INTRODUCTION:
Intra-articular administration of viral vectors has a great potential in gene therapy for joint diseases. Although Adenoviral (Ad) vector remains a strong candidate for joint gene therapy due to its efficient and rapid transgene expression, use of adeno-associated viral (rAAV) vector may be considered advantageous because of safety and greater penetration into articular cartilage. Self-complementary AAV (scAAV) may expedite and enhance the transgene expression compared to rAAV. The objectives of this study were to evaluate relative tropism of Ad, rAAV, scAAV vectors in equine chondrocytes and synovial cells in vitro, and to assess biologic response to intra-articular administration of the vectors in vivo. We hypothesized that rAAV, particularly scAAV, would induce greater and faster in vitro transgene expression, and in vivo, less inflammatory/immune response, than Ad vectors.

METHODS:
Equine chondrocytes and synovial cells were isolated, cultured, and transfected by Ad, rAAV, and scAAV vectors encoding green fluorescent protein (GFP) or bone morphogenetic protein-2 (BMP2) genes. The GFP transgene expression and BMP2 protein production were assessed over 6 weeks. In 10 healthy adult horses, the metacarpo/tarsophalangeal joints were bilaterally treated by intra-articular injections of saline (GBSS), Ad-GFP (5e11 particles/joint), Ad-BMP2 (5e11 particles/joint), rAAV-GFP (5e11 DRP/joint), rAAV-BMP2 (5e11 DRP/joint), scAAV-GFP at low (5e11 DRP/joint) or high dosages (1e13 DRP/joint), or scAAV-BMP2 at low (5e11 DRP/joint) or high dosages (1e13 DRP/joint). The injected joints were evaluated weekly by palpation and radiography. Joint fluid were collected weekly and analyzed for cell count and concentrations of total protein, BMP2, and IL-1beta. Neutralizing antibody (NAb) titer for Ad/AAV vectors were measured for the weekly serum and joint fluid samples. The joint tissues were harvested at 8 weeks after injections and evaluated by RT-PCR for GFP/BMP2 gene expression, PCR detection of CMV-promoters, CT-scan, and histology for an assessment of ectopic soft tissue mineralization.

RESULTS:
In vitro, equine chondrocytes and synovial cells were efficiently transduced by Ad/rAAV/scAAV-vectors, and scAAV showed significantly greater and more sustained GFP expression and BMP2 production compared to Ad/rAAV-vectors (Fig1 and Fig2). In vivo, detectable level of BMP2 was induced by an intra-articular injection of Ad-BMP2 at day 2 and 7 (Fig3). The Ad-GFP/BMP2 vectors induced greater articular inflammation than rAAV/scAAV-GFP/BMP2 vectors measured as swelling, pain, and synovial fluid parameters, including IL-1beta concentration; although, all parameters were recovered in normal values within 4 weeks (Fig3). The NAb titer for Ad/AAV vectors were increased in the serum and joint fluid from the Ad-AAV-injected joints as well as the un.injected contralateral joints (Fig4). The CMV-promoters were detected only in Ad-injected joints, and ectopic soft tissue mineralization was not evident in CT-scan and histology.

DISCUSSION:
Direct joint injection of rAAV/scAAV vectors, even at high MOI (1e13 DRP/joint), was unable to produce sufficient transduction in vivo in this model despite excellent results in vitro, potentially due to the inhibitory effect by synovial fluid. Therefore, delivery method will need to be optimized for efficient and reliable gene transduction to articular/synovial tissues. Direct intra-articular administration appeared to be an efficient vector delivery method for Ad vectors producing robust BMP2 in joint fluid, inducing a transient inflammatory response, and without adverse soft tissue mineralization. The effects of repetitive gene therapy application may be diminished by the increased neutralizing antibody titers in both the Ad-AAV-injected joints and the distant joints.

REFERENCE: