

Comparative and Experimental Medicine Research Symposium

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Abstract Proceedings

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1. Optimizing seeding density for MC3T3-E1 precursor osteoblast cells

Austin J. Bow, Jake Samsel, David Anderson, Madhu Dhar

Comparative and Experimental Medicine (Bow, Anderson, Dhar), Biochemistry and Cellular and Molecular Biology (Samsel), Large Animal Clinical Sciences (Anderson, Dhar)

Determination of an optimal cell seeding density to an adherable surface is vital to promoting healthy growth and proliferation of cells during experimentation. In evaluating the optimal seeding density for 3T3-E1, a mouse precursor osteoblast cell line, the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was used. A proliferation assay was performed on a range of seeding densities over a set period of time. In the presence of metabolically active cells, the reagent produced a formazan product, which was then analyzed by taking absorbance readings at 490 nm. Readings exceeding the control (only media) denoted cell presence, while an increase in absorbance readings between separate time points indicated cell proliferation. The MTS assay therefore affords a definitive means of assessing cell viability and proliferation relative to the readings taken at the first time point. Optimal density readings presented as a linear increase in output readings over time points without values plateauing at later stages. In addition to evaluating seeding densities of 5, 10, 20, 40, and 60 thousand cells over a 21-day period using the MTS assay, a supplementary staining analysis with calcein-acetoxymethyl (Calcein-AM) was also performed. The staining agent was readily transported into the cytoplasm of live cells and fluoresces green, allowing for visual confirmation of cell viability and proliferation. Despite MTS assay and live/dead staining being common, well-documented methods for observing cell viability and proliferation, evaluating their efficacy in analyzing cell adherence and proliferation on a biomaterial is crucial to the development of accurate characterization experiments for that material.

2. Xenogeneic implantation of equine synovial fluid-derived mesenchymal stem cells promotes cartilage regeneration in a rat subchondral defect model

Mohammed Zayed, Steven Newby, Nabil Misk, Robert Donnell, Madhu Dhar

Large Animal Clinical Sciences (Zayed, Misk, Dhar), Comparative and Experimental Medicine (Newby, Dhar), Biomedical and Diagnostic Services (Donnell)

Recent reports suggest that synovial fluid-derived mesenchymal stem cells (SFMSCs) may have the potential to aid in the regeneration of cartilage defects. The purpose of this study was to evaluate the potential value of SFMSCs for cartilage repair in a standardized and established rat subchondral defect model. We hypothesized that equine SFMSCs have chondrogenic potential for cartilage tissue engineering, which can be evaluated using this rodent model in a time- and cost-effective manner. To prove our hypothesis, 3.0×10^6 SFMSCs were labeled for cell tracking by fluorescent CM-Dil, and then loaded onto agarose scaffolds for implantation. Under anesthesia, 2 mm diameter, full-thickness articular cartilage defect was created in trochlear grooves of the distal femur using a 2-mm power drill. The cells and scaffold constructs were then implanted into the defect. In each animal, the left knee was treated, whereas the contralateral joint was used as a control and was filled with the scaffold (agarose) only. At 12 weeks after treatment, knee joints were first evaluated macroscopically and then, histologically by hematoxylin and eosin, and Masson's trichrome stains and by immunofluorescence for collagen type-II protein. The macroscopic and histological scores were better in the SFMSCs group than in the control. The Dil fluorescence of cells was observed in specimens 1 week after treatment. In conclusion, our data suggest that equine SFMSCs maybe a viable option for tissue engineering in chondral defects. Future studies to investigate tissue and species specificities, optimal cell numbers, and scaffolds are needed.

3. Cytotoxicity resulting from use of hydrogen peroxide gas for sterilization of carbon-based biomaterials

Richard Steiner, David Anderson, Madhu Dhar, Alex Biris

Steiner (Comparative and Experimental Medicine), Large Animal Sciences (Anderson, Dhar), Center for Integrative Nanotechnology Sciences, University of Arkansas at Little Rock (Biris)

Peripheral neuropathies are a problem in the medical field and diminish the overall quality of life for patients. The use of biomaterials to regenerate damaged peripheral nerve presents a possible solution to healing severely damaged peripheral nerve segments. Previous studies have shown biomaterial characteristics of carbon-based materials (graphene, graphene oxide), indicating their potential in applications for regenerating damaged peripheral nerves. Previous studies have also shown that biomaterials crosslinked with biocompatible polymers such as PEG can improve the regenerative properties of biomaterials. Our studies focus on the assessment of the in vitro capability of novel carbon-polymer biomaterial designs as candidates for regeneration of damaged peripheral nerves. Specifically, graphene-based materials crosslinked with PEG polymers will be studied for suitability as a biomimetic peripheral nerve graft. As a first step, we designed a study to assess the ability of graphene-PEG biomaterials to support an immortalized neural cell line (PC12). Initial studies revealed un-adhesion and rapid cytotoxicity with cell death. This led us to perform a systems analysis of the components, steps, and protocols associated with the in vitro system. System analysis revealed that the use of hydrogen peroxide gas to sterilize the biomaterials immediately prior to cell culture experiments resulted in generation of cytotoxic radicals. Additional studies are required to more fully assess the factors associated with cytotoxicity and determine protocols for safe and effective sterilization of carbon-based biomaterials. This may have a profound effect on future manufacturing processes and hospital protocols for preparation of graphitic scaffolds for implantation in the body.

4. Comparison of methods for determining prevalence of *Macrorhabdus ornithogaster* in a flock of captive budgerigars (*Melopsittacus undulatus*)

Patrick J. Sullivan, Cheryl Greenacre, Edward Ramsay, Michael Jones

Small Animal Clinical Sciences

Macrorhabdus ornithogaster, known as avian gastric yeast, is a yeast organism that infects multiple species of birds, both captive and wild, although it appears to disproportionately affect Australian psittacine species. Diagnosis in vivo has historically proven difficult, as shedding can be intermittent, and not all positive animals are clinically ill. Ill animals can show a myriad of symptoms, including regurgitation, loose stool with undigested food material, marked weight loss, and acute death. In this study we compared fecal gram's staining (FGS) and polymerase chain reaction (PCR) of cloacal swabs to evaluate prevalence of active shedding of *M. ornithogaster* in a captive flock of budgerigars (*Melopsittacus undulatus*). Of the 100 birds sampled, 34 were positive for *M. ornithogaster* by FGS and 57 were positive by PCR. All FGS-positive results correlated to a positive PCR, which helped to act as an internal control for this study. Based on the PCR results, the prevalence of *M. ornithogaster* shedding in this flock was 59.6%. One bird that had previously tested negative on both FGS and PCR, and found to be in good body condition at sampling, was found dead 26 days post testing. The patient was emaciated, and necropsy revealed abundant *M. ornithogaster* at the isthmus, as well as marked koilin disruption with multifocal to coalescing lymphoplasmacytic and granulocytic infiltrates and gram-positive bacterial colonization. Cloacal swab PCR is a more sensitive predictor of the presence of *M. ornithogaster* compared to FGS in budgerigars, but negative PCR does not appear to preclude infection.

6. Associations among perceived motor competence, motor competence, physical activity, and health-related physical fitness of children ages 10–15 years old

Emily M. Post, Eugene C. Fitzhugh, Jeffrey T. Fairbrother, Dawn P. Coe
Kinesiology, Recreation, and Sport Studies

The purpose of this study was to examine the associations among perceived motor competence (PMC), motor competence (MC), physical activity, and health-related physical fitness during middle childhood and early adolescence. Participants were 47, 10–15-year-old youth. Each participant completed two visits in East Tennessee or northwest Ohio. During these visits, the participants completed the Bruininks-Oseretsky Analysis Test for Motor Proficiency (BOT-2), Harter's PMC questionnaire, and the FITNESSGRAM battery for health-related physical fitness. The Actigraph GT3X+ accelerometer was used to measure physical activity. There were significant associations among health-related physical fitness and both motor percentile ($r_s=0.44$, $P<.01$) and PMC ($r_s=0.32$, $P<.05$). A relationship was found among PMC and MC ($r_s=0.47$, $P<.05$). There were no significant associations among average daily MVPA and any of the other variables. High MC and PMC appear to be associated with higher levels of health-related physical fitness. It is important for children to learn fundamental motor skills to possibly participate in more complex motor skills related to physical fitness and for children to be encouraged in a positive manner while participating in physical activity to possibly increase their PMC.

