

# PHI ZETA

The Honor Society of Veterinary Medicine  
Epsilon Chapter



November 1, 2023

Research Emphasis Day

AUBURN UNIVERSITY  
COLLEGE OF VETERINARY MEDICINE



**PHI ZETA  
EPSILON CHAPTER  
COLLEGE OF VETERINARY MEDICINE  
AUBURN UNIVERSITY**

welcomes you to our

**PHI ZETA RESEARCH DAY FORUM  
November 1, 2023**

**We want to thank all the presenters, their co-investigators and mentors for their participation in this annual event.**

**We also want to thank the following departments for their support:**

**Office of the Dean  
Office of the Assoc. Dean for Research and Graduate Studies  
Department of Anatomy, Physiology, and Pharmacology  
Department of Clinical Sciences  
Department of Pathobiology  
Scott-Ritchey Research Center**



## **PHI ZETA RESEARCH DAY FORUM**

**NOVEMBER 1, 2023 – VETERINARY EDUCATION CENTER**

**8:00: Opening Statement**

Dr. Frank F. Bartol,  
Alumni Professor and Associate Dean for Research & Graduate Studies,  
Auburn University College of Veterinary Medicine

**8:15-12:00 MORNING Presentations - Overton Auditorium**  
**Moderator (Morning Sessions):**  
**Dr. Daniel Kroeger**

**Veterinary Students**

- 8:15 Alonza Klopfer Evaluating antimicrobial properties of canine platelet lysate *in vitro*
- 8:30 Sarah Lutz Pharmacokinetics of ceftiofur crystalline free acid in the first hour after single dose intramuscular administration in swine
- 8:45 Madison Smith Regulation of canine Neutrophil Extracellular Trap formation by immune-mediated hemolytic anemia therapeutics

**Graduate Students**

- 9:00 Andrew Aitken Crosstalk of Angiotensin II and Oxidative Stress Leads to Neuroinflammation and Neurogenic Hypertension in Rats
- 9:15 Leonie Bertram Small Animal Veterinary Clients' Preferences for Communication and Decisions during CPR
- 9:30 Nikolia Darzenta Evaluation of the immunoregulatory effects of Mesenchymal Stem Cell derived Extracellular Vesicles on mixed glial cells
- 9:45 Patricia Egli Characterization of platelet lysate for nebulization in the horse
- 10:00 Courtney Garrett Treatment of feline GM1 gangliosidosis by cerebrospinal fluid delivery of AAV9 or AAVrh10

**10:15 Break**

- 10:30 Oscar Huertas-Molina Comparison of passively transferred serum total protein (STP) levels in dairy calves fed maternal colostrum (MC), colostrum replacement (CR), or a combination of both
- 10:45 Nneka Iduu First report of virulent *Theileria orientalis* genotype Ikeda in cattle, Alabama, USA



## PROGRAM

- 11:00 Shari Kennedy Validation of Commercially Available Antigen-Capture ELISA in Bovine Viral Diarrhea Testing of Heterologous Species
- 11:15 Steven Kitchens Use of a Chicken Embryo Assay to Investigate Virulence of *Salmonella* from Poultry Environmental Sources
- 11:30 Avery Loyd Effect of fluorescence biomodulation on dermal healing in an equine experimental wound model – a preliminary study
- 11:45 Miguel Saucedo Effect of time and serum IgG levels on the diagnosis of persistent infection (PI) with BVDV in neonatal calves

### **12:00–1:00 POSTER Presentations**

### **1:15-3:30 AFTERNOON Presentations – Overton Auditorium Moderator (Afternoon Sessions): Dr. Bruce F. Smith**

#### **Graduate Students (continued)**

- 1:15 Troy LoBue Comparison of PacBio HiFi Long-read and Illumina Short-read Sequencing and the Identification of Genetic Risk Factors for Breast Cancer
- 1:30 Patricia Titos Novel Murine Models to Study the Pathogenesis and Possible Therapies for the Liver Disease in Niemann-Pick Disease Type C1
- 1:45 Brittany Whitt Evaluating ancestry in African Americans with ties to breast cancer
- 2:00 Jyoti Yadav Antibody Gene Therapy for Rabies Encephalitis

#### **Faculty/Postdoctoral/Staff**

- 2:15 Subarna Barua Comparative Evaluation of GS-441524, Teriflunomide, Ruxolitinib, Molnupiravir, Ritonavir, and Nirmatrelvir for In Vitro Antiviral Activity against Feline Infectious Peritonitis Virus
- 2:30 Lindsey Boone Safety Study of Leucoreduced Allogeneic Freeze-Dried Pooled Platelet-Rich Plasma (PrecisePRP™ Equine) in Normal Equine Subjects
- 2:45 Douglas Castro Comparison of “Blind” and Ultrasound-Guided Sciatic Nerve Injection Techniques in Laboratory Rat Cadavers
- 3:00 Amanda Gross A Vaccine to Mitigate Vampire Bat Predation and Rabies Transmission
- 3:15 Alex Jenkins Quantitative analysis of Perampanel in feline plasma by High Performance Liquid Chromatography (HPLC) and UV detection



**4:00**  
**5:00**

**KEYNOTE LECTURE-Overton Auditorium**  
**INDUCTION AND AWARDS ANNOUNCEMENT**

**INDUCTION of new Phi Zeta Members**

**Research Awards Presentations**

**PHI ZETA KEYNOTE SPEAKER**

**“Tick-borne Diseases Impacting Animal and Human Health: Building Bridges from Basic Research to Vaccine Development”**



**Roman R. Ganta, MSc, PhD**

McKee Endowed Chair Professor

Department of Veterinary Pathobiology

College of Veterinary Medicine, University of Missouri

Dr. Roman Ganta is currently the McKee Endowed Chair Professor in the Department of Veterinary Pathobiology, College of Veterinary Medicine at University of Missouri. Prior to 2023, he was a University Distinguished Professor in the Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine at Kansas State University. Dr. Ganta established the Center of Excellence for Vector-borne Diseases at Kansas State University in 2015 and served as its founding Director until his move to the University of Missouri early this year.

Dr. Ganta has over 25 years of internationally well-recognized research program focused on several tick-borne rickettsial diseases of global public health and animal health importance. Dr. Ganta has consistently secured competitive grant funding which amounted to over \$20 million in research support. He currently has three active NIH R01 grants and a USDA grant focused on basic and translational research goals investigating various tick-borne diseases. He authored or co-authored more than 100 peer-reviewed research articles & book chapters, delivered over 100 invited lectures, and also secured several patents. Dr. Ganta served on various study sections and editorial boards. Over the years, he mentored numerous graduate students, postdoctoral researchers, and faculty members.



## **Posters**

### **Undergraduate Students**

Phoebe Bunting	Characterizing Pain in DRGs
Hannah Eady	Comprehensive immunohistochemical analysis of various immune checkpoint molecules in canine cancers
Luke Eller	Development and characterization of CAR T cells against B7-H3 surface antigen
Ashli Evans	Alzheimer's Disease Histopathological Phenotype in Feline GM1 and GM2 Gangliosidosis
Emma Hruska	AAV-mediated Anti-hormone Antibody Therapy as a Treatment for Alzheimer's Disease
Ellie Hundley	Assessing the Dosage Effect of AAV Gene Therapy in Intravenous Treatment of Feline GM1 Gangliosidosis
Henry Limbo	Ultrasound-induced Sleep in Mice
Nathan Newman	Designing a Powerful Bispecific Fusion Protein for PD1/PD-L1 Blockade and OX40 Agonism
Caroline Parrish	Optimizing the PCR for the STK11 and MSK1 genes in canine mammary cancer
Julia Peterson	Assess an orexin 2 receptor (OX2R) agonist's efficacy in alleviating the secondary symptoms of narcolepsy in orexin knock-out (OX-KO) mice
Isabella Shimko-Lofano	Development of Next-Generation Oncolytic Canine Adenovirus Type 2

### **Veterinary Students**

Kristen Hoehler	Effect of soy isoflavones on hormone secretion in the male gonad
Startus Hutcherson	Assessing the immunoregulatory effect of mesenchymal stem cell extracellular vesicles on stimulated mixed glia
Myles McAtee	Developing a Canine-Specific 4-1BB Agonist for Cancer Immunotherapy
Jenna Sanossian	Circumductal anal sacculectomy in 63 dogs
Anna Welsh	The Effects of FOXL2 Overexpression in the Adrenal Cortex
Charlotte Whitmer	Developmental toxicity of dichloroacetic acid (DCA) in zebrafish ( <i>Danio rerio</i> )



**Graduate Students**

Samantha Bradley	Legacy and emerging per- and polyfluoroalkyl substances (PFAS) regulate steroidogenesis in the male gonad
Jeremy Brozek	Ultrasound-guided cerebrospinal fluid collection at the atlanto-axial space in mature cattle with a comparative evaluation of lumbosacral cerebrospinal fluid collection
Victoria Caravaggio	Isolating exosomes from in vitro cultured trophoblast cells
Sheniqua Glover	Functional investigation of a rare <i>MAD1L1</i> variant in African American hereditary breast cancer risk
Deepika Goyal	Optimization of rtPCR Targeting a Frameshift Mutation in CEACAM24
Natasha Grabau	Effects of sleep on the progression of Multiple Sclerosis
Theresa Higgins	CAR T Cells as Targeted Immunotherapy for Osteosarcoma
Aliaa Ismail	Optimizing Single Nuclei Isolation from Canine Mast Cell Tumors: Enhancing Nucleus Quality for Comprehensive Molecular Analysis
Gurjinder Kaur	Interleukin-27 regulates the metabolic reprogramming of macrophages during Herpes Simplex Virus-1 (HSV) infection
Alana Kramer	Maximizing Therapeutic Potential: Tailoring a PD-1-Dependent 4-1BB Agonist for Combination Immunotherapy
Pia LaPorte	A Cross-Species Comparison of Mammalian Nasal Cavity Microenvironments
Vander LeKites	Sleep as a tool: using chemogenetic activation of the Ventral Medial Midbrain/Pons to examine the effect of sleep on cognitive behavior
Xiaolei Ma	Metagenomic analysis reveals associations between working memory and Bifidobacterium abundance in canine gut microbiome
MelikaSadat Mollabashi	Evaluation of Canine Platelet Lysate Antimicrobial Activities in Vitro
Kira Noordwijk	Use of tigilanol tiglate in the treatment of perineal squamous cell carcinoma in a horse
Chetan Pundkar	$\beta$ -catenin blockade in the tumor microenvironment-conditioned macrophages promotes immunogenicity
Jiayi Ren	Inhibiting mitogen-activated protein kinase kinase (MEK) signaling suppresses immune and inflammatory responses in macrophages and fibroblasts after HSV-1 infection
Damien Ruiz	Humanizing Nanobody-Based ARC Platform for Cancer Immunotherapy



J. Coleman Wahlborg	Friction knot Terminology is Inconsistent in Veterinary Surgery
Xiao Xiong	Genome evolution of the microsporidian pathogen genus <i>Nosema</i>
Taylor Yenrick	Developmental Exposure to Trichloroethylene (TCE) Alters Expression of DNA Methyltransferases in Zebrafish ( <i>Danio rerio</i> )
Ying Zhang	Genome assembly, annotation, and genomic imprinting of the blackstripe livebearer <i>Poeciliopsis prolifica</i> shed light on fish placental evolution
Yue Zhang	Transcriptome landscape of the canine zonary placenta

**Faculty/Postdoctoral Fellows/Staff**

Lindsey Boone	Effect of long term, repeat administration of Clodronate disodium (OsPhos®) on bone turnover in horses
Christine Charvet	Translating Time: an online tool for biomedical sciences
James Gillespie	Prediction of tissue-homing peptides in a mouse model using a machine learning classification model
Samantha Hagerty	Chemical Characterization of Detection Canine Training Aid Volatile Headspace using Proton Transfer Reaction-Time of Flight-Mass Spectrometry
Jessica Klabnik	Case series: Surgical success and reproductive performance for correction of penile deviations in 10 bulls
AbbiLynn Lanier	Heritable anophthalmia/microphthalmia in a marsupial biomedical model





## Veterinary Student Platform Presentations

### Evaluating antimicrobial properties of canine platelet lysate *in vitro*

Alonza Klopfer<sup>1,2,3</sup>, Melika Mollabashi<sup>1,2</sup>, Matt Murray<sup>1,2</sup>, Ann Cochran<sup>1</sup>, Thaina Lunardon<sup>1,2</sup>, Scarlett Sumner<sup>3</sup>, Maria C. Naskou<sup>1,2</sup>

<sup>1</sup>Scott-Ritchey Research Center CVM Auburn University, <sup>2</sup>Department of Pathobiology CVM Auburn University, <sup>3</sup>Department of Clinical Sciences CVM Auburn University, Auburn, AL 36849

**Introduction.** Platelet lysate (PL) is a platelet derived acellular product, that is rich in growth factors and attachment factors. Topical application of PL has shown promising benefits for promoting wound healing. Moreover, PL has also shown antimicrobial properties *in vitro* and *in vivo*, which is an attractive attribute for wound therapies since bacterial contamination is one of the most serious complications impairing the wound healing process. The objective of this *in vitro* study was to compare the antimicrobial activity of PL generated by different methods against bacteria that are commonly present in canine wounds.

**Methods.** Two different centrifugation methods (leukocyte poor versus leukocyte rich) were used to isolate the platelets from canine whole blood. Additional treatment groups were created by performing plasma depletion (PD) and/or heat inactivation (HI) on the canine PL as plasma and complement inactivation pathways have unique impacts on bacterial growth. Each treatment group was tested via a spiking assay against bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. Colony counts were determined after 3 and 24 hours of incubation. Log reductions were calculated for each treatment group compared to the standard media; log reduction >1 is considered significantly relevant inhibition of bacterial growth.

**Results.** For *Escherichia coli*, PL log reduction in leukocyte poor method was 3.54 at 3 hours and >6.4 at 24 hours and in leukocyte rich method was 4.12 at 3 hours and >6.4 at 24 hours. Additionally, PL PD log reduction in leukocyte poor method was 1.27 and in leukocyte rich method was 1.0 at 24 hours. For *Staphylococcus aureus*, PL log reduction in leukocyte poor method was 2.57 at 3 hours and 2.2 at 24 hours and in leukocyte rich method was 3.29 at 3 hours and 1.95 at 24 hours. In leukocyte poor methods, log reduction for PL HI was 1.27 and PL PD HI was 1.31 at 3 hours. Lastly for *Staphylococcus aureus*, PL HI log reduction in leukocyte rich method was 1.11 at 24 hours. For *Enterococcus faecalis*, PL log reduction in leukocyte poor method was 2.0 and PL PD in leukocyte rich method was 1.07 at 24 hours.

**Conclusions.** Plasma and the complement system likely play a key role in reducing bacterial growth given reduced antimicrobial efficacy in plasma depleted and heat inactivated treatments. PL shows promise as a topical antimicrobial and additional research is warranted in evaluating best manufacturing methods and clinical use.

**Acknowledgments.** Research Grant: Scott Fund, Scott-Ritchey Research Center, Auburn University College of Veterinary Medicine  
Student Support: Boehringer Ingelheim Veterinary Scholars Program

**Pharmacokinetics of ceftiofur crystalline free acid in the first hour after single dose intramuscular administration in swine**

Sarah Lutz<sup>1</sup>, Micah Jansen<sup>2</sup>, Sherry Cox<sup>3</sup>

<sup>1</sup>College of Veterinary Medicine, Auburn University, Auburn, AL; <sup>2</sup>Zoetis, Parsippany, NJ;

<sup>3</sup>Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN

**Introduction** Swine practitioners consider many factors when implementing antibiotic regimens, including labeled pathogens, clinical breakpoints or minimum inhibitory concentrations, cost, ease of implementation, and withdrawal times. Duration of therapy is another factor to consider, especially with today's challenges in labor. Ceftiofur crystalline free acid (CCFA, Excede for Swine®, Zoetis) has proven duration of therapy of seven days in an APP challenge model. Previous studies also showed that CCFA reaches plasma concentrations of 2.23 ug/mL at 1 hour post-injection. This is above the MIC90 for *Streptococcus suis*, which is 2.0 ug/mL. However, little is known about the pharmacokinetics of CCFA at shorter time periods post-injection, specifically in the first hour. To understand how quickly CCFA reaches concentrations at or above MIC values for targeted bacteria, the objective of this trial was to study the pharmacokinetics of CCFA in the first hour after a single intramuscular dose.

**Materials and Methods** Six, 21-day old pigs of mixed sex were selected, weighed, and enrolled from two litters. A labeled dose of 1 ml/44 lb (5.0 mg/kg) of CCFA was calculated for each pig. Blood was collected from each pig at 0, 5, 10, 15, 30, 45, and 60 minutes following administration of a single intramuscular dose of CCFA. All blood samples were collected into heparinized tubes and centrifuged to obtain heparinized plasma. The concentration of ceftiofur and its active metabolite, desfuroylceftiofur, in plasma were determined using a derivatization method and reversed phase high pressure liquid chromatography.

**Results** Plasma concentrations are shown in Table 1.

Table 1: Lower confidence limit (LCL), mean, upper confidence limit (UCL), standard deviation, and range of plasma concentrations of ceftiofur and its metabolites at each time point

<b>Time (min)</b>	<b>95% LCL (ug/mL)</b>	<b>Mean (ug/mL)</b>	<b>95% UCL (ug/mL)</b>	<b>Std dev (ug/mL)</b>	<b>Range (ug/mL)</b>
0	0	0	0	0	0
5	0.98	1.37	1.76	0.37	1.14-2.12
10	1.56	1.90	2.24	0.32	1.65-2.50
15	1.80	2.44	3.08	0.61	1.97-3.58
30	2.50	3.83	5.16	1.27	2.23-6.04
45	3.55	5.16	6.78	1.54	3.84-8.17
60	4.12	6.71	9.30	2.47	4.64-11.55

**Conclusions** Based on MIC90 values for *Streptococcus suis* (2.0 ug/mL), CCFA achieved therapeutic plasma concentrations in all pigs by 30 minutes post-injection. Most pigs were above the threshold after only 15 minutes. Producers and practitioners can be confident that CCFA not only has a long duration of therapy, but also reaches therapeutic levels rapidly.

**Acknowledgements** The authors would like to thank Iowa State University's Swine Veterinary Internship Program for the opportunity to conduct research, Four Star Veterinary Service for hosting this project, and Zoetis for sponsoring this project.

**Regulation of canine Neutrophil Extracellular Trap formation by immune-mediated hemolytic anemia therapeutics**

Madison Smith<sup>1</sup>, Liming Shen<sup>1</sup>, Terri Higgins<sup>1</sup>, Rie Watanabe<sup>2</sup>, Dana LeVine<sup>1</sup>

<sup>1</sup> Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

<sup>2</sup> Department of Pathobiology, Auburn University, AL

**Introduction.** Immune-mediated hemolytic anemia (IMHA) is an autoimmune disease in dogs resulting in life-threatening anemia. IMHA has a tragic mortality rate of up to 80% due to fatal thromboemboli (TE). One probable cause of TE in IMHA is exuberant neutrophil extracellular trap (NET) formation; NETs are extracellular strings of DNA, histones, and granular proteins released by activated neutrophils. NETs are prothrombotic and increased circulating NET markers are associated with death in IMHA dogs. There is little evidence to support the ideal immunosuppressant or antithrombotic regimens for IMHA. We hypothesized that, based on previous human data and the drugs' mechanisms of action, some IMHA therapies (heparins, aspirin, cyclosporine, mycophenolate, ticagrelor), but not others (dexamethasone, azathioprine, rivaroxaban), would inhibit NETosis, providing a rationale for their preferred use.

**Methods.** In an *ex vivo* model, neutrophils from healthy dogs were isolated and treated with each drug at various concentrations prior to stimulation with a NET inducer, phorbol-12-myristate-13-acetate (PMA). Cells were stained with a mixture of cell permeable dye (SYTO green), staining intracellular DNA, or intact neutrophils, and impermeable dye (SYTOX orange), staining extracellular DNA, or released NETs. Using a fluorescence microscope, the percent NET forming neutrophils was determined for each drug and compared to the percent NETs induced by PMA alone. Depending on normality, data was analyzed by repeated measures one-way ANOVA followed by Tukey's multiple comparisons or a Friedman's test followed by Dunn's multiple comparisons. As SYTOX orange staining is nonspecific for NETs, significant NET inhibition was confirmed qualitatively with immunofluorescence microscopy where NET-specific antibodies were employed to stain granular and citrullinated H3 components of NETs.

**Results.** Of the antithrombotics tested, enoxaparin (low molecular weight heparin) at 160 U/ml significantly reduced PMA-induced NETosis ( $p < 0.0001$ ) as did unfractionated heparin (UFH) at 0.7 U/ml ( $p = 0.0368$ ). High concentrations of aspirin (200 ug/ml) also inhibited PMA-induced NETosis ( $p = 0.0466$ ). The other tested antithrombotics, rivaroxaban and ticagrelor, did not impact NETosis. None of the tested immunosuppressants (dexamethasone, azathioprine, mycophenolate, cyclosporine) impacted NETosis.

**Conclusions.** Heparins and aspirin may be superior antithrombotics in dogs with IMHA due to their capacity to inhibit NETosis, hopefully improving the prevention of fatal TE. Future studies *in vivo* in healthy dogs and dogs with IMHA are warranted.

**Acknowledgements.** Boehringer Ingelheim Veterinary Research Scholars Program provided support to Madison Smith.



## Graduate Student Platform Presentations

### Crosstalk of Angiotensin II and Oxidative Stress Leads to Neuroinflammation and Neurogenic Hypertension in Rats

Andrew V. Aitken<sup>1</sup> and Vinícia C. Biancardi<sup>1</sup>

<sup>1</sup>Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

**Introduction.** Oxidative stress is a central mechanism underlying Angiotensin II (Ang II)-induced enhanced sympathetic outflow within brain cardio-regulatory nuclei in neurogenic hypertension. Yet, a causal association between Ang II, Nrf2 (nuclear factor erythroid 2-related factor 2 - the master regulator of the antioxidant response), and sympathetic activity in neurogenic hypertension has not been explored. We hypothesized that increased Ang II in neurogenic hypertension impairs the Nrf2 signaling pathway within the paraventricular nucleus of the hypothalamus (PVN), leading to increased oxidative stress, inflammation, and consequent sympathoexcitation.

**Methods.** We used 12–13-week-old male spontaneously hypertensive rats (SHRs) and normotensive Wistar Kyoto rats (WKYs) confirming blood pressure levels indirectly through tail-cuff recording [Kent Scientific]. We measured mRNA expression in PVN punches (real-time PCR) of the Nrf2 negative regulator Keap1 and Nrf2-based antioxidant defense genes (Gpx1 and NQO1). We then measured the protein expression of Nrf2, obtained by immunofluorescence (IF), and detected by high-magnification confocal imaging in the subcellular localization of neurons and astrocytes within the PVN. The neuronal marker NeuN and the astrocytic marker GFAP were used for subcellular identification and image J analyses. To dissect the cause-effect of Ang II-Nrf2 dysregulation other than the MAP increase in hypertension, we treated cohorts of SHR via oral gavage with the Ang II-type-1 receptor blocker Losartan (SHR-Los 20mg/kg, 4 weeks) or the vasodilator Hydralazine (SHR-Hyd 10mg/kg, 10 days).

**Results.** Initially, hypertension was confirmed in SHR vs. WKY with greater in mean arterial pressure (MAP) (162.8±3.6mmHg vs. 107.8±2.1mmHg, respectively). We found increased mRNA levels of Keap1 (2.1±0.2) and reductions in Gpx1 (0.7±0.1) and NQO1 (0.5±0.1 fold change) in SHR (P<0.05 vs. WKY), suggesting lower Nrf2 and associate antioxidant genes expression. Relative to WKYs, Nrf2 IF density was reduced in SHR neurons (whole cell: -43.3±4.0, nucleus: -33.3±6.7, cytosol: -63.9±6.8% vs. WKY, P<0.001) and astrocytes (cell: -55.2±5.0, nucleus: -71.9±4.0, cytosol: -54.1±5.7% vs. WKY, P<0.001). Following treatment, compared to WKY, SHR-Los normalized MAP (154.9±1.7 to 103.6±2.4 mmHg, p<0.0001) and Nrf2 protein expression in both neurons (cell: -2.8±6.8, nucleus: -5.8±6.5, cytosol: -2.8±13.9%) and astrocytes (cell: -3.9±9.0, nucleus: +1.2±11.9, cytosol -4.1±10.2%). In SHR-Hyd, despite normalization of MAP (154.9±1.7 to 105.2±3.8 mmHg, p<0.0001), no reduction in Nrf2 protein expression was observed in neurons (cell: -39.3±3.8, nucleus: -28.4±4.2, cytosol: -61.7±5.3%) and astrocytes (cell: -51.5±4.3, nucleus: -68.4±3.8, cytosol: -52.1±5.7%).

