JOINTS AND OSTEOARTHRITIS — LARGE ANIMAL

NOVEMBER 2, 2020 | 10:00–NOON ET
HOLLY STEWART
Development of an experimental model of bone marrow lesions using the ovine femoral condyle

Holly L. Stewart, Jeremiah T. Easley, Kurt T. Selberg, Brad B. Nelson, Christian M. Puttlitz, Christopher E. Kawcak

2020 ACVS Surgical Summit
Conflict of Interest Disclosure

I hereby certify that, to the best of my knowledge, no aspect of my current legal, personal or professional situation might reasonably be expected to affect my views on the subject on which I am presenting.
Osteoarthritis: Translational Impact

- Osteoarthritis (OA) in humans $\rightarrow$ high economic burden
  - Disability, co-morbidities, expense of treatment
- Osteoarthritis in horses $\rightarrow$ economic impact
  - 60% equine lameness related to OA
  - 7.3 million horses (US)
Osteoarthritis & Subchondral Bone

- Osteoarthritis
  - Traditional focus: articular cartilage
  - Contemporary focus: articular cartilage ⇔ subchondral bone

- Osteochondral unit:
  - Articular + calcified cartilage + subchondral + trabecular bone
Subchondral Bone

- Specialized bone, underlying calcified cartilage layer
- **Function**: attenuate forces secondary to locomotion
- Highly adaptable → response following Wolff’s Law
- **Subchondral bone disease** = delay in resorption/formation
  - Limited ability to compensate → pathologic changes
Bone Marrow Lesions (BMLs)

- “Bone edema,” “bone bruise,” “bone contusion”
- Early indicator of OA/maladaptive change
- Clinically recognized to cause pain
- Diagnosis = increased fluid signal within bone on MRI
- Variety of histological findings → multiple etiologies
- Paucity of information about biological behavior → experimental model lacking

Olive 2009, Biggi 2012, Barrett 2018
Study Objectives: Experimental BML Model

- Translational experimental model of bone marrow lesions
- Layers of osteochondral unit stimulated → bone marrow lesion formation
Hypothesis

• It is possible to create an experimentally-induced bone marrow lesion that mimics naturally-occurring lesions.
Methods

• Direct/invasive method using 1.1 mm Steinmann pin
  – *Red*: Articular + calcified cartilage
  – *Yellow*: Articular + calcified cartilage + subchondral bone plate
  – *Green*: Articular + calcified cartilage + subchondral bone + trabecular bone
  – *Blue*: Trabecular + subchondral bone
Methods

- Direct/invasive method using 1.1 mm Steinmann pin
  - Red: Articular + calcified cartilage
  - Yellow: Articular + calcified cartilage + subchondral bone plate
  - Green: Articular + calcified cartilage + subchondral bone + trabecular bone
  - Blue: Trabecular + subchondral bone
Methods

Surgical induction: medial femoral condyle
N = 6 sheep; 8 mm pin depth

Imaging performed every 2 weeks
• MRI (3 T) – fluid-specific sequences
• CT – standard, dual-energy techniques

30-, 60-, or 90-day sacrifice

MicroCT

Decalcified histology
Analysis

• Descriptive results
• Image grading
• Total bone and trabecular bone analysis on microCT
• Histological grading
Results: MicroCT & Histology

- Gross evaluation
- MicroCT
  - Decreased trabecular spacing
  - Increased trabecular number
- Histology
  - Modeling changes around the pin tract
  - Hemorrhage, inflammatory cells, fibrosis, sclerosis

Martel-Pelletier 2007
Conclusions

• Consistent, reproducible experimental BML model
• Volumetric imaging effective for identification of increased fluid signal
• BMLs actively changing over time
• Structural changes to underlying trabecular bone
• Histological evidence of inflammation and local modeling of bone
Discussion & Future Directions

- Limitations
- Long(er)-term investigation of BMLs → osteoarthritis
- Explore mechanisms that create/perpetuate BMLs

**Ultimate goal:** understand how BMLs perpetuate joint inflammation
Acknowledgements

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• PSRL: Kim Lebsock, Cat Hersch, Niki Adams, Katie Bisazza, Erin McCready, Lisa Mangin, Meaghan Monahan, Ki Goodman, Amy Morts, Heather Troyer, Leah Stopper, Mikala Balthazor, Wendy Villavicencio, Andres Bonilla, PSRL Students/Staff, LAR veterinarians