7. Effect of fentanyl on the minimum infusion rate of propofol preventing movement in dogs

Carrie Davis, Reza Seddighi, Christine Egger, Sherry Cox, Xiaocun Sun, Thomas Doherty
Small Animal Clinical Sciences (Davis, Egger), Large Animal Clinical Sciences (Seddighi, Doherty), Biomedical and Diagnostic Sciences (Cox), Office of Information and Technology (Sun)

Propofol is commonly combined with fentanyl for total intravenous anesthesia. The study objective was to determine the effect of fentanyl on the minimum infusion rate (MIRNM) of propofol preventing movement in response to noxious electrical stimulation in dogs. Six male beagles (12.6 ± 0.44 kg) were studied on three occasions, at weekly intervals, using a randomized crossover design. Dogs were pre-medicated with 0.9% saline, fentanyl $5 \mu\text{g} \cdot \text{kg}^{-1}$ or fentanyl $10 \mu\text{g} \cdot \text{kg}^{-1}$, administered IV over 5 min. Anesthesia was induced with IV propofol as a loading dose ($2 \text{mg} \cdot \text{kg}^{-1}$) over 15 sec followed by $1 \text{mg} \cdot \text{kg}^{-1}$ every 15 sec until endotracheal intubation was possible. Anesthesia was maintained initially with a propofol continuous rate infusion (CRI) of $0.5 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (P), or propofol $0.35 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with low-dose fentanyl $0.1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (PLDF), or propofol $0.3 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with high-dose fentanyl $0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (PHDF). Propofol MIRNM determination was initiated after 90 min, and the CRI was increased or decreased by $0.025 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ based on whether the response was positive or negative. Data were analyzed using a mixed-model ANOVA. Mean \pm SE propofol MIRNM was 0.60 ± 0.04 , 0.29 ± 0.02 , and $0.22 \pm 0.02 \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for P, PLDF, and PHDF, respectively. Treatments PLDF and PHDF decreased ($P<.05$) propofol MIRNM by $51 \pm 3\%$ and $62 \pm 2\%$, respectively. Fentanyl, at the doses studied, caused statistically significant and clinically important decreases in the MIRNM of propofol.

8. Vesico-preputial anastomosis for treatment of obstructive urolithiasis in small ruminants

Elizabeth Erin Cypher, Sarel R. van Amstel, Ricardo Videla, Kyle Force Clark, David E. Anderson
Large Animal Clinical Sciences

The objective of this clinical case series was to describe a novel surgical technique for the treatment of obstructive urolithiasis in four castrated male goats. Medical records of male goats having undergone vesico-preputial anastomosis (VPA) as a treatment for obstructive urolithiasis were reviewed for history, signalment, clinical signs, and intra- and post-operative complications. Long-term follow-up was obtained by telephone interviews with owners or by clinical examination. Four castrated male goats underwent VPA for the surgical correction of obstructive urolithiasis. All goats

had undergone at least one surgical procedure (mean 2.5 ± 1.5) to correct obstructive urolithiasis before undergoing VPA. Post-operative complications included premature removal of the tube from the bladder (1), bacterial cystitis (2), and infection caudal to the surgical site (1). One goat suffered stricture of the anastomosis site 3 months following the original procedure, and 1 goat died 7 months after surgery due to severe, acute hydronephrosis and renal failure. Long-term survival was good with 3/4 (75%) having unobstructed urination 8 months following surgery. Vesico-preputial anastomosis should be considered a surgical option for the correction of recurrent obstructive urolithiasis in male small ruminants.

9. Evaluation of the effect of oral omeprazole on serum electrolytes, cobalamin, and bone mineral homeostasis in cats

Emily N. Gould, Casey Clements, Ann Reed, Luca Giori, Jörg M. Steiner, Jonathan A. Lidbury, Jan S. Suchodolski, Mabre Brand, Tamberlyn Moyers, Lee Emery, M. Katherine Tolbert
Small Animal Clinical Sciences (Gould, Clements, Reed, Giori, Brand, Moyers, Emery, Tolbert), Texas A&M University, Gastrointestinal Laboratory, College Station, TX (Steiner, Lidbury, Suchodolski)

Chronic proton pump inhibitor administration has been associated with electrolyte and cobalamin deficiency, disrupted bone homeostasis, hypergastrinemia, and rebound acid hypersecretion in humans. It is unknown if this occurs in cats. Prolonged oral omeprazole resulted in altered bone mineral density or content, serum calcium, magnesium, cobalamin, and gastrin concentrations in six healthy adult DSH cats. In a within-subjects, before and after design, cats received placebo followed by omeprazole (0.83–1.6 mg/kg, PO q 12 h) for 60 days each. Analysis of serum calcium, magnesium, cobalamin, and gastrin concentrations was performed on days 0, 30, and 60. Bone density and content were evaluated on days 0 and 60 of each intervention. Continuous data were analyzed using a two-way ANOVA ($P=.006$). On day 60 of omeprazole administration, continuous intragastric pH monitoring was performed in two cats to evaluate the effects of abrupt withdrawal of omeprazole. No significant changes were detected between treatments for any variables, except serum gastrin, which was significantly higher during omeprazole treatment in comparison to placebo ($P=.002$). Evidence of gastric hyperacidity was seen in both cats in which intragastric pH monitoring was performed following cessation of omeprazole. Although further studies with larger populations of cats will be needed in order to draw any definitive conclusions, these preliminary results suggest that prolonged PPI treatment results in hypergastrinemia, and abrupt PPI withdrawal may result in rebound acid hypersecretion in cats.

10. Comparison of penetrating vs. non-penetrating captive bolts in horned goats

Sam Collins, Marc Caldwell, Silke Hecht, Brian Whitlock
Large Animal Clinical Sciences (Collins, Caldwell, Whitlock), Small Animal Clinical Sciences (Hecht)

The objective of this study was to use magnetic resonance imaging (MRI) and computed tomography (CT) to compare the amount of tissue damage (both soft tissue and bone) to the head of goats after non-penetrating and penetrating captive bolt administration. Twelve 1–5-year-old, mixed breed goats were euthanized with an overdose of pentobarbital as part of an unrelated study. A captive bolt device with an interchangeable non-penetrating and penetrating attachment was used to impact 12 goat cadavers on midline over the external occipital protuberance. The heads were then removed, and CT and MRI studies were conducted on each head. One animal was imaged prior to captive bolt administration to serve as a normal control. Following imaging, each skull was transected along the sagittal plane to permit gross evaluation of central nervous tissue and obtain digital photographic images. In addition, a specimen receiving a non-penetrating captive bolt was further evaluated via blunt dissection and removal of adnexa from the external surface of the calvarium. Based on MRI, CT, and dissection of goat skulls, severe skeletal and soft tissue damage was induced after impact with both the penetrating and non-penetrating captive bolt devices. MRI, CT, and gross dissection were used to suggest that the non-penetrating captive bolt causes similar

tissue damage to the cranium and soft tissues of the head in goats as the penetrating captive bolt. This damage suggests that the non-penetrating captive bolt may be an acceptable method of euthanasia in goats.