**Conclusions.** These data support that the compromised Nrf2 dynamics observed are due to Ang II signaling rather than elevations in blood pressure alone. Our ongoing studies aim to understand the Nrf2 contribution to inflammation and autonomic dysfunction in hypertension.

**Acknowledgments.** This work was funded by AHA 953524 to VCB.



### **Small Animal Veterinary Clients' Preferences for Communication and Decisions during CPR**

Leonie Bertram<sup>1</sup>, Kendon Kuo, Eric Hofmeister<sup>1</sup>, Katherine Gerken<sup>1</sup>

<sup>1</sup>Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

**Introduction.** The objectives of this study were to identify the preferences of small animal veterinary clients for the timing of communication during CPR and whether these clients prefer the veterinarian or pet owner decide on the termination of resuscitation.

**Methods.** This cross-sectional observational study used an anonymous internet-based electronic survey distributed to 28,000 clients of the Wilford and Kate Bailey Small Animal Teaching Hospital. 1468 surveys were included in the final analysis. The survey included 16 questions asking for the respondents' demographics, healthcare profession status, supplemental questions pertaining to CPR, preference for timing of communication during CPR and veterinary team members to speak to, as well as the decision on termination of resuscitation. An optional comment section was provided.

**Results.** Of the respondents, 56% and 63% (when asked using a short or long scenario question, respectively) preferred to be informed about their pet undergoing CPR after CPR has ended. Most clients (84%) wanted the veterinarian to decide when to stop CPR. In the comments section, clients predominantly emphasized that patient care should always be prioritized over client communication.

**Conclusions.** This study contributes to a better understanding of veterinary clients' preferences and may help improve client communication and decision-making during CPR. More studies are warranted to reach a wider population before broad recommendations can be made.

**Evaluation of the immunoregulatory effects of Mesenchymal Stem Cell derived Extracellular Vesicles on mixed glial cells**

Nikolia Darzenta<sup>1,2</sup>, Ann Cochran<sup>1</sup>, Emily Edelman<sup>1,2</sup>, Douglas R. Martin<sup>1,3</sup>, Maria C. Naskou<sup>1,2</sup>

<sup>1</sup>Scott-Ritchey Research Center, <sup>2</sup>Department of Pathobiology, <sup>3</sup>Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL.

**Introduction.** Novel investigations in regenerative medicine highlight that the immunoregulatory properties of Mesenchymal Stem Cells are attributed to the secretome and specifically to their Extracellular Vesicles (MSCs-EVs). Extracellular Vesicles (EVs) consist a heterogeneous population of cell-free double layer membrane – bound carriers enriched with parental cell-derived active cargos that contribute to intracellular communication through the delivery of signal molecules. More specifically, stem cell derived EVs have been suggested to alleviate neuroinflammation and neurodegeneration via suppression of astrogliosis and microgliosis. In addition, they can cross the brain - blood barrier, making them a promising tool to fight neuroinflammation. However, the characteristics and the immunomodulatory properties of MSC-EVs are dependent on the cell source and the culture conditions from which they are isolated. Common practices to produce EVs include culture of MSCs under serum free (SF), EV-depleted FBS and inflammatory conditions (IF) and their altered effects must be considered when designing potential therapeutics with clinical value. Sandhoff disease (SD) is a GM2 Lysosomal storage disease (LSD), caused by mutations of the N-acetyl- $\beta$ -hexosaminidase (Hex), that causes progressive, rapid, and fatal neurodegeneration. Moreover, this genetic disease is characterized by inflammatory features typical of all neurodegenerative diseases such as astrogliosis and microgliosis. Therefore, using an animal model characterized by a single gene disorder allows us to evaluate the anti-inflammatory effects of MSC-EVs on the main effector cells that are involved in neuroinflammation. Our objective was a) to compare the phenotype of mixed glia from normal and SD cats, and b) to evaluate the immunosuppressive properties of different isolates of MSCs-EVs on neuronal mixed glia derived from SD cats and normal cats.

**Methods** Phenotypical characterization was performed in mixed glia cultures from normal and SD cats via flow cytometry and immunofluorescence. Extracellular Vesicles isolated from umbilical cord derived MSCs were isolated under different culture conditions including a serum free environment (SF-EVs) or in the presence of FBS (FBS-EVs) or following priming of MSCs with IFN- $\gamma$  and TNF- $\alpha$  (IF-EVs). Subsequently, different isolates of EVs were added to naïve and LPS-stimulated cells for 24 and 48 hours. Cell culture supernatants were collected and analyzed for pro- and anti-inflammatory cytokines, such as IL-6 and IL-10, through ELISA while mRNA expression of inflammatory cytokines was assessed via reverse transcriptase quantitative PCR.

**Results.** Isolated mixed glia cells from SD and normal cats were confirmed as a mixed glia population while expression of specific markers seemed different between normal and SD cats. We found that the addition of EVs to mixed glia population from SD and normal cats suppressed the production and the mRNA expression of IL-6, IL1- $\beta$ , TNF- $\alpha$  compared to those without the addition of EVs. The culture conditions of MSCs seemed to affect the suppressive capacity of their EVs.

**Conclusions.** These findings provide novel insights on how the MSCs-EVs consist a promising tool against neuroinflammation in the feline SD model and their potential use in translational studies to fight diseases that are characterized by neuroinflammation.

**Acknowledgments.** Animal Health and Disease Research program.

**Characterization of platelet lysate for nebulization in the horse**

Patricia Egli<sup>2</sup>, Laura Huber<sup>1</sup>, Lindsey Boone<sup>2</sup>, Maria Naskou<sup>1</sup>, John Peroni<sup>3</sup>, Kara Lascola<sup>2</sup>

<sup>1</sup>Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

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<sup>3</sup>Department of Large Animal Medicine, University of Georgia Veterinary Medicine, GA

**Introduction.** Respiratory disease remains a significant cause of morbidity in the horse. Treatment relies heavily on antimicrobial and anti-inflammatory therapy. Emerging antimicrobial resistance along with side effects associated with anti-inflammatory therapy emphasize the need to identify novel treatment therapies. Platelet lysate (PL), an acellular biologic product, demonstrates anti-inflammatory and antimicrobial properties. Nebulized PL has been proposed to treat respiratory disease in humans. To date, nebulization of equine PL has not been described. The objectives of this study are to characterize nebulized equine PL according to particle size and flow rate, protein composition, and *in vitro* antimicrobial activity against clinically relevant respiratory pathogens.

**Methods.** Pooled equine PL (3 horses) underwent nebulization using a Flexineb E3® equine commercial mesh nebulizer equipped with a sealed, sterile receptacle to collect condensate. All analysis was performed in duplicate or triplicate to compare pre- and post-nebulized PL. Flow rate (ml/min) and quantification of aerosolized particles  $\leq 5\mu\text{m}$  and  $\leq 10\mu\text{m}$  was determined via Spraytec® spray particle and droplet size analyzer. Cytokine concentrations (IL-1 $\beta$ , IL-4, IL-6, IL-10, IFN- $\gamma$ , TNF- $\beta$ ) were measured with an equine multiplex magnetic bead immunoassay (MILLIPLEX® MAP). Bacterial growth inhibition parameters [maximum growth ( $\mu$ ); carrying capacity ( $K$ )] were evaluated for *Escherichia coli*, *Streptococcus equi* subsp *zooepidemicus*, and *Rhodococcus equi* (susceptible and MDR) isolates using pre- and post-nebulized PL concentrations of 50% (PreN50, PostN50) and 100% (PreN100, PostN100) with BHI alone as reference group.

**Results.** Nebulized flow rate was 0.8 ml/min. Median nebulized particle size was 4.991  $\mu\text{m}$  with 52% of particles  $\leq 5\mu\text{m}$  and 86 %  $\leq 10\mu\text{m}$ . For all cytokines measurable concentrations were detected with no differences identified between pre- and post-nebulization ( $p = 0.39$ ). Negative affects of PL on  $K$  were noted for *E. coli* (PreN50;  $P = 0.026$ ) and *S. zooepidemicus* (all PL treatments;  $P < 0.05$ ). Negative effects of PL on  $\mu$  were noted for *R. equi*-susceptible for PostN100 ( $P=0.013$ ), PostN50 ( $P=0.006$ ) and PreN100 ( $P=0.021$ ) treatments. No effect of treatment was observed on  $\mu$  for *E. coli* and *S. zooepidemicus* or on  $K$  for *R. equi*-susceptible. For *R. equi*-MDR,  $K$  was positively affected by all PL treatments ( $P < 0.05$ ) while  $\mu$  was only negatively affected by the PostN50 treatment ( $P = 0.048$ ).

**Conclusions.** Nebulized PL provides adequate particle sizes for deposition into the lower airways and can be delivered at clinically relevant flow rates. Nebulization does not appear to significantly effect cytokine composition or the antimicrobial properties of PL, however overall antimicrobial activity is organism dependent. Further research is recommended to better characterize antimicrobial activity and to describe growth factor and antimicrobial peptide content of PL before and after nebulization. Evaluation of the *in vivo* efficacy of nebulization of PL in horses with respiratory disease is also warranted.

**Acknowledgments.** Funding for this study provided by VCRS Research Foundation

**Treatment of feline GM1 gangliosidosis by cerebrospinal fluid delivery of AAV9 or AAVrh10**

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**Introduction.** Feline GM1 gangliosidosis is a fatal neurodegenerative disease caused by a deficiency of lysosomal  $\beta$ -galactosidase ( $\beta$ -gal). The feline disease faithfully emulates the pathology of GM1 in humans, making cats an ideal animal model for studying this disease. Adeno-associated viral (AAV) gene therapy for GM1 aims to restore  $\beta$ -gal activity and minimize disease progression. Choosing the appropriate AAV serotype and delivery route is critical for GM1 therapy since the vector must display tropism for cells that can achieve therapeutic effect and reach the areas most affected. Intracranial injection of AAV into the thalamus and deep cerebellar nuclei resulted in a 7.5-fold increase in the lifespan of GM1 cats, but the procedure is invasive and requires a craniotomy for delivery to the brain parenchyma. Injection into the CSF via the cisterna magna (CM) is less invasive and could potentially improve biodistribution to certain parts of the CNS and peripheral organs. This study compares the effects of AAV serotype on survival,  $\beta$ -gal distribution, viral distribution, and disease progression in GM1 cats following CM injection using serotypes AAV9 and AAVrh10.

**Methods.** Each cohort received  $1.5 \times 10^{13}$  vector genomes/kg body weight at  $2.2 \pm 0.3$  months of age (symptom onset occurs at  $\sim 4$  months). 4-MU enzyme assays were used to assess  $\beta$ -gal enzyme activity in various tissue samples in the CNS and peripheral organs. 7T Magnetic resonance imaging was used to track neurodegeneration across cohorts. The two vectors were directly compared using empty to full capsid ratios and aggregation rates.

**Results.**  $\beta$ -gal activity increased in the cerebellum and the spinal cord for both treatment cohorts; however, activity remained minimal in the cerebrum for both cohorts (untreated animals exhibit little to no  $\beta$ -gal activity in the brain and spinal cord). Both cohorts also displayed improved  $\beta$ -gal activity in peripheral tissues, such as the heart and cervical dorsal root ganglion. MRI showed delayed neurodegeneration in both cohorts, which correlated with delayed clinical symptoms such as inability to stand. These symptoms are reached at approximately 8 months in untreated animals, but were delayed by 2+ months in both treatment cohorts. Ultimately, untreated GM1 cats ( $n=9$ ) survived  $7.9 \pm 0.3$  months, GM1+AAV9 ( $n=3$ ) cats survived  $13.9 \pm 1.9$  months, and GM1+AAVrh10 ( $n=3$ ) cats survived  $11.9 \pm 0.9$  months. Statistical significance was found between the survival of untreated GM1 cats treated cats in both cohorts ( $p=0.0089$ ) but there was no statistical significance found between AAV9 and AAVrh10 treated cats ( $p=0.1966$ ).

**Conclusions.** This study indicates efficacy of AAV therapy in both treatment cohorts and suggests that CM-injected AAV9 delays disease progression compared to AAVrh10 for the feline model.

**Acknowledgments.** This research was supported by Lysogene and Scott-Ritchey Research Center.



**Comparison of passively transferred serum total protein (STP) levels in dairy calves fed maternal colostrum (MC), colostrum replacement (CR), or a combination of both**

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**Introduction.** Passive immunity in calves is evaluated or quantified by measuring serum IgG or serum total proteins (STP) during the first 7 days of age. Serum total protein is highly correlated with serum IgG and can be used as a predictor of dairy calf passive immunity, health, and performance during the pre-weaning period. In previous studies, the suggested STP cut-off values for failure in the transfer of passive immunity varied from  $\leq 5.2$  g/dL to  $\leq 5.5$  g/dL. When maternal colostrum (MC) is unavailable, commercially available colostrum replacement products (CR) are used to ensure adequate transfer of passive immunity. However, a recent study suggested that the mean STP at 24 hours was lower in calves fed CR compared with calves fed MC (5.16 vs. 5.84 g/dL, respectively). The objective of this study was to determine if feeding a higher dose of CR (300 g of IgG total), a combination of CR (100 g of IgG) with 2-3 L of high-quality MC ( $>21\%$  Brix), or 4 L of high-quality MC within the first 4 hours of life resulted in comparable STP and IgG levels in dairy calves.

**Methods.** Seventeen Holstein dairy calves were randomly assigned to 1 of 3 treatments after birth. Group MC (n=5) received 4 L of MC  $> 21\%$  Brix, group CR (n=9) received 300 g of IgG from a CR powder mixed according to the manufacturer's recommendations, and group MCCR (n=3) received 100 g of IgG from a CR powder in addition to 2-3 L of MC  $> 21\%$  Brix. All treatments were administered within 4 hours of birth. Serum samples were collected from all calves between 24 and 48 hours of life to determine STP and IgG concentrations. Descriptive statistics and Kruskal-Wallis analysis were performed to analyze and compare results between groups.

**Results.** The mean  $\pm$  SD STP of calves from the CR group ( $5.83 \pm 0.41$  gr/dL) was significantly lower compared with the mean  $\pm$  SD STP from MC and MCCR calves ( $7.1 \pm 0.43$  and  $6.7 \pm 0.6$  gr/dL, respectively  $p < 0.003$ ). The mean STP between MC and MCCR calves was not significantly different ( $p > 0.05$ ).

**Conclusions.** Although a high dose of CR resulted in adequate levels of STP based on current herd recommendations for dairy calves, high-quality MC or a combination of CR and high-quality MC resulted in greater levels of STP. Greater STP could be associated with greater serum IgG and lower pre-weaning morbidity.

**Acknowledgments.** Funding from this research was partially provided by the Saskatoon Colostrum Company Ltd. (SCCL), Saskatoon, Ca., and the Department of Clinical Sciences, Auburn University School of Veterinary Medicine.

**First report of virulent *Theileria orientalis* genotype Ikeda in cattle, Alabama, USA**

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**Introduction.** *Theileria orientalis*, a hemoprotozoan parasite, affects cattle with adverse effects, including anemia and abortion. Researchers have identified eleven genotypes, with Buffeli, Chitose, and Ikeda being common, the latter two more virulent. Recently, *T. orientalis* Ikeda has become endemic in Virginia and has been detected in seven other U.S. states since 2017. This study investigates its prevalence in Alabama's cattle population.

**Methods.** Whole blood samples were collected from two sources: cattle (n=72) at Auburn University Large Animal Teaching Hospital and bovine blood samples (n=147) from Alabama farms. DNA extraction was performed using the High-Pure PCR Template Preparation Kit. Two validated quantitative PCRs were used to detect *Theileria* DNA: a TaqMan PCR targeting the MPSP gene and an rRNA FRET-PCR. *Theileria*-positive PCR products were sequenced, and nucleotide sequences were submitted to NCBI for GenBank Accession numbers and used for phylogenetic analysis. Additionally, *Anaplasma* DNA detection was conducted following a published protocol in Auburn University's Molecular diagnostic laboratory.

**Result.** The genotyping results revealed that the MPSP gene sequences (639-660 bp) from two positive cases from cattle in Lee and Mobile Counties of Alabama (ALP-1 and ALP-2) exhibited a 100% match with those of recognized *T. orientalis* Ikeda strains. These sequences also displayed similarities ranging from 76% to 88% with ten other *T. orientalis* genotypes. In the blood of infected cattle, a high copy number of *T. orientalis* Ikeda was detected, with values of  $1.7 \times 10^5$  and  $1.3 \times 10^6$ /ml whole blood for ALP-1, and  $7.1 \times 10^6$ /ml whole blood for ALP-2. Importantly, these two positive samples were confirmed free of *Anaplasma* spp and showed no significant clinical abnormalities.

**Conclusion.** *T. orientalis* Ikeda infection and the detection of a high pathogen burden in seemingly healthy cattle in this study suggest that other tick species, as well as shared needles and dehorning procedures, could facilitate pathogen transmission within the herd. Continued investigations are necessary for the surveillance of *T. orientalis* Ikeda and *Haemaphysalis longicornis* ticks in Alabama and other U.S. states, along with assessing the pathogenicity of *T. orientalis* Ikeda infections in cattle.



## Validation of Commercially Available Antigen-Capture ELISA in Bovine Viral Diarrhea Testing of Heterologous Species

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**Introduction.** Commercially available antigen-capture enzyme-linked immunosorbent assays (ACE) are a mainstay in diagnosing bovine viral diarrhea virus (BVDV) in cattle and are often used in heterologous, or non-cattle, species. However, these tests are validated only for use in cattle. The objective was to validate results of commercially available ACE on ear-notch samples from white-tailed deer by performing reverse-transcription quantitative polymerase chain reaction (RT-qPCR) on pooled lymph nodes from the same animals.

**Methods.** Ear-notch samples from 576 and 589 hunter-harvested white-tailed deer from the 2007-2008 and 2008-2009 (respectively) hunting seasons in Alabama were provided by the Thompson Bishops Sparks Diagnostic laboratory. Three-hundred ear-notch samples and 196 lymph nodes were provided between 2020-2022 by the United States Department of Agriculture (USDA) National Wildlife Disease Program. Performed ACE testing on ear-notch samples per manufacturer's instructions and confirmed results of the 2020-2022 samples by RT-qPCR on lymph nodes collected from the same animals.

**Results.** There were seven positive and 13 suspect animals during the 2007-2008 season and three positive and four suspects during the following hunting season. Antigen-capture ELISA suspect and positive results between 2007-2009 were unable to be confirmed by RT-qPCR. No samples were positive or suspect during the 2020-2022 sampling period and negative results were verified through pooled lymph node RT-qPCR.

**Conclusions.** Positive or suspect ACE results should be confirmed through secondary methods such as virus isolation or PCR. Negative results appear reliable as all negative ACE results were confirmed through RT-qPCR on same animal lymph node samples.

**Acknowledgments.** Thank you, Dr. Nuehring at the Thompson Bishop Sparks State Diagnostic Laboratory (Auburn, Alabama), for collecting the ear-notch samples during the 2007-2009 years of this project. A huge thank you to Emily Handley, Thompson Bishop Sparks State Diagnostic Laboratory, for performing all BVDV antigen-capture ELISA assays in this study. Our sincere appreciation for Kelly Douglas, Stacey Coggins, James Freye, and Michael Milleson, Wildlife Disease Biologists from the USDA/APHIS/WS/ National Wildlife Disease Program for sample collection and submission. Funding was provided by the USDA ARS NBAF Workforce Development Fund.

**Use of a Chicken Embryo Assay to Investigate Virulence of *Salmonella* from Poultry Environmental Sources**

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**Introduction.** *Salmonella* is a zoonotic enteropathogen that causes significant disease in humans and livestock animals. It is important to understand this pathogen in host organisms and in the environment. Our research group is investigating prevalence of *Salmonella* in the poultry production chain from farm through processing. A *Salmonella* isolate lacking the O-antigens used for serotyping was isolated from a poultry farm environmental sample. The purpose of this project was to determine the serotype of this "rough" isolate and evaluate the virulence of the "rough" isolate.

**Methods.** The rough isolate was sequenced on an Illumina MiniSeq. SeqSero 1.2 and KmerFinder 3.2 from the Center for Genomic Epidemiology (<http://genomicepidemiology.org/>) was used to analyze whole-genome sequencing data to determine or predict the "rough" isolate's serotype. To better understand this wild "rough" isolate, a chicken embryo lethality assay was used to assess virulence of this isolate. Specific-pathogen free (SPF) eggs were incubated until day 11 of development. SPF eggs were inoculated with the *Salmonella* rough isolate (10<sup>2</sup> CFU/egg) into allantoic fluid. Eggs were candled daily to monitor embryo mortality up to day 17 of development. SAS Studio v.3.81 (SAS Institute Inc., Cary, NC, USA), Kaplan–Meier curves and log-rank test were used to analyze data.

**Results.** The "rough" isolate could not be serotyped with SeqSero. Utilizing KmerFinder 3.2, the reference sequence for *Salmonella enterica* subsp. *enterica* serovar Infantis strain R21.1147 was contained, which predicts that the rough isolate serotype should be *Salmonella* Infantis. The survival of chicken embryos was significantly higher ( $p=0.0043$  and  $p=0.0291$ ) between eggs inoculated with the "rough" isolate versus *S. Typhimurium*. There was no significance found between survival of embryos administered the "rough" isolate or a monophasic *Salmonella* serovar 4,[5],12:i:-. Significant ( $p=0.0395$ ) difference in survival was found between *Salmonella* Infantis and the "rough" isolate, but significance ( $p=0.0942$ ) was not found when eggs were inoculated with 10 times (10<sup>3</sup> CFU/egg) the regular dose of the "rough" isolate when compared to *Salmonella* Infantis.

**Conclusions.** This work shows that the chicken embryo lethality assay is beneficial when investigating attenuation of virulence of *Salmonella* isolates without having to sacrifice laboratory animals.

**Acknowledgments.** This work was supported by the U.S. Department of Agriculture, Agricultural Research Service

**Comparison of PacBio HiFi Long-read and Illumina Short-read Sequencing and the Identification of Genetic Risk Factors for Breast Cancer**

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**Introduction.** PacBio High Fidelity (HiFi) long read sequencing is a recent contribution to the continuously evolving genetic sequencing industry. HiFi sequencing generates long reads around 15,000 base pairs in length compared to the 100-150 base pair reads generated by Illumina and similar short read sequencers. The access to HiFi long reads provides researchers with the accuracy of short reads while also facilitating more complete genome coverage at lower sequencing depths. Ultimately, HiFi reads have led to easier library preparation, faster assembly time, and more confident detection of all variant types including structural variants. In this study, identical samples are sequenced using HiFi long read and Illumina short read systems and are compared for general sequencing statistics and their ability to identify different variant types associated with breast cancer susceptibility.

**Methods.** PacBio HiFi reads were generated using the Sequel® II SMRT® Cell 8M platform. Illumina short reads were generated with the NovaSeq 6000 system. HiFi and Illumina reads were both processed using the Genome Analysis Tool Kit (GATK v.4.1.9) Best Practices for short variant identification. HiFi reads were also processed using PacBio SMRTtools for both short variant (DeepVariant) and structural variant identification. Structural variant calling program Manta was used to call copy number variants in Illumina data. Sequencing metrics were collected using functions included with GATK and Samtools.

**Results.** Initial sequencing metrics showed that when using Burrows-Wheeler Alignment tool (BWA) both the HiFi reads and Illumina short reads were aligned to the hg38 reference genome in similar fashions. HiFi reads showed 99.78% reads mapped to the reference while Illumina short reads showed 99.76% reads mapped. Alignment of the PacBio reads was slightly improved when using their recommended alignment tool pbmm2, which showed 100% alignment. The average total short variants called was also similar between PacBio and Illumina data when using GATK protocols. There was no significant difference in the average total short variants called for the PacBio data when using DeepVariant. Additionally, the average total copy number variants called was similar between the PacBio data, 11,675 called using SMRTtools, and the Illumina data, 10,840 called using Manta.