• OBRL: Dr. Ben Gadomski, Lucas Nakamura, Cecily Broomfield, Jimmy Johnson

• TMI/ORC/VTH: Dr. Brad Nelson, Dr. Kelly Zersen, Dr. Katie Sikes, Chrissy Battaglia, Mindy Meyers, Bill Brock, Jeff Stewart

• American College of Veterinary Surgeons Foundation

• College Research Council, College of Veterinary Medicine and Biomedical Sciences, Colorado State University

• Preclinical Surgical Research Laboratory

Colorado State University

Preclinical Surgical Research Laboratory
Thank you
KATHRYN SEABAUGH
Examining the Effects of an Extract of *Biota Orientalis* in the Osteochondral Fragment-Exercise Model of Osteoarthritis

Kathryn A. Seabaugh, DVM, MS, DACVS, DACVSMR, Myra F. Barrett, DVM, MS, DACVR, DACVR-EDI, C. Wayne McIlwraith, BVSc, PhD, DACVS, DACVSMR, David D. Frisbie, DVM, PhD, DACVS, DACVSMR

Colorado State University
Fort Collins, Colorado
I hereby certify that, to the best of my knowledge, no aspect of my current legal, personal or professional situation might reasonably be expected to affect my views on the subject on which I am presenting, other than the following:

No Conflict of Interest
Disclosures

• This project was funded by Interpath Global, Ballarat, Australia.

• This study was approved by the institution’s Animal Care and Use Committee
Introduction to oral joint supplements

- "Nutraceuticals"
- Commonly used to maintain joint health
- Countless products available
- Clinically, it is the most common question I receive from owners
In a perfect world...

- Reliable ingredients
- Repeatable Results
- Scientific Evidence
- Veterinarian and Owner Satisfaction
Introduction to Epiitalis®

- Proprietary extract from the seeds of *biota orientalis*
  - Highly bioavailable fatty acids
- Reduce arachidonic acid release from the liver
  - Ikeda I et al. Lipids 1992
- Reduce production of prostaglandin E2 in a cartilage explant model
  - Pearson W et al. AJVR 2008
- 4CYTE Canine was found to be at least as effective as carprofen in relief of pain and lameness in dogs suffering from OA
  - Richards L et al, JVIM 2013
Hypothesis/Objectives

• Oral treatment with Epiitalis® would result in disease modifying and symptom modifying effects in a proven model of osteoarthritis
Post-traumatic model of osteoarthritis

- OA induction
  - Distal radial carpal bone fragment

- 16 healthy, 2-5 year old horses

- Two treatment groups
  - BO-treated – 2.5 mL liquid gel suspension containing 2.2 mL of active ingredient (*biota orientalis*, Epiitalis®) + 0.3 mL of excipients
    - n = 8 horses
  - Placebo treated – 2.5 mL of liquid gel suspension containing excipients only
    - n = 8 horses
Materials and Methods

Baseline data
- Radiographs
- Lameness exam

Day 0
- Synovial fluid
- OA induction
- Treatment initiation

Day 7
- MRI

Day 14
- Radiographs
- Synovial fluid
- Lameness exam
- Exercise induction

Weekly - Day 21-56
- Synovial fluid
- Lameness exam

Endpoint
- Lameness exam
- Radiographs
- MRI
- Synovial fluid
- Gross inspection
- Histology
Materials and Methods

• Treadmill exercise
  – 6 minutes a day
    • Trot-Gallop-Trot
  – 5 days a week for 8 weeks
Outcome measures – In Life

• Lameness exams
  – Subjective (0-5) and objective assessments
  – Effusions scores (0-4)
  – Range of motion grade (0-4)
  – Flexion response – carpal flexion (0-4)

• Synovial fluid analysis
  – Prostaglandin E$_2$ concentration
  – Glycosaminoglycan concentration
  – Fluid analysis

• Radiographic assessment
  – Baseline and Day 14

• Magnetic resonance imaging
  – Day 7 (baseline)
Outcome Measures - Endpoint

- Lameness Exam
- Radiographs
- MRI
- Synovial fluid
- Gross Inspection
- Histology
  - Hematoxylin and Eosin (H&E)
  - Safranin O and Fast Green (SOFG)
  - Dimethylmethylene Blue (DMMB) assay
  - Hoechst assay
  - Cell viability
  - Apoptosis
Statistical analysis

• Difference based analysis
  – Baseline values deleted from all subsequent values

• Linear mixed-models for repeated measures
  – Week and treatment group (placebo or Epiitalis) were included as fixed, additive, effects