11. Gadoxetate disodium (Eovist) contrast-enhanced magnetic resonance imaging characteristics of hepatocellular carcinoma in dogs

Chase Constant, Silke Hecht, Linden E. Craig, Cassie N. Lux, Claire M. Cannon, Gordon A. Conklin
Small Animal Clinical Sciences (Constant, Hecht, Lux, Cannon, Conklin), Biomedical and Diagnostic Sciences (Craig), Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota (Cannon)

Hepatocellular carcinoma is the most common primary hepatic tumor in dogs and is amenable to surgical resection in many cases. Unfortunately, overlap of sonographic findings between benign and malignant hepatic lesions typically requires more invasive diagnostic tests to be performed (i.e. biopsy for histopathology). The availability of a non-invasive diagnostic test to identify hepatocellular carcinoma would be beneficial. The use of a liver-specific magnetic resonance imaging contrast agent such as gadoxetate disodium (Eovist) has improved lesion detection in human patients. Gadoxetate disodium (Eovist) contrast-enhanced magnetic resonance imaging characteristics were evaluated in seven dogs (total of eight lesions). The imaging characteristics were variable, with the exception of all lesions being hypointense to surrounding normal hepatic parenchyma on 3D T1-weighted gradient recalled echo images at all post-contrast time points. All lesions displayed signal intensity ratios less than 1, consistent with retained but impaired hepatocyte function. Hepatic lesions not identified on previous imaging were found in 3/7 patients, which may affect surgical planning. In two patients, several hepatic nodules were identified during surgery; these nodules had not been visible on magnetic resonance imaging and were found to be benign on histopathology. This descriptive study reports the magnetic resonance imaging characteristics of hepatocellular carcinoma in dogs using the liver-specific contrast agent gadoxetate disodium.

12. Improving surveillance and screening of La Crosse virus in eastern Tennessee

Cassandra Urquhart, Doris D'Souza, Amy Lambert, David Paulsen, Rebecca Trout Fryxell
Entomology and Plant Pathology (Urquhart, Paulsen, Trout Fryxell), Food Science and Technology (D'Souza), CDC Division of Vector-borne Diseases (Lambert)

La Crosse virus (LACV), the leading cause of arboviral encephalitis in children in the United States, is endemic to the Appalachian region. Without a vaccine, mosquito control is crucial to disease prevention. The objective of this study was to improve detection of LACV by improving vector surveillance. Field studies were conducted to identify the best trapping method and timing for each mosquito vector, and molecular methods were improved for LACV detection. We compared five traps: CDC questing traps baited with dry ice, CDC questing traps baited with dry ice and a chemical lure, gravid traps, BG sentinel traps, and resting traps. Then the most effective traps were used around Knox County, TN, to identify when mosquitoes are actively questing (biting) and ovipositing (laying eggs). Additionally, a novel molecular method for virus detection was evaluated. CDC questing traps baited with dry ice and the chemical lure were effective at collecting *Aedes albopictus* and *Ae. triseriatus*, while *Ae. japonicus* was most efficiently collected in gravid traps. *Aedes albopictus* exhibited questing and oviposition behaviors at all hours, whereas *Ae. triseriatus* and *Ae. japonicus* were significantly more likely to quest and oviposit between 1700 and 900 hr. We also developed an inexpensive and efficient method for detection of LACV. Together, the information gathered in this study improves the cost and efficiency of LACV surveillance, makes it more accessible, and protects public health.

13. An unseen enemy: the ticks that parasitize Tennessee beef cattle

David Theuret, Rebecca Trout Fryxell

Entomology and Plant Pathology

Beef cattle are Tennessee's largest agricultural commodity and are currently threatened by ticks and tick-borne pathogens such as *Anaplasma marginale*, the causative agent of bovine anaplasmosis. However, many Tennessee producers express little concern regarding ticks. This is problematic because this could allow for the establishment of invasive ticks and tick-borne pathogens that will cause significant damage to the beef cattle industry, such as *Rhipicephalus annulatus*, which transmits the agents causing Texas cattle fever. In order to prepare for these invasive threats, it is imperative to identify surveillance strategies for current tick issues. The goals of this study were to (1) develop baseline information on ticks parasitizing beef cattle across time and space, (2) compare surveillance methods to identify the best means of monitoring beef cattle for potential invasive tick species, and (3) educate producers and extension agents about ticks parasitizing beef cattle. Baseline information was generated from collections at three University of Tennessee research and education centers, from producers via extension agents, and at livestock auctions and sales. Thus far, four distinct tick species have been collected in 2016, with collections peaking in spring. Surveillance methods were compared to identify which source is best for monitoring invasive species. An educational training video was made to demonstrate tick removal from cattle. Producer education has been anecdotally noted as increasing, although a post-study retroactive survey will be conducted for verification. Ultimately, investigating tick diversity and collection procedures will improve existing control efforts and prepare cattle producers for invasive ticks and pathogens.

14. Exploring granuloma formation in Johne's disease with in vitro and in silico modeling

Hunter Rice, Alyson Marek, Eunhye Lee, Margaret McDaniel, Sarah Alouani, Judy Day, Shigetoshi Eda

Microbiology (Rice, Marek, Lee, Eda), Mathematics (Day), Forestry, Wildlife, and Fisheries (McDaniel, Alouani, Eda)

Johne's disease (JD) is a chronic illness of cattle and other ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map), resulting in significant losses in the agricultural industry each year. In order to understand the pathology of Map infection that results in latency and long-term shedding, we focused on the interaction of Map with its preferred host, the intestinal macrophage. In this in vitro study of the induction of formation of granuloma-like cell clusters associated with JD, bovine monocyte-derived macrophages were infected with Map at various concentrations, and the number of clusters were quantified over a period of 2 weeks. Cluster formation peaked at day 8 post-infection at a concentration equivalent to one bacterium per four macrophages, while cluster formation in bacteria-free wells remained negligible throughout the time-course. The viability of infected macrophages was observed to be significantly greater than uninfected macrophages over the incubation period as observed by counting macrophages that remained adhered to the vessel. Additionally, the proportions of infected macrophages were determined at each concentration by counting cells with internalized, fluorescently-stained bacteria. Results from the in vitro study were incorporated into an in silico model of granuloma formation coded in NetLogo, an open source, agent-based, modeling program. Parameters such as macrophage movement speed, death rate of infected and uninfected cells, and initial proportion of infected cells were derived from in vitro experiments, and the number of aggregates formed in silico were compared to experimental data with the aim of elucidating the macrophage interactions that result in granuloma formation.

15. Identification of host-bacterial factors induced during experimental infection of dairy cows with *Staphylococcus aureus*

Oudessa Kerro Deago, Paulina A. Pacha-Becerra, Barbara E. Gillespie, Raul A. Almeida, Mark J. Lewis, Charlie Young
Animal Science

Mastitis is the most costly disease affecting dairy cows throughout the world. Mastitis is primarily caused by bacteria, of which *Staphylococcus aureus* is the most frequent and major pathogen of mammary glands of dairy animals. *S. aureus* is not only a pathogen of mammary glands of dairy animals but also a zoonotic pathogen that can cause mild to life threatening diseases in humans. Intramammary infusion of *S. aureus* is a reliable method in terms of causing infection, but it is a very much unrealistic method in terms of mimicking naturally occurring intramammary infections because a large number of bacteria are directly delivered into the intramammary area, bypassing non-specific natural defenses as well as innate and acquired immune mechanisms that could have prevented infection. Therefore, it is important to develop a teat dip challenge method that is relatively similar to natural infection. The overall aim of this study was to evaluate host-bacterial responses to experimental teat dip challenge with suspension of *S. aureus*. A total of eight cows were divided in to two groups: $n=5$ cows to be challenged with *S. aureus* and $n=3$ cows to be dipped in PBS as control. Of challenged cows, 90% were sub-clinically infected, whereas 10% were clinically infected. We developed a teat dip challenge model that is similar to natural infection. This teat dip infection model is of paramount importance to evaluate a protective vaccine against *S. aureus* infection.