**Conclusions.** There are slight advantages observed in this comparison study between HiFi reads and Illumina short reads. HiFi reads showed a slightly higher percent of reads mapped and average number of short variants and structural variants called compared to the short read data. Also, using the PacBio recommended alignment tool (pbmm2) and short variant caller (DeepVariant) increased the percent of mapped reads and number of variants called, respectively, when compared to BWA and GATK. HiFi reads are different from short reads and using the recommended and specialized programs designed for long reads improves downstream analysis, potentially revealing novel genetic risk factors for breast cancer.

**Acknowledgments.** PacBio 2022 HiFi for Accuracy SMRT Grant, Auburn University HPC administrators, all participants of the Alabama Hereditary Cancer Cohort, Arizona Genomics Institute at the University of Arizona.



### **Effect of fluorescence biomodulation on dermal healing in an equine experimental wound model – a preliminary study**

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**Introduction.** Distal limb injuries can result in significant tissue loss leading to the need for them to heal by second intention. Additionally, exuberant granulation tissue formation can complicate wound healing. Fluorescence biomodulation (FBM) is a form of photobiomodulation that relies on light absorbing molecules to convert light from LED sources to wavelengths and energy that can penetrate skin and stimulate healing. The objectives were to evaluate the macroscopic and microscopic effects of FBM on experimentally induced, full-thickness wounds in the horse.

**Methods.** Six adult horses were used for dorsal metacarpal full thickness wound models. Two wounds were created on the dorsum of each metacarpus. One forelimb received FBM and contralateral limb served as control. One wound per limb was used to grade granulation tissue and digital wound area measurements. The second wound was biopsied for histopathologic scoring. Treatment was performed as recommended by the manufacturer every seven days for a total of four treatments. Statistical analyses were performed using linear mixed model, generalized linear mixed models, and Satterthwaite degrees of freedom method. Significance was set as  $p < 0.05$ .

**Results.** No statistically significant differences found on wound area, granulation tissue grading, or histopathology scoring. One horse produced exuberant granulation tissue of all four wounds regardless of treatment, requiring debridement following the study.

**Conclusions.** FBM did not have any statistically significant effects on wound healing by second intention. However, it did not appear to have adverse effects on experimentally created equine distal limb wounds.

Limitations included small sample size, acute surgical wound model, and short-term treatment (28 days).

These data support future study of FBM on equine chronic and infected wound models

**Acknowledgments.** This study was funded by Vetoquinol S.A.

The Authors are very grateful to the Auburn University Large Animal Teaching Hospital and would like to thank Brittany Tipton and Dr. Deborah Keys their contributions to the study.

**Effect of time and serum IgG levels on the diagnosis of persistent infection (PI) with BVDV in neonatal calves.**

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**Introduction.** Bovine viral diarrhea virus (BVDV) infection continues to cause major economic losses to cattle producers in the United States (US) due to pregnancy loss and increased morbidity resulting from viral infection. Identification and elimination of calves persistently infected (PI) with BVDV shortly after birth to prevent exposure to pregnant cattle is of outmost importance in BVDV control and eradication programs across the globe. Antigen detection tests such as antigen-capture ELISA (ACE), reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry (IHC), and chute-side ELISA snap test (i.e., IDEXX Snap BVDV antigen test®, IDEXX, Fort Collins, CO) are commonly used by producers and veterinarians for the identification of PI cattle. Although the majority of BVDV PI antigen tests are highly sensitive, colostrum-derived BVDV antibodies can interfere with test performance and lead to false negative results.

**Methods.** Ten Black Angus, 18-month-old, pregnant heifers were inoculated intranasally with BVDV 1b strain AU526 between 70 and 90 days of gestation. After calving, serum, whole blood [white blood cells (WBC)], nasal swabs (NS), and skin (ear notch) samples were collected from newborn calves before colostrum intake (T0), as well as at 12h (T1), 24h (T2), 7 days (T3), 14 d (T4), and 28 d (T5) following birth. At each time point, ear notch samples were tested for BVDV PI by antigen capture ELISA (ACE) and the calf-side IDEXX Snap BVDV antigen test®. Serum samples were tested for PI with ACE, RT-PCR, and virus isolation. Additionally, serum IgG levels were evaluated at each time point by single radial immunodiffusion (SRID).

**Results.** Between 12 and 24 hours of life, the false negative rate for the IDEXX Snap BVDV antigen test®, ACE, VI and PCR in serum samples from this population of neonatal BVDV PI calves varied from 10% to 100% depending on diagnostic test and testing time. Between 12 and 24 hours of life, the false negative rate for the ACE and IDEXX Snap BVDV antigen test® in skin (ear notches) varied from 20% to 40%. The mean serum levels of total IgG increased in all calves up to 24 hours. There was a moderate but significant negative correlation between serum IgG and ACE results in serum and skin samples.

**Conclusion.** Significant interference of colostrum-derived immunity on BVDV PI diagnostic test performance can negatively affect current BVDV controls programs worldwide.

**Acknowledgments.** Funding for this study was provided by the American Association of Bovine Practitioners (AABP) Foundation.



**Novel Murine Models to Study the Pathogenesis and Possible Therapies for the Liver Disease in Niemann-Pick Disease Type C1.**

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**Introduction.** Niemann-Pick disease type C (NPCD) is a lysosomal storage disorder characterized by disrupted cholesterol transport and other lipids, leading to its accumulation within the lysosomes. It is caused by mutations in NPC1 or NPC2 genes. Current mouse strains used for NPC1 studies are useful for the evaluation of the neurodegenerative phenotype; however, its rapid progression limits the development of disease in other organs, including the liver and spleen. Our goal was to create a liver-specific knockout to characterize the liver disease in NPC1 and evaluate possible therapies.

**Methods.** We developed a liver specific knockout model using the *Cre-lox* tissue specific knockout system by breeding a liver-specific or Kupffer cell-specific with a homozygous NPC1 "floxed" mouse. The genotype was confirmed by PCR. Mice were euthanized, and the liver was removed, post-fixed in 4% paraformaldehyde, paraffin embedded, and 4  $\mu$ m sections were prepared for H&E staining.

**Results.** We have successfully developed a hepatocyte-specific and Kupffer cell-specific NPC1 mouse knockout models. Our preliminary results show that both knockout models have enlarged liver.

**Conclusions.** We expect our mouse models provide a valuable tool for studying liver disease in NPC1. The creation of this liver-specific knockout model provides a significant contribution to the field of NPC1 research and could lead to improved understanding of the disease, which may ultimately lead to the development of better therapies for patients.

**Acknowledgments.** University of Michigan, the Jackson Laboratory and Auburn University, College of Veterinary Medicine.



**Evaluating ancestry in African Americans with ties to breast cancer**

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**Introduction.** Ancestry analysis is a powerful tool, which can be utilized in order to gain insight into the geographic roots of a person's lineage. This analysis can be used to also provide evidence in risk for certain inherited diseases. These connections are critical to understanding and better assessing certain ailments, such as breast cancer genetic risk in African American populations – a focus of our laboratory. Performing ancestry analyses on the whole genome sequencing data of our African American breast cancer-affected study participants holds the potential to unveil critical links between ancestral populations and single nucleotide polymorphisms (SNPs) found in our research. To align with the goals of the American Cancer Society grant, we want to make sure our study participants are showcasing at least a 70% African American ancestry. Having this threshold allows for accurately assessing if a mutation has any relevance from ancestry or if the cause is located elsewhere.

**Methods.** The program used to run ancestral analyses on sequenced data is called Admixture. In order to utilize the program correctly with our data, it must be run in a "supervised" manner, which means reference data with preassigned populations must be used. For this, we are working with data from 1000 Genomes 30x on GRCh38. This dataset comes with roughly 3200 samples and includes 26 populations covering a large variety of geographic areas. This data is combined with 5 of our cases at a time and converted into Plink files, which is the required formatting of files for the Admixture program. Admixture is then able to run its analysis to estimate the likelihood of our samples having ancestry from the prefixed 26 populations.

**Results.** As of now, over one-half of our cases (60 of 120) have been analyzed using this method. This process is incredibly time consuming and has yielded the challenge of using quite a large amount of data on Auburn's Supercomputer, the Easley clusters. We have determined of the 26 population groups provided by the 1000 Genomes data, 7 of those would qualify as African due to their geographic regions covered. Many of our samples are showcasing a high percentage of ancestry from these areas, and none so far have fallen below the seventy percent threshold. We are presently trying to ensure the process is as reproducible as possible and are solving some minor discrepancies in values obtained from different iterations of our methodology.

**Conclusions.** Ancestry analysis is a powerful tool that is proving to be a major help for the work being done by our team. Though this is an ongoing project, and much work is still needed to smooth out our process, the results we have obtained are alluding to some major breakthroughs in the near future. Finishing up ancestral analyses and coupling that with upcoming association analysis work will start painting a clearer picture into what is going on in the genetics of our study participants.

**Acknowledgments.** We would like to thank our study participants as well as East Alabama Health and all of our gracious community partners.

**Antibody Gene Therapy for Rabies Encephalitis**

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**Introduction.** Rabies virus is one of the most crippling neurotrophic viruses with almost 100% fatality rate worldwide. Vaccination strategies that are currently used to prevent rabies infection primarily rely on the generation of the host serum neutralizing antibodies against rabies virus. The major caveat of serum neutralizing antibodies-based therapy is that while the rabies virus can easily breach blood-brain barrier, the antibodies cannot, making them largely ineffective in the treatment of symptomatic brain encephalitis. There is a need for antibody-induced protection in the central nervous system, independent of the host immune system. We propose to use the recombinant AAV9 (rAAV9) viral vector to traverse the blood-brain barrier and express neutralizing human immunoglobulin against rabies glycoprotein in the brain.

**Methods.** A rAAV9 vector expressing a broadly neutralizing human antibody against rabies (CR57) was used to treat C57BL/6J mice intravenously for dose escalation study ( $1 \times 10^{10}$  to  $1 \times 10^{13}$  vg/kg) and longitudinal study ( $6 \times 10^{13}$  vg/kg dose and monitored for 60 days). We analyzed the expression of human CR57 in blood samples and brain tissue by Rapid Fluorescent Focus Inhibition Test (RFFIT), ELISA, and IHC. Additionally, 4-month-old cats were treated with low ( $2 \times 10^{12}$  vg/kg) or high ( $1 \times 10^{13}$  vg/kg) doses of rAAV9 intravenously and tissues were further analyzed.

**Results.** The rabies neutralizing antibody level in mouse serum was >200 times higher than the protective titer suggested by the World Health Organization (WHO; 0.5 IU/ml) 1-3 months after the administration of  $1 \times 10^{12}$ ,  $1 \times 10^{13}$  and  $6 \times 10^{13}$  vg/kg rAAV9 vector. The expression of rabies neutralizing antibodies was further confirmed in neurons and brain endothelial cells by immunohistochemistry (IHC). The level of CR57 was persistent in mouse serum for 60 days post-injection. However, neutralizing antibodies were absent in the lowest dose treatment group ( $1 \times 10^{10}$ vg/kg). In rAAV9-treated cats, the high-dose treatment group showed significant levels of rabies neutralizing antibodies in serum for at least 15 months and in CSF for 3 months post-injection. Antibodies were also observed in cat serum of the low-dose cohort for 15 months after treatment. The decline in rabies neutralizing antibodies in feline serum of both high and low-dose treatment groups after 15-months is attributed to the development of cat immune response against human CR57. Nonetheless, rabies neutralizing antibodies were detected in the feline brain of the high-dose treatment group at endpoint (24-months post-treatment) by IHC.

**Conclusions.** An AAV9 vector injected intravenously in two species expresses broadly neutralizing antibodies in brain cells and thereby may provide protection against, or treatment of, viral encephalitis.

**Acknowledgments.** Studies were supported by the Scott-Ritchey Research Center and the Intramural Grants Program of Auburn University. We gratefully acknowledge the expertise of Dr. Chengming Wang and Terri Wood for RFFIT data.



## Faculty/Postdoctoral/Staff Platform Presentations

### Comparative Evaluation of GS-441524, Teriflunomide, Ruxolitinib, Molnupiravir, Ritonavir, and Nirmatrelvir for In Vitro Antiviral Activity against Feline Infectious Peritonitis Virus

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**Introduction.** Feline infectious peritonitis (FIP), caused by feline coronavirus (FCoV), is considered one of the most enigmatic diseases in cats. Developing effective drugs for FIP is crucial due to its global prevalence and severity. However, limited data is available for the antiviral efficacy and toxicity of antiviral drugs against FIPV. In this study, six drugs were tested for their cytotoxicity, cell viability, and antiviral efficacies in Crandell-Reese feline kidney (CRFK) cells.

**Methods.** Six drugs (GS-441524, teriflunomide, ruxolitinib, molnupiravir, ritonavir, and nirmatrelvir) used in this study were purchased from commercially available sources. The cytotoxicity and cell viability of these drugs were examined in CRFK cells using commercially available CellTox Green Cytotoxicity Assay Kit and Cell Proliferation Kit I (MTT) respectively. Finally, antiviral efficacies of six drugs were evaluated using quantification of FIPV by qRT-PCR in CRFK treated with six drugs.

**Results.** A cytotoxicity assay demonstrated that these drugs were safe to be used with essentially no cytotoxicity with concentrations as high as 250  $\mu$ M for ruxolitinib; 125  $\mu$ M for GS441524; 63  $\mu$ M for teriflunomide, molnupiravir, and nirmatrelvir; and 16  $\mu$ M for ritonavir. GS441524 and nirmatrelvir exhibited the least detrimental effects on the CRFK cells, with 50% cytotoxic concentration (CC50) values of 260.0  $\mu$ M and 279.1  $\mu$ M, respectively, while ritonavir showed high toxicity (CC50 = 39.9  $\mu$ M) in CRFK cells. In the dose-response analysis, GS441524, nirmatrelvir, and molnupiravir demonstrated promising results with the selectivity index values of 165.54, 113.67, and 29.27, respectively, against FIPV.

**Conclusions.** Our study suggests that nirmatrelvir and molnupiravir hold potential for FIPV treatment and could serve as alternatives to GS441524. Continued research and development of antiviral drugs are essential to ensure the well-being of companion animals and improve our preparedness for future outbreaks of coronaviruses affecting animals and humans alike.

**Safety of Study of Leucoreduced Allogeneic Freeze-Dried Pooled Platelet-Rich Plasma (PrecisePRP™ Equine) in Normal Equine Subjects**

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**Introduction.** Osteoarthritis (OA) is one of the most significant causes of chronic pain and disability of both human and equids. Clinical use of autologous blood-derived intra-articular therapies as autologous platelet-rich plasma has increased due to their proposed disease-modifying effects. Lack of uniformity and standard characterization of autologous PRP have left concerns in previously published studies for varied outcomes and a need for a product that may provide more standardized study conditions. A freeze-dried, pooled PRP product has been developed for equine use that may help provide a standardized product. Compare the safety of intra-articular injection of leucoreduced pooled allogeneic equine PRP to a placebo control. in a masked, randomized two period, two treatment, two joint cross-over study. We hypothesized that intra-articular injection of leucoreduced pooled allogeneic equine PRP will not cause significant adverse reactions compared to a placebo control.

**Methods.** A masked, randomized two period, two treatment, two joint cross-over study. The treatment and joint treatment sequence (Radiocarpal RCJ or Tarsocrural TCJ) were randomized for each subject. Twelve healthy adult horses with naturally occurring lameness, but free of radiographic abnormalities in joints selected for treatment were included in the study. Horses were treated with either saline placebo or leucoreduced allogeneic freeze-dried pooled platelet rich plasma, LR-fdPRP. Horses were evaluated by physical examination every 12-24 hours, CBC/Chemistry, and synovial fluid analysis and cytology before study start then every 7 days during the treatment period. Joints were evaluated for heat, effusion, and circumference daily. Lameness was subjectively and objectively evaluated daily. Synovial fluid was evaluated by standard ELISA for IL-1 $\beta$ , TNF $\alpha$ , and IL-1RAP. Data was analyzed using generalized linear mixed models.

**Results.** No significant differences were observed for physical exam parameters (HR, RR, Temperature). One horse in the control group experienced colic that resolved medically. No significant differences were observed for blood parameters contained in the cbc and chemistry analyses. For joint evaluations, there was a trend for higher scores for 6-12 hours post injection, but no significant differences between the groups. There was a trend for greater joint swelling in the first 6-12 hours post injection with the control group experiencing this with the first injection and the LR-fdPRP group after the second injection. There were no significant difference in joint circumference or scoring for passive flexion. For lameness, the score worsened in the control group but improved in the LR-fdPRP group following treatment. No difference in synovial fluid analyses were observed. ELISA analysis of synovial fluid is pending.

**Conclusions.** Administration of a pooled leucoreduced allogeneic PRP product did not elicit systemic or local inflammatory response and appears safe for clinical use.

**Acknowledgements.** Funding for this study provided by VetStem Cell Therapy. The authors would like to thank Ms. Jessica Brown for her technical assistance during this project.



## Comparison of "Blind" and Ultrasound-Guided Sciatic Nerve Injection Techniques in Laboratory Rat Cadavers

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**Introduction.** The increasing use of ultrasound-guided nerve techniques in regional anesthesia research has gained popularity. Given that laboratory rats are commonly used as a model for pharmacological studies, and sciatic nerve injections have traditionally relied on "blind" techniques using anatomical landmarks, this study aims to compare the success rate and extent of sciatic nerve staining achieved with a bupivacaine dye solution using two different approaches: the conventional "blind" approach and the ultrasound-guided technique in cadaveric rats.

**Methods.** In this prospective, randomized experimental study, 24 Wistar rat cadavers were utilized, each with an approximate weight of  $349 \pm 31.05$  g. The sciatic nerves from these cadavers were randomly assigned to one of two groups: the "blind" approach (Group B) or the ultrasound-guided technique (Group US). A uniform 0.1 mL injection of bupivacaine dye solution was administered to each nerve. Subsequently, a comprehensive gross anatomical dissection was conducted to observe and categorize nerve staining as either "positive" or "negative." Additionally, the study involved measurements of the length of nerve staining and visual inspection for potential nerve damage. To compare the presence or absence of nerve staining, Fisher's exact test was applied, and the length of staining between the two groups was compared using the Wilcoxon Signed Rank test. All statistical comparisons adhered to a significance level of  $p < 0.05$ .

**Results.** Group B, bupivacaine dye solution successfully stained 16 out of 24 sciatic nerves, resulting in a success rate of 67%. In contrast, all 24 sciatic nerves in Group US exhibited successful staining, achieving a 100% success rate, which was statistically significant ( $p < 0.0039$ ). Furthermore, the length of nerve staining in Group B had a median of 2 mm (IQR, 2 to 3 mm). In comparison, Group US displayed significantly longer staining, with a median length of 5 mm (IQR, 4 to 6 mm) ( $p < 0.0001$ ). It's worth noting that an outlier was observed in Group B, with one sciatic nerve displaying a dye solution spread over 16 mm, suggesting a potential intraneural injection. Importantly, no visual indications of laceration or nerve damage were observed in either group.

**Conclusion.** The "blind" approach for sciatic nerve injection, when compared to the ultrasound-guided technique, exhibited a lower success rate and inferior dye solution distribution. Therefore, whenever feasible, it is recommended to opt for ultrasound guidance over the "blind" approach for sciatic nerve injection in rats.

**Acknowledgments.** Our sincere gratitude extends to Pat Rynders and Bettina Schemera, veterinarian/director, and associate director of laboratory animal health at Auburn University College of Veterinary Medicine, for their invaluable assistance and coordination in facilitating the provision of rat cadavers for this study. We also wish to express our thanks to Trisha Kendall, the manager of laboratory animal health, for her indispensable support in managing the disposal of rats as biological waste.

**A Vaccine to Mitigate Vampire Bat Predation and Rabies Transmission**

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**Introduction.** Vampire bats are obligate blood feeding mammals abundant in the Americas, ranging from South Texas to Argentina. Vampire bat predation causes major losses in livestock production efficiency in countries that produce 30% of the world's protein of animal origin. Millions of domestic livestock, humans, and wild animals are exposed to rabies and other zoonoses by bat predation. Vampire bats feed by creating wounds that bleed continuously caused by anticoagulants present in the bat's saliva. Cattle repeatedly exposed to vampire bats' saliva develop resistance to anti-coagulation. The goal of this study is to determine if vaccines containing purified vampire bat salivary anticoagulant proteins generate specific antibodies and disrupt vampire bat feeding.

**Methods.** Molecularly defined vampire bat salivary anticoagulants Draculin, a factor X inhibitor, and DSPAα1, a plasminogen activator were synthesized as antigens in vaccines combined with CpG molecular adjuvant and Emulsigen D™. Weanling beef calves were assigned randomly to groups immunized intramuscularly with Draculin (n=3) or DSPAα1 (n=3) antigens, or a vaccine without antigen (n=2). The initial vaccination was followed by two booster doses 21 days apart. Draculin and DSPAα1 antibody titers were measured by ELISA. Antibody neutralization of anticoagulation was assayed with chromogenic substrates and clot-based assays.

**Results.** Preliminary data demonstrated that the Draculin vaccine generated elevated neutralizing antibody titers, while DSPAα1 is less antigenic. Draculin generated an antibody response after 2 doses of the vaccine, while DSPAα1 required 3 doses.

**Conclusions.** Anti-bat salivary vaccines have the potential to disrupt vampire bat feeding, decrease zoonotic disease transmission and increase productivity of cattle living in areas burdened by vampire bat predation.

**Acknowledgments.** This research was funded by Auburn University's College of Veterinary Medicine Animal Health and Disease Research funds and the Scott-Ritchey Research Center.

**Quantitative analysis of Perampanel in feline plasma by High Performance Liquid Chromatography (HPLC) and UV detection.**

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**Introduction.** Perampanel is an anti-seizure medication that can be used in humans to treat seizures. It is thought to function by non-competitively antagonizing AMPA-glutamate receptors in the brain. This study describes the development and validation of a specific, reversed-phase high performance liquid chromatographic (HPLC) method with UV detection for the identification and quantification of perampanel in feline plasma.

**Methods.** Previously reported perampanel studies describing chromatographic conditions, physical properties of the drug, sample types, sample size, sample preparation, and expected level of accuracy and precision were considered to develop this HPLC method with UV detection. Various different methods to develop the optimal chromatographic conditions for identification and quantification of perampanel were tested. Different chromatographic columns (Luna C18, Sunfire C18) and mobile phases (50 mM potassium dihydrogen phosphate with 0.1% acetic acid: acetonitrile and water with 0.1% 85% phosphoric acid: acetonitrile) with different ratios were tested. The pH (4.0, 2.3), flow rate (0.8 mL/min, 1.5 mL/min), column temperature (40° C, 50° C), and UV detection (254 nm, 300 nm, 320 nm) were also tested. For sample preparation, three different methods were tested. Protein precipitation and solid-phase extraction (SPE) with two different cartridges (Oasis HLB, Oasis Prime HLB) were compared. With optimal conditions established, the next step was to validate the method to get robust quantification in terms of accuracy and precision.

**Results.** Feline plasma samples were analyzed for perampanel concentrations by HPLC using a reversed-phase Sunfire C18, 5 $\mu$ m, 4.6 x 150 mm column at 50° C. The mobile phase was water with 0.1% 85% phosphoric acid pH 2.3 and acetonitrile with a flow rate of 1.5 mL/min and UV detection at 300 nm. SPE with Oasis HLB cartridges was used to extract the perampanel and remove the matrix, followed by drying under a gentle stream of nitrogen, and reconstitution of the residue with mobile phase. The injection volume was 50  $\mu$ L. The linear coefficient was 0.99. The lower limit of detection (LOD) for perampanel in feline plasma samples was 10 ng/mL. The lower limit of quantification (LOQ) for perampanel in feline plasma samples was 20 ng/mL. The precision (CV %) for perampanel in feline plasma samples at 20, 30, 50, 100, and 300 ng/mL was 14.18%, 8.27%, 6.03%, 4.91%, and 2.66%, respectively. The accuracy (% recovery) for perampanel in feline plasma samples at 20, 30, 50, 100, and 300 ng/mL was 112.54%, 108.67%, 101.57%, 100.59%, and 101.70%, respectively.

