INTRODUCTION
Injection of intra-articular and local anesthetics and steroids in the orthopaedic office setting is common practice to provide analgesia and decrease local inflammation, as well as a treatment for many tendonopathies. Previous studies have demonstrated that local anesthetics have cytotoxic effects on chondrocytes and have also been implicated in post-arthroscopic glenohumeral chondrolysis (1). The effect of anesthetics and steroids, however, on tendon health and viability remains largely unknown. We aim to compare the cytotoxic nature of Lidocaine and Ropivicaine in conjunction with Dexamethasone on cultured bovine tenocytes. We hypothesize that Ropivicaine will be less cytotoxic than Lidocaine and the addition of Dexamethasone will lead to greater cell death.

METHODS
Cell Culture: Patellar tendons were harvested from bovine calves and placed in sterile PBS. They were then minced in DMEM and digested with collagenase IV (Sigma) for 8-12 hours in a 37°C incubator shaker. The tenocytes were filtered through a 100-μm nylon mesh cell filter and were cultured in DMEM containing 10% FBS for 3-5 days.

Drug exposure: Cells were plated in a 96 well plate at a density of 1x10^4 per well and allowed to rest for 24 hours before being subjected to one of ten different conditions. Cells were subjected to (1) Normal Saline (NS), (2) 1% Lidocaine, (3) 2% Lidocaine, (4) 0.2% Ropivicaine, (5) 0.5% Ropivicaine (6) Dexamethasone (Dex), (7) 1% Lidocaine+Dex, (8) 2% Lidocaine+Dex, (9) 0.2% Ropivicaine+Dex, (10) 0.5% Ropivicaine+Dex for 30 minutes and then allowed to recover in DMEM containing 10% FBS for 24 hours.

Cell Viability: Treated cells then underwent CellTiter-Glo viability assay (Promega), fluorescence-activated cell sorting (FACS), and live/dead assay. FACS was performed by pooling 12 samples and running the assay on a FACScan flow cytometry machine (BD Biosciences) using calcein to stain live cells and 7-AAD to stain dead cells. Live/dead assay was performed using a 2x reagent of calcein-AM/ethidium homodimer-1 (EthD-1) (Invitrogen). Random images were taken at 10x of each well and live and dead cells were automatically counted using ImageJ.

Statistical Analysis: CellTiter-Glo results are exhibited as mean luminescence (representing relative cell viability) +/- standard error. FACS data is shown as the percentage of dead cells seen in the overall cell sorting. Live/dead assay is shown as the percentage of dead cells per reaction well. Statistically significant differences were determined with the Student t test with significance set at p<0.05.

RESULTS
We found decreased tenocyte cell viability with increasing concentrations of local anesthetics. Overall, the greatest decrease in cell viability was seen with 2% Lidocaine. 0.2% Ropivicaine had the least cytotoxic effect of the anesthetics (Figures 1-3). When tenocytes were treated with both Dexamethasone and anesthetic, cell viability further decreased compared to when the anesthetic was used alone. FACS data counting and live/dead assay for showed similar results with increased dead cells with higher anesthetic concentrations (Figures 2-4). Interestingly, Dexamethasone had a greater detrimental synergistic effect on cell viability and dead cell numbers with Ropivicaine than with Lidocaine (Figures 1-3). This was consistent with all assays.

DISCUSSION
As seen with bovine and human chondrocytes, local anesthetics demonstrate significant cytotoxic effects on bovine tenocytes. 30 minute exposures with Lidocaine and Ropivicaine exhibited a dose dependent response with decreased tenocyte viability and increased dead cell counts with increased anesthetic concentration. Dexamethasone alone failed to demonstrate a statistically different effect than our NS control (p=0.22) but when used in concert with the anesthetics, the combination proved to have a greater cytotoxic effect. Further studies will need to be done to see the toxicity of local anesthetics and steroids on human tenocytes.

SIGNIFICANCE
Use of local anesthetics and steroids can have detrimental effects on human tenocytes. Given the high volume of local injections performed for a variety of tendonopathies in the orthopaedic office setting, the toxicity seen from local anesthetics and steroids on bovine tenocytes will aid in proper administration of the agents in post operative orthopaedic procedures.

REFERENCES