16. Effect of heat stress on adherence to and internalization of *Streptococcus uberis* into bovine mammary epithelial cells

Raul Almeida
Animal Science

Considerable research has been conducted on the effects of heat stress (HS) on reproduction and the immune system, but there is no information on how HS affects the pathogenesis of intramammary infections (IMI). In this project, HS bovine mammary epithelial cells (BMEC) were co-cultured with *Streptococcus uberis*, and viability, adherence, and internalization of *S. uberis* to BMEC were evaluated. Adherence of *S. uberis* to HS BMEC was 85.9% higher than to non-HS BMEC. Similarly, internalization of *S. uberis* into HS BMEC was 85.7 % higher than into non-HS BMEC. As far as Trypan blue exclusion and lactate dehydrogenase (LDH) activity, reduction of viability was detected in HS BMEC, with the highest number of non-viable epithelial cells detected in HS BMEC co-cultured with *S. uberis*. No significant reduction in viability was observed in non-HS BMEC cultured alone or co-cultured with *S. uberis*. These results suggest that a reduction in cell viability and cell membrane damage was initially induced by the HS treatment and later worsened by the adherence and internalization of *S. uberis* into HS BMEC. It was shown that HS alters the phosphorylation activity of mTOR signaling factors in MAC-T cells, which may lead to cell membrane damage and impairment of early bactericidal intracellular mechanisms, thus allowing the intracellular survival of *S. uberis*. In conclusion, adherence to and internalization of *S. uberis* into HS BMEC was significantly higher than into non-HS BMEC, which may explain the increased susceptibility to IMI observed in HS cows.

17. Phenotypic and genetic characterization of *Staphylococcus aureus* isolates from cases of bovine mastitis

Oudessa Kerro Deago, Barbara E. Gillespie, Sarah A. Staley
Animal Science

The overall aim of this project was to identify and characterize virulence factors of *Staphylococcus aureus* that enable this bacterium to be the most prevalent and dominant mammary gland

pathogen of dairy animals. A total of 300 *S. aureus* isolates from cases of bovine mastitis were screened genetically for known virulence factors by PCR and phenotypically for production of hemolysins and slime on blood and Congo red agar plates, respectively. Of 300 strains tested, most of them caused strong hemolysis of α -toxin type and were strong slime producers. Based on frequency of isolation and number of virulence factors present in the strain, predominant strains ($n=2$) were selected and further evaluated by RNA-sequencing and quantitative real-time PCR (qRT-PCR) after co-cultured with bovine mammary epithelial cells in vitro. From this analysis, we identified some virulence-associated genes that were differentially expressed when *S. aureus* was co-cultured with bovine mammary epithelial cells. These genes may be responsible for initial colonization of bovine mammary glands and are good potential targets for development of protective vaccine against *S. aureus* mastitis.

18. Variation of immune response to *Staphylococcus pseudintermedius* surface-exposed peptides among dogs with pyoderma

Mohamed A. Abouelkhair, Manasi Balachandran, David Bemis, Stephen Kania
Comparative and Experimental Medicine (Abouelkhair, Balachandran, Bemis, Kania), Biomedical and Diagnostic Sciences (Bemis, Kania), Bacteriology, Mycology and Immunology, University of Sadat City, Egypt (Abouelkhair)

Little is known about the immune response to *S. pseudintermedius*, an important canine pathogen that has developed a high prevalence of methicillin resistance. Understanding the nature of the protective immune response would facilitate the design of new therapeutics. The aim of this study was to characterize the nature of the immunoglobulin G (IgG) response in 12 dogs with pyoderma to surface antigens from two genetically distinct *S. pseudintermedius* isolates. Surface proteins of two strains of *S. pseudintermedius* (NA45 [ST84] and 06-3228 [ST68]) were cleaved using soluble trypsin. The staphylococcal proteins were analyzed using two techniques: Western blot and enzyme-linked immunosorbant assay (ELISA) using 12 serum samples from different dogs with pyoderma. The results indicated significant differences in reactivity between infected dogs, as revealed by ELISA. Seven of 12 dogs' sera showed high reactivity with trypsin-cleaved antigen, while the remaining five sera showed very low reactivity. In addition, the surface proteome of these two strains of *S. pseudintermedius*—NA45 (ST84) and 06-3228 (ST68)—was studied using biotinylation and soluble trypsin approaches combined with mass spectrometry that pave the way toward a new level of understanding strain virulence and antigenic variation, opening new avenues for infection-related research on this crucial pathogen.

19. Identification of *Staphylococcus pseudintermedius* sortase A inhibitors

Manasi Balachandran, David Bemis, Jerome Baudry, Stephen Kania
Comparative and Experimental Medicine (Balachandran), Biomedical and Diagnostic Sciences (Bemis, Kania), Biochemistry, Cellular and Molecular Biology (Baudry), Center for Molecular Biophysics, Oak Ridge National Laboratory (Baudry)

Conventional antibiotic-based therapies against *Staphylococcus pseudintermedius* infections have failed in recent years due to the increased prevalence of methicillin resistance and multidrug resistance. Therefore, alternate strategies for treatment have to be explored. Anti-infective therapy based on sortase inhibition holds potential to address this shortcoming. Sortase A (srtA) is a transpeptidase commonly produced by gram-positive bacteria. It has specificity for proteins that harbor the LPXTG domain and anchors them on the bacterial cell wall. They include numerous virulence factors that may overcome the host immune response. The objectives of the present study were to (a) clone and express the srtA gene from *S. pseudintermedius* in *Escherichia coli* and (b) use the recombinant protein to screen for potential inhibitors against sortase A. A srtA gene from *S. pseudintermedius* was synthesized, inserted into an expression vector, and expressed in *E. coli*. A FRET-based assay using the synthetic substrate Abz-LPETG-K(Dnp)-NH₂ showed that sortase had high

specific activity. Using molecular dynamic simulations, a 3-D model of sortase A was created to screen a library of compounds from the National Cancer Institute (NCI) for potential inhibitors. From the top 20 most active inhibitors, 10 were selected and tested for their ability to inhibit sortase in vitro. Four of these compounds showed ~50% inhibition of sortase A in the FRET-based functional assay. The effect of these compounds in an in vivo model is yet to be investigated. The results indicate the possibility of sortase A inhibition as a potential therapeutic strategy in the treatment of staphylococcal infection in both humans and animals.

20. Finished genome sequences of three dominant *Staphylococcus pseudintermedius* strains reveal differences in virulence and antibiotic resistance

Matthew C. Riley, Stephen Kania, David Bemis

Comparative and Experimental Medicine (Riley), Biomedical and Diagnostic Sciences (Kania, Bemis)

Staphylococcus pseudintermedius is a gram-positive pathogen of both veterinary and human concern, and is increasingly methicillin- and multidrug-resistant. Here we present the complete, finished genome sequences of three *S. pseudintermedius* clinical isolates utilizing Solexa, Illumina MiSeq, Ion Torrent, Roche 454, and Pacific Biosciences next generation sequencing technologies in parallel with Argus whole genome mapping. Genomes were assembled using a variety of algorithms in the software package Geneious and automatically annotated using the Rapid Annotation using Subsystem Technology server. Isolates 063228, 081661, and NA45 represent the top three dominant methicillin-resistant *S. pseudintermedius* sequence types in North America, and comparative genomics on these isolates reveals roughly 2800 predicted coding sequences, 76 RNAs, including 6 rRNA operons, and a total size of 2.8 megabases each. Genetic differences in virulence genes such as *spa* and multiple antibiotic resistance genes, including some carried on transposons and extrachromosomal elements, were observed between these isolates. While no current technology can independently assemble an accurate closed genome for these isolates, a combination of techniques can reveal the small yet significant differences between incomplete and complete genome sequences.