**Conclusions.** An analytical method by reversed-phase HPLC with UV detection for perampanel in feline plasma was developed and validated. This assay will be used to accurately quantify perampanel in a pharmacokinetic study in feline plasma and may be used for future research projects and/or therapeutic drug monitoring applications.

**Acknowledgments.** Department of Anatomy, Physiology, and Pharmacology.



## Undergraduate Student Poster Presentations

### Characterizing Pain in DRGs

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**Introduction.** Pain is a complex problem affecting millions of people worldwide. The dorsal root ganglia (DRGs) are a cluster of cells found within the dorsal root of the spine. DRGs are responsible for transmission of sensory messages such as thermoreceptors, proprioceptors, chemoreceptors, and nociceptors. The nociceptors are the first neurons in the pain pathway and have been shown to contribute to chronic pain disorders. The current therapies to reduce pain are limited as many treatment options inadequately address the causes of pain and are addictive. Nuclear receptors are a family of transcription factors that control gene expression for multipole biological processes. Our lab has previously shown that nuclear receptor REV-ERB agonism alleviates inflammatory pain in vivo. The aim of this project is to explore the potential of REV-ERB agonist STL1267 in treating pain, and to develop in-vitro systems for understanding its mechanism of action in DRGs.

**Methods.** Three male 6-8 weeks old mice were either dosed with 50mg/kg STL 1267 or vehicle (DMSO) intraperitoneally. The mice were euthanized per IACUC guidelines using deep anesthesia. The mice were then transcardially perfused with 10ml PBS. A dorsal laminectomy was performed. The spinal cord was accessed via the usage of Friedman-Pearson Rongeur and spring scissors. The DRGs were carefully dissected and put into 1ml of 1x HBSS. A TRIZOL extraction was run to make RNA, and cDNA was synthesized using BioRad cDNA synthesis kit according to manufacturer's instructions. QPCR was performed using SYBR green. DRG culture - The DRGs were homogenized with a Dounce homogenizer and transferred into a 1.5 ml tube. The homogenizer was rinsed with 500 microliters of HBSS. Spun the homogenized DRGs at 1000 rpm for 25 minutes and had pellet formation. The pellet was resuspended in 800 microliters of the culture media and plated in 2 laminin coated wells of a six well plate. There were 625 microliters of cells and 2ml of culture media in each well. After five days of cell growth, the old media was aspirated off and 2ml of neurobasal media was added to each well. Papain was diluted in PBS (99 microliters PBS to 1 microliter of papain). 0.5 microliters of the papain dilution were placed into one well. After incubating, the media was pipetted into labeled tubes and froze at -80°C. A substance P ELISA was then run.

**Results.** We show here an optimized protocol for the isolation and culture of DRGs from mice. We confirmed the viability of the ex vivo system by characterizing its ability to secrete substance P, which is a neuropeptide that transmits pain signals. We showed that papain treatment induced the secretion of substance P in these DRGs as validated by ELISA. We also show in vivo that upon dosing with nuclear receptor REV-ERB agonist STL 1267, DRGs have reduced inflammatory and pain pathway gene expression as validated by qPCR.

**Conclusions.** STL1267 treatment in-vivo is able to reduce inflammatory and pain signatures in DRGs, suggesting its potential use as an anti-pain therapeutic. We also define a novel optimized method for DRG isolation and culture and characterize it. Allowing for its use in functional studies of sensory neurons, and to explore the mechanism of action of various therapeutics.

**Acknowledgments.** This project was supported by NIH 1R01NS126204.



**Comprehensive immunohistochemical analysis of various immune checkpoint molecules in canine cancers**

Hannah Eady, Jonathan Marable, Alana Kramer, Damien Ruiz, Payal Agarwal, Bruce F. Smith, Maninder Sandey

Cancer is a leading cause of death in dogs over ten years of age. Although current treatments such as surgery, chemotherapy, and radiation therapy are effective in early-stage cancers, they tend to be ineffective in advanced-stage diseases. However, recent advances in immunotherapy have revolutionized the treatment of human cancer patients. The use of monoclonal antibodies to block immune checkpoint pathways (CTLA4 and PD1) has become the standard of care for certain tumor types, including advanced melanoma. Tumor cells often exploit immune checkpoint pathways, such as cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed cell death-1 (PD-1), to evade the immune system and promote their own growth. There have been few studies on the expression of these immune checkpoints in canine cancers. Our study aims to use canine-specific nanobodies to detect PD1, PD-L1, and CTLA4 expression in various types of canine cancers, including oral melanoma, osteosarcoma, glioma, and hemangiosarcoma. The nucleotide sequences for anti-PD1 and anti-PD-L1 nanobodies were cloned into the periplasmic expression vector pET22b (+) containing the C-terminal Strep II Tag and 6 X histidine tag. The anti-CTLA4 Nbs were expressed in *E. coli* (DE3) and purified from the periplasmic fraction by affinity chromatography on AKTA explorer. Our purified nanobodies specifically bind to cells expressing canine PD1 and PD-L1. Our next goal is to perform flow cytometry and immunohistochemistry analysis to examine the expression of CTLA4, PD1, and PD-L1 on both tumor-infiltrating lymphocytes and tumor cells in various canine cancers.

**Acknowledgments.** This study was supported by research funding from the National Cancer Institute (NCI-NIH). We would also like to thank Rie Watanabe for flow cytometry support.

**Development and characterization of CAR T cells against B7-H3 surface antigen**

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**Introduction.** Osteosarcoma (OS) is a malignant bone tumor arising in the long bones of children and adolescents and is the third most frequent bone tumor in adults in the United States. OS is characterized by a high tendency for pulmonary metastasis and poor prognosis. OS is responsible for 3-5% of pediatric cancer cases; incidence rates have risen by 0.4% per year over the last decade. Current treatment regimens consist of preoperative neoadjuvant chemotherapy and surgical resection. Over the last 20 years, the survival rates of OS are unchanged, and there are still no therapies targeted explicitly to OS patients. We need novel treatment options and more extensive research to treat all solid tumors, especially osteosarcoma, which offers attractive targets for immunotherapy. Cancer immunotherapy is a treatment modality that stimulates innate and adaptive immunity against tumors in the tumor microenvironment (TME). Chimeric antigen receptor (CAR) T cell therapy is the **reinforcement** of cytotoxic T cells against cancer cells. CAR T cells are designed to express recombinant receptors against specific tumor cell surface antigens to promote T cell-mediated cancer cell death without limiting MHC binding. There is a lack of research on CAR T cell therapy in solid tumors. This begs the question: can CAR T cells be developed to bind to osteosarcoma cells and kill solid tumors? We are developing GFP/ffluc<sup>+</sup> and B7H3 knockout osteosarcoma cell lines to assess the CAR T cells' effects on osteosarcoma cell lysis.

**Methods.** Canine osteosarcoma cell line D17 was modified to express GFP and ffluc (firefly luciferase). Cells were grown and infected with lentivirus encoding GFP and luciferase. After one week of lentivirus infection, single cells were sorted based on GFP expression in 8, 96 well plates. The clonal growth of cells from each cell was monitored using the Keyence bz-x800 imaging system. The most proliferous colonies were transferred to 6 wells plates, and eventually, the best two colonies were selected for luciferase expression analysis. The selected clone cells were grown in 96 wells plates (flat bottom) and analyzed for luciferase expression using Steady-Glo Luciferase assay. Non-modified parental D17 cells were used as negative. In the future, we will knockout the B7H3 gene using CRISPR/Cas9 digestion in cells.

**Results.** We have successfully modified D22 cells to express GFP and ffluc genes. We expect to be able to knock out the B7-H3 receptor in the near future.

**Conclusion.** To quantify CAR T cells induced cell lysis, we now have a better in vitro system. GFP and ffluc expression will be very helpful in quantifying the cell lysis and reduction of tumor spheroids post CAR-T treatment.

**Acknowledgments.** The authors would like to acknowledge Isabella Shimko-Lofano, Dr. Rie Watanabe, Dr. Emily Graff, Dr. Maninder Sandey, Dr. Rimas Orentas, and Dr. Bruce F. Smith for collegial support. This project is funded by the Scott Ritchey Research Center, CVM-ORGS, and NIH R15.

**Alzheimer's Disease Histopathological Phenotype in Feline GM1 and GM2 Gangliosidosis**

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**Introduction.** Alzheimer's Disease (AD) is a degenerative brain disorder and the most common form of dementia. The presence of amyloid-beta ( $A\beta$ ) plaques, neurofibrillary tangles (NFTs), and loss of neuronal connections are hallmarks of this disease. While current therapies may reduce symptoms of AD, they do not slow AD progression, and the overwhelming majority of new, potential therapies fail in clinical trials. Major roadblocks for development of new, effective therapeutics for the treatment of AD have been the lack of translatable animal models and our incomplete understanding of the pathological onset and progression of the disease. In recent years, much evidence has shown that aged cats may represent a natural, sporadic model for the study of AD. Additionally, there is a growing body of literature showing a link between gangliosides and the development of  $A\beta$  plaques and NFTs in certain cases of AD. We hypothesize that gangliosidosis-affected cats are a novel model for studying progression of AD pathogenesis and for testing current and new AD therapies. Therefore, in the present study, we examine feline models of both GM1 and GM2 gangliosidosis for the presence of  $A\beta$  plaques and NFTs.

**Methods.** We examined four different brain regions (corona radiata, parietal cortex, temporal cortex and thalamus) using immunohistochemistry (IHC) in gangliosidosis-affected cats as well as age-matched controls for the presence and subtypes of  $A\beta$  plaques and NFTs. Brain tissue from cats aged 2-8 months for GM1 cats, 0.5-4 months for GM2 cats and aged-matched controls was examined in this study. Whole brain images were scanned at 20X using the Olympus Evident VS200 slide scanner.

**Results.** Results show both age-related and region-specific changes in the presence, number and subtype of  $A\beta$  plaques as well as changes in the expression pattern of phosphorylated tau (pTau-Ser422). Results also suggest that changes in  $A\beta$  accumulation and pTau expression in gangliosidosis cats correlate spatially and temporally with AD progression in human patients. Changes appear earlier in regions affected during early stages of AD, and increase in frequency during the later stages of gangliosidosis in a manner comparable to later stages of AD. In addition, the progression of  $A\beta$  plaque subtypes throughout the brain of gangliosidosis-affected cats may model the same pattern of progression in AD.

**Conclusions.** Overall, we show that gangliosidosis-affected cats could be a novel model for the study of AD pathogenesis as they present with the hallmarks of AD,  $A\beta$  plaques and NFTs. We assert that GM1 and GM2 cats could represent a significant advancement for studying disease progression of an AD-like phenotype in a naturally-occurring, sporadic animal model. In future studies, we will compare  $A\beta$  and pTau expression patterns in gangliosidosis-affected cats to that of aged-cats, a documented model of human AD.

**Acknowledgments.** We acknowledge financial support from the Scott-Ritchey Research Center.

**AAV-mediated Anti-hormone Antibody Therapy as a Treatment for Alzheimer's Disease**

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**Introduction.** Alzheimer's disease (AD) is the most common form of dementia and is marked by abnormal accumulation of beta-amyloid proteins and the hyperphosphorylation of tau proteins that together lead to neuronal degeneration. It is estimated that by 2025, 7.2 million people aged 65 and older will have AD, an 11% increase from the 6.5 million people affected in 2022. Additionally, almost two-thirds of Americans with AD are women, and evidence suggests that menopause is a clear driver for AD development. During this period, hormone levels change due to lack of estrogen, and previous studies have implicated altered hormone levels as a potential factor for AD development. Thus, we hypothesize that altered hormone levels may contribute to the neuropathology and memory loss associated with AD.

**Methods.** We used an innovative adeno-associated virus (AAV)-mediated anti-hormone antibody treatment in APP/PS1 hemizygous mice. APP/PS1 mice express a chimeric mouse/human amyloid precursor protein (APP) (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9), and are commonly used for AD studies as they exhibit memory loss and develop neuropathological hallmarks of AD. To test the biological efficacy of our anti-hormone antibody treatment, we examined changes in estrous cyclicity and serum hormone levels. In addition, we examined an innate, olfactory-mediated memory and learning behavior in mice known as the social transmission of food preference (STFP), which is known to deteriorate with neurodegeneration.

**Results.** Here, we report that treatment with our novel anti-hormone antibodies significantly disrupts estrous cyclicity, specifically leading to an increased time spent in estrus. Mice also show alterations in serum hormone levels following treatment as compared to control mice. Additionally, treated APP/PS1 hemizygous mice appear to show improvement in learning and memory using a food choice test that measures STFP.

**Conclusions.** Overall, AAV-mediated anti-hormone antibody treatments appear to not only disrupt estrous cyclicity, likely due to alterations in hormone levels, but also restore learning and memory performance during the STFP. Ongoing studies will examine changes in other memory and learning behaviors to determine which anti-hormone antibody treatment provides the most benefit for slowing and/or preventing AD progression.

**Acknowledgments.** We acknowledge financial support from the Center for One Health Research Seed Grant Program through the Edward Via College of Osteopathic Medicine and Auburn University College of Veterinary Medicine as well as funds from the Scott-Ritchey Research Center.



## Assessing the Dosage Effect of AAV Gene Therapy in Intravenous Treatment of Feline GM1 Gangliosidosis

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**Introduction.** GM1 gangliosidosis is a neurodegenerative disease and a lysosomal storage disorder, which is caused by the lack of  $\beta$ -galactosidase ( $\beta$ -gal). A lack of  $\beta$ -gal leads to the collection of GM1 ganglioside, which leads mainly to defects in the brain. However, both feline and human patients suffer from profound peripheral disease. Felines were used for this study because of their similarities to human disorders. Adeno-associated viral (AAV) gene therapy restores  $\beta$ -gal, which leads to the breakdown of GM1 ganglioside. Treatments were administered intravenously at high and low dosages to GM1 cats to effectively treat the brain as well as the peripheral organs. The goal of this study is to compare the effectiveness of high and low dosage treatments in peripheral organs.

**Methods.** One cohort received a high dosage IV treatment of  $6 \times 10^{13}$  vg/kg. Of four cats treated in this cohort for long-term follow-up, two remain alive. A fifth cat was followed to the short-term time point of sixteen weeks after treatment. A second cohort received a low dosage IV treatment of  $1.5 \times 10^{13}$  vg/kg with three animals following long-term (humane endpoint) and four animals following short-term. Both cohorts were treated at  $1.71 \pm 0.4$  months of age. Clinical assessments were performed, which included neurological exams. Postmortem assessments included vector distribution via qPCR and enzyme distribution via specific activity assays.

**Results.** Untreated GM1 cats survive approximately  $8.0 \pm 0.6$  months, while the treatment increased the lifespan to  $27.3 \pm 4.4$  months in the high dosage cohort, with two animals ongoing at 29.3 and 30.1 months of age. The low dose cohort survived to  $32.6 \pm 13.5$  months of age. Additionally, all treated animals had an increase in quality of life, as determined by neurological assessment. The specific activity of  $\beta$ -gal increased in the treated cats, and in some cases, the increase in activity was greater in the cohort that received the high dosage treatment than the low dosage treatment. Surprisingly, there was more enzyme activity increase in some peripheral organs in the low dose cohort than in the high dose cohort. In the high-dose cohort, the average  $\beta$ -gal activity for liver, pancreas, heart, skeletal muscle, and spleen was  $0.2 \pm 0.1$ ,  $0.9 \pm 1.0$ ,  $6.0 \pm 0.9$ ,  $4.3 \pm 0.7$ , and  $0.05 \pm 0.06$  fold normal, respectively. In the low-dose cohort, the average  $\beta$ -gal activity for liver, pancreas, heart, skeletal muscle, and spleen is  $0.9 \pm 0.4$ ,  $0.5 \pm 0.7$ ,  $13.7 \pm 10.3$ ,  $3.3 \pm 3.6$ , and  $1.1 \pm 0.3$  fold normal, respectively.

**Conclusions.** This study demonstrated the effectiveness of IV treatment of GM1 gangliosidosis with AAV gene therapy. AAV gene therapy was effective in restoring  $\beta$ -gal in both dosages, however, the two dosage cohorts improved enzyme activity levels differently in the peripheral organs. The high dose treatment would increase activity more than the low dose, and in others, the low dose treatment was more beneficial to raising activity levels. With two animals in the high dose cohort ongoing, it remains to be determined which dose will prove most effective in ameliorating symptoms and extending lifespan.

**Acknowledgements.** This study was funded by Porter's Fund, private donations, and the Scott-Ritchey Research Center.

**Ultrasound-induced Sleep in Mice**

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**Introduction.** Although researchers have not come to a consensus on the function(s) of sleep, there is strong evidence that sleep is necessary for many physiological and cognitive processes including learning and memory. Previously, sleep enhancement in mice was either performed with pharmacological agents or required the use of transgenic mice (expressing the protein cre-recombinase in certain neuronal populations) and invasive techniques such as optogenetics or chemogenetics to activate the neurons generating sleep. To facilitate the translational application of this research to humans, we utilized focused ultrasound (fUS) to non-invasively activate neurons in the nucleus accumbens (NAc) which has been shown to be one of the centers involved in sleep modulation.

**Methods.** In an initial step, we sought to characterize the dimensions of the focal point of the ultrasound waves to calibrate the fUS transducer. We used chicken meat and high amplitude stimulation parameters for this step to induce a lesion in the tissue. Once calibrated for the correct distance to target, we used lower intensity fUS parameters to activate the NAc in anesthetized mice. Brains of mice with fUS stimulation were harvested and for immunohistochemistry analysis and stained for cFos, a protein indicating recent neuronal activation. We compared the amounts of cFos found on the ipsilateral fUS stimulation hemisphere with the contralateral control side for each mouse using a counting box over the NAc area.

**Results.** cFos staining revealed a greater number of cFos positive neurons on the fUS stimulated side of the brain compared to the contralateral control hemisphere, suggesting that fUS can indeed be used to non-invasively activate neurons located in the NAc.

**Conclusions.** The current experiments produced proof of concept data showing that neurons in the NAc can be stimulated non-invasively using focused ultrasound. However, in future experiments it will be important to selectively stimulate only sleep-activating neurons in the NAc, as opposed to the current non-discriminant stimulation affecting all cells within the area. To this end, we will employ calcium imaging of specific neuronal populations in the NAc via fiber-photometry in transgenic mice. Once we have determined the precise fUS parameters to effectively activate the desired neuronal populations in anesthetized mice, we will implant mice with fUS transducers for awake animal fUS stimulation. The goal is to enhance sleep non-invasively in free-roaming mice. Finally, we will assess their performance in the novel object recognition (NOR) test to determine the relationship between sleep enhancement and memory performance. We hypothesize that mice who receive fUS stimulation of the NAc will sleep better and will perform better on the NOR test than unstimulated control mice.

**Designing a Powerful Bispecific Fusion Protein for PD1/PD-L1 Blockade and OX40 Agonism**

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The difficulty of providing effective treatment for cancer makes it a leading cause of death in the United States. With the harmful effects of chemotherapy, the medical field has been exploring novel therapeutics to combat cancer, such as immunotherapy. Immunotherapy utilizes the body's immune system to target and kill cancer cells. Immune checkpoint receptors, such as the PD1/PD-L1 pathway, prevent overactivation of the immune system to avoid damage to healthy body cells. However, cancer cells can overexpress the PD-L1 receptor, thus hijacking the PD-1/PD-L1 pathway to prevent destruction by CD8<sup>+</sup> T lymphocytes. Immune checkpoint blockade therapy using antagonistic monoclonal antibodies targeting PD-1/PD-L1 receptors can overcome this tumor cell-mediated immunosuppression. However, the problem with this method of cancer therapy is that its effectiveness is too inconsistent between different patients. Only 15-20% of the human cancer patients respond to PD-1/PD-L1 monotherapies. The therapeutic efficacy of the PD-1/PD-L1 mAbs can be enhanced by combination immunotherapy with an OX40 agonist. When activated, OX40 signaling promotes activation and survival of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, thus generating a robust anti-tumor immune response. Our lab has already synthesized a bispecific molecule, aPD1-Fc-OX40L, which combines PD-1 blockade with OX40 activation into a single therapeutic. This project aims to find the most efficient version of the aPD1-Fc-OX40L molecule, which elicits the most potent PD-1/PD-L1 blockade and causes OX40 activation. To achieve this, we synthesized, cloned, and expressed six versions of aPD1-Fc-OX40L protein that contain different clones of anti-PD1 nanobodies. We also synthesized, cloned, and expressed seven versions of aPD-L1-Fc-OX40L protein containing different clones of anti-PD-L1 nanobodies. The gene sequences coding for various aPD1-Fc-OX40L or aPD-L1-Fc-OX40L proteins were cloned into pCDNA3.4 Topo vector and transfected into Expi293-F cells. The transfected cells were then plated in the presence of geneticin to select the colonies that successfully integrated the plasmid. Colonies were then chosen from these plates and expanded to grow in suspension culture to purify recombinant proteins. The conditioned media was collected from these cultures, and protein was purified using affinity chromatography with HiTrap Protein G column. Once purified, the proteins were run on an SDS-PAGE to test for the purity of the bispecific molecule. Once production was confirmed on all the clones, flow cytometry was run to test the binding of aPD-1/aPD-L1 and OX40L to their respective receptors on hPD-1/hPD-L1 and OX40 effector cells. In future studies, we will purify the remaining aPD1-Fc-OX40L and aPD-L1-Fc-Ox40L proteins. We will compare the ability of these various bispecific proteins to block the PD-1/PD-L1 pathway in a PD1/PD-L1 blockade Bioassay.

**Acknowledgments.** This study was supported by research funding from the National Cancer Institute (NCI-NIH). We would also like to thank Rie Watanabe for flow cytometry support.

**Optimizing the PCR for the STK11 and MSK1 genes in canine mammary cancer**

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**Introduction.** MSK1 and STK11 are key regulatory genes in the signal transduction cascade controlling cell proliferation in cancer cells. The open reading frames of the genes encoding MSK1 and STK 11 expressed in mammary cancers of domestic dogs, *Canis lupus familiaris*, were analyzed to find highly conserved sequences for designing an rtPCR reaction that can specifically amplify the targeted region of each of the coding sequences, as well as using gel electrophoresis to verify the results.

**Methods.** To optimize the PCR protocol for amplifying the targeted sequences, first, rtPCR primers were created by using the genomic sequences of the MSK1 and STK11 genes in *Canis lupus familiaris*. The canine genome was then analyzed to identify primer pairs also conserved in *Homo sapiens* that were within protein coding sequence and spanned an intron-exon junction. Once the successful primers were synthesized, they were utilized in an rtPCR reaction to find the targeted sequences. RNA from several independent canine mammary tumor cell lines was extracted after successful cell culturing and passing of the cells with no contamination and roughly 80% confluency. The rtPCR protocol was optimized by analyzing the amount of RNA and MgSO<sub>4</sub> added to each reaction, and then changing these amounts based on the results obtained when the bands were observed through non-denaturing agarose gel electrophoresis. Additionally, DMSO was added to the reaction to improve the specificity of the primers. The annealing temperatures of the primers were also adjusted accordingly by increasing incrementally by 0.5° C along with cycle number to optimize amplification.

**Results.** There are various parameters and factors that went into optimizing the rtPCR reaction. The successful cell culturing of the canine mammary tumor cell lines that would be used to extract RNA was done through careful passage and allowing the cells to go through a few passages before extracting the RNA at 80% confluency. This allowed us to obtain a relatively high concentration and purity of RNA to be used in rtPCR reactions. When the MgSO<sub>4</sub> concentration was changed from 1 mM to 1.5, this resulted in a brighter and sharper band when analyzed. The addition of DMSO, at just 1 µL allowed the primers to be more specific in this binding. The theoretical length determined from the sequence of the MSK1 gene is between 203-230 nucleotides and for STK11 is between 230-336 nucleotides in length. The gel electrophoresis results showed this length of nucleotides for each gene sequence.

**Conclusions.** This approach has produced a highly optimized rtPCR protocol that will allow specific amplification of the target sequences encoding MSK1 and STK11 and can then be used further for gel extraction and sequencing to verify the results.





### **Assess an orexin 2 receptor (OX2R) agonist's efficacy in alleviating the secondary symptoms of narcolepsy in orexin knock-out (OX-KO) mice**

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**Introduction.** Narcolepsy is a sleeping disorder resulting from the loss of orexin neurons in the lateral hypothalamus and the consequent lack of orexin signaling in the brain. Narcolepsy patients typically exhibit the following primary symptoms: severe sleepiness during the day, fragmented sleep during the night, hypnagogic hallucinations, and cataplexy (a sudden loss of skeletal muscle tone). The secondary symptoms include memory impairments, anxiety, and depression. Current therapies utilize stimulants and/or antidepressants to enhance wakefulness and to address the primary symptoms, but symptoms rarely fully resolve. Orexin knock-out (OX-KO) mice are exemplary because they lack the gene that encodes the protein prepro-orexin (a precursor molecule for the orexin peptides), resulting in a loss of orexin signaling and symptoms of narcolepsy. Previous research utilizing OX-KO mice demonstrated that an orexin agonist targeting the orexin 2 receptor (OX2R) could be used as an effective treatment for the primary symptoms of narcolepsy. However, thus far it was unclear whether this agonist would also address the secondary symptoms in narcolepsy. Here we used OX-KO mice treated with the agonist and assessed their performance in a battery of behavioral tests to investigate the effect of restored orexin signaling on cognition.

**Methods.** We implanted three groups of 12 OX-KO mice (and three groups of 12 wild-type control mice) with EEG/EMG leads (to monitor sleep/wake states). Following a two-week acclimation period, one group of each genotype received the OX2R agonist treatment, a vehicle treatment, or Modafinil (a current narcolepsy treatment). One hour after administration, we assessed performance in the novel object recognition test (memory), open field test (anxiety), and forced swim test (depression).

**Results.** Our results indicate that 1) modafinil-treated OX-KO and WT mice tend to show less anxiety-like behaviors in the open field test compared to mice treated with vehicle or the OX2R agonist. 2) Vehicle-treated OX-KO mice exhibit increased depression-like symptoms in the forced swim test. 3) Agonist treatment of OX-KO mice tends to increase short-term working memory as compared to vehicle treated OX-KO mice.

**Conclusions.** Our data is consistent with previous research indicating that modafinil treatment of Narcolepsy results in hyperactivity and reduced anxiety. Moreover, our results indicate that treatment of OX-KO mice with the new OX2R agonist tends to improve working memory, therefore indicating that this treatment can improve some of the secondary symptoms of Narcolepsy.

**Acknowledgments.** Auburn University Office of Undergraduate Research – Undergraduate Research Fellowship

**Development of Next-Generation Oncolytic Canine Adenovirus Type 2**

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**Introduction.** Conditionally replicative adenoviruses (CRAds) are promising cancer therapeutic tools due to selective replication in tumor cells, efficient gene delivery, robust transgene expression, broad tissue tropism, and their ability to induce a potent immune response against tumor cells. Our research team has previously created two oncolytic viruses: CAV2-AU-M1 and CAV2-AU-M2. CAV2-AU-M1 has the dsRed gene (red fluorescence protein) inserted into its genome under the control of the CMV promoter. CAV2-AU-M2 was developed by substituting the E3 gene to express a single-domain antibody (sdAb) against PD-1 under the control of a CMV promoter. CAV2-AU-M2 is an efficient oncolytic virus that infects and kills the cancer cells but does not secrete anti-PD1 sdAb outside the cells. The secretion of anti-PD1 sdAb is dependent on cell lysis. Therefore, we are developing a next-generation CAV2 with no dsRed and a secretory signal in the anti-PD1 sdAb expression cassette inserted between Fiber and E4. Meanwhile, we are also developing similar CAV2 oncolytic virus by replacing E3 with a secretory anti-PD1 sdAb expression cassette. Both oncolytic modifications are done using CRISPR/Cas9 digestion. In another unrelated project, we explore the stem cell markers (NANO-G, OCT-4, and SOX-2) mRNA expression in four osteosarcoma cell lines (CF11, MCKOS, D17, and D22)

**Methods.** We used the CRISPR/Cas9 digestion system to cut pICOCAV15 to insert the secretory anti-PD1 sdAb expression cassette. The secretory anti-PD1 sdAb expression cassette was amplified using high-fidelity Taq polymerase. Yeast homologous recombination was used for recombining cut plasmid and insert sequence. The recombined plasmid was transformed into 10-beta competent cells. PCR and gel electrophoresis were used to determine the size of the insert sequence and the correct location. PCR-amplified DNA was sent for sequencing to verify the sequence. RNA isolation and Quantitative PCR were used for STEM cell marker PCRs as well as sequencing for PCR verification

**Results.** We were successful in making one next-generation recombinant oncolytic virus plasmid. Endpoint PCR data and sequencing data from the new virus plasmid show that the secretory anti-PD1 sdAb expression cassette was successfully inserted and is in the correct place. We are currently in the stage of packaging the virus in packaging cell line Dkcre. Our PCR showed that markers for STEM Cells are expressed in all four cell lines CF11, D17, D22, and MCKOS at varying degrees

**Conclusions.** Upon the success of our next-generation CAV2 oncolytic virus with secretory anti-PD1 sdAb, we will be able to test the efficacy of oncolytic virus in the cell lysis of osteosarcoma cells *in vitro* and *in vivo*. Our virus will also be able to upregulate the anti-tumor immune response in tumor microenvironment (TME). The STEM cell markers expressed by the four osteosarcoma cell lines indicate that all our cell lines contain STEM cells. Based on the PCR data, we can test the cytotoxic effect of our oncolytic viruses on STEM cells. This will help us provide the efficacy of oncolytic viruses for potential cancer treatment and their potential to slow target metastasis.

**Acknowledgments.** Authors would like to acknowledge Aliaa Ismail, Dr. Maninder Sandey, Jonathan Marable, Terri Higgins, Luke Eller, Dr. Rebecca Nance, and Dr. Bruce F. Smith. Authors are thankful for generous financial support from AUCVM AHDR and NIH R15.



## **Veterinary Student Poster Presentations**

### **Effect of soy isoflavones on hormone secretion in the male gonad**

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**Introduction.** Soy isoflavones are a class of chemicals known as phytoestrogens which are present in common food items such as soybeans and other legumes, tofu products, and soy-based infant formula. Our two primary isoflavones of interest are genistein and daidzein. Preliminary research indicates that these estrogen-mimicking compounds interfere with male reproductive development and function by disrupting male testosterone and estradiol production. However, the specific sites of this interference in the male endocrine system have yet to be determined.

**Methods.** To identify the proteins affected by soy isoflavones, 21-day old Long-Evans rats were fed either a control casein diet, a control casein diet plus 300 ppm genistein and 200 ppm daidzein (G+D), or a control casein diet plus 600 ppm genistein and 400 ppm daidzein (2G+2D). Leydig cells were isolated upon sacrifice via the Percoll-gradient density method, and radioimmunoassays were performed to measure testosterone (T) and estradiol (E2) concentrations. Subsequent western blots were performed to assess gene expression of specific proteins involved in the male reproductive system.

**Results.** Decreased T concentrations were identified in the G + D group in serum ( $p < 0.05$ ), basal testes ( $p < 0.05$ ), and in both basal and LH stimulated Leydig cells ( $p < 0.05$ ). Increased T concentrations occurred in the 2G + 2D group in serum ( $p < 0.05$ ), basal testes ( $p < 0.001$ ), LH stimulated testes ( $p < 0.05$ ), and basal Leydig cells ( $p < 0.05$ ). Additionally, E2 concentrations in the 2G + 2D group were found to significantly increase in basal testes ( $p < 0.05$ ) and LH stimulated testes ( $p < 0.001$ ), as well as the G + D group in LH stimulated testicular cells ( $p < 0.05$ ). E2 concentration significantly decreased in basal Leydig cells in the 2G + 2D group ( $p < 0.05$ ). The results of Western blot analyses indicate a significant increase in Estrogen receptor  $\alpha$  expression in the 2G + 2D group ( $p < 0.05$ ), a significant decrease in Cathepsin D expression in the G + D group ( $p < 0.001$ ) and the 2G + 2D group ( $p < 0.0001$ ), and a significant increase in Sirtuin 1 expression in the G + D group ( $p < 0.0001$ ) and 2G + 2D group ( $p < 0.00001$ ).

**Conclusions.** These observations imply that soy isoflavones target the estrogenic mechanisms within the male HPG axis and may impact reproductive function.

**Acknowledgements.** Research grant from the Animal Disease and Health Research Program at Auburn University College of Veterinary Medicine. Student support from the Boehringer Ingelheim National Veterinary Summer Scholars Program.

**Assessing the immunoregulatory effect of mesenchymal stem cell extracellular vesicles on stimulated mixed glia**

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**Introduction.** Neurodegenerative conditions (NCs) such as Alzheimer's, Parkinson's, and Lysosomal storage disorders (LSDs) are characterized by diverse pathogenic mechanisms. Nonetheless, neuroinflammation remains a common feature in many NCs, making it a target of therapeutic interest. Recent advancements in stem cell technology have highlighted its potential to mitigate inflammation. Notably, extracellular vesicles (EVs) derived from stem cells have demonstrated the capacity to modulate immune cell phenotypes and reduce the production of inflammatory cytokines. This study aims to explore the immunomodulatory effects of human mesenchymal stem cell (hMSC) EVs on a cell population comprising both astrocytes and microglia, the primary immune cells of the central nervous system (CNS), isolated from the brains of LSD-afflicted cats.

**Methods.** Isolated glial cells from cat brain tissue were subjected to lipopolysaccharide (LPS) treatment to induce the production of inflammatory cytokines, followed by treatment with hMSC-derived EVs. Subsequent assessments involved enzyme-linked immunosorbent assays (ELISAs) and quantitative polymerase chain reaction (qPCR) to evaluate changes in the expression and production of inflammatory cytokines.

**Results.** Preliminary findings indicated a notable reduction in both gene expression and production of inflammatory cytokines.

**Conclusion.** These findings suggest that hMSC-derived EVs possess the capability to downregulate the production of inflammatory cytokines. Further *in vivo* investigations are warranted to evaluate their potential efficacy in mitigating neuroinflammation in live LSD-afflicted cats.

**Acknowledgments.** This research project received support from the College of Veterinary Medicine Animal Health and Disease Research Program through a research grant, and student support was provided by the Boehringer Ingelheim Summer Scholars Program.

**Developing a Canine-Specific 4-1BB Agonist for Cancer Immunotherapy**

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Cancer is one of the leading causes of death in the older dog population. Traditional approaches to cancer treatment, including surgery, radiotherapy, and chemotherapy combinations, are marked by invasiveness, prolonged recovery times, non-specific cytotoxicity, and undesirable suppression of the immune system. Over the last decade, the evolution of immunotherapies has transformed our cancer treatment strategies. Costimulatory receptors such as GITR, OX-40, and 4-1BB have emerged as promising therapeutic targets to overcome non-responsiveness to immune checkpoint inhibitor (ICI)-based immunotherapies. These transmembrane proteins signal alongside various cytokines and T-cell receptor (TCR) stimulation to prime and activate T-lymphocytes. The 4-1BB costimulatory receptor, a member of the TNFRSF, is highly expressed or upregulated on natural killer cells and T-cells following TCR stimulation. The primary ligand for the 4-1BB receptor, 4-1BBL, is expressed on activated B cells, dendritic cells (DCs), and macrophages. Upon 4-1BB ligation, a signaling cascade culminates in the secretion of interferon-gamma (IFN- $\gamma$ ) and interleukin 2 (IL-2). Additionally, anti-apoptotic Bcl-2 family members are upregulated, offering robust protection against activation-induced T-cell death. Post-TCR stimulation, co-signaling from 4-1BB enhances T-cell priming, activation, differentiation, effector function, and survival. Preliminary preclinical evidence of anti-4-1BB agonistic monoclonal antibodies suggests their excellence as candidates for cancer immunotherapy. Consequently, this project's primary objective was to develop a canine-specific 4-1BB agonist. The nucleotide sequence coding for canine 4-1BBL<sub>ECD</sub> was fused *in silico* to the Fc domain of subclass D (functional analog of human IgG4) of canine IgG via coiled-coil trimerization domain derived from yeast GCN4 (ILZ). We placed a secretion signal sequence from the V-J2-C region of the mouse Ig Kappa-chain at the N-terminus for efficient secretion of the recombinant protein after transfection. The open reading frame coding for the cFc-4-1BBL<sub>ECD</sub> sequence was commercially synthesized and cloned into the pCDNA3.4/Topo vector. The recombinant plasmid coding for cFc-4-1BBL<sub>ECD</sub> was transfected into Expi293-F cells. The expression of cFc-4-1BBL was confirmed by western blotting. An Expi293-F-based stable cell line was constructed to express and purify cFc-4-1BBL protein. The conditioned media was collected, and the recombinant protein was purified using AKTA explorer by HiTrap protein G column. The purity of the purified protein was confirmed by SDS-PAGE. The purified recombinant protein was evaluated for its ability to bind to canine 4-1BB receptors. Our results demonstrate that we successfully cloned the open reading frame coding for cFc-4-1BBL<sub>ECD</sub> in the pCDNA3.4 Topo Vector. The cFc-4-1BBL<sub>ECD</sub> was actively secreted into the conditioned media, as confirmed by western blotting. The recombinant protein was purified to high levels using affinity chromatography. The reduced form of the cFc-4-1BBL migrated at the predicted molecular weight of ~55kDa, while the protein formed dimers (110 kDa) under non-reducing conditions. The purified cFc-4-1BBL<sub>ECD</sub> binds to the canine 4-1BB receptor with high affinity and specificity. In future studies, we will investigate the ability of the cFc-4-1BBL protein to bind and activate native 4-1BB receptors on canine natural killer (NK) cells, CD4+ and CD8+ T lymphocytes.

**Acknowledgments:** This study was supported by funding from Animal Health and Disease Research (AHDR) by AUCVM. We would also like to thank Rie Watanabe for flow cytometry support.

**Circumductal anal saccullectomy in 63 dogs**

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**Introduction.** The anal sacs are paired secretory structures within the anal sphincter, each with an excretory duct that opens at the 4 and 8 o'clock regions. Each sac is lined by stratified squamous epithelium and contains both apocrine and sebaceous glands. Anal saccullectomy is the surgical removal of the anal sac and is performed for many reasons, including anal sacculitis, neoplasia, and anal sac impaction or abscessation. Currently, there are four described techniques to perform this procedure: standard open, modified open, closed, and inside out. The most common malignant anal sac tumor in dogs is anal sac adenocarcinoma (ASAC), and the recommended technique for removal is the closed anal saccullectomy. This technique leaves behind varying amounts of ductal tissue which could potentially result in post operative complications, as some secretory tissue might remain. If complete removal of the ductal tissue could be achieved, this could potentially lower the risk of post-operative recurrence, draining tracts, and other complications. The aim of this study is to describe a circumductal (removal of anal sac, duct, and ductal orifice) anal saccullectomy in dogs diagnosed with apocrine gland anal sac adenocarcinoma (AGASACA) and to report survival times, complication rates, and recurrence rates.

**Methods.** Medical records from two specialty hospitals were retrospectively reviewed to identify dogs that undergone circumductal anal saccullectomy for removal of ASAC between November 2011 and December 2022. The dogs included had a histological diagnosis of ASAC. If a bilateral, staged anal saccullectomy was performed, the survival analysis was calculated from the date of the second surgery.

**Results.** There were 63 cases (64 surgeries) that met the inclusion criteria for this study. Intraoperative complications related to the primary surgical site occurred in 3 cases: inadvertent entry into the anal sac (n=1), liquid stool contamination of the surgical site (n=1), and defecation of suspected proglottids into the surgical site (n=1). These dogs did not develop post-operative complications. Post-operative complications occurred after 3 surgeries: incisional inflammation (n=2) and incisional discomfort/pain more than 7 days after surgery (n=1). One surgery was classified as a failure to cure, as gross disease was left because it was determined to be inoperable due to invasion with the rectum, and this case was excluded from analysis of local recurrence. No cases developed local recurrence during the follow up period. The median overall survival time was 677 days and the 1-year survival probability was 77%.

**Conclusions.** The circumductal anal saccullectomy is an appropriate surgical approach with potentially lower complications and recurrence rates than previously reported. Survival times are similar to those previously reported. The intraoperative complication rate of 4.6% is comparable to previous reports involving the closed approach, being 9%, 3.5%, and 3%. The post operative complication rate in our case was 3.4%, which is lower than previously reported rates of 17%, 42.1%, and 20%.

**Acknowledgments.** Thank you to Dr. Keys for statistical analysis and to the Department of Clinical Sciences for financial support.



## **The Effects of FOXL2 Overexpression in the Adrenal Cortex**

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**Introduction.** Unique to mice, the X-zone is a population of fetal cortical cells at the cortical-medullary boundary that exhibits sexually dimorphic regression. The sexual dimorphism of X-zone regression implies a possible role of sex hormones on the fate of the aged cell population.

FOXL2 regulates ovarian development from the adrenal-gonadal primordium through the repression of male sex determination. In the adrenal gland, FOXL2 expression is extremely low. If normal levels of FOXL2 suppress the male gene pathway in ovarian development, further expression of FOXL2 in the adrenal gland cortex could affect X-zone regression/proliferation given the presumed role of sex hormones in these processes. How does overexpression of FOXL2 in the adrenal cortex alter adrenal organization and adrenocortical cell fate?

**Methods.** Sf1-Cre and ROSA-FOXL2 mice were bred to harvest adrenal glands with FOXL2 overexpression in Sf1+ cells from progeny. Mutant and control adrenal samples from 3-week, 8-week, and 28-week old female and male mice underwent two double immunohistochemistry (IHC) staining protocols. To assess adrenal organization, an IHC protocol utilizing 3BHSD and tyrosine hydroxylase as primary antibodies was followed. To assess adrenocortical cell fate via X-zone regression, an IHC protocol utilizing 20aHSD and tyrosine hydroxylase as primary antibodies was followed.

**Results.** FOXL2 overexpression disrupted adrenal organization in male and female mice as early as 3 weeks of age and into adulthood. Overexpression of FOXL2 delayed X-zone regression in males with the aged cell population persisting until 28 weeks of age.

**Conclusions.** Overexpression of FOXL2 in the adrenal gland cortex leads to adrenal disorganization and affects adrenocortical cell fate by delaying X-zone regression.

**Acknowledgments.** Research mentor: Dr. Jeff Huang, Lab manager: Dr. Yuan Kang, Funding: National Institutes of Health, Boehringer Ingelheim Veterinary Scholar Program, Collaborator: Dr. Humphrey Yao (NIEHS, NIH)



### **Developmental toxicity of dichloroacetic acid (DCA) in zebrafish (*Danio rerio*)**

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**Introduction.** Dichloroacetic acid (DCA) is a by-product of drinking water chlorination and is a major metabolite of Trichloroethylene (TCE) and Tetrachloroethylene (PERC). TCE and PERC are major environmental toxicants commonly used as industrial solvents and metal degreasers during the mid-twentieth century. TCE, PERC, and DCA can be found in numerous ground water sources, which is concerning since TCE and PERC are linked to short- and long-term health effects. One of the major health effects is developmental toxicity in mammals and aquatic life, with reports of cardiac abnormalities, stunted growth, and fetal death. The zebrafish (*Danio rerio*) biomedical model organism was used to test the hypothesis that DCA contributes to TCE and PERC related developmental toxicity.

**Methods.** An LC50 assay was performed and no lethal toxicity was observed at concentrations up to 100 parts per millions (ppm). To evaluate toxicity at environmentally relevant concentrations, wild-type zebrafish embryos were statically exposed to 0, 5, 50, or 500 parts per billion (ppb;  $\mu\text{g/L}$ ) DCA from 1-120 hours post fertilization (hpf). Embryo survival and hatching were monitored every 24 hours for 120 hpf and larval morphology, heart rate, and behavior, via a visual motor response assay, were evaluated at 120 hpf.

**Results.** There were no changes in survival and hatching between groups; however, the 5 ppb exposure group had significantly decreased body length and head length compared to controls on morphological assessment. All DCA exposures had a significantly increased heart rate compared to controls. Additionally, the visual motor response assay showed the 50 ppb exposure group had a decrease in distance moved and velocity compared to controls and the 50 and 500 ppb exposure groups had decreased time spent moving.

**Conclusions.** This data indicates that DCA does significantly contribute to TCE and PERC related developmental toxicity and continued investigation is warranted.

**Acknowledgments.** Research funding was provided by the National Institute of Environmental Health Sciences (R15 ES033361), the Boehringer Ingelheim Vetmedica, Inc. Veterinary Scholars Program and the Auburn University College of Veterinary Medicine.





## Graduate Student Poster Presentations

### Legacy and emerging per- and polyfluoroalkyl substances (PFAS) regulate steroidogenesis in the male gonad

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**Introduction.** Per- and polyfluoroalkyl substances (PFASs) are a ubiquitous group of man-made chemicals that are used in the manufacture of fire-safety foams, non-stick dishware, and several cosmetics. Because unregulated disposal of PFASs into landfills contaminates groundwater sources for both drinking and agriculture, toxicity concerns have resulted in regulations prohibiting the use of legacy (long-chain) PFASs. Emerging (short-chain) PFASs, such as perfluorobutanoic acid (PFBA) and perfluorobutanesulfonic acid (PFBS), are thought to be safer alternatives to legacy perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) due to decreased toxicity and more rapid environmental degradation. However, PFBS and PFBA are known to bioaccumulate and persist in body tissue. Interestingly, there is little information on endocrine-disrupting properties of emerging PFASs. Our objective is to compare the effects of legacy and emerging PFASs in the male neuroendocrine axis.

**Methods.** In the first experiment, male Long-Evans rats were administered test chemicals (PFOA, PFOS, PFBA, PFBS) in drinking water at 1, 10, 100, and 1000 ng/L from 21 to 35 days of age (14-day exposure). In the follow-up experiment, male Long-Evans rats (n=12) were administered PFOA, PFOS, PFBA, and PFBS in drinking water at 10 or 100 ng/L from postnatal days 21 to 49 (28-day exposure). In both cases, we obtained testicular tissue and isolated Leydig cells at sacrifice, which were then incubated in DMEM/F-12 culture medium without (basal) and with 100 ng/mL ovine LH (NIDDK, NIH; LH-stimulated) for 3 h. Basal and LH-stimulated testicular and Leydig cell testosterone (T) and 17 $\beta$ -estradiol (E2) concentrations were determined by RIA. Western blot analysis was performed to determine protein gene expression in testes, Leydig cells, and pituitary glands from the second experiment.

**Results.** Acute exposures to PFBA at 10 ng/L increased basal and LH-stimulated testicular T production. Similarly, basal testicular T was increased by PFBS at 1 ng/L and PFOS at 1000 ng/L, but LH-stimulated testicular T was decreased by PFOS at 10 ng/L. Chronic exposures to both PFOS and PFBS at 10 and 100 ng/L decreased basal testicular T, while LH-stimulated testicular T was increased by PFBA at 10 ng/L. Western blot analyses showed that testicular MIS protein was increased by PFOS and PFBA at 100 ng/L and PFOS at 10 ng/L when compared to control. Testicular inhibin- $\beta$  protein was decreased by all chemicals at the 10 and 100 ng/L doses, except the low dose of PFBA, which had no effect. In addition, both PFOA at 10 ng/L and PFBS at 100 ng/L increased pituitary Luteinizing hormone- $\beta$  subunit expression compared to control, while PFBA at 100 ng/L and PFBS at 10 ng/L increased FSH- $\beta$  concentrations. In Leydig cells, PFOA at 100 ng/L increased, while PFBA at 100 ng/L decreased StAR protein concentrations.

**Conclusions.** Together, these data demonstrated that both legacy and emerging PFASs regulate testicular steroidogenesis. Interestingly, PFASs increased as well as decreased gonadal androgen secretion in male rats, which has implications for testicular function, as both androgen insufficiency and excess impact germ cell development. Also, investigation of additional exposure paradigms will help to further identify differences in testicular toxicity due to legacy and emerging PFASs.

**Acknowledgments.** This work was done as part of the Environmental Contaminants Research Group (ECRT) and supported in part by a PAIR award at Auburn University.