• Linear models were used where only the change at endpoint data was considered for analysis
  – Treatment group specified as a fixed effect

• P < 0.05 was considered significant
Results - General

- No significant difference in lameness exam parameters
  - Subjective and objective lameness grades
  - Effusion scores
  - Range of motion scores
  - Flexion grades

- No significant treatment effect in MRI results

- No significant treatment effect in macroscopic grades
Results – Prostaglandin E2

- Significantly lower concentrations of PGE$_2$ in synovial fluid of Epiitalis-treated horses ($p = 0.043$)
Results – Radiographic changes

- Significant reduction in radiographic changes in Epiitalis-treated horses
  - Subchondral lysis of the radial carpal bone \((p = 0.019)\)
  - Osteophyte formation \((p < 0.001)\)
  - Subchondral sclerosis of the radial carpal bone \((p = 0.012)\)
  - Total radiographic score \((p = 0.001)\)
Radiographic changes – Day 70

- Orange arrow – osteochondral fragment
- Pink arrow – sclerosis of 3rd carpal bone
- Red arrow – sclerosis of radial carpal bone
- Green arrow – enthesopathy of joint capsule
- Blue arrow – osteophytosis
- White arrow – soft tissue swelling
Discussion

• Osteoarthritis significantly impacts not only performance but the comfort of the horse over its lifetime

• Oral joint supplements are one of the most frequently fed supplements to horses
Discussion

- Prostaglandin E2
  - Pro-inflammatory mediator
  - Consistently identified as a marker of both experimentally induced and naturally occurring osteoarthritis in horses


  - The reduction of PGE2 in the current study suggest a profound anti-inflammatory effect
Discussion

• Radiographic changes are known to lag behind other clinical symptoms of OA.

• In the current model, radiographic changes are often subtle
  – Low sensitivity for evidence of disease modification

• This finding is strongly supportive of a disease modifying effect of treatment with Epiitalis®.
Conclusion

• Disease modifying effects
  – Radiographic changes

• Anti-inflammatory effects
  – Prostaglandin $E_2$
  – Symptom modifying effects
Study Limitations

- Timing of baseline MRI

- This was a short term study (10 weeks) making long-term predictions of therapy more difficult.
Clinical Relevance

- This study demonstrates significant anti-inflammatory and disease modifying effects following prophylactic treatment with Epiitalis® in induced equine OA

- This confirms positive results of previous studies.

- This investigators feel this is some of the most compelling evidence of therapeutic effects that have been seen with an oral supplement using this model
Thank You

- Co-authors
- Horses
- ORC Staff
- Interpath Global
Metabolism & Glycosylation in Equine OA

ACVS Annual Surgery Summit
Dr. Heidi Reesink, VMD, PhD, DACVS-LA
hlr42@cornell.edu

November 2, 2020
Conflict of Interest

I hereby certify that, to the best of my knowledge, no aspect of my current legal, personal or professional situation might reasonably be expected to affect my views on the subject on which I am presenting, other than the following:

Dr. Reesink has a provisional patent on the production and use of recombinant glycoproteins related to lubricin and similar mucins.
Joint disease and OA are leading causes of wastage in equine athletes.

• Early diagnosis remains a challenge
• Treatment options are limited
Synovial fluid metabolomics has shown promise for differentiating between healthy & OA joints in humans.

Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis

A.K. Carlson †‡, R.A. Rawle †, C.W. Wallace †, E.G. Brooks †, E. Adams †, M.C. Greenwood †, M. Olmer §, M.K. Lotz §, B. Bothner †, R.K. June †

Carlson+ Osteoarthritis Cartilg., 2019
Synovial fluid is an ultrafiltrate of plasma composed of many distinct biomolecules:

From: Clish+ Cold Spring Harb Mol Case Stud., 2015
Metabolomic analyses of equine SF have been performed, but have been limited to:

- Targeted lipidomics\(^1\)
- Septic vs. non-septic joint disease\(^2\)
- Fetlock palmar osteochondral disease\(^3\)

*Metabolomic analysis of synovial fluid from Thoroughbred 2nd racehorses diagnosed with palmar osteochondral disease using magnetic resonance imaging*

R. J. T. Y. GRAHAM\(^*\), J. R. ANDERSON\(^\d\), M. M. PHELAN\(^\$\), E. CILLAN-GARCIA\(^\d\), B. M. BLADON\(\d\)\(^\d\) and S. E. TAYLOR\(^\d\)

\(^1\) de Grauw+ *Arthritis Res Ther.*, 2011; \(^2\) Anderson+ *J Proteome Res.*, 2018; \(^3\) Graham+ *Equine Vet J.*, 2019
Differences in eicosanoid release were detected in SF between NSAID and placebo-treated horses in an LPS synovitis model.

