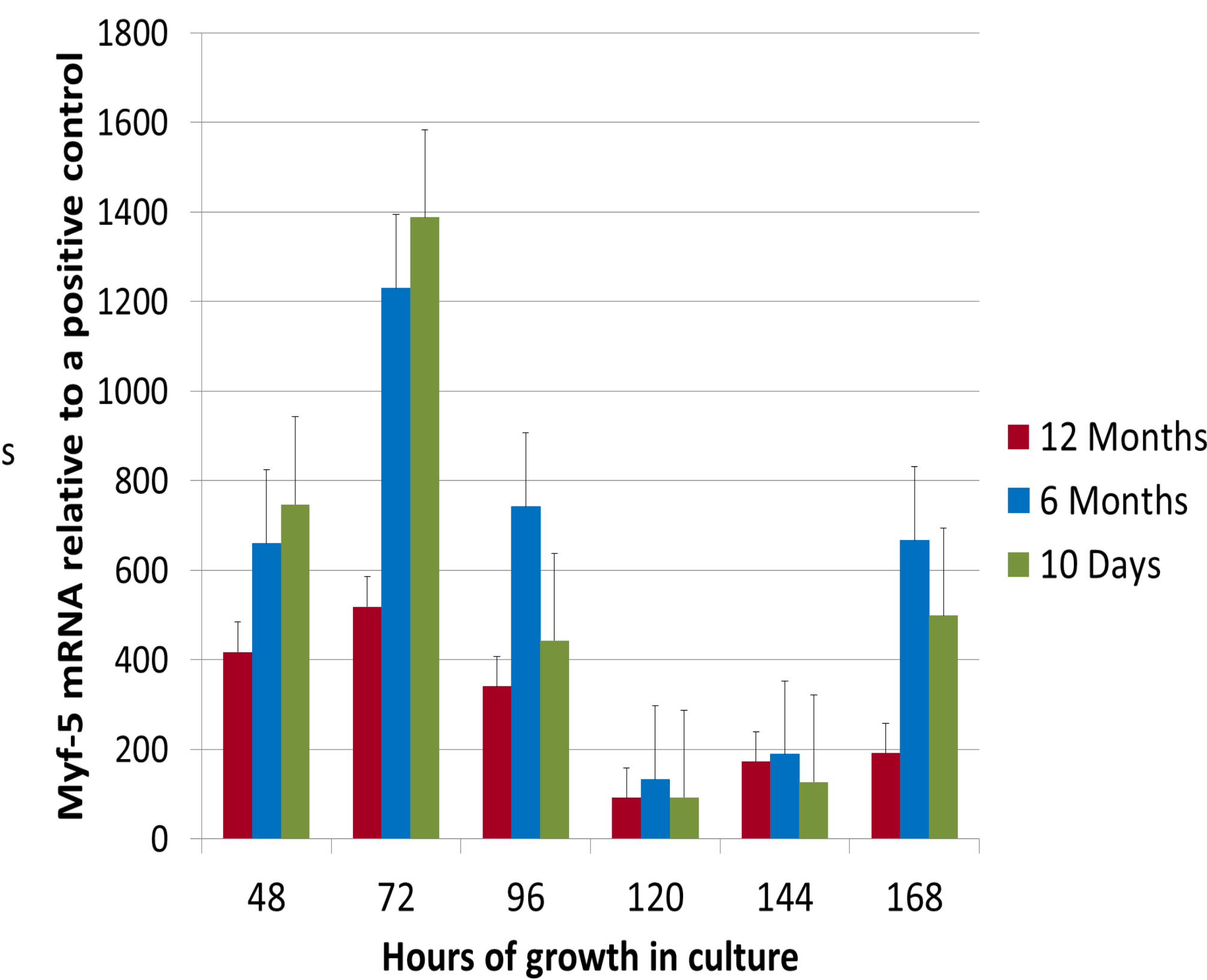
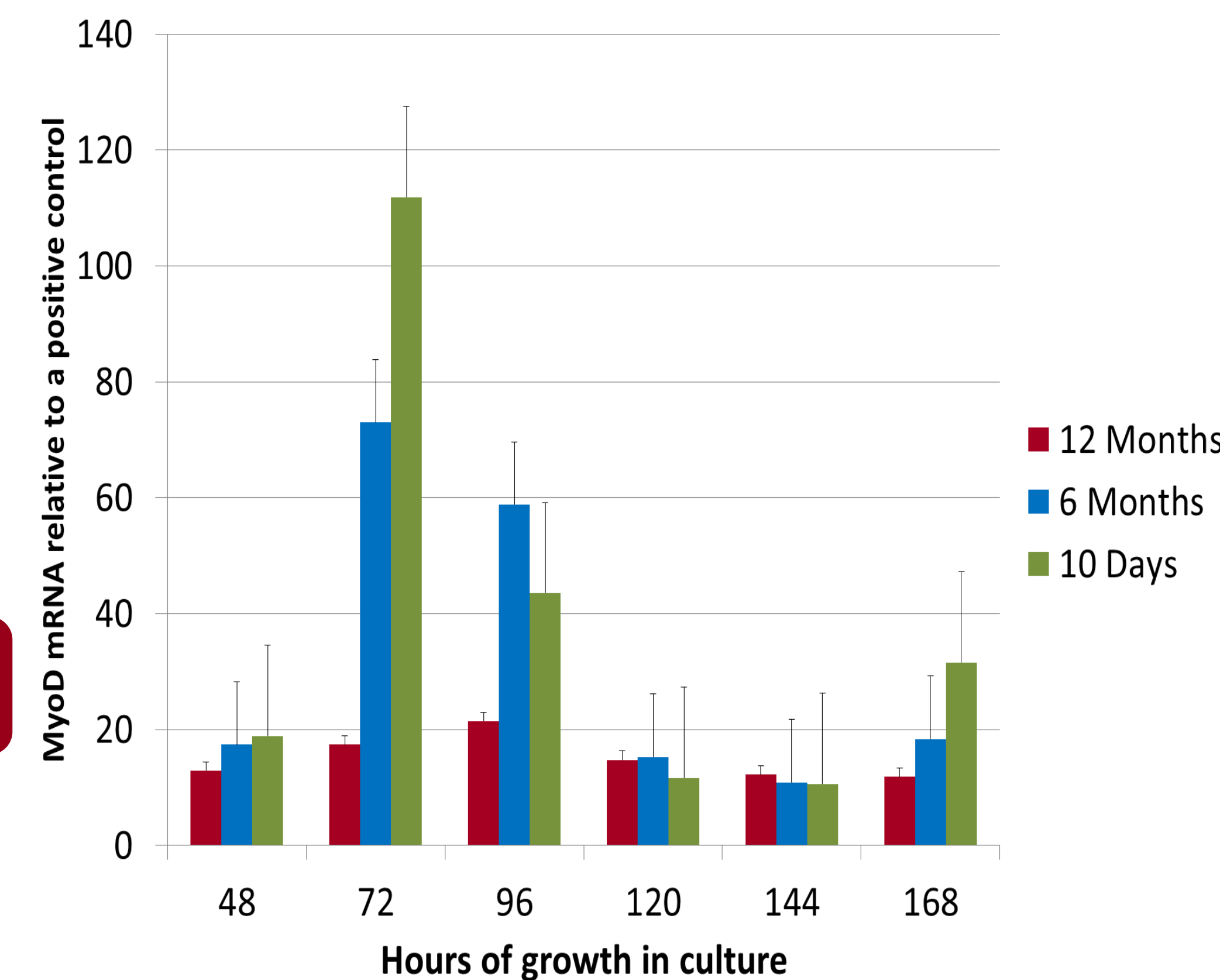
Methodology

- Equine satellite cells isolated from 3 horses aged 10 days, 6 months, and 12 months were grown in culture.
- Total RNA was isolated after 48, 72, 96, 120, 144, and 168 hours in culture.
- RNA was reverse-transcribed into cDNA, then used as a template in Real Time Polymerase Chain Reaction.
- Another set of cultures from each time point was Giemsa stained and photographed.

Works Cited

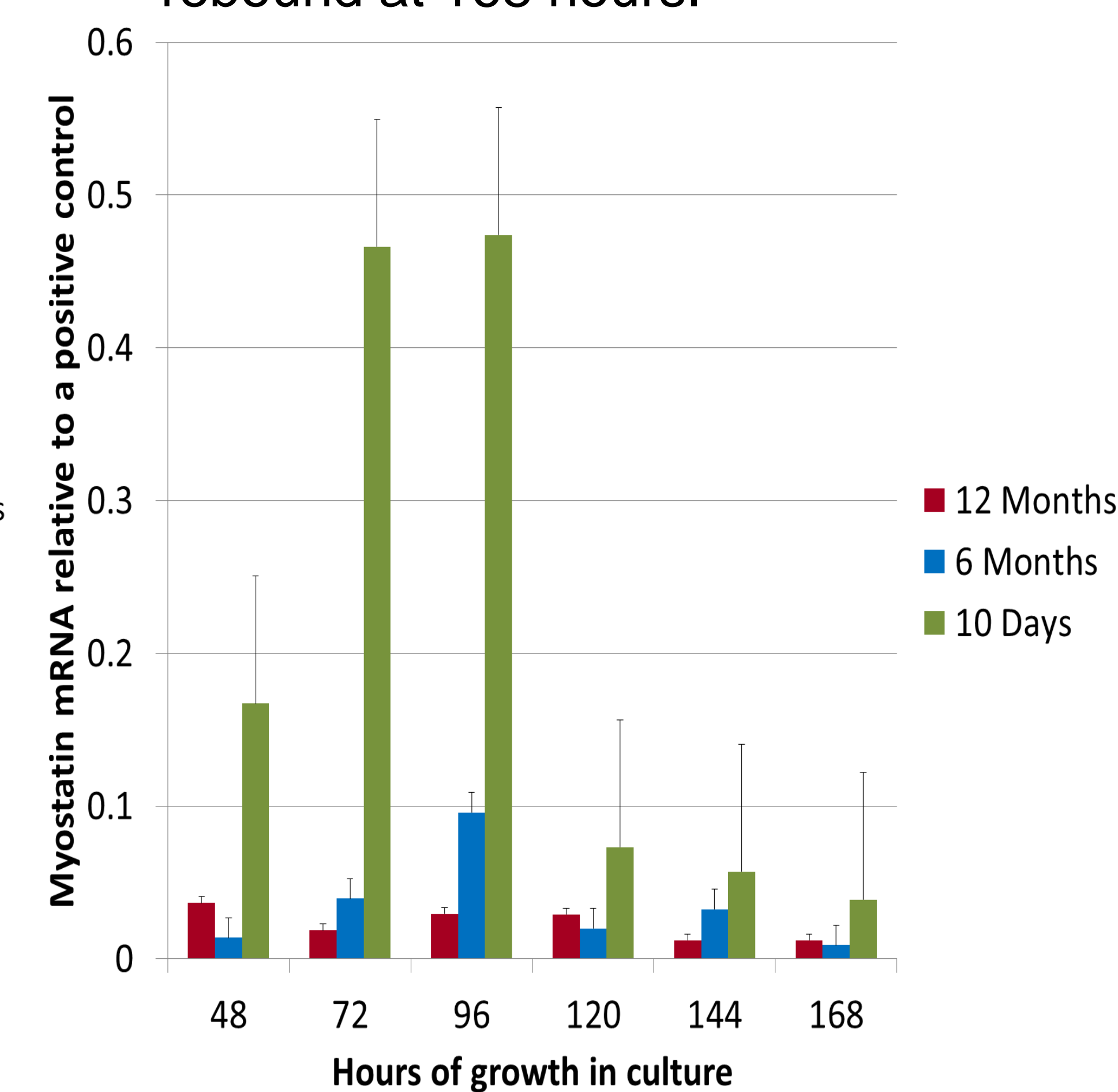
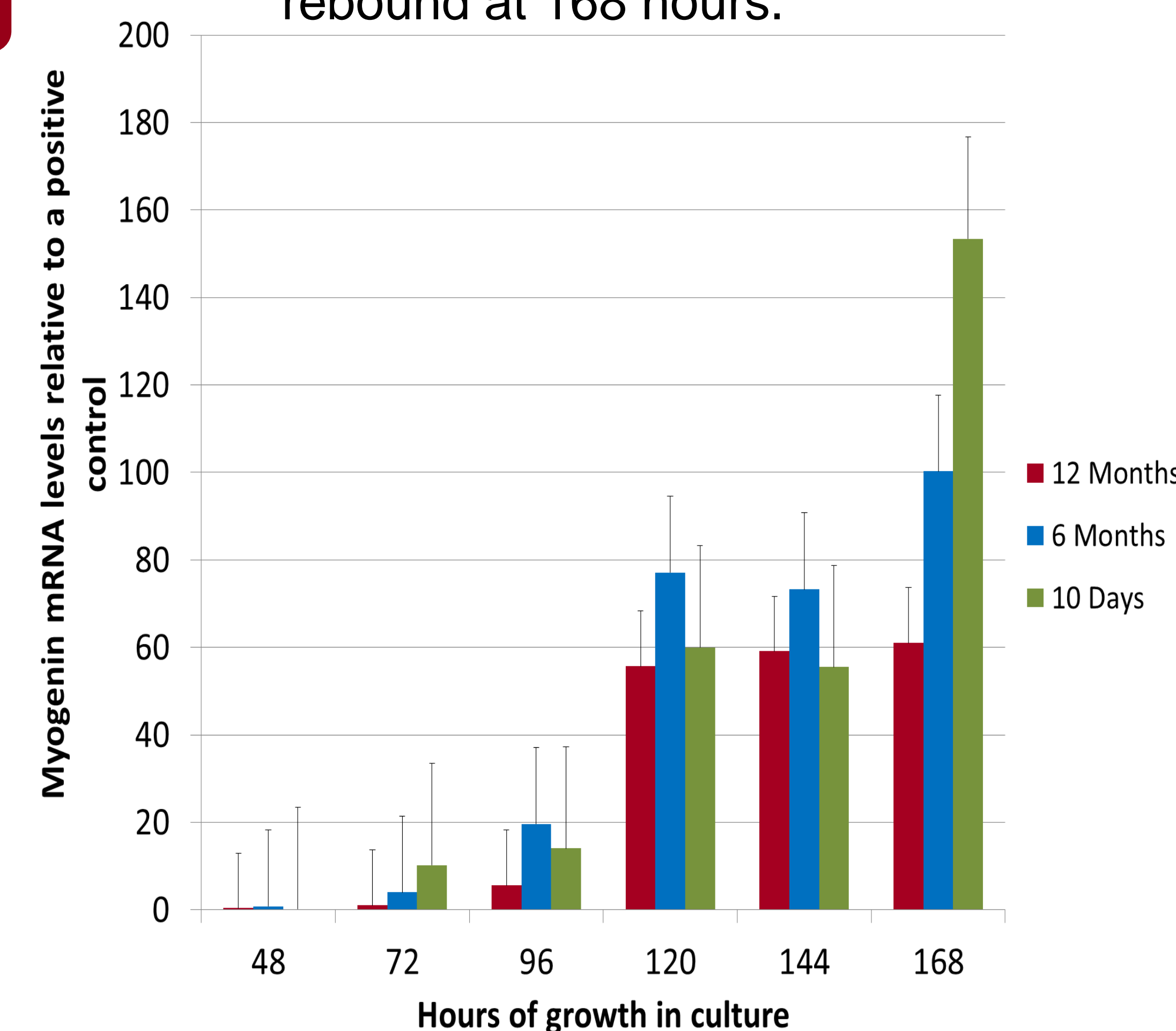
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Results



- MyoD mRNA levels rise to a peak at 72 hours while the satellite cells are actively proliferating. After, the levels gradually decline during differentiation until they rebound at 168 hours.

- Myf-5 mRNA levels rise to a peak at 72 hours while the satellite cells are actively proliferating. After, the levels gradually decline during differentiation until they rebound at 168 hours.



- Myogenin mRNA levels are low when satellite cells are proliferating but increases during differentiation.

- Myostatin mRNA levels were highest at 96 hours, the end of the proliferation phase of growth, after which they decreased. Myostatin showed the most variability among animals

Discussion

- Interest in myostatin has increased since its connection with ideal racing distance in thoroughbreds.
- Recent work with myostatin has only focused on the presence of myostatin genes, not the protein expressed. Since protein expression is linked to mRNA levels, knowing when myostatin mRNA levels peak will help in targeting a stage of cell growth for future protein studies.
- MRF levels over the time course will serve as a baseline for future studies on effects of other factors on myogenic gene expression.
- MyoD, Myf-5, and myogenin mRNA levels showed expected patterns, and validated the stage of cell growth associated with myostatin peaks.
- Suggested further research would include comparing the effects of animal age on myostatin expression.