### **Ultrasound-guided cerebrospinal fluid collection at the atlanto-axial space in mature cattle with a comparative evaluation of lumbosacral cerebrospinal fluid collection.**

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**Introduction.** Currently, two CSF collection sites are used in cattle: the cisterna magna at the atlanto-occipital (AO) joint on the neck and the lumbar cistern at the lumbosacral (LS) junction on the back. The AO centesis site has greater risk due to needle placement near the brainstem and requires general anesthesia. LS centesis is performed in a minimally sedated standing patient restrained in a squeeze chute. However, the LS technique is difficult in heavily muscled animals; low yields and contamination of the sample are not uncommon. Interpretation of grossly hemorrhagic CSF samples is difficult and can preclude the identification of an etiological diagnosis. An alternative ultrasound-guided CSF sampling technique was recently developed in horses, in which CSF is obtained from the cervical region C1-C2 (atlanto-axial space) in a standing sedated animal. This new technique was demonstrated to be a safe alternative to LS centesis, but it has not been described in cattle. The specific aims of the study were: (1) Describe the technique of ultrasound-guided atlanto-axial CSF collection in healthy cattle. (2) Compare the technical aspects of CSF collection between the two approaches. (3) Compare CSF cytology results (sample characteristics) in samples collected at the C1-C2 and LS centesis sites.

**Methods.** Ten healthy mixed-breed beef cows from the AU research herd were enrolled in the pilot study. Using a randomized, crossover study design, each operator performed both sampling techniques on each animal. Sampling events were completed every 7 days for 4 weeks. Cows were sedated with 2mg of acepromazine intravenously for both collection approaches. C1-C2 collection was completed under ultrasound guidance to visualize needle placement within the dorsolateral subarachnoid space at the C1-C2 window, while LS collection was completed blinded, driven by palpation alone. The total time (min) from initial spinal needle insertion to complete sample acquisition and the number of repositioning events, gross sample appearance, and animal behavior/reactivity were recorded. A score was assigned to each sampling procedure (1-4). The presence of blood in the sample or behavior precluding continuing the procedure was also recorded. Clinicopathological analyses included total nucleated cell count (TNCC), total protein (TP), and red blood cell (RBC) concentrations performed by an unblinded clinical pathologist. Statistical tests were used to compare the cytology results and performance metrics of the two techniques. Significance was set at  $p < 0.05$ .

**Results.** No significant differences between the two operators were found in cytological and performance data, and data was averaged for subsequent analyses. Means for sample acquisition time (C1-C2 5.4min, LS 6.2min), repositions (C1-C2 2.9, LS 3.1), and difficulty score (C1-C2 2.7, LS 3.1) were found, with no significant differences found between the different approaches. The degree of RBC contamination within the samples was not significantly different between techniques ( $p = 0.087$ ), though the C1-C2 samples had significantly lower TNCC and TP concentrations compared to the LS samples ( $p < 0.01$  and  $0.005$ , respectively).

**Conclusions.** This study demonstrated that the ultrasound-guided C1-C2 CSF technique can be successfully and safely used in cattle with no negative impact on the quality of the collected sample. This may enhance the clinician's ability to obtain an etiological diagnosis of neurologic disease in cattle and further research into the application of this technique in clinically affected cattle is warranted.

**Acknowledgments.** The authors would like to thank the LAVTH technical staff for husbandry support. Special thanks to the ACVR for providing funding for the project.

**Isolating exosomes from in vitro cultured trophoblast cells**

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**Introduction.** The interaction between the uterus and placenta is critical for the establishment and maintenance of a pregnancy. This communication is, at least, partially facilitated by placental release of membrane bound vesicles called exosomes. These exosomes travel to the uterus and modulate the maternal immune response for the close interaction of the foreign tissue of the placenta. Our goal was to isolate exosomes from in vitro cultured trophoblast cells and characterize their function.

**Methods.** Using the BeWo immortalized cell line, we isolated exosomes from in vitro cultured trophoblast cells. Cells were cultured for 24 hours in exosome free media made with 90% F12-K and 10% exosome free fetal bovine serum. After 24 hours, media was collected and cleared of cell debris by centrifugation at 400 x g for 15 minutes at 4° Celsius. Media was moved to a new conical tube and additional large particles were cleared from the sample by centrifugation at 10,000 x g for 30 mins at 4° Celsius. Media was transferred to ultra-centrifuge tubes and spun at 22,000 rpm for 120 minutes at 4° Celsius using a SW 41 Ti rotor. The media was then decanted off and the sample was resuspended in PBS before undergoing a second round of ultracentrifugation under the same conditions as the first cycle. PBS was removed, and the isolated exosomes that remained at the bottom of the tube were resuspended in 200 microliters of cold PBS.

**Results.** Particle size analysis using a Zetaziser molecular size analyzer was used to determine the size of the exosomes. We found distinct populations of particles distinguishable by size. Populations of particles ranged in size from 174.1 – 5590 nanometers. The polydispersity index (PdI) value for the samples was 0.256 and 0.634. The samples with a PdI value of greater than 0.5 were indicative of particle aggregation.

**Conclusions.** Extracellular vesicles released from the placenta are a critical link to understanding communication between the placenta and the uterus in the establishment and progression of pregnancy. In this study, we have determined that BeWo immortalized trophoblast cells do release membrane bound exosomes. Additionally, we can collect these exosomes from spent culture media and particles obtained using our ultracentrifugation isolation protocol appear to contain populations that meet the criteria exosome size. To further verify the presence of exosomes, further analysis will need to be done; including western blotting for membrane surface proteins CD9, CD81, and CD63 as well as transmission electron microscopy for particle imaging.

**Acknowledgements.** We would like to acknowledge Dr. Richard Bird and Dr. James Gillespie for their assistance with the ultracentrifuge and Zetasizer and Dr. Robert Judd for his critical assessment of the abstract. This project was funded by the Auburn University Animal Health and Disease Research Fund awarded to Dr. West.

**Functional investigation of a rare *MAD1L1* variant in African American hereditary breast cancer risk**

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**Introduction.** Breast cancer (BC) is the most diagnosed cancer among women in United States. Historically, African American (AA) women have been underrepresented in BC research, despite having higher incidence rates under the age of 40 and triple negative breast cancer - hallmarks of hereditary disease. Currently, mutations in known BC susceptibility risk genes account for roughly 30% of all hereditary breast cancer cases, which leaves ~70% cases unexplained. It is important to note that this data is based on research conducted with participants of European descent; therefore, these numbers are not a clear representation of other ethnic groups, including AAs. Recognizing AA health and research disparities, a better understanding of AA BC risk is needed. Our group has established the Alabama Hereditary Cancer Cohort (AHCC) to aid in identifying risk variants associated with AA hereditary breast cancer.

**Methods.** Participants of the AHCC enrolled in the study through hospital- or community-based recruitment, and genetic variants were identified using next-generation sequencing. The FASTQ files were processed for variant calling using the Genome Analysis ToolKit (GATK) Best Practice Pipeline. Variants were annotated using ANNOVAR. Both single variant and gene-based statistical analyses were carried out to identify significant p-values. Variants of interests were validated through polymerase chain reaction and Sanger sequencing (SS). To functionally study a variant, we use a novel gene editing technique called prime editing. For prime editing, we digested bacterial plasmids and constructed mutation specific oligonucleotides building a new plasmid with golden gate assembly. After vector transfection and bacterial propagation, plasmid constructs are validated via SS. Upon confirmation, plasmids will be transfected into human cell lines for gene editing and functional analyses.

**Results.** Sequencing has identified several potential BC risk variants. Of these variants, we chose to functionally investigate frameshift deletion variant, *MAD1L1* c.1579delG; p.E527Sfs\*19. The *mitotic arrest deficient-like 1 (MAD1L1)* gene plays an important role in regulating the cell cycle. During metaphase of mitosis, *MAD1L1* acts as a checkpoint to ensure proper chromosomal alignment before progressing to anaphase/chromosomal segregation. Furthermore, *MAD1L1* is associated with prostate cancer. This rare variant has an allele frequency of 0.0042 in AA BC cases in the AHCC compared to allele frequency of 2.38 E-5 in the general AA population.

**Conclusions.** Functionalizing *MAD1L1* c.1579delG; p.E527Sfs\*19 and other rare or low frequency variants are important to demonstrate breast cancer pathophysiology, especially to bridge the knowledge gap in AA hereditary breast cancer cases. Our group has begun to employ prime editing to incorporate our *MAD1L1* mutation into cancer cell lines. After prime editing, we will utilize assays to assess cellular characteristics to understand the functional role of this variant.

**Acknowledgments.** We would like to thank the College of Veterinary Medicine, Auburn University, National Institutes of Health T32 Training Grant, as well as our community partners and study participants.

**Optimization of rtPCR Targeting a Frameshift Mutation in CEACAM24**

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**Background.** The glycoprotein encoding genes, *Carcinoembryonic Antigen –Related Cell Adhesion molecule* (CEACAM), are members of the large and highly conserved carcinoembryonic antigen (CEA) gene family. They play important roles in multiple cell pathways including cell adhesion, cell signaling, angiogenesis, inflammation, tumor development, and cancer progression. A recent study has found a relationship between a spontaneously occurring frameshift mutation in canine CEACAM24 in canine mammary tumors in particular breeds.

**Objective.** To optimize Real-time PCR (rtPCR) to selectively amplify a rare protein-truncating variant of CEACAM24 in canine cell lines independently derived from individual spontaneous canine mammary tumors.

**Methods.** rtPCR with RNA extracted from Canine mammary tumor cell line CMT28 was conducted using three distinct primer sets designed for the canine CEACAM24 gene, targeting the open reading frame region encompassing the c.247dupG mutation (p.(Val83Glyfs\*48)). Subsequently, agarose gel electrophoresis was performed to evaluate the outcome, size, and purity of the PCR products. Due to the presence of numerous transcript variants, a significant background signal was observed with all three primer sets. To mitigate this background noise, adjustments were made to the annealing temperature and magnesium concentration. Additionally, 2 percent DMSO was introduced into the final reaction mix. After multiple rounds of rtPCR with various combinations of magnesium and temperature, successful rtPCR was achieved with only one primer set, yielding two distinct bands. One band, measuring 333 base pairs, corresponded to the original transcript, while the other, spanning 869 base pairs, is anticipated to represent a transcript variant.

**Results.** CEACAM24 has six different transcript variations that made it challenging to specifically amplify the original CEACAM24. However, after multiple rounds of rtPCR with varying magnesium concentrations and higher temperatures, we have successfully optimized the PCR results. Gel electrophoresis showed two distinct bands: one at 333 base pairs, corresponding to the original transcript, and another at 869 base pairs, likely representing a transcript variant.

**Conclusions.** In summary, the study successfully optimized rtPCR to selectively amplify the rare CEACAM24 variant in canine mammary tumors. The results revealed the presence of the protein-truncating variant associated with a frameshift mutation, offering insight into genetic factors in canine cancer. Further DNA sequencing will be conducted to validate the sequence and confirm the presence of the c.247dupG mutation (p.(Val83Glyfs\*48)).

**Effects of sleep on the progression of Multiple Sclerosis**

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**Introduction.** Recent discoveries suggest sleep is essential for the maintenance and optimal functioning of the immune system. We hypothesized that manipulation of sleep via stimulation of sleep active neurons of the Ventral Medial Midbrain/Pons (VMP), a brain center known for its modulating role in sleep in mice, can affect immune responses. In other words, we sought to determine whether increased sleep or decreased sleep could affect how the body responds to infections. For this, we used a mouse model of the autoimmune disease Multiple Sclerosis (MS), manipulated their sleep behaviors, and assessed the effects of different levels of sleep on the disease progression.

**Methods.** We used a mouse model for MS called Experimental Autoimmune Encephalomyelitis (EAE) in which the symptoms and pathophysiology of MS are recapitulated. The model works by temporarily opening the blood brain barrier and immunizing the system with myelin peptides in mice (n=10). We then employed chemogenetic activation of sleep-active neurons in the VMP region, by specifically stimulating neurons using the inhibitory neurotransmitter GABA to increase sleep for 4 hours daily in the first of the three groups of mice (n=4). Mice (n=3) in the second group were left to sleep normally, while the third group (n=3) were sleep deprived for 4 hours each day. Neurological symptoms of MS progression were scored daily at dark onset according to an EAE clinical severity scale. When mice reached severity score of 4 (complete hind limb paralysis), the study was terminated and the draining lymph nodes (DLN) and spleen were collected to analyze the percentages and numbers of CD45, CD4, CD8, Treg, Th1 and Th17 using flow cytometry.

**Results.** Our data thus far shows a trend indicating that mice with sleep deprivation may have more inflammatory Th1 cells in the DLN and conversely, less anti-inflammatory Treg cells when compared to mice with normal sleep. This was also reflected in the EAE score of clinical symptoms where sleep deprived mice exhibited more severe scores than mice in the normal sleep group. Interestingly, mice that received extra sleep also displayed more severe clinical symptoms with higher EAE scores.

**Conclusions.** This preliminary data suggests that there is a trend toward increasing pathogenic Th1 cells and a reduction of Treg cells in mice with MS following sleep deprivation, implying that sleep deprivation may lead to aberrant immune responses leading to autoimmune diseases. To confirm this and establish the effects of extra sleep on MS, an extended study with more animals is necessary.

**CAR T Cells as Targeted Immunotherapy for Osteosarcoma**

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**Introduction.** Osteosarcoma (OS) is a highly aggressive and metastatic bone malignancy. Immunotherapy has shown promise in treating a variety of cancers. The tumor microenvironment is immune suppressive due to the inhibition of T cell proliferation and infiltration. We propose to develop and characterize chimeric antigen (CAR) T cells to target OS via the B7H3 receptor.

**Methods.** Two lentiviruses encoding anti-B7H3 single-chain variable fragment (scFv) and GFP/ffluc (firefly luciferase), were packaged in HEK293T/17SF cells using pALD-Lenti system. Lentiviruses were purified and titrated for functional virus particle number using canine lymphoma cell line, OSW, via flow cytometry. Four canine OS cell lines (D17, D22, CF11, and MC-KOS) were genetically modified via lentiviral infection to express GFP and firefly luciferase genes to assay cell killing by CAR T cells. Peripheral blood mononuclear cells (PBMCs) were isolated from canine whole blood to develop CAR T cells. T cells were activated from PBMCs using anti-CD3/28 activation beads and were maintained in media supplemented with IL-2. Activated T cells were transfected with anti-B7H3 lentivirus. T cell activation and anti-B7H3 were quantified using flow cytometry. GFP/ffluc+ cell lines will be modified further to knock out B7H3 gene using CRISP/Cas9 digestion to be used as a negative control. Anti-B7H3 CAR T cells will be incubated with all four canine GFP/ffluc+ and B7H3- OS cell lines. Cell death will be measured by GFP expression and luciferase secretion assays. Additionally, IFN $\gamma$ , TNF $\alpha$ , and IL-2 cytokine panels will be performed to assay the immunogenic role of CAR T cells in tumor microenvironment (TME).

**Results.** All four canine OS cell lines (D17, D22, CF11, and MC-KOS) express B7H3. B7H3 expression was confirmed by PCR and flow cytometry. Anti-B7H3 scFv and GFP-Luciferase lentiviruses have been successfully packaged and quantified. Canine OS cell lines D17, D22, CF11, and MC-KOS have been successfully modified to express GFP and firefly luciferase genes. T cells were successfully activated from PBMCs using anti-CD3/28 beads.

**Discussion.** We expect to construct B7-H3 binding CAR T cells successfully and demonstrate the efficacy of CAR T therapy against osteosarcoma. We expect cell lysis only in osteosarcoma cell lines and not in B7H3 negative cell lines. And, lastly, we expect that IFN $\gamma$ , TNF $\alpha$ , and IL-2 cytokine levels will be elevated in OS cell lines post-CAR T therapy.

**Acknowledgments.** The authors would like to acknowledge Daniel Patton, Aliaa Ismail, and Rimas Orentas for their support and collaboration. This research is generously funded by SRRC, CVM-ORGS, and NIH R15.

**Optimizing Single Nuclei Isolation from Canine Mast Cell Tumors: Enhancing Nucleus Quality for Comprehensive Molecular Analysis**

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**Introduction.** Mast cell tumors (MCTs) are the most common cutaneous neoplasms in dogs, presenting a significant clinical challenge due to their variable behavior and diverse outcomes. Due to the complex cellular diversity within each tumor, a fundamental understanding of their genetic and epigenetic heterogeneity is essential for advancing both diagnostic and therapeutic approaches. When tissue samples are studied in bulk, without consideration for the presence of different cell types, results can be biased due to the combination of cellular gene expression data from multiple cell types. In order to unravel the genetic intricacies of canine MCTs, we have undertaken a study of cell type-specific RNA expression profiles within MCT tissues. This approach was chosen to account for the heterogeneity that exists between different cells within the same tumor, which can significantly influence tumor behavior and treatment responsiveness. Our objective was to isolate nuclei from frozen canine MCTs to study RNA expression and ATAC (Assay for Transposase-Accessible Chromatin) analysis profiles using single-nuclei RNA sequencing (snRNA-seq). In this context, we have carefully optimized the 10x Genomics' nuclear isolation protocol for nuclei extraction from archived frozen MCTs cells suitable for downstream snRNA-seq and ATAC analyses.

**Methods.** Canine mast cell tumors were sampled after surgical excision from dogs presenting to the Auburn University Bailey Small Animal Hospital. Sterile biopsy punch cores (5mm) were obtained from excised fresh tumor and trimmed of attached skin. The tissues were flash frozen in liquid nitrogen and stored at -135 degrees C. For nuclei preparation, frozen pieces were cut into smaller pieces with a scalpel. Samples of 42 mg were homogenized in 10X Genomics lysis buffer using a mechanical blade homogenizer. The amount and strength of the buffer were varied, as was the incubation time post-homogenization. Nuclei were purified by nuclei isolation column. Assessment of nuclear quality was done using the Keyence bz-x800 imaging system, with a 60x oil objective. Samples were stained with acridine orange (AO) and propidium iodide (PI) to distinguish between intact and ruptured nuclei. Isolated nuclei were quantified by trypan blue staining and counting viable (unstained) and non-viable (blue-stained) nuclei on a Bio-Rad TC20 automated cell counter and Invitrogen Countess II Automated Cell Counter.

**Results.** Preliminary findings demonstrated a considerable enhancement in nuclear quality, integrity, and minimized cellular debris. By fine-tuning these critical parameters, we achieved an improvement in the purity and quality of isolated nuclei. This is of paramount importance, as high-quality nuclei are essential for robust downstream analysis. Healthy, good quality nuclei displayed vibrant and uniform staining patterns along with clear, well-defined boundaries in bright-field microscopy. When stained with AO/PI, good quality nuclei typically fluoresce green (stained with AO), indicating they are healthy with intact membranes. Our results indicated that less aggressive methods of tissue homogenization and lysis resulted in removal of cell debris providing nuclei of sufficient quality for snRNA-seq and ATA-seq.

**Conclusion.** Optimization resulted in production of high-quality single nuclei suspensions from frozen canine MCTs in ~ 20 minutes for downstream gene expression analysis. Through this approach, we aim to unravel the genetic and epigenetic variations among cells within the same MCT, shedding light on the underlying factors driving tumor progression and response to therapy.



**Interleukin-27 regulates the metabolic reprogramming of macrophages during Herpes Simplex Virus-1 (HSV) infection.**

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**Introduction.** Herpetic Stromal Keratitis (HSK) is a painful and vision-impairing disease caused by recurrent HSV-1 infections of the cornea. Current antiviral and corticosteroid therapies, while partially effective, induce immunosuppression and viral latency. Understanding the molecular and cellular events are crucial for novel therapies. Macrophages play a key role in HSV-1 clearance during HSK progression. Activated macrophages exhibit increased glycolysis, TCA cycle disruption, and elevated IRG1 expression. Our prior research shows that HSV-1 induces IL-27 production, an immunoregulatory cytokine, in macrophages. Thus, we hypothesize that IL-27 modulates macrophage metabolism to regulate inflammation and enhance antiviral immunity.

**Methods.** We utilized IL27r<sup>-/-</sup> mice in our study. To understand macrophage biology, we used bone marrow-derived macrophages (BMDMs) differentiated from bone marrow of wild-type and IL27r<sup>-/-</sup> mice. We assessed the production of inflammatory cytokines and IRG1 expression by qPCR following HSV-1 infection in BMDMs. To investigate the metabolic status of the macrophages, we measured glycolytic uptake, flux and oxygen consumption rates using an XFp analyzer.

**Results.** In IL27r<sup>-/-</sup> BMDMs, we observed increased production of Irg1 and inflammatory cytokines. Moreover, we noted an increased glycolysis rate, further supporting the presence of inflammation. In terms of mitochondrial respiration, we detected a reduced oxygen consumption rate, signifying a decrease in oxidative phosphorylation (OXPHOS) within the mitochondria, along with an increase in dysfunctional mitochondria.

**Conclusions.** The absence of IL-27 signaling is associated with an intensified inflammatory environment. We observed increased glycolysis and mitochondrial dysfunction in macrophages from IL27r<sup>-/-</sup> mice. Thus, IL-27 could play a key role in regulating the inflammation in the cornea through the metabolic reprogramming of macrophages.

**Acknowledgments.** This research work is funded by National Eye Institute Grant EY035057 and National Institute of Allergy and Infectious Diseases Grant AI153794.

**Maximizing Therapeutic Potential: Tailoring a PD-1-Dependent 4-1BB Agonist for Combination Immunotherapy**

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**Introduction.** Malignant cancer cells employ diverse strategies to evade the immune system within the tumor microenvironment (TME), thereby facilitating tumor growth. While immune checkpoint inhibitor (ICI) therapies targeting PD-1, PD-L1, and CTLA4 aim to counteract some of these immunosuppressive mechanisms, limitations become apparent in patients undergoing single-agent therapy. The integration of ICIs with co-stimulatory receptors has emerged as a strategy to enhance patients' immune responses. One notable receptor in this context is 4-1BB (CD137), a member of the TNF receptor superfamily (TNFRSF), which activates CD8+ T cells, induces IFN- $\gamma$  secretion, and triggers a potent anti-tumor immune response. Optimal signaling through 4-1BB requires the trimerization of the receptor at the cell surface. Unfortunately, the 4-1BB agonist monoclonal antibody, Urelumab, induced severe hepatotoxicity in human cancer patients. This is associated with the systemic activation of 4-1BB receptors on monocytes, which in turn recruit CD8+ T cells that cause widespread hepatocellular damage. Therefore, we hypothesize that restricting 4-1BB activation to the tumor microenvironment (TME) will mitigate hepatotoxicity while simultaneously triggering a robust antitumor immune response. To achieve this goal, we designed three distinct bispecific fusion proteins. These proteins utilize an anti-PD-1 nanobody to cross-link and trimerize the 4-1BB receptor. Since PD1-expressing T cells are most abundant in the TME, this approach will prevent the systemic activation of 4-1BB receptors, limiting hepatotoxicity, and simultaneously triggering a potent anti-tumor immune response within the tumor itself.

**Methods.** Three murine-specific formats of aPD-1-4-1BBL were synthesized and cloned into pCDNA3.4 Topo vector. Expi293F cell-based stable cell lines were constructed to produce and purify these three proteins. Conditioned media containing all mu-aPD1-4-1BBL formats was collected. The expression of all formats was confirmed by Western Blot analysis. Protein purification was performed by AKTA explorer using a HiTrap protein A column for formats 1 and 2 and Strep Tag II column for format 3. The configurations of the three formats are as follows: Format 1: 4-1BBL, isoleucine zipper domain (ILZ), Fc IgG, and anti- PD-1 nanobody (130 kDa). Format 2: Analogous to format 1 with replacement of ILZ domain with a glycine linker (~122 kDa). Format 3: Analogous to format 2 with replacement of Fc domain with a second anti PD-1 attached to a glycine linker (~100 kDa). Stable cell lines expressing murine 4-1BB or PD-1 were used to investigate the binding ability of three formats to these receptors by flow cytometry (CytoFlex LX, Beckman Coulter) using Goat-anti mouse IgG highly antibody Alexa Fluor 680 (1:1200) and Monorab rabbit anti-camelid VHH secondary antibodies (1:250).