Eicosanoid release was not limited to PGE$_2$ or to the early inflammatory phase.
SF metabolites were able to differentiate between septic & non-septic joint disease in horses.

Glucose was the predominant discriminator between septic & non-septic pathologies.
SF $^1$H-NMR spectroscopy was unable to differentiate between TB racehorses with POD and healthy control fetlocks.

Glucose and lactate were the most influential metabolites.
Objective: To identify differentially expressed metabolites & global protein glycosylation between healthy & OA equine synovial fluid.

- Untargeted metabolomics
- Untargeted glycomics
Methods—Experimental Design.

• Metabolomics study (Cohort 1)
  – n = 12 horses (n = 6 control, n = 6 OA) of racing pedigree
  – Carpal radiography & arthroscopy
  – SF analyzed using high-resolution LC-MS/MS
    • Negative and positive electrospray ionization modes

• Statistical Analysis
  – Multivariate analysis: Log transformation & Pareto scaling
  – PCA and PLS-DA (MetaboAnalyst 4.0)
  – KEGG pathway analysis by hypergeometric test; FDR adjusted p-values
Study Demographics—Metabolomics.

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| Age range | Control | 3-5 yrs | OA  | 2-6 yrs |
| Median age | 4.5 yrs | 3 yrs   |

n=12 horses; 6 control, 6 OA
Methods—Experimental Design.

- **Glycomics study (Cohort 2)**
  - n = 38 horses (n = 18 control, n = 20 OA)
  - Carpal radiography & arthroscopy OR gross dissection
  - Carpal SF analyzed using lectin microarrays & custom lubricin sandwich ELISA

- **Statistical Analysis**
  - Glycomics: univariate & multivariate analyses; unpaired t-tests between healthy & OA joints and between N- and O-glycans
  - Lubricin ELISA: unpaired t-test
## Study Demographics—Glycomics.

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**Age range** 2-11 yrs 2-19 yrs  
**Median age** 5 yrs 3 yrs
Results—SF Metabolomics (S-Plot & Heatmap).

- 84 differentially expressed metabolites

n=12 horses; 6 OA, 6 control

Noordwijk, Qin+ In review
Results—SF Metabolomics (PCA, PLS-DA and VIP Plots).

n=12 horses; 6 OA, 6 control
Results.

1) Inflammation (histidine, tryptophan metabolism)

2) Oxidative stress (arginine biosynthesis)

3) Collagen metabolism (lysine degradation)

n=12 horses; 6 OA, 6 control

Noordwijk, Qin+ In review
Results—SF Glycomics (Volcano Plot & Heatmap).

n=38 horses; 20 OA, 18 control

↑ in OA
↓ in OA

p = 0.05

Core 1 O-glycosylation
α-2,3 sialylation
α-1,2 fucosylation

Noordwijk, Qin+ In review
Results—SF Glycomics (Lectin Microarrays, Lubricin ELISA).

- $N$-Glycans predominate in SF
- Lubricin is ↑ in OA SF

n=38 horses; 20 OA, 18 control

Noordwijk, Qin+ In review
Conclusions.

• Metabolite & glycan expression differentiate between healthy & OA equine carpal SF
  – Pathways: Inflammation, oxidative stress & collagen metabolism

• O-glycans predominate over N-glycans in SF

• ↑ Core 1 O-glycosylation & ↑ α-2,3 sialylation in OA SF
  – Could be associated with ↑ lubricin in OA?
Limitations.

• Limited sample size
  – \( n = 12 \) horses in metabolomics cohort (Cohort 1)
  – \( n = 38 \) horses in glycomics cohort (Cohort 2)

• Naturally occurring OA
  – No standardized medication/Rx regimens prior to hospital admission
  – Variation in injury severity & duration
Clinical Relevance.