21. Feeding low crude protein diets in lactating dairy cows during summer months: Improvements in energy metabolism

Jeffrey Kaufman, Kimberly Kassube, Agustin Rius

Animal Science

A study was conducted to evaluate the effect of feeding low rumen degradable protein (RDP) and rumen undegradable protein (RUP) levels on energy metabolism in cows during summer months. Forty-eight Holstein cows were assigned to treatments using a complete randomized block design in a 2 × 2 factorial arrangement of treatments ($n=12/\text{treatment}$). Treatments included two levels each of RDP (10% and 8%) and RUP (8% and 6%). A common diet (10% RDP–8% RUP) was fed from day 1 to 21 followed by the respective treatment diets from days 22 to 42. Cows were exposed to the intense summer climates with no supplemental cooling. Blood samples were collected from cows at day 42. Plasma was harvested for analysis of glucose, insulin, non-esterified fatty acids (NEFA), and beta-hydroxybutyrate. Main effects and their interaction were tested using the mixed procedure of SAS. Compared with a.m., p.m. temperatures increased ($P<.001$) rectal temperatures and respiration rates. The 10% RDP treatment decreased ($P<.04$) glucose concentrations compared with the 8% RDP treatment. The 10% RDP treatment increased ($P<.01$) insulin concentrations compared with the 8% RDP treatment. The 8% RUP treatment tended to increase ($P<.08$) insulin concentrations compared with the 6% RUP treatment. The 8% RUP treatment decreased ($P<.01$) NEFA concentrations compared with the 6% RUP treatment. Compared with 10% RDP, the 8% RDP treatment decreased β -hydroxybutyrate concentrations in the 8% RUP treatment but increased β -hydroxybutyrate

concentrations in the 6% RUP treatment ($P < .01$). In conclusion, lower RUP diets improved energy metabolism and milk production in heat-stressed dairy cows.

22. Hen diet enriched in long chain n-3 polyunsaturated fatty acids reduces adiposity in chicks

Ronique Beckford, Sarah Howard, Suchita Das, Abigail Tester, Shawn Campagna, Jay Whelan, Jeanna Wilson, Brynn Voy

Animal Science, Chemistry (Tester, Campagna), Nutrition (Whelan), Department of Poultry Science, University of Georgia (Wilson)

In avian species, the yolk supplies fatty acids to developing tissues, and the fatty acid composition of the yolk can be manipulated by altering the hen's diet. We used this model to test the hypothesis that enriching developing adipose tissue in omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) reduces adiposity compared to enrichment in n-6 LCPUFA. Sixty Cobb500 broiler-breeders were fed diets containing fat (5%) from either fish oil (FO) or corn oil (CO). After 4 weeks, fertilized eggs were collected and hatched, and all chicks were fed the same CO-based broiler starter diet. At days 7 and 14 chicks were weighed and euthanized, and blood, liver, breast, and subcutaneous and abdominal fat samples harvested. Gas chromatography and mass spectrometry were used to determine effects of diet on fatty acid profiles of tissue triglyceride and phospholipid pools. Mixed model analysis of variance and least square means were used to identify effects of maternal diet and age \times diet interactions on chick phenotypes (SAS v9.4, Cary NC). Hen dietary fat source did not significantly affect growth. Phospholipids and total lipids in muscle and adipose tissue of FO-chicks were significantly enriched in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) species relative to CO-chicks, with corresponding reductions in n-6 LCPUFA ($P < .05$). FO-chicks had significantly lower abdominal and subcutaneous fat deposition ($P < .05$). Maternal diet did not significantly affect offspring glycemia or lipolysis at either age ($P > .05$). These results suggest that enriching the maternal diet in long chain n-3 LCPUFA programs offspring toward reduced adiposity.

23. Subjects with and without osteoarthritis alter their muscle coordination to achieve beneficial knee moments while cycling

Rachel Thompson, Jacob Gardner, Songning Zhang, Jeffrey Reinbolt

Mechanical, Aerospace and Biomedical Engineering (Thompson and Reinbolt), Kinesiology (Zhang), Biola University (Gardner)

Osteoarthritis (OA) is a degenerative joint disease destroying the quality of life for 27 million adults. Cycling is an alternative exercise for people with OA, but there is a lack of scientific knowledge characterizing benefits for these individuals. The purpose of this study was to evaluate muscle force changes for subjects with and without knee OA during cycling using different pedal conditions. Our hypothesis was that lateral pedal wedge modifications of 5° and 10° would change mean muscle forces in both cohorts compared to neutral (0°). Subjects were two females, one with knee OA (1.8 m; 99.55 kg) and a healthy subject (1.7 m; 93.18 kg). Data was collected in three cycling pedal conditions: 1) neutral, 2) 5° lateral pedal wedge, and 3) 10° lateral pedal wedge. Simulations were created using OpenSim. Musculoskeletal models were scaled based on skin marker positions. Inverse kinematics was used to derive joint kinematics from experimental data. Static optimization was carried out to estimate muscle forces to generate inverse dynamic moments. We evaluated our hypothesis regarding differences in mean muscle forces in three pedal conditions with a 2-sample t test ($P < .01$). The subject with OA decreased mean muscle forces by 53% (Iliacus) and 47% (Psoas) with the 5° lateral pedal wedge condition compared to neutral. The healthy subject increased mean muscle forces by 357% (quadratus femoris) and 784% (adductor magnus) in pedal conditions. There were fewer significant differences in OA subject; this could be due to adaptations in the subject analyzed. Future work is needed to investigate changes in muscle forces and joint contact loads.

24. Biomechanics and anomalies in subadult bone development: A gateway to understanding fracture patterns in imperfect bone

Christina Tengelin

Tengelin (Comparative and Experimental Medicine), Dentistry (Marks), Pathology (Mileusnic), Small Animal Clinical Sciences (Millis)

This project addresses the cellular, molecular, and biomechanical properties of bone development in order to attempt to address if there are differing mechanisms at play during remodeling and healing in normal versus imperfect bone. The focus is aimed at assisting medical examiners when determining manner of death in subadults, where fractures are present, by providing a foundation for microscopic and histological comparison. This project is also aimed at providing a foundation for future research into the remodeling/healing properties of imperfectly developed bone.

25. Evaluation of calcium deposition in calcified aortic heart valves using laser-induced breakdown spectroscopy (LIBS)

Seyyed Ali Davari, Shirin Masjedi, Dibyendu Mukherjee

Mechanical, Aerospace and Biomedical Engineering

Calcific aortic valve disease (CAVD) is the most prevalent disorder among the elderly population in the developed countries. In early stages of CAVD, diagnosis is difficult; in later stages when CAVD can be diagnosed via imaging, the only viable treatment is valve replacement surgery. Laser-induced breakdown spectroscopy (LIBS) is a non-destructive method that uses a laser to excite a sample and ultimately collect a spectrum from the sample. Recently, LIBS has shown promising results in medical diagnosis. The aim of this study was to test the ability of calcium atomic detection within calcified valvular interstitial cells (VICs) using LIBS. VICs were isolated from freshly dissected aortic valves. To induce calcification, osteogenic media was added on the VICs for 10 and 14 days. Then, the cells were removed, washed, and lyophilized overnight. Finally, the calcium intensity of the dry cell pellets was detected by LIBS. Calcium deposition was also assessed using a calcium assay via arsenazo dye absorbance. Our LIBS measurements showed a 1.5-fold increase in the calcium intensity within the calcified cells after 14 days of osteogenic culture compared to the normal cells. Similarly, the calcium assay results showed increased calcium deposition among calcified VICs. In conclusion, the level of calcium elevation within the calcific aortic valves can be detected using LIBS as a non-destructive medical diagnosis tool.

26. Evaluation of pulse oximetry in healthy brachycephalic dogs

Shiara Arulpragasam, Cassie Lux, Adesola Odunayo, Jeff Biskup, Xiaocun Sun

Small Animal Clinical Sciences (Arulpragasam, Lux, Odunayo, Biskup), Office of Information and Technology (Sun)

This prospective study evaluated pulse oximetry (SpO₂) and arterial blood gas values in 18 healthy brachycephalic dogs (BD) and compared these values to those obtained from 18 healthy mesocephalic and dolichocephalic dogs (MDD). All dogs in both groups were assigned a brachycephalic airway syndrome (BAS) score based on an owner questionnaire. Inclusion criteria included presentation to the hospital for a problem unrelated to the respiratory system, a normal complete blood count, biochemistry profile, and physical examination. SpO₂ values were obtained in awake dogs from a minimum of two sites. Dogs were sedated, and SpO₂ values were again obtained concurrently with an arterial blood gas sample. Healthy BDs had significantly lower SpO₂ readings than healthy MDDs, but there were no statistically significant differences between BD and MDD for any arterial blood gas parameters. Based on the BAS score, BDs that were moderately affected ($n=5$), had significantly lower saturation of hemoglobin with oxygen (SaO₂) values on arterial blood gas when compared to MDD dogs ($n=18$). These results suggest that even healthy BDs,

which are not presenting for respiratory disease, may have decreased oxygenation compared to healthy MDDs.