**Results and Conclusions.** The SDS-PAGE and western blot analysis confirmed the expression of all mu-aPD1-4-1BBL proteins in the conditioned media. All mu-aPD1-4-1BBL proteins were successfully purified. The purity and ability to form dimers by mu-aPD1-4-1BBL proteins was confirmed on SDS-PAGE analysis. All mu-aPD1-4-1BBL protein formats successfully bind to murine PD1- and 4-1BB receptors. In future studies, we aim to investigate the ability of these proteins to activate murine 4-1BB in the presence or absence of PD1-expressing cells in an *in vitro* bioassay. Subsequently, we will explore the therapeutic efficacy and toxicity profiles of various mu-aPD1-4-1BBL proteins in mouse melanoma tumor xenograft model.

**Acknowledgments.** This study was supported by research funding from Animal Health and Disease Research (AHDR) by AUCVM. We would also like to thank Rie Watanabe for flow cytometry support.

**A Cross-Species Comparison of Mammalian Nasal Cavity Microenvironments**

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**Introduction.** Rats represent the quintessential mammalian model in biological research. Studies of these Eutherian mammals have revealed information from a vast array of topics including physiology, pharmacology, behavior, nutrition, and learning. It is unknown how differences in the distinct respiratory and olfactory nasal cavity microenvironments ranging from structure to microbiota can influence function within and across species. The microenvironment of other mucosal sites demonstrate an integral relationship in regards to the function of epithelial development, immunological regulation, and overall homeostasis. Distinct characterization of the olfactory and respiratory systems within the nasal cavity are proposed to show similar significance.

**Methods.** To address this limited understanding of the distinct olfactory and respiratory nasal cavity microenvironments are being addressed through anatomical, histological, immunohistochemical (IHC) and microbial comparisons between the nasal cavities of rats, cats, and dogs. The nasal cavity of representatives of each species were dissected for the collection of Respiratory Epithelium (RE) and Olfactory Epithelium (OE) from the nasal septum and ethmoidal turbinates. Tissues were preserved, embedded in paraffin, sectioned, and stained. Staining methods include H&E, PAS, and OMP (IHC). Initial microbial characterization was performed by PCR-amplified DNA with the 16S universal Eubacterial primers. Amplicons were sequenced by the PacBio Sequel platform for identification of each member of the microbiota.

**Results.** These mammalian models all have distinct anatomical differences, histological discrepancies, and immunohistochemical reactivities. Although these animals have distinctly shaped rostra and turbinates within their nasal cavities, they all have a clear dividing line between the Olfactory Epithelium (OE) and Respiratory Epithelium (RE) along the nasal septum. Histology and IHC of the OE and RE of these animals has revealed differences in gland structure, neuronal presence, and relative epithelial thickness. Pilot microbiota characterization of the rat revealed unique distinctions in composition between the olfactory and respiratory systems.

**Conclusions.** Although many features of the evolutionarily ancient sense of olfaction are similar across taxa, discrepancies exist between species. These differences likely have wide-ranging impacts on epithelial development, Olfactory Sensory Neuron turnover, olfactory ability, and even the general health of the organism. Further studies will include comparative analyses of the microbiome of the nasal cavity across species and seek to determine how differences in microbial community structure in the OE influence olfactory function.

**Acknowledgements.** We would like to acknowledge the contributions of Dr. Gross and the faculty and staff of the Scott-Ritchey Research Center, Dr. Biancardi and the staff of the Biancardi Lab, and Dr. Akingbemi and the staff of the Akingbemi Lab. The financial support of laboratory start-up funding through Auburn University.

**Sleep as a tool: using chemogenetic activation of the Ventral Medial Midbrain/Pons to examine the effect of sleep on cognitive behavior**

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**Introduction.** Sleep is important for many bodily functions, but the underlying neural circuits regulating sleep/wake states are still poorly understood. A recent study by Takata and co-workers (2018) identified the Ventral Medial Midbrain/Pons (VMP) as a potential candidate area containing sleep-active neurons. Specifically, inhibitory GABAergic neurons in the VMP are thought to be active during sleep. Whether activation of these neurons induces restorative sleep or simply increases the quantity of sleep such as current pharmaceutical aids is unknown. For this study we use chemogenetics to specifically activate GABAergic neurons in the VMP of mice and perform an in-depth analysis of sleep-wake parameters, as well as behavioral testing to assess the effects of sleep on cognition.

**Methods.** We used chemogenetics to target sleep-active GABAergic neurons in the VMP. In short, we employed an adeno-associated virus (AAV) to transfect GABAergic neurons of the VMP in VGAT-cre transgenic mice, in which cre-recombinase is expressed in inhibitory GABAergic neurons, which then drive expression of the chemogenetic receptor (hM3Dq) in these neurons. After 3 weeks of viral expression and acclimation of the mice to the recording chambers, we activated the hM3Dq receptors with its exogenous ligand, Clozapine-N-Oxide (CNO), in varying concentrations (0.1, 0.3, 0.9 and 2.7 mg/kg) and recorded EEG/EMG signals for subsequent analysis of the quality and quantity of sleep. Specifically, we analyzed the percentages and durations of rapid eye-movement (REM sleep), non-REM (NREM) sleep and wakefulness.

**Results.** In a first step, we show that CNO administration, but not saline administration, causes an increase in c-Fos expression (c-Fos is a marker of recent neuronal activation) in VGAT neurons in the VMP, suggesting that chemogenetic stimulation activates these neurons. Moreover, we find that administration of CNO (2.7 mg/kg) increased NREM sleep over 200% as compared to saline administration during the first 4 hours. Similarly, REM sleep is increased during the same time period. Further analysis is required to determine exactly how sleep is increased through the activation of GABAergic neurons in the VMP.

**Conclusions.** Our results suggest that chemogenetic stimulation of hM3Dq receptors on GABAergic neurons in the VMP increased both NREM sleep and REM sleep, while simultaneously decreasing the amount of wake. We conclude that activation of GABAergic neurons in the VMP can be used as a model system to increase sleep in laboratory mice, to e.g. investigate the effects of sleep on cognitive behaviors in mice.

**Acknowledgements.** Kroeger lab, Auburn University - College of Veterinary Medicine

**Metagenomic analysis reveals associations between working memory and Bifidobacterium abundance in canine gut microbiome**

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**Introduction.** Working memory in non-human animals is defined as short-term memory for stimuli within a specific experimental trial or session. It is the least heritable among all cognitive traits in dogs, indicating potential roles of nongenetic factors. Existing studies investigating the link between gut microbiome and memory used client-owned dogs and detected only phylum-level differences, which suffered from confounding factors such as variable age, diet, breeds, housing, and body conditions with decreased statistical power.

**Methods.** To address these issues, we evaluated working memory performance in 27 dogs at the puppy (3-month), juvenile (6-month), and young adult (12-month) stages, and discovered profound variations in overall memory scores (OMS) using WGS metagenomic sequencing. The dogs were brought to a designated start position, and an experimenter placed a reward beneath one of two containers placed upside down. The handler then removed the dog from the room for the duration of the delay of 10 or 40 seconds. Upon return, the dog had 15 seconds to make a choice. The number of correct trials was recorded as the overall memory score (OMS). To ensure dogs were not using odor cues, an odor control test was performed. The sequencing library constructions were performed using NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). Finally, the pooled library was sent for sequencing on an Illumina NovaSeq6000 machine at 150-bp paired-end mode at Novogene (Novogene Corporation Inc., Sacramento, CA, USA). The alpha- and beta-diversity of taxonomy profiles were performed using the vegan in R. LEfSe (Linear discriminant analysis Effect Size) version 1.1.2 was utilized to discover the most featured orders, families, genera, and species between the high and low memory performance groups. Spearman's correlation test was performed on the OMS and the abundance levels of the taxa across all 80 measurements using the R software. Random forest model (Breiman 2001) was used to predict the working memory score.

**Results and Conclusions.** Linear discriminant analysis of shotgun metagenomic data from dogs that displayed low and high OMS (n=8 each) revealed that a single bacterial species in the gut microbiome, *Bifidobacterium* spp, is associated with high OMS (LDA score>3), which was subsequently confirmed with a significant correlation in all 80 samples using qPCR quantification ( $P<0.0001$ ). A total of 36 bacterial taxa with significant abundance differences in the high-OMS vs. low-OMS comparison and significant correlations with OMS were modeled by random forest regression, together with other factors. Age, litter, sex, and breed displayed little to no predictive value, and abundance of 17 bacterial taxa in the gut microbiome were highly predictive for memory performance (Spearman's rho=0.472).

**Acknowledgments.** This study is supported by a laboratory start-up fund from Auburn University College of Veterinary Medicine and Auburn University Intramural Interdisciplinary Team Research Grant 190559 to XW. XM is supported by an Elanco Animal Health Fellowship.

**Evaluation of Canine Platelet Lysate Antimicrobial Activities in Vitro**

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**Introduction.** Platelet lysate (PL) is an acellular platelet derived product which is rich in growth factors, cytokines, and chemokines among other molecules. Research has shown that topical application of PL accelerates the process of wound healing in canine. Additionally, PL holds antimicrobial properties, which could have a profound effect on the clinical management of bacterial infected wounds. However, the preparation process and composition of the final product can affect their antimicrobial potential. Our goal was to compare the antimicrobial activity of canine PL produced by two methods against bacteria that are often seen in canine wounds. Our hypothesis was that the leukocyte concentration in the final product as well as the presence of plasma and complement will affect bacteria growth *in vitro*.

**Methods.** Whole blood was collected from three healthy dogs. Platelets were separated using two distinct centrifugation methods. For the leukocyte-poor method (method A), whole blood was centrifuged at 1000g for five minutes followed by a second centrifugation at 1500g for 15 minutes. For the leukocyte-rich method (method B), whole blood was centrifuged at 180g for 20 minutes followed by a second centrifugation at 650g for 15 minutes. Platelet-Poor Plasma (PPP) was isolated after the second centrifuge in both methods which was considered as control group. Subsequently a portion of the generated Platelet-Rich Plasma (PRP) and PPP underwent plasma depletion (PD) by centrifugation at 3000g for 30 minutes. PPP, PPP-Plasma Depleted (PPP-PD), PRP and PRP Plasma Depleted (PRP-PD) pooled equally between three donors. PRP-lysate was generated from PRP and PRP-PD via five freeze-thaw cycles and complement was inactivated (HI) for a portion of PRP-lysate via heating at 56°C for 30 minutes. The same steps were taken for generating PPP-lysate, PPP-Lysate-PD and PPP-Lysate-HI. The antimicrobial effect of different platelet preparations was evaluated via a bacterial spiking assay against *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa* and *Methicillin-resistant Staphylococcus aureus* (MRSA) at 3 and 24 hours of incubation. The Log reduction was calculated based on the number of bacteria colonies cultured in Brain Heart Infusion (BHI) media. A difference of less than one log was considered to be of no significance relevance.

**Results.** PRP-Lysate produced with either method A or B resulted in a similar log reduction in *P.aeruginosa* at 3 hours. On the contrary, a higher log reduction (Method B, 1.77 vs. Method A, 0.99) was noted for PRP-Lysate produced via method B on *S. pseudintermedius* at 3 hours. PRP-Lysate produced with either method led to similar log reduction on *S. pseudintermedius* growth at 24 hours while PPP-Lysate from method B demonstrated higher log reduction (Method B, 3.28 vs. Method A, 2.61) at that time point. Although PRP-Lysate, PPP-Lysate and PPP-Lysate-PD from method B inhibited MRSA growth after 3 hours, all the treatment samples from both methods led to the same log reduction after 24 hours remarkably.

**Conclusions.** Our results show that canine PL has potent antibacterial action against bacterial strains that are often encountered in canine wounds. However, the generation method and presence of complement can affect these antimicrobial properties. Future studies evaluating the growth curve dynamics of bacteria exposed to platelet products as well as comparison with topical antibiotics used for canine wounds are needed.

**Acknowledgments.** Scott Fund, Scott-Ritchey Research Center, Auburn University College of Veterinary Medicine.

**Use of tigilanol tiglate in the treatment of perineal squamous cell carcinoma in a horse.**

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**Introduction.** Cancers of the skin account for approximately half of all equine neoplasms. The three most common forms of skin cancer are equine sarcoid, squamous cell carcinoma, and melanoma. Although each are distinct types of cancers with different etiologies and signalments, all are difficult to treat and often refractory to treatment. Recently, tigilanol tiglate (Stelfonate®), a medication licensed for the treatment of canine mast cell tumors, has been used in the treatment of a small equine sarcoid and small periocular squamous cell carcinoma. Tigilanol tiglate works by breaking down tumor cell walls, restricting blood supply to the tumor, as well as stimulating a local immune response to the tumor. The drug has not been used in the treatment of larger, aggressive squamous cell carcinoma. The case treated with tigilanol tiglate in this report was refractory to previous aggressive attempts of debridement, treatment with cisplatin injection, and electrochemotherapy.

**Methods.** The mare was sedated, administered 1.1 mg/kg flunixin meglumine, and a local ring block with mepivacaine hydrochloride was performed. The volume of tigilanol tiglate was calculated as the following: tumor volume [(height cm) x (width cm) x (length cm) x 0.5] x 0.35 based on previous report<sup>1</sup>. Six milligrams were injected into the tumor region using 23-gauge needles in a fanning motion to evenly distribute the drug throughout the tumor tissue. The larger nodular section of tumor was injected along the base of the tumor, approximately 50% of the tumor mass, to conserve volume of tigilanol tiglate utilized.

**Results.** Twenty-four hours following injection, the treated site became markedly inflamed and the tumor quickly developed a large necrotic region. Subsequently the local inflammation decreased, and the tumor started to shell out from the surrounding healthy tissue. Five days post injection the surrounding tissue began to develop healthy granulation tissue.

**Conclusions.** Few cases have been reported using tigilanol tiglate as a treatment for equine skin neoplasias. The necrotizing response of the tumor to injection with tigilanol tiglate was profound, despite not responding to previous therapies, showing great promise for future application in cases of equine skin neoplasia.

**Acknowledgments.** We would like to acknowledge Dr. Jarred Williams from the University of Georgia College of Veterinary Medicine for providing the dosing recommendations and consultation on this case.

**Citations.**

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 **$\beta$ -catenin blockade in the tumor microenvironment-conditioned macrophages promotes immunogenicity**

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**Introduction.** Melanoma cells activate Wnt/ $\beta$ -catenin signaling in the tumor microenvironment (TME) to promote tumor progression and suppress dendritic cell-mediated anti-tumor immunity. However, the role of  $\beta$ -catenin in macrophages and its effect on anti-tumor immunity is largely undefined.

**Methods.** In this study, using XAV939, a tankyrase inhibitor encapsulated in nanoparticles, we explored whether targeting  $\beta$ -catenin in macrophages can promote immunogenicity in TME. We used flow cytometry to analyze the macrophages' activation and inhibitory markers. Pro and anti-inflammatory cytokine levels were measured using Enzyme-linked immunosorbent assay (ELISA).

**Results.** Our data show that the co-culture of macrophages with melanoma cell supernatants or melanoma cells in the presence of XAV939 nanoparticles significantly promotes the expression of CD80, CD86, and suppresses the expression of PD-L1 and CD206 compared to macrophages treated with control nanoparticles. Similarly, XAV939 nanoparticle treatment of macrophages in co-culture conditions resulted in significantly increased TNF- $\alpha$  and IL-6 and reduced IL-10 production by macrophages compared to control groups.

**Conclusions.** Our data suggest that targeted inhibition of  $\beta$ -catenin in tumor-associated macrophages could overcome tolerance and promote anti-tumor immunity.

**Acknowledgments.** This study was supported by research funding from the Intramural Grants Program, Auburn University Research Initiative in Cancer (AURIC), and start-up funds from the Department of Pathobiology, College of Veterinary Medicine, Auburn University.



**Inhibiting mitogen-activated protein kinase kinase (MEK) signaling suppresses immune and inflammatory responses in macrophages and fibroblasts after HSV-1 infection**

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Herpes simplex virus-1 (HSV-1) is a very successful and highly prevalent human pathogen. Globally 3.7 billion people are estimated to be seropositive for HSV-1 infection. HSV-1 is a neurotropic virus that, after an initial lytic replication in the epithelial cells, enters sensory neurons and undergoes life-long latency in the trigeminal ganglion (TG). The clinical outcome of HSV-1 infection largely depends on the site of recurrent HSV-1 replication and the host's immune status. HSV-1, depending on the site of lytic HSV-1 replication, can cause herpes labialis, herpetic stromal keratitis (HSK), meningitis, and herpes simplex encephalitis (HSE). There is no cure for latent HSV-1 infection or successful vaccine to prevent new infections. The conventional therapeutic management of HSV-1 infections and associated immunopathologies relies on long-term anti-viral (acyclovir or ACV) treatments to suppress HSV-1 replication and corticosteroids to provide symptomatic relief from pain and acute inflammation. However, long-lasting anti-viral treatments are a significant risk factor for developing drug resistance and the emergence of ACV-resistant HSV-1 strains. Although corticosteroids effectively relieve symptoms, they also suppress protective anti-viral immune responses. Therefore, there is an unmet clinical need to develop novel, safe, and effective immunotherapies that selectively induce anti-viral responses and suppress inflammation to treat and prevent latent and recurrent infections caused by multi-drug-resistant HSV-1 strains. This study investigates the anti-viral and anti-inflammatory functions of Trametinib, an FDA-approved MEK inhibitor. HSV-1 infection through pattern-recognition receptor signaling activates MEK-extracellular signal-related kinase (ERK)1/2 pathway to induce anti-viral and inflammatory responses. Our data using *in vitro* (bone marrow-derived macrophages (BMDMs) and mouse corneal stromal fibroblast (MK/T-1) cells show that Trametinib treatment of HSV-1-stimulated BMDMs suppresses the expression of CD80 and CD86 (co-stimulatory ligands). Further, we also show that pre-treatment of HSV-1-infected BMDMs and MK/T-1 cells suppresses the production of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ . However, Trametinib did not significantly upregulate IFN-mediated anti-viral responses [IFN- $\alpha$ , Interferon stimulated genes (ISGs: MX1, ISG-15, OAS) mRNA expressions] in macrophages. In future studies, we are exploring the role of MEK in the induction of type I IFN responses during ongoing HSV-1 infection using a mouse model of corneal HSV-1 infection. Collectively, our findings suggest that MEK inhibition using Trametinib could suppress HSV-1-induced inflammatory cytokine production by macrophages and corneal fibroblasts.

**Humanizing Nanobody-Based ARC Platform for Cancer Immunotherapy**

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Biologics such as monoclonal antibodies (mAbs) and fusion proteins have emerged as a promising alternative to conventional cancer treatment options. However, initial biological therapeutics posed an immunological risk to human patients due to their xenogeneic origins. Previously, we have developed a novel canine-specific anti-cancer biologic that combines OX40 agonism and immune checkpoint blockade into a single therapeutic. Our novel Agonist Redirected Checkpoint (ARC) molecule employed an anti-PD-1 nanobody (Nbs) which binds and blocks the PD1 co-inhibitory T-cell receptor and the extracellular domain (ECD) of canine OX40L to stimulate the OX40 receptor. An isoleucine zipper (ILZ) domain was used to facilitate trimerization of OX40L, a requirement for binding and initiating signal transduction through the OX40 receptor. The canine ARC molecule successfully disrupted the PD-1/PD-L1 axis and initiated signal transduction of the NF- $\kappa$ B pathway via the OX40 receptor. Together, these lead to an enhanced anti-tumor immune response through T-cell expansion, survival, and effector function. In this study, it was our goal to determine if our canine ARC molecule would retain its biochemical and functional properties following the humanization process. To construct the humanized variant, canine OX40L was replaced with human OX40L, the ILZ domain was replaced with a TRAF2 trimerization domain, the canine Fc IgG(d) region was replaced with Fc region of human IgG4, and the camelid nanobody was partially humanized by introducing amino acid substitutions in framework 1, 3, and 4 of the variable domain. The genetic alterations for Hu-aPD1-Fc-OX40L were designed *in silico* and cloned into a pCDNA3.4 Topo vector. Hu-aPD1-Fc-OX40L was expressed and purified from Expi-293F cells using affinity (HiTrap Protein G) and size-exclusion chromatography. Hu-aPD1-Fc-OX40L demonstrated the ability to bind HEK 293 cells that stably express its cognate receptors, human PD1 and OX40. To determine if Hu-aPD1-Fc-OX40L could disrupt the PD1/PD-L1 axis, HEK 293-PD1 cells were pre-incubated Hu-aPD1-Fc-OX40L and, after washing, incubated with Zenon-647 labeled human PD-L1-Fc protein. Hu-aPD1-Fc-OX40L completely inhibited the binding of human PD-L1 to the PD-1 receptor. Using a luciferase Jurkat-based OX40 reporter cell line, Hu-aPD1-Fc-OX40L strongly displayed the ability to initiate signal transduction of the NF- $\kappa$ B pathway upon OX40 binding. Signal transduction was further enhanced when the humanized ARC molecule was co-incubated with OX40 effector cells in the presence of 293-PD1 cells. To sum up, the ability of our humanized nanobody-based ARC molecule to abrogate immunosuppressive checkpoint binding and initiate co-stimulatory T-cell signaling following humanization was confirmed. The evaluations herein will help us determine if the molecular adaptations made to the original ARC platform are suitable for designing future therapeutics for human cancer patients.

**Acknowledgments.** This study was supported by research funding from the National Cancer Institute (NCI-NIH). We would also like to thank Rie Watanabe for flow cytometry support

**Friction knot Terminology is Inconsistent in Veterinary Surgery**

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**Introduction.** Knots are an essential method of obtaining reliable hemostasis and secure closure of tissue layers in surgery. Friction knots other than the surgeons' knot are preferred by some surgeons to increase knot security by increasing tension. Common friction knots used include the constrictor, miller's, modified miller's, and strangle knots. The names of these friction knots may be unclear because of the origin of their use, as almost all knots are traced back to an occupation rather than being specifically developed for surgical use. There is a deficit of dependable resources concerning friction knots - published literature is not only sparse, but also inconsistent and incomplete. There is limited information available on instructions to tie these knots even in traditional texts used to teach surgery courses. This lack of consistency in instruction can lead to inaccurate knot tying or the use of a contraindicated knot. The purpose of this study was to determine what terms board-certified veterinary surgeons use for different friction knots. We hypothesized that a majority (>50%) of veterinary surgeons would call each knot configuration by the same name, but that there would be unfamiliarity with friction knot nomenclature.

**Methods.** An anonymous survey was developed using Qualtrics. Surgeons were recruited through email listings available on veterinary school websites and the ACVS website. Participation was also solicited through the ACVS Facebook page, the Society of Veterinary Soft Tissue Surgery, and the Veterinary Orthopedic Society. The survey included questions regarding 4 knot tying videos.

**Results.** Responses from 160 veterinary surgeons were received. The modified miller's knot and the miller's knot were correctly named by the majority of surgeons (61% and 63% respectively). There was no consensus for the constrictor knot, with the greatest number of respondents identifying it as the constrictor knot (38%) or the miller's knot (31%). There was also no consensus for the strangle knot, with respondents split between identifying it as the miller's knot (39%), modified miller's knot (34%), or strangle knot (21%).

**Conclusions.** There is apparent disagreement on the names of surgical friction knots among veterinary surgeons. A large number of participants indicated they are not familiar with the demonstrated knots. There are inconsistencies in knot terminology across the veterinary literature and among veterinary surgeons. This study can serve as a resource to improve consistency and familiarity with these knots.

**Acknowledgments.** The authors would like to thank Brad M. Matz, DVM, MS, DACVS-SA for his surgical expertise and review of the final version.



## Genome evolution of the microsporidian pathogen genus *Nosema*

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**Introduction.** *Nosema* is a diverse genus of microsporidian parasites, which are all unicellular, obligate fungal symbionts and pathogens of insects and other arthropods. *N. muscidifuracis* infects parasitoid wasp species of *Muscidifurax zaraptor* and *M. raptor* (Hymenoptera: Pteromalidae). In this study, we report high-quality assemblies (14,397,169 bp) of *Nosema muscidifuracis* genomes and comparisons to other *Nosema* genomes.

**Methods.** Using the combination of *de novo* prediction with TRIP pipeline and PacBio long-read sequencing technology, a novel telomeric repeat was determined in *N. muscidifuracis* genome. Gene prediction and functional annotation were performed by FunGAP pipeline. The sequence motif was predicted using MEME software in regulatory regions, and the presence of the motif in different expression genes was identified to investigate the potential function. Comparative genomic analysis was performed to construct the phylogenetic relationship between *N. muscidifuracis* and other microsporidia.