- SF metabolites and protein glycosylation differentiate between healthy & OA equine joints
  - Biomarkers
  - New therapeutic / druggable targets

- Lectin microarrays may be a potential screening tool for equine OA
  - “First in horse” application
  - First systematic evaluation of SF protein glycosylation in any spp.
Acknowledgements

• Reesink Lab
  – Kira Noordwijk
  – Jin Su

• Collaborators
  – Cornell Proteomics & Metabolomics Facility
    • Dr. Sheng Zhang, Maria Elena Diaz-Rubio
    – Dr. Lara Mahal, University of Alberta
  • Rui (Ric) Qin

• Cornell Veterinary Biobank
  – NIH R24 GM082910
Noordwijk KJ, Qin R, Diaz-Rubio ME, Zhang S, Su J, Mahal LK, Reesink HL
O-Linked Glycosylation.
O-Linked Glycosylation.

O-glycosylation pathway of eye mucins

From: Brockhausen+ *Carbohydrate Res.*, 2018
O-Linked Glycosylation.
Synovial fluid is an ultrafiltrate of plasma composed of many biomolecules:

- Proteins
- Metabolites
  - Lipids
  - FFAs, bile acids, lipid mediators
  - Amines & cationic metabolites
  - Central metabolites
  - Sugars, organic acids, purines/pyrimidines

Adapted from: Clish+ Cold Spring Harb Mol Case Stud., 2015
Differences in eicosanoid release were detected in SF between NSAID and placebo-treated horses in an LPS synovitis model.

Eicosanoid release was not limited to PGE$_2$ or to the early inflammatory phase.
Future Directions.

- Prospective, longitudinal study design
  - Prediction of OA progression
  - Assessment of Rx response
- Injury-specific markers
  - OA phenotypes/endotypes
Dogma is that lubricin is decreased in joint disease.

n=38 studies; 12 human studies, 26 animal studies

Watkins+ Osteoarthritis Cartilg., 2020
However, many human studies and translational animal studies suggest that lubricin is increased.

n=38 studies; 12 human studies, 26 animal studies

Watkins+ Osteoarthritis Cartilg., 2020
However, there is a citation bias for studies reporting decreased lubricin in joint disease.

n=38 studies; 26 animal studies, 12 human studies

Watkins+ *Osteoarthritis Cartilg.* 2020
Evaluation of a modified Subchondroplasty technique for osteochondral defects in the medial trochlear ridge of the femur in 3 horses: A pilot study

Lauren Smanik, Kurt Selberg, Holly Stewart, Laurie Goodrich, Christopher Kawcak

Resident in Equine Surgery
Colorado State University
Conflict of Interest

I hereby certify that, to the best of my knowledge, no aspect of my personal or professional situation might reasonably be expected to significantly affect my views on the subject on which I am presenting.
Osteoarthritis & The Osteochondral (OC) Unit

• Deterioration of the OC unit → Loss of tissue integrity and biomechanical functionality

• Bone Marrow Stimulation
  – Penetration of subchondral bone (SCB) plate → mesenchymal clot supporting fibrocartilage formation
  – First-line treatment for symptomatic cartilage defects
    • Minimally invasive, cost-effective, limited surgical morbidity
  – Gold standard for comparison of emerging cartilage repair technologies
Bone Marrow Stimulation

- Long-term alterations of the SCB \( (\text{Seow et al., Cartilage 2019}) \)
  - Reduced density of deep SCB
  - Altered force transduction and fluid flow

- Subchondral bone plate advancement and central osteophyte formation \( (\text{Fortier et al., J Knee Surg 2012; Mithoefer et al., AJSM 2016}) \)

- Formation of subchondral bone cysts \( (\text{Orth et al., AJSM 2012}) \)
Subchondroplasty® (SCP)

- Percutaneous subchondral injection of calcium phosphate bone substitute material (AccuFill®) into bone marrow lesions identified in humans with significant knee osteoarthritis (Cohen & Sharkey, J Knee Surg 2016; Byrd et al., OJSM 2017)
- Significant improvement in pain and function
- **Modification for treatment of cartilage defects in horses**

![Pre-op, 9mo post-op, 14mo post-op images](Nevalainen et al., Clin Imaging 2016)

(Zimmer Biomet, 2017)
Objective

To perform a modified Subchondroplasty technique as a proof of concept pilot study for potential treatment of osteochondral disease in horses

**Specific Aim:** Evaluate the administration of a common biologic matrix to osteochondral defects, with and without a bone substitute material (BSM), using a modified Subchondroplasty technique.
Hypothesis

- Use of the modified Subchondroplasty technique will be a safe, simple and reproducible surgical technique for the filling of osteochondral defects in the medial trochlear ridge of the femur, with minimal adverse effects on host tissues
Study Design