27. Pelvic limb reflex changes in dogs with acute focal thoracolumbar myelopathy

Amy Hodshon, William Thomas

Small Animal Clinical Sciences

The objective of this prospective case series was to document the prevalence of decreased pelvic limb reflexes and changes in these reflexes over time in 34 dogs with naturally occurring acute thoracolumbar myelopathies. Dogs presented for acute pelvic limb paralysis caused by an intervertebral disk herniation (IVDH), acute non-compressive nucleus pulposus extrusion (ANNPE), or fibrocartilaginous embolism (FCE) between the levels of the third thoracic and third lumbar vertebrae and who underwent cross-sectional imaging were included. Dogs whose pelvic limb reflexes (patellar and/or withdrawal) were assessed as decreased by two examiners on admission were considered affected. Affected dogs underwent additional testing (complete blood count, serum chemistry, pelvic limb electromyography, and imaging through the lumbosacral junction) and were assessed every 12 hr for return of reflex function. Follow-up neurologic exams were performed at 4 and 8 weeks in a subset of dogs. Twenty-eight paraplegic and six monoplegic dogs were enrolled. Diagnoses were IVDH in 30 dogs, FCE in 3 dogs, and ANNPE in one dog. Nine of 34 dogs (26%) were considered affected, including five dogs with IVDH (17%) and all four dogs with FCE or ANNPE. Withdrawal reflex was decreased in all affected dogs, and patellar reflex was decreased in two. The median time for return of normal withdrawal reflex was 60 hr (range 12 to 156 hr). Pelvic limb reflexes, especially the withdrawal reflex, can be transiently depressed in a significant percentage of dogs presenting for acute transverse myelopathy cranial to the lumbosacral intumescence. Awareness of this phenomenon is important for correct localization, diagnostic workup, and prognosis.

28. Detection of carcinogen-induced bladder cancer by fluorocoxib A in mice

Jennifer Bourn, Maria Cekanova

Genome Science and Technology (Bourn), Small Animal Clinical Sciences (Cekanova)

Sensitivity and specificity of tumor detection can be significantly improved by the specific uptake of targeted fluorescence probes by tumors. Fluorocoxib A, a rhodamine-conjugated analog of indomethacin, selectively targets cyclooxygenase-2 (Cox-2)-expressing cancers. In this study, we evaluated fluorocoxib A, an optical imaging agent, for detection of early stages of carcinogen-induced bladder cancer in mice. Specific uptake of fluorocoxib A by tumors was detected using the IVIS optical imaging system *in vivo*. After imaging of mice, histopathology of the bladder was assessed using H&E and immunohistochemistry staining for detection of Ki67, uroplakin-1A, and COX-2 expression. Mice treated with the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine developed bladder cancer that strongly resembles human invasive urothelial neoplasia. The progression of bladder cancer from normal urothelium to carcinoma was confirmed by H&E staining and by increased expression of Ki67 and decreased expression of uroplakin-1A proteins. The specific uptake of fluorocoxib A correlated with the progression of bladder carcinogenesis and also with increased Cox-2 expression in urothelial cells. No fluorocoxib A uptake was detected in bladder tissue from the control group. These results, taken together, indicate fluorocoxib A detected early stages of bladder tumorigenesis in the mouse model. Fluorocoxib A specifically detected Cox-2-expressing bladder tumors with no detectable uptake by normal bladder urothelium. This mouse cancer model offers a unique translational application for the evaluation of novel imaging agents to detect early stages of bladder cancer *in vivo*.

29. Reactive oxygen species-mediated synergistic and selective induction of cell death and reduction of clonogenic resistance in breast cancer cells by combined cisplatin and FK228

Lenora Ann Pluchino, Shambhunath Choudhary, Hwa-Chain Robert Wang

Biomedical and Diagnostic Sciences (Pluchino, Choudhary, Wang), Charles River Laboratories (Choudhary)

Although combination chemotherapy has shown significant promise in cancer treatment, safe and effective combination chemotherapy regimens that specifically target breast cancer are currently lacking. To address this issue, we used our cellular system, consisting of the non-cancerous human breast epithelial MCF10A cell line paired with its derived tumorigenic, oncogenic H-Ras-expressing MCF10A-Ras cell line, to investigate the effectiveness of a novel combination chemotherapy regimen targeting breast cancer cells. We chose a combination of two FDA-approved agents, cisplatin and FK228, that have previously been shown to synergistically and selectively kill bladder cancer cells, to examine their effectiveness against breast cancer cells. Results showed that combined cisplatin and FK228 significantly, synergistically, and selectively induced death and reduced clonogenic survival/drug resistance of cancerous MCF10A-Ras versus noncancerous MCF10A cells. Enhancement of the already aberrantly-upregulated Ras-ERK-Nox pathway by combined cisplatin and FK228 significantly increased reactive oxygen species (ROS) levels, leading to selective induction of death, reduction of drug resistance, and induction of DNA damage and oxidation in cancerous MCF10A-Ras cells. The Ras-ERK-Nox-ROS pathway played a major role in both synergistic cell death induction and GSH-level reduction, which contributed to the synergistic suppression of drug resistance in breast cancer cells. Our study demonstrates, for the first time, that combined cisplatin and FK228 should be seriously considered in clinical trials as a new regimen for therapeutic control of human breast cancers.

30. The protease activity of separase is required for both chromosome segregation and membrane trafficking during anaphase

Xiaofei Bai, Diana Mitchell, Lindsey Klebanow, Joshua Bembenek

Biochemistry & Cellular and Molecular Biology

Chromosomal segregation and cytokinesis are tightly regulated processes involved in cell division. Separase cleaves a subunit of the protein complex called cohesin, which keeps the sister chromatids together until the end of metaphase. In addition, separase is a key player in meiotic cortical granule exocytosis and vesicle trafficking during mitotic cytokinesis. RAB-11 is known to regulate both cortical granule exocytosis and exocytosis during cytokinesis. This raises the question of whether separase's protease activity regulates RAB-11 vesicle exocytosis. To address this question, we generated a catalytically inactive separase, SEP-1PD::GFP. We found that expression of SEP-1PD::GFP causes chromosomal nondisjunction. Depletion of cohesin rescues this defect, indicating that cohesin cleavage is prevented by the inactive protease, possibly by a substrate trapping mechanism. To test whether SEP-1PD::GFP also impairs RAB-11 vesicular trafficking during cytokinesis, we imaged the embryos that express RAB-11::mCherry and SEP-1PD::GFP. Interestingly, expression of SEP-1PD::GFP caused an abnormal accumulation of RAB-11 vesicles at the cleavage furrow site, similar to depletion of separase by RNAi. Moreover, we found that RAB-11 co-localized with both wild type separase and our protease dead mutant on cortical granules during meiotic anaphase I. Our results indicated that SEP-1PD::GFP expression delayed the completion of cortical granule exocytosis. Moreover, we found an additive increase in embryonic cytokinesis failure relative to t-SNARE syntaxin-4 syx-4 depletion or SEP-1PD::GFP expression alone. These findings suggest that the protease activity of separase is required for the exocytosis of RAB-11 vesicles during mitotic cytokinesis and cortical granule exocytosis during meiosis I.