**Results.** A novel composite 4-bp (TAGG)<sub>n</sub> and 5-bp (TTAGG)<sub>n</sub> telomeric repeat motif was discovered at the ends of chromosomes, which represent the first identified telomeres for *Nosema* (and other microsporidia). In total, 2,782 protein-coding genes were annotated, of which 449 shared orthologous genes with six other genome-sequenced *Nosema* species. Comparative phylogenomic analyses revealed incongruency in the *Nosema* and host species trees, indicating a host switch event between parasitoid wasps and bees. In *N. muscidifuracis*, a highly significant ACCC motif was found within 20 bp upstream of the translation start codon ATG. This motif is present in 90% of highly expressed genes, in sharp contrast to ~20% in lowly expressed genes, and therefore serves as a candidate *cis*-element for positive regulation of gene expression. Strikingly, similar (C)<sub>3</sub> and (C)<sub>4</sub> motifs were also discovered in other distant related *Nosema* species, suggesting a conserved *cis*-regulatory mechanism. The unusually low GC-content (22.6%) drives a substantial A-T bias in the third codon position in protein-coding genes in *N. muscidifuracis*. Cytogenetic analyses revealed substantial *Nosema* load within the ovaries of *M. raptor* and *M. zaraptor*, consistent with a heritable component of infection and per ovum vertical transmission.

**Conclusions.** *Nosema* are widespread pathogens, including inducing epizootics in honeybees. The parasitoid-*Nosema* system is laboratory tractable, and therefore can serve as a model to inform future genome manipulations of *Nosema*-disease microsporidian pathogen as potential cures for this disease. Our study also provides novel insights into the genetic architecture, gene regulation, and genome evolution of *Nosema* species and will enhance the understanding of host-parasite interactions.

**Acknowledgments.** This project was supported by an Alabama Agriculture Experiment Station ARES Agriculture Research Enhancement, Exploration and Development (AgR-SEED) award, and USDA NIFA Hatch program (ALA05-2-18041).

**Developmental Exposure to Trichloroethylene (TCE) Alters Expression of DNA Methyltransferases in Zebrafish (*Danio rerio*)**

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**Introduction.** Trichloroethylene (TCE) is a significant legacy environmental toxicant due to its known adverse health effects. TCE is a volatile organic compound (VOC) and has been found at over half of the sites on the US EPA's National Priorities List. TCE has been linked to epigenetic alterations, but the extent of these alterations remains unclear. It is thought that TCE exposure, especially during developmental periods could alter DNA methylation status, resulting in epigenetic toxicity. This study tests the hypothesis that zebrafish exposed to TCE during a developmentally critical period have altered expression of DNA methyltransferases, which could explain the mode of action of epigenetic toxicity.

**Methods.** Zebrafish embryos were exposed to a static exposure of 0, 5, 50, or 500 parts per billion (ppb; µg/L) TCE from 1 to 120 hours post fertilization (hpf). Following the exposure, larval zebrafish were rinsed, pooled together, and homogenized. RNA was extracted and isolated using TRIZol and Qiagen RNeasy Mini Kits. The Invitrogen SuperScript First-Strand System was utilized to synthesize and the relative expression of seven DNA methyltransferase genes (*dnmt1*, *dnmt3aa*, *dnmt3ab*, *dnmt3ba*, *dnmt3bb.1*, *dnmt3bb.2*, and *dnmt3bb.3*) was evaluated on an Applied Biosystems QuantStudio 3 system. β-actin was used as a reference gene and six biological replicates were tested in triplicate according to MIQE guidelines.

**Results.** Of the seven genes analyzed, two demonstrated a significant change in expression. *dnmt1* had an increase in relative expression in the 5 and 50 ppb exposure groups, as compared to the controls (p=0.041). *dnmt3bb.2* had decreased relative expression in the 500 ppb exposure group as compared to the controls (p=0.042). All remaining genes of interest had no significant change in relative expression.

**Conclusions.** The results indicate that developmental exposure to TCE does change the expression of genes related to maintenance methylation and *de novo* methylation. Further investigation into the epigenetic effects of TCE exposure is warranted.

**Acknowledgments.** I would like to thank the Auburn University Institutional Grants Program, the Auburn University College of Veterinary Medicine Animal Health and Disease Grants Program, and the National Institutes of Health-National Institute of Environmental Health Sciences (R15 ES033361) for funding this research.

**Genome assembly, annotation, and genomic imprinting of the blackstripe livebearer *Poeciliopsis prolifica* shed light on fish placental evolution**

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**Introduction.** The placenta is a transient organ for nutrient transport and gas exchange between mother and fetus that performs essential functions in physiological, endocrine, and immune interactions at maternal-fetal interface. All individuals in diploid species have two copies of each gene, one inherited from each parent. Genomic imprinting is a phenomenon that turns one of the two copies off so that the individual is effectively haploid for that gene. Genomic imprinting has only been found in therian mammals, which coincides with evolution of the placenta, and placenta is a hotspot for genomic imprinting. In the fish family Poeciliidae, placentation has independently evolved at least nine times. We aim to investigate whether genomic imprinted is present in the fish placenta.

**Method.** Two inbred lines (line A and line B) were developed in Dr. Reznick's lab. We sequenced the genome of a single male in line A of *P. prolifica* using PacBio long-read sequencing and 10× Genomics linked-read sequencing and sequenced the transcriptome of juvenile whole-body, embryo and placenta of *P. prolifica* in line A and line B using Illumina sequencing. After trim, the PacBio reads and 10× Genomics linked-reads were assembled and merged to obtain the scaffold assembly. To achieve high-quality annotation, the final gene model was generated by merging the gene sets from MAKER annotation pipeline based on the transcriptome evidence and homology evidence and the GeMoM annotation pipeline based on the *Poecilia reticulata* genome, the closely related species to *P. prolifica*. For SNP and imprinting analysis, RNA-seq reads were mapped to our genome assembly and alignment BAM files were merged into a combined BAM using SAMtools. Exonic SNP positions were called using GATK and the effect of the SNPs was annotated using SNPEff.

**Results and Conclusions.** The final size of *P. prolifica* genome is 674,152,735 bp including 504 contigs in 415 scaffolds. The BUSCO completeness score against the Actinopterygii database is 97.2% with 4.6% duplicated BUSCO score. The contig N50 is 7.7 Mb, which suggests high quality for fish genomes. Based on the repeat-masked genome, we totally got 27,227 protein coding genes with a start codon and a stop codon after merging two gene sets and obtained 5549 non-coding RNA genes including tRNA, rRNA, snRNA, snoRNA, miRNA. The synteny analysis showed a high level of synteny between the genome of *P. prolifica* and *P. reticulata*. From the RNA-seq data of juvenile whole-body, embryo and placental samples, 485,487 exonic SNPs were identified, covering 19,337 genes with an average SNP count of 5.43 per gene, which provides a truly transcriptome-wide coverage for the inference of allele-specific expression. We were able to detect significant parent-of-origin effects in F1 hybrids between Line A and Line B, and validated four imprinted genes of interest. None of these genes are imprinted in mammals, yet they share the same general functions as those that are imprinted in mammals. The key functions include the modulation of growth and metabolism and the regulation of cell proliferation, adhesion and differentiation.

**Acknowledgments.** This project is supported by the National Science Foundation EPSCoR RII Track-4 award to XW (1928770), an Alabama Agriculture Experiment Station ARES Agriculture Research Enhancement, Exploration and Development (AgR-SEED) award.

**Transcriptome landscape of the canine zonary placenta**

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**Introduction.** The mammalian placenta is an organ connecting the mother with the developing conceptus in utero. Despite its critical role in conceptus survival and growth, the placenta is the most morphologically diverse organ in mammals. The dog has a unique zonary placenta, which is a belt-shaped structure surrounding the fetus. Characteristic structures include the transfer zone (TZ) and pigmented zone (PZ), which are unique features compared to other placental forms highlighted in reproductive biology textbooks. However, the functions and gene expression profiles in these distinct zones have not been explored. This research aims to characterize the full-length transcriptomes and functional enrichment of gene networks in different placental tissues of the canine zonary placenta.

**Methods.** Canine placental tissue samples on the rough side and smooth side of transfer zone (TZr and TZs), the pigmented zone (PZ), allantochorion (AC) and umbilical cord (UC) (total N=28) were dissected from gestation day 60 term placenta immediately after birth through C-sections. Total RNA samples were extracted for Illumina stranded RNA sequencing. Filtered reads were mapped to the dog CanFam3.1 genome reference using TopHat. Then differential gene expression and functional enrichment analyses were performed to explore functions of different tissues of the canine placenta. We also employed PacBio HiFi sequencing to characterize the full-length cDNA transcripts of AC, PZ, and TZ of one male and one female dog respectively (total N=6).

**Results.** Over 14,700 expressed genes were detected in the RNA-seq data in all four tissue types. Comparisons showed that TZr and TZs had almost identical transcriptome profiles, suggesting there is no functional differentiation between maternal and fetal sides, and they were combined as a single group (TZ) in subsequent analysis. Compared to PZ, there are a total of 249 upregulated genes in TZ (logFC>2 and FDR<0.01, same below) and they are enriched in multiple transmembrane transport functions (14 significant terms, FDR<0.05) and vasculature development and angiogenesis pathways. In sharp contrast, upregulated genes in PZ are only enriched for one transport function, which is ion transmembrane transport. The majority of PZ-enriched genes are related to nervous system development, neuron differentiation, and synaptic signaling (11 significant terms). Overexpressed genes in AC (N=1493, compared to TZ) are enriched for immune functions.

**Conclusions.** We discovered drastic functional specialization among different placental tissues inside zonary placenta, which provides new insights into canine placental biology.

**Acknowledgements.** This research is supported by an Auburn University Intramural Grant Program and an NSF award to XW (1928770). YZ, XX, WC are supported by the Auburn University Presidential Graduate Research Fellowship and College of Veterinary Medicine Dean's Fellowship. XM is supported by an Elanco Animal Health Fellowship.



## Faculty/Staff Poster Presentations

### Effect of long term, repeat administration of Clodronate disodium (OsPhos®) on bone turnover in horses

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**Introduction.** Bisphosphonates (BP) inhibit bone resorption, suppress bone turnover, and interfere with bone remodeling in humans. BP use in equine practice is increasing, yet concern remains regarding safety. The objectives of the study were to evaluate blood-based biomarkers of bone resorption and formation in horses and evaluate changes in bone quality due to bisphosphonate administration. Repeat administration of clodronate disodium (Osphos®) to horses will decrease bone cell activity overtime.

**Methods.** Eight adult horses were treated with either saline (Control, n=3) or clodronate disodium (BP, Osphos® 1.8 mg/kg IM) every 3 months for 12 months. Blood pulled every 3 months was measured for biomarkers of bone formation (osteocalcin and N-terminal propeptide of type 1 procollagen, P1NP) and bone resorption (tartrate resistant acid phosphatase, TRAP-5b and C-terminal telopeptide, CTX). Bone biopsy was performed at 0 and 12 months for histomorphometry and FTIR.

**Results.** There was a significant decrease CTX, but no difference osteocalcin, and P1NP were observed in the BP group over time. There was a slight trend for TRAP5b to increase over the study period. When data were analyzed with AI, a significant difference in TRAP5b was observed at 3 months that was maintained throughout the study period when compared to the control group. A small difference was also observed with P1NP. Histomorphometry showed no difference between groups.

**Conclusions.** Long-term, repeat administration of BP (clodronate disodium) in horses caused a reduction in only one marker of bone resorption, CTX and no change in markers of bone formation. Therefore, we conclude there is little change to bone quantity following long-term repeat administration of BP in horses.

**Acknowledgments.** Funding for this study was provided by the HHMA Foundation. We would like to thank Dr. Sandra Zetterström for her assistance with initial sampling and Ms. Jessica Brown for her technical assistance.



**Translating Time: an online tool for biomedical sciences**

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Many researchers use model systems to make inferences about biological processes or dysfunctions in humans. To bridge the gap across these systems, we created an online resource called Translating Time ([www.translatingtime.org](http://www.translatingtime.org)). This online tool equates corresponding ages across humans and 19 mammalian model systems, including several species of rodents, carnivores, and primates. Here, I discuss past methods used to find corresponding ages across species. I also discuss new research where we employed an integrative approach that uses machine learning tools to compile 573 time points across select species. Specifically, we captured time points from temporal variations in gene expression, anatomy, and behavior across humans and some nonhuman primates to map ages across the lifespan. The Translating Time resource has many applications. For instance, our resource can be used to predict biological processes that are challenging to study in key species. I discuss how we can use data on hippocampal neurogenesis from multiple model systems to predict the decline in human hippocampus neurogenesis at postnatal ages. Collectively, these examples emphasize the importance of integrating results from a variety of model systems to address issues in biomedical research, which benefit human and animal health.

**Acknowledgments.** This work was supported by an INBRE pilot grant from NIGMS (P20GM103446) to [C.J.C], an R21 from NICHD (R21HD101964) to [C.J.C], and NIGMS COBRE (5P20GM103653).

**Prediction of tissue-homing peptides in a mouse model using a machine learning classification model**

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**Introduction.** Landscape phage display libraries have been extensively studied for their ability to generate unique molecular landscapes across their surface based on a displayed fusion peptide. Phage libraries have been used to identify peptide ligands interacting with different mammalian cell targets *in vitro* and *in vivo* in a specific and selective manner. Based on our previous study of a landscape phage library in mice, we discovered comprehensive panels of trimer peptides enriched within specific tissues following intravenous injection of a random clone library. From these data, we hypothesized that trimer elementary binding units (EBUs) would accumulate within a single displayed peptide and evolve into functional Short Linear Motifs (SLiMs) interacting with protein domains of physiologically relevant proteins. Here, we extend our previous work to study the evolution of displayed 9-mer peptides and discover motifs responsible for tissue-specific accumulation of phages.

**Methods.** In this study, we examined the evolution of random 9-mer peptides displayed on the landscape phage library to migrate to different tissues in a mouse model. Nu/nu mice with or without an implanted MDA-MB-231 human breast cancer xenograft were injected via tail vein with different doses of the f8/9 phage library. Mice were euthanized and tissues recovered to extract the DNA and infectious phage particles accumulating within each tissue. Peptide structures for each tissue were determined using p8-targeted amplicon sequencing. Sequence reads were quantified and normalized according to the number of transcript-reads per sample. Resulting peptide libraries were split into training and testing datasets for development of a supervised machine learning model to predict tissue accumulation.

**Results.** Translated NGS data for each mouse and target tissue over two different time points were aggregated into a summary table for each peptide based on the number of unique transcripts quantified per million sequence reads. Overall, 4.8 million different peptide sequences were analyzed. To reduce the number of peptide sequences, single occurrence reads were removed from the analysis leaving 42,559 peptide sequences that were clustered into families using CD-Hit clustering software. Approximately 39.0% (16,615/42,559) peptides were clustered into a corresponding peptide family. Peptide families were scored for their average correlation to quantify the similarity of peptides within each family. Given the number of peptides analyzed was limited to those recovered in the analysis, we developed a machine learning classification model to predict the tissue distribution of previously unseen peptides.

**Conclusions.** We demonstrate a proof-of-concept framework for constructing a machine learning classification model to discover novel peptide sequences that may be enriched within specific tissues. Accumulation of peptides discovered using this classification model will need to be further studied to determine their utility for development of novel nanomedicines. We envision that new models to predict distribution of peptides would incorporate gene expression data.

**Acknowledgments.** Project funding was provided by an Auburn University Research Initiative in Cancer (AURIC) Major Grant Program (MGP) to V.A.P. We are grateful for the support of the Auburn University Easley Cluster and the Massachusetts General Hospital (MGH) Next Generation Sequencing Core for their assistance with this work.

**Chemical Characterization of Detection Canine Training Aid Volatile Headspace using Proton Transfer Reaction-Time of Flight-Mass Spectrometry**

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**Introduction.** Current detection methods of pathogenic threats are limited in their ability to readily and non-invasively identify widespread biological agents. While canine employment in defense-related detection is well-established, training for biological targets presents unique challenges. The risk of handling infected materials possibly resulting in pathogenic outbreak limits the availability of such materials for use in canine training outside of controlled facilities. Furthermore, the chemical composition of volatilomes associated with disease and microbes are not well understood due to the metabolic complexity of cellular interactions. Previous work introduced the use of polymer-based odor capture and release (POCR)<sup>™</sup> training aids to deliver true odor composites of viral-based biological materials without contamination risk and demonstrated canine generalization from training to operational phases. Building upon this concept, current work aims to supplement canine discriminatory performance in POCR<sup>™</sup> systems with chemical analysis of training aids to determine volatile biomarkers that may be indicative of positive alerts. Through a real-time, soft-ionization method of mass spectrometry known as Proton Transfer Reaction Mass Spectrometry (PTR-MS), sample headspace is analyzed for characterization of volatile composition and dissipation over time. We aim to develop odor signatures that are complimentary to detection performance to advance canine and instrument-based biosensor technology.

**Methods.** This multidisciplinary work incorporates foundational phases of both laboratory and canine-based tasks. Initial POCR<sup>™</sup> task learning begins with non-relevant odors such as a detection calibrant compounds (DCC) and distractors. POCR<sup>™</sup>s were prepared with a polymer-based mixture followed by polymerization, heat treatment, and charging with aqueous samples. A series of dilutions were prepared from 10<sup>-3</sup>-10<sup>-12</sup> DCC in mineral oil. All concentrations of DCC-1 were analyzed by PTR-MS for optimization and development of background odor profile. We are using a PTR-MS 1000 Ultra (Ionicon Analytik). The sampling apparatus includes glass jars with rubber septa through which the inlet line and VOC-free compressed air line are pierced for sampling and intersample cleaning.

**Results.** Preliminary stages have focused primarily on PTR-MS method optimization to gather background VOC signatures of POCR<sup>™</sup> training aids and initial task training DCC-1. Initial sampling of aqueous and gas phase DCC-1 charged for either 1 or 24 hours resulted in a selection of masses associated with background materials (glass jar, POCR<sup>™</sup> material), matched blank diluent (mineral oil), and distractor compounds, but the chemical nature of DCC-1 does not react with hydronium or oxygen ion sources. However, charging time inversely correlated with intensity of selective peaks by an average difference of 0.5 ppbv. Uncharged POCR<sup>™</sup>s produced spectra at masses 105, 107, 369, and 388 amu. Mineral oil was associated with mass peaks at 59, 73, and 149 amu. The most concentrated dilution of DCC-1 at 1x10<sup>-3</sup> resulted in reproducible peaks at 131, 69, and 75 amu.

**Conclusions.** Background VOCs were readily identified and associated with materials such as the glass jar and the POCR<sup>™</sup> material or the diluent. This is important for sample control and odor signature relevance modeling. The most concentrated dilution of DCC-1 at 1x10<sup>-3</sup> resulted in reproducible peaks consistent with referenced gas chromatography-mass spectrometry spectra for 1-bromoperfluorooctane using electron ionization. Additional analyses are needed for specific chemical identification of collected masses. However, by characterizing the training aid material for culture and sample-based charging, this allows for subtraction of background and reduction of spectral noise. As the canine cohort progresses to the next task training phase, the next steps of this work will include preparation and analysis of the next DCC and advancement into viral-based samples complimenting the prior viral-based POCR<sup>™</sup> studies.

**Acknowledgments.** This research is funded by the United States Department of Agriculture G00016151.

**Case series: Surgical success and reproductive performance for correction of penile deviations in 10 bulls**

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**Introduction.** Information regarding the expected outcomes, such as surgical complications and successful return to natural service, of surgery to correct penile deviation of bulls are limited. Therefore, making evidence-based recommendations and managing client expectations regarding this condition is difficult for veterinarians. The objective of this study is to provide retrospective outcomes of return to reproductive performance of bulls after surgery to correct penile deviation.

**Methods.** Medical records at 4 teaching hospitals from 2008 to 2022 were reviewed. Cases from 3 teaching hospitals and results of owner surveys were included in the study. The outcomes of bulls impotent due to a spiral or ventral deviation of the penis were evaluated by performing a breeding soundness examination after surgery or a phone interview with the owner at least 1 year post-operatively. The surgery was considered successful if the bull's penis grossly appeared to no longer deviate to a degree that would prohibit intromission during natural service. Reproductive success was defined as the ability of the bull to produce a pregnancy in 1 or more cows via natural service after surgery. The owner's satisfaction with the surgery considered positive if the owner would request the surgery to be performed again on another similarly affected bull, or negative if the owner would choose to cull another similarly affected bull of the same value. Of the 28 bulls diagnosed with a deviation, only 10 bulls, from 3 of the veterinary teaching hospitals, had sufficient follow-up information to be reported in the study.

**Results.** The bulls' age, at presentation, ranged from 3 to 5 years old. Seven of the bulls had a spiral deviation, and 3 bulls had a ventral deviation. None of the bulls experienced surgical complications. Seven of the bulls' deviations were corrected following surgery (i.e., considered a surgical success). In 5 of the 7 cases in which surgery was successful, the bull post-surgery sired pregnancies via natural service. All owners except one stated that they would have the surgery performed on a future bull if they had a valuable bull that was impotent because of a penile deviation.

**Conclusions.** Overall, the retrospective data presented here provides valuable insights into the surgical and reproductive outcomes of bulls undergoing surgery to correct a penile deviation. Surgical correction of a penile deviation of a bull appears to have a low risk for complications but the success of surgery in returning the bull to reproductive soundness is inconsistent.

**Acknowledgments.** Authors greatly thank Dr. Jim Schumacher, Mary Passmore, Simon Taylor, and Dr. Adrienne DiFoggio for their contributions in either manuscript reviewer or towards acquiring owner follow-up.

**Heritable anophthalmia/microphthalmia in a marsupial biomedical model**

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**Introduction.** Anophthalmia and microphthalmia (A/M) are conditions in which the eye is absent from the orbit or exceptionally small, respectively. It is estimated that between 1 and 3 out of every 10,000 births in the United States are affected by A/M. The A/M phenotype can be further categorized as true/complete anophthalmia, clinical anophthalmia, or microphthalmia, with completely missing the eye being extremely rare. The grey, short-tailed opossum, *Monodelphis domestica*, is an established marsupial model for biomedical research. A laboratory opossum colony is maintained at Auburn University College of Veterinary Medicine (AUCVM), and ten cases of A/M have been identified within this colony. Our aim is to understand the biological mechanisms of A/M and establish a new genetic model for human anophthalmia.

**Methods.** Mating of the affected individuals with female carriers resulted in offspring with an increasingly severe presentation of A/M, with clinical anophthalmia being observed. Comprehensive ophthalmic examinations and ocular ultrasound were performed for all affected individuals, confirming the absence of eyeballs and lens. Histopathological analysis was performed for N=4 individuals, including N=3 affected individuals and N=1 unaffected control individual. Using the information gathered in ophthalmic examinations, ocular ultrasound, and histopathological analysis, a pedigree was constructed to determine the mode of inheritance. Whole-genome resequencing of N=8 affected, carrier, and unaffected individuals has been completed, and the data analysis is underway.

**Results.** The results obtained from comprehensive ophthalmic examinations, ocular ultrasound, and histopathological analysis confirmed true anophthalmia in one orbit of each affected individual. The phenotype of the contralateral eye in affected individuals varied, ranging from unaffected to extreme microphthalmia. Additionally, histological analysis of other major tissues/organs was performed and provided confirmation that no other systemic defects are present, indicating true, non-syndromic anophthalmia in the AUCVM *Monodelphis domestica* colony.

**Conclusions.** All affected individuals have true anophthalmia in one eye, with the contralateral eye phenotype being unaffected, extreme microphthalmia, or retinal degeneration. Pedigree analysis revealed an X-linked mode of inheritance for this true, non-syndromic anophthalmia phenotype. A search of the OMIM (online Mendelian Inheritance in Man) database revealed there are currently no identified causal genes for such anophthalmia that follow an X-linked recessive pattern of inheritance. The AUCVM *Monodelphis domestica* colony provides a unique opportunity to study true, non-syndromic anophthalmia with an x-linked recessive mode of inheritance.

**Acknowledgments.** This study is supported by a laboratory start-up fund from Auburn University College of Veterinary Medicine.