- **Randomized, controlled pilot study**
  - Bilateral hind limb preclinical model
  - Exploratory/proof of concept → descriptive statistics for analysis

- **3 clinically normal research horses**
  - No evidence of stifle disease

- **4 full-thickness cartilage defects per horse**
  - Each defect filled with 1 of 4 treatments
  - Random assignment of treatments
Bilateral Femoropatellar Joint Arthrotomy

- Medial trochlear ridge of the femur  
  (Frisbie et al., AJSM 2006; Frisbie et al., AJSM 2009)  
  - Two 15mm OC defects
- Subchondral microfracture 
  - 2.0mm awl
- Percutaneous approach 
  - 2.0mm drill bit  
  - Radiographic guidance
- Biologics for Injection 
  - Fibrin glue matrix (75%)  
  - Calcium phosphate BSM (AccuFill®)
Defect Treatments

1. Fibrin glue via SCP
2. Direct injection of fibrin glue (no SCP)
3. AccuFill® via SCP + direct injection of fibrin glue
4. Untreated (negative control)
Lameness Examination – lameness, ROM, flexion
Stifle Effusion
Digital Radiographs
Magnetic Resonance Imaging
Computed Tomography
Gross Evaluation – tissue retention, defect filling
Histology – H&E and SOFG stains
MicroCT – trabecular morphometry
Results
Lameness Evaluation

LEFT HINDLIMB

- Effusion
- Lameness
- ROM
- Flexion

Day 0
Day 14
Day 0
Day 14
Day 0
Day 14

Horse 1
Horse 2
Horse 3

RIGHT HINDLIMB

- Effusion
- Lameness
- ROM
- Flexion

Day 0
Day 14
Day 0
Day 14
Day 0
Day 14

Horse 1
Horse 2
Horse 3

Colorado State University
Digital Radiography

By day 14 → widening of microfracture holes with a more obvious rim of sclerosis

C = control, A = AccuFill® + FG, F = SCP with FG only, D = direct injection of FG
Intermediate signal tissue (arrow head) within all defects regardless of treatment

A) Hyperintense fibrin glue extending from drill tract (white arrow) to defect (*), with focal dispersion through the trabecular bone.
B) Circumferential distribution of hypointense BSM (circle) from drill tract to defect.
C) Bone substitute material as seen on CT.
Magnetic Resonance Imaging & Computed Tomography

- Horse #3 → Focal resorption of the subchondral plate associated with the microfracture holes
  - All defect treatments except for that treated with AccuFill®
- Iatrogenic damage to medial patellar ligament: 5/6 stifles
- Joint capsule dehiscence at the arthrotomy site
## Gross Examination

100% of the defect base covered for all samples

### Defect Filling (Grade)

1. 0-25% filled
2. 25-50% filled
3. 51-75% filled
4. 76-100% filled

<table>
<thead>
<tr>
<th></th>
<th>Fibrin glue via SCP</th>
<th>Direct injection of fibrin glue</th>
<th>AccuFill® via SCP + Direct FG</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse 1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Horse 2</td>
<td>4</td>
<td>3</td>
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<tr>
<td>Horse 3</td>
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## MicroCT

<table>
<thead>
<tr>
<th></th>
<th>Fibrin glue via SCP</th>
<th>Direct injection of fibrin glue</th>
<th>AccuFill® via SCP + Direct injection of fibrin glue</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horse 1</strong></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<td><img src="image9.png" alt="Image" /></td>
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</table>
MicroCT

- **Trabecular Morphometry** $\rightarrow$ 15mm x 10mm VOI
  - BV/TV
  - Trabecular number, spacing, and thickness
  - BS/BV

- **Varying degrees of resorption of the subchondral plate**

<table>
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<th>AccuFill® via SCP + Direct injection of fibrin glue</th>
<th>Negative control</th>
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</thead>
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<td><img src="accufillSCP.png" alt="Image" /></td>
<td><img src="negative_control.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**AccuFill® treated defects:**
- Higher BV/TV, Tb.N, Tb.Th
- Lower Tb.Sp, BS/BV
Histopathology

- Early subchondral plate advancement
  - Control, n=2
  - AccuFill®, n=1
  - Direct injection of FG, n=0
  - SCP with FG, n=1

- Defects primarily filled with fibrous tissue
  - Variable amounts of fibrin and/or fibrocartilage

- Variable retention of repair tissue post-processing
  - No effect of treatment
Discussion

• Proof of concept that osteochondral defects treated with microfracture can be filled with a common biologic via modified Subchondroplasty
  – Adjustments in surgical technique required