31. Spatiotemporal regulation of Cdc42 coordinates cytokinetic events in *Schizosaccharomyces pombe*

Brian Hercyk, Bin Wei, Mikayla Clark, Julius Habiyaremye, Maitreyi Das
Biochemistry Cellular and Molecular Biology

Cytokinesis is the terminal phase of cell division that physically separates daughter cells to maintain genome integrity. Defects in this process contribute to developmental disorders and may lead to the onset of cancer. Following chromosome segregation, cytokinesis occurs in a sequential process that involves contractile ring assembly, ring constriction, furrow ingression, and cell abscission. In yeast, furrow ingression is concurrent with septum (cell wall) formation. It is not clear how these steps are temporally organized to ensure proper cell separation. The conserved GTPase Cdc42 is a major regulator of growth in eukaryotes. We demonstrated that the Cdc42 GEFs (guanine nucleotide exchange factors), Gef1 and Scd1, sequentially activate Cdc42 at the site of cell division and promote distinct cytokinetic events. Gef1 localizes to the cytokinetic ring and promotes ring constriction, while Scd1 localizes to the ingressing membrane and promotes primary septum formation. Interestingly, Scd1 also appears to restrict secondary septum formation. Finally, Cdc42 needs to be inactivated for successful cell abscission. This is likely mediated through the Cdc42 GAP (GTPase-activating protein) Rga4, which localizes later to the ingressing membrane. This suggests that spatiotemporal regulation of Cdc42, as established by the localization of its effectors (Gef1, Scd1, and Rga4), allows temporal organization of the different cytokinetic events. Our findings define principles that help establish temporal organization of multi-step cellular processes using cytokinesis as a paradigm. GTPase regulatory patterns are emerging to be critical for diverse cellular processes including polarity, trafficking, and cytokinesis.

32. Nicastrin is required for APP but not Notch processing, while Aph-1 is dispensable for processing of both APP and Notch

Chen Hu, Linlin Zeng, Ting Li, Michael A. Meyer, Mei-Zhen Cui, Xuemin Xu
Biomedical and Diagnostic Science (Hu, Cui, Xu), Life Science, Jilin University (Zeng), Cell Biology, Tianjin Medical University (Li), Sisters Hospital, Buffalo, NY (Meyer)

The gamma-secretase complex is composed of at least four components: presenilin-1 or presenilin-2, nicastrin (NCT), anterior pharynx-defective 1 (Aph-1), and presenilin enhancer 2. In this study, using knockout cell lines, our data demonstrated that knockout of NCT, as well as knockout of presenilin enhancer 2, completely blocked gamma-secretase-catalyzed processing of C-terminal fragment CTF alpha and CTF beta, the C terminal fragments of beta-amyloid precursor protein (APP) produced by alpha-secretase and beta-secretase cleavages, respectively. Interestingly, in Aph-1-knockout cells, CTF alpha and CTF beta were still processed by gamma-secretase, indicating Aph-1 is dispensable for APP processing. Furthermore, our results indicate that Aph-1 as well as NCT is not absolutely required for Notch processing, suggesting that NCT is differentially required for APP and Notch processing. In addition, our data revealed that components of the gamma-secretase complex are also important for proteasome- and lysosome-dependent degradation of APP and that endogenous APP is mostly degraded by lysosome while exogenous APP is mainly degraded by proteasome.

33. *Staphylococcus pseudintermedius* efflux pump detection and characterization

Chris Millis, Stephen Kania, Manasi Balachandran, David Bemis
Biomedical and Diagnostic Sciences (Millis, Kania, Bemis), Comparative and Experimental Medicine (Balachandran)

The development of *Staphylococcus pseudintermedius* resistance to methicillin and common biocides has been a concern to veterinary medicine and the general public due to potential infections spreading through veterinary hospitals and clinics, many of which can be life threatening if

untreated. Strains of the bacteria have been found to be resistant to erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and levofloxacin. *S. pseudintermedius* is similar to methicillin-resistant *Staphylococcus aureus*, commonly known as MRSA, affecting humans. It is of concern in veterinary medicine because *S. pseudintermedius* is the most common cause of pyoderma in dogs and is associated with invasive disease in dogs and cats. It is also prevalent in the food industry. In this study, we report a strong correlation between the presence of a plasmid encoded QacG multidrug efflux pump and a higher resistance profile to certain antibiotics and biocides for *S. pseudintermedius*. Detection and characterization of the QacG efflux pump was made possible by PCR and comparing DNA results to those found in the National Center for Biotechnology Information (NCBI) database. Samples that tested positive for the known efflux pump were less susceptible to treatments of benzalkonium chloride (a common biocide) in drug titration experiments.

34. Commercially available citrus-based and Quillaja extracts to reduce Tulane virus titers

Sukriti Ailavadi, P. Michael Davidson, Doris D'Souza

Food Science and Technology

Human noroviruses cause numerous gastrointestinal illnesses and deaths annually. In the absence of human norovirus vaccines, alternate preventive and control strategies are being studied. Tulane virus (TV) is recognized as a novel cultivable human norovirus surrogate to determine inactivation efficacy. Natural plant components that contain bioflavonoids and organic acids (e.g., citric acid) and *Quillaja* bark extracts (QE) that contain quillaic acid glycosides and saponins are known to have antimicrobial effects. QE has antiviral effects against Aichi virus. The objective of this study was to determine the effect of a commercially available citrus-based formula (CX) and QE on the infectivity of TV at room temperature (RT). TV (200 μ l) at 7 log PFU/mL was mixed with equal volumes of undiluted antimicrobial (CX), QE, or water (control) for 30, 60, 120, and 180 min at RT. After each time point, treatments were stopped in cell culture media supplemented with 10% fetal bovine serum and serially diluted in cell culture media supplemented with 2% fetal bovine serum. Infectivity was measured using standard plaque assays on confluent LLC-MK2 cells in 6-well plates in comparison to controls. Each treatment was replicated thrice and assayed in duplicate, and data were statistically analyzed. Treatment with CX at RT resulted in TV reductions of 0.75 ± 0.07 , 1.04 ± 0.24 , 2.40 ± 0.1 , and 2.97 ± 0.15 log PFU/mL after 30, 60, 120, and 180 min, respectively, while QE (50% solution) showed only 0.98 ± 0.10 log PFU/mL reduction after 180 min. Results indicate that the commercial citrus-based formulation shows promise as an alternate control strategy against TV. Further studies with CE using carrier tests and organic load against TV are needed for application as routine sanitizing washes.

35. Use of mycophenolate mofetil to treat immune-mediated skin disease in 14 dogs-A retrospective evaluation

Amanda L. Ackermann, Elizabeth R. May, Linda A. Frank

Small Animal Clinical Sciences

Mycophenolate mofetil (MMF) is a lymphocytotoxic immunosuppressive agent used in human and companion animal medicine for the treatment of immune-mediated disease. Mycophenolate mofetil is reported to have reduced myelotoxicity and hepatotoxicity when compared to azathioprine. It was hypothesized that treatment with MMF as a second agent to glucocorticoids would be effective in treating immune-mediated skin disease. A retrospective review of medical records from 2010 to 2015 were used to identify dogs with immune-mediated skin disease that were treated with MMF. The study included 14 dogs. All dogs were treated with MMF (mean dose 14.7 mg/kg q 12 h) in conjunction with glucocorticoids. Ten of 14 cases showed positive results, with complete remission in eight cases and partial remission in two cases. Mean time to remission was 5.7 weeks. Therapy was discontinued in one case (perianal fistula) due to lack of response. Adverse events were noted in six cases and included diarrhea ($n=6$), hematochezia ($n=2$), vomiting ($n=3$), and

papilloma formation ($n=1$). Therapy was discontinued in two cases with diarrhea. Mycophenolate mofetil was discontinued in an additional case because of a diagnosis of neoplasia. All other adverse events were self-resolving or easily medically managed. No hepatotoxicity or bone marrow suppression was seen. This study supports the use of MMF as a second line immunotherapeutic in immune-mediated skin disease in dogs.

