We hypothesize that supplemental vitamin C will bolster collagen synthesis and act as a cytoprotective agent against negative anti-inflammatory glucocorticoid consequences in tendon proper (TP) and peritenon (PERI) stem/progenitor cells of the equine superficial digital flexor tendon (SDFT).

**METHODS**

**Cells**

Cells were harvested from SDFT of horses and grown to 95% confluence.

**TREATMENTS**

- Cells seeded in 6 well plates and treated with vitamin C in 3D constructs and in 2D for dexamethasone challenge.
- Each treatment was challenged with 10μM dexamethasone for 3 days in 2D for qPCR and biochemical assays.

**qPCR**

- Reverse Transcription with High-Capacity Reverse Transcription Kit
- TaqMan RT-qPCR

**RNA Isolation of Vitamin C treated cells in 3D**

Vitamin C treated cells challenged with 10 μM treatment dose equivalent of dexamethasone in 2D

**Apoptosis Assay**

- Added 100 μl of Apo-ONE Caspase-3/7 Reagent to each well of a black 96-well plate seeded in 2D after 72 hour treatment with 10 nM of Dexamethasone.
- Incubated for ~30min
- Measured fluorescence with a spectrophotometer

**Hydroxyproline Assay**

Dried tendon treated 3D tendon constructs in a series of acid and boiling steps

- Generated L-hydroxyproline standards for standard curve analysis
- Plated in a clear 96-well plate and read in a spectrophotometer

**Hydroxyproline Assay**

- Vitamin C concentration
- MxK
- COL1A1
- PLD01
- LARP6
- CSPG4

**Caspase 3/7 Assay**

- 30 min - Apoptosis
- TP Constructs
- PERI Constructs

**RESULTS**

- Vitamin C supplementation increased the overall collagen content in tendon constructs with TP and PERI cell populations. (A): Levels of collagen for constructs of TP-deranged (BLUE) and PERI-deranged cells trended upward with increasing amounts of vitamin C. (B): Constructs of PERI-derived (RED) progenitor cells treated with vitamin C showed a significant increase in collagen content relative to the control in 100 nM, 200 nM, and 400 nM (p<0.01). Statistical significance was found via a paired t-test utilizing GraphPad Prism.

In conclusion, vitamin C affected the genetic expression of TP and PERI derived 3D tendon constructs. More specifically, TP-derived cells seemed to increase tenogenic genes while PERI-derived cells increased both cellular proliferation and matrix assembly genes. Vitamin C also caused an overall decrease in apoptotic gene expression as well as apoptotic enzyme activity, mainly for TP cells. From these findings one can conclude that vitamin C not only caused an overall increase in tenogenic effects but also plays a cytoprotective role in dexamethasone challenged cells.