• Lameness similar to previous studies
  – Perform arthroscopically in clinical cases → eliminates complications associated with arthrotomy

• **Theory** – The presence of BSM within the trabecular bone may help mitigate some of the bone resorption associated with microfracture in the early postoperative stages
Limitations

- Limited sample size with individual variability
- Short study duration – cartilage healing not evaluated
- Use of normal joints
Future Direction

• **Foundation for future cartilage- and bone-healing studies**
  – Improved support of SCB
  – Further testing of impact on host tissues
  – Longer study duration in a larger sample population
  – Evaluation of osteochondral mechanics

• Standardize targeting system

• Incorporation of biologic adjuncts
Acknowledgments

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The evaluation of beta defensins as a biomarker of septic arthritis in horses

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CONFLICT OF INTEREST STATEMENT

I hereby certify that, to the best of my knowledge, no aspect of my current personal or professional situation might reasonably be expected to affect significantly my views on the subject on which I am presenting.
Introduction

• Septic arthritis
  • Debilitating disease
  • Affects horses of all ages

• Bacterial invasion
  • Rapid, robust inflammation → acute pain
  • Prolonged exposure to inflammatory products
    • Breakdown of normal joint
    • Development of osteoarthritis
    • Chronic pain

• Rapid diagnosis important
Introduction

• Diagnosis not straightforward
  • Clinical signs
  • Cytologic analysis
  • Synovial fluid culture

• Synovial fluid analysis
  • Concurrent disease
  • Pathogens involved (Gustafson et al 1989)
  • Administration of intra-articular medications (Tulamo et al 1989)

• Synovial fluid culture
  • Positive in 50-60% (Schneider et al 1992, Madison et al 1991)
Introduction

• Human periprosthetic joint infection similar
  • Clinical signs
  • Diagnostic testing
  • Suggestive of disease

• Accurate diagnosis important → biomarkers increasing used
Introduction

• Defensins
  • Cysteine rich antimicrobial peptide (Bruhn et al 2007)
  • Innate immune response
  • Utility in diagnosis of periprosthetic joint infection

• Alpha defensin
  • Sensitive (100%) and specific (98%) (Deirmengian et al 2014)
  • In horses, only found in Paneth cells

• Beta defensin
  • Increased in bone and synovial tissue (Liu et al 2014)
  • In horses, presence in synovial fluid not investigated
Objective: Identify and compare expression of beta defensins in synovial fluid from normal joints, those with aseptic inflammation, and joints with septic arthritis

Hypothesis: Beta defensin expression would be higher in synovial fluid from septic arthritis cases when compared to synovial fluid from normal joints and those with aseptic inflammation
Methods

• IACUC approval

• Synovial fluid collected
  • Normal joints
  • Aseptic inflammation
  • Septic inflammation

• Sepsis → two of following criteria:
  • Marked neutrophilic inflammation (neutrophil percent >80%)
  • Increased nucleated cell count (> 20 x 10^3 cells/uL)
  • Elevated total protein (> 4 g/dL)
  • Positive bacterial culture
Methods

• Following collection
  • Samples stored at -80C

• Equine beta defensin -1, -2, -3 identified
  • Commercially available ELISA
  • Ran in duplicates
  • Samples diluted with one-part assay buffer to fall within standard OD$_{450}$ curve
  • Validated for equine use using a spike and recovery assessment
Methods

• Statistics
  • Normality assessed Shapiro-Wilk test
  • One-way ANOVA with Kruskal-Wallis post-hoc statistical test
    • Significance P < 0.05
Results

• Normal joints n=6
  • Tarsocrural, n=3
  • Metacarpophalangeal joint, n=1
  • Middle carpal, n=1
  • Radiocarpal, n=1

• Aseptic inflammation n=4
  • Tarsocrural, n=3
  • Distal interphalangeal, n=1

• Septic joints n=11
  • Tarsocrural, n=7
  • Metacarpo- and metatarsophalangeal joint, n=2
  • Distal interphalangeal, n=1
  • Coxofemoral, n=1
Results

Normal and aseptic inflammation
  • Cell count 1,771 cells/uL
  • Protein 2.5 g/dL

• Septic joints
  • Cell count 61,287 cells/uL
  • Protein 4.4 g/dL
Results

• Equine beta defensin 1
Results

• Equine beta defensin 2
Results

• Equine beta defensin 3

P < 0.01
Discussion

• We identified beta defensin expression in equine synovial fluid

• Higher expression of beta defensin 1 and 3 in aseptically inflamed samples

• Results do not support our hypothesis
Discussion

• Paulsen et al 2014
  • Synovium from osteoarthritic joints had increased beta defensin 1 and 3
  • Synovium from joints with pyogenic inflammation had increased beta defensin 3

• Gollwitzer et al 2013
  • Beta defensin 3 significantly increased in periprosthetic joint infection
    • Serum and synovial fluid
  • Beta defensin 2 significantly increased in periprosthetic joint infection
    • Serum ONLY
Discussion

- Varoga et al 2005
  - TNFa, IL-1 strong inducers of beta defensin 3 in human chondrocytes

- Reasons for disparity:
  - Variation among species in beta defensin function
  - Variation among clinical cases
  - Synovial fluid vs synovium, cartilage, or bone
Study limitations

• Low case numbers
• Including a variety of cases that induce inflammation in the aseptic inflammation group (ie. OA and cellulitis)
Conclusions

• Identified beta defensin expression patterns in samples from equine synovial fluid

• Beta defensins do not appear to be useful as biomarkers of equine septic inflammation

• May be useful for diagnosis or therapeutic intervention in equine aseptic inflammatory joint disease, such as osteoarthritis

• Further studies to elucidate role of beta defensins in equine joint physiology warranted
References


AMANDA WATKINS
Comparison of synovial fluid lubricants and inflammatory cytokines following repeated arthrocentesis in equine synovitis and joint lavage models

Amanda Watkins, VMD
Conflict of Interest

I hereby certify that, to the best of my knowledge, no aspect of my current legal, personal or professional situation might reasonably be expected to affect my views on the subject on which I am presenting.
Background

Synovitis

• Decreased lubrication ability of inflamed synovial fluid [1]

Interleukin 1β-induced synovitis

• Joint inflammation [2]
• Increased pro-inflammatory mediators [3]
• Cartilage degradation

[2] Colbath+ 2018
Background

Intra-articular lavage

• Removal of healthy synovial fluid [4]
• Increased cartilage surface friction [5]
• Decreased histological staining of lubricin [6]

Lubricants

• Lubricin
• Hyaluronic acid

[5] Starke+ 2018
Objectives

Compare the effects of IL-1β-induced synovitis and intra-articular lavage models on:

• Lubricating molecules and viscosity of equine synovial fluid

• Inflammatory cytokines and chemokines in synovial fluid
Hypotheses

IL-1β-induced synovitis and intra-articular lavage would

• Increase inflammatory molecules
• Decrease HA
• Decrease lubricin
Materials & Methods

Randomized crossover study design (n=6)

• Middle carpal joint
  • IL-1β OR phosphate buffered saline (PBS)
  • 5 week sampling period

30 day washout period

• Tarsocrural joint
  • Intra-articular lavage OR arthrocentesis
  • 5 week sampling period
Materials & Methods

- Lubricin (9G3 peanut agglutinin sandwich ELISA)
- sGAG
- HA
- Viscosity (microrheology)
- PGE$_2$
- CCL2, CCL3, CCL5, CCL11, TNF$\alpha$, IL-1$\beta$
Statistical Analysis

• Multi-level mixed-effects linear regression
  • Groups fixed effects

• $\alpha = 0.05$
Lubricin

* = different from baseline
△ = different from contralateral limb
Lubricin

sGAG

* = different from baseline
Δ = different from contralateral limb
* = different from baseline
△ = different from contralateral limb
HA

Viscosity

* = different from baseline
Δ = different from contralateral limb
CCL2

* = different from baseline
Δ = different from contralateral limb
**PGE$_2$**

---

* = different from baseline

$\Delta$ = different from contralateral limb

---
Inflammatory Cytokines

- TNF$\alpha$ increased
- IL-1$\beta$ no significant change
- CCL3 no significant change
- CCL5 no significant change
- CCL11 mild increase
Study Limitations

• Small sample size

• New assay
  • Multiplex ELISA for cytokines/chemokines
Conclusions

• Lubricin increase in response to joint inflammation

• Chemokine increase

• Repeated arthrocentesis
  • Caution
Future Directions

• Lubricin and CCL2 testing as possible biomarkers

• Mechanism of increased SF lubricin

• Effect of changes in lubricants on synovial fluid lubrication and superficial zone chondrocytes
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References


JOINTS AND OSTEOARTHRITIS — LARGE ANIMAL

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