36. Consideration of the fungal phosphatidylserine synthase as a drug target

Chelsi Cassilly, Abigail Farmer, Jerome Baudry, Shawn Campagna, Todd Reynolds

Microbiology (Cassilly, Reynolds), Chemistry (Farmer, Campagna), Biochemistry, Cellular, and Molecular Biology (Baudry)

The past few decades have seen increases in the prevalence of nosocomial infections within immunocompromised individuals. *Candida albicans* is responsible for the majority of nosocomial fungal infections, which include oral, vulvovaginal, and life-threatening systemic infections. Only three drug classes are used to treat systemic *C. albicans* infections, and rising drug resistance within the genus and drug toxicity have made these drugs less effective, creating a need for new antifungals. Previous research identified the fungal phosphatidylserine (PS) synthase (Cho1p) as a potential drug target because it is: 1) required for virulence, 2) conserved across fungi, and 3) absent from mammalian genomes. If found, Cho1p inhibitors could be broad range, non-toxic antifungals. Thus, characterizing Cho1p as a drug target became the focus of our study. We began by using mass spectrometry to create steady state phospholipid profiles of wild-type and PS synthesis mutant strains of *C. albicans*. We then assessed Cho1p enzyme activity via an in vitro PS synthase assay and compared these with the phospholipid profiles. Through this same enzyme assay, we found that Cho1p shows high specificity for L-serine over other tested amino acids or structural analogs of serine. Last, we elucidated the enzyme kinetics of both substrates of Cho1p. Serine was found to have a K_m of 1.24 mM and CDP-DAG a K_m of 0.043 mM. We are continuing this work by looking for small molecule inhibitors of Cho1p as well as beginning efforts to map the substrate binding sites on the enzyme.

37. Decreased dissolved oxygen as a possible mechanism of increased persistence of *Trichomonas gallinae* in the presence of organic material

Kathryn Purple, Richard Gerhold

Comparative and Experimental Medicine (Purple), Biomedical and Diagnostic Sciences (Purple, Gerhold)

The transmission of *Trichomonas gallinae*, the avian protozoan parasite, may be facilitated by backyard birdbaths. *T. gallinae* persists up to 20 hr in distilled water with organic material, mimicking soiled birdbaths. We hypothesized that decreased dissolved oxygen could be a key factor leading to increased persistence of this microaerophilic trichomonad. Using plastic containers to simulate birdbaths, we determined 1) the levels of dissolved oxygen in distilled water created by various amounts of organic material, 2) the amount of the enzyme Oxyrase needed to re-create those various oxygen levels, and finally, 3) the persistence of two *T. gallinae* isolates in distilled water with artificially lowered dissolved oxygen. Oxyrase was added to 500 mL distilled water in three treatment concentrations: 0%, 0.5% and 1%, which created dissolved oxygen levels of ~8–9 ppm, 1–2 ppm, and 0–1 ppm, respectively. The latter concentrations of dissolved oxygen were representative of those created with organic material. We evaluated one isolate from a Cooper's hawk (*Accipiter cooperii*) and one from a broad-winged hawk (*Buteo platypterus*) by adding $\sim 1 \times 10^6$ trichomonads to 500 mL distilled water containers (representing birdbaths) in triplicate. Aliquots of 0.5 mL taken at various time points from containers were inoculated into Hollander Fluid media, incubated at 37°C, and read by light microscopy every other day for 5 days. Isolates persisted up to 18 and 30 hr in the 0.5% and 1% Oxyrase treatments, respectively. In contrast, in unaltered distilled water, neither isolate persisted past 4 hr. These results offer a possible mechanism to explain the persistence of trichomonads in water with organic material.

38. Next generation sequencing and genomic analysis of *Tritrichomonas foetus* species of bovine and feline origin

Ellen Fleetwood, Rebecca Wilkes

Comparative and Experimental Medicine (Fleetwood, Wilkes)

Tritrichomonas foetus is a bovine and feline protozoan parasite with substantial clinical and economic significance. Bovine and feline isolates of this parasite have long been considered the same species; however, recent genome profiling work from our lab has shown that bovine and feline *T. foetus* exhibit markedly different properties that may indicate a speciation event has occurred. The question of whether these isolates are now different species holds implications for pathogen-host relationships, as both of these types of parasites demonstrate different host specificity and tissue tropism. To answer this question, we conducted next generation sequencing (NGS) of clinical isolates from each type. This is the first DNA sequencing project for *T. foetus*, and few closely related organisms have been sequenced or thoroughly characterized. As such, both bovine and feline *T. foetus* proved to be refractory to standard molecular biology, biochemical, and sequencing protocols. In order to successfully sequence these organisms, we were required to extensively troubleshoot and optimize cell culture, DNA extraction, and NGS library preparation. Currently, we have been able to produce enough sequences with sufficient coverage to produce two draft genomes using a combination of Ion Torrent and Illumina MiSeq NGS platforms. Through comparative genomics analyses, we hope to identify differences in virulence pathways that might lead to future therapeutic targets, as well as elucidating the general mechanisms of host recognition and pathogenesis.

39. Identification and role of cysteine protease 30 in feline *Tritrichomonas foetus*: A novel target for preventive and/or therapeutic strategies for feline trichomonosis

Emily N. Gould, Mabre Brand, Stephen Kania, M. Katherine Tolbert

Comparative and Experimental Medicine (Gould), Biomedical and Diagnostic Sciences (Brand, Kania, Tolbert)

Tritrichomonas foetus (Tf) is a protozoan parasite recognized as a significant cause of diarrhea in domestic cats, but efficacious therapeutics are lacking. Cysteine proteases (CPs) have been identified as mediators of Tf-induced adhesion-dependent cytotoxicity to the intestinal epithelium. As cats also produce CPs as part of life-critical systems, selective targeting is necessary to reduce potential host toxicity. Previous studies have demonstrated the importance of a specific CP, CP30, in mediating bovine and human trichomonad cytopathogenicity. Aims of this study were to evaluate the presence of CP30 in feline Tf and the effect of targeted inhibition of CP30 on feline Tf-induced cytotoxicity. The presence of CP30 in feline Tf isolates ($n=4$) was evaluated by indirect immunofluorescence (IF) and flow cytometry using an α -CP30 polyclonal antibody. Tf treated with rabbit IgG and secondary Ab in the absence of α -CP30 Ab served as negative controls, with bovine Tf representing a positive control. The effect of CP30 inhibition on feline Tf adhesion and cytotoxicity in a co-culture model with intestinal epithelial (IPEC-J2) monolayers was evaluated by fluorescence quantification and crystal violet spectrophotometric assays. Data were analyzed using Systat software. CP30 expression was identified in all feline isolates, with inhibition of CP30 resulting in significantly decreased Tf adhesion to and cytotoxicity towards IPEC-J2 monolayers compared to control-treated groups ($P<.05$). These studies establish that CP30 might be an important virulence factor in feline Tf cytopathogenicity and provide evidence-based justification for investigation of CP30 as a novel target for the prevention and/or treatment of feline trichomonosis.

40. Role of IL-28A in herpes stromal keratitis

Ujjaldeep Jaggi, Siddheshvar Bhela, Barry T Rouse

Biomedical and Diagnostic Sciences

Ocular infection with herpes simplex virus (HSV) can result in a chronic immune-inflammatory lesion that is a significant cause of human blindness. A key to controlling stromal keratitis (SK) lesion severity is to identify cellular and molecular events responsible for tissue damage. Interferons (IFNs) are key cytokines in the establishment of a multifaceted antiviral response. Although all IFNs are important mediators of antiviral protection, their roles in antiviral defense vary. The IFN lambda, which is a type III cytokine, is capable of inducing an antiviral response both in vitro as well as in vivo. This family consists of three structurally related interferon lambda subtypes called IFN- λ (IL-29), IFN- λ 2 (IL-28A) and IFN- λ 3 (IL-28B). We determined that IFN- λ 2 (IL-28A) has a prominent role in HSK. The binding of IFN- λ to their cognate receptors induces a common signaling cascade that results in the activation of STAT1 and STAT2, which promotes MX1, OAS1, and IRF7, which in turn mediate the antiviral activity of the type-III IFNs. We determined that IL-28A appears to specifically target neutrophil function and cause a major reduction in the neutrophil number at the target site and also targets Th1 cells, the major orchestrators of HSK. Administration of IL-28A effectively reduces HSK lesions and can prove to be a very reliable therapeutic agent.

41. The role of resistance training in influencing insulin resistance among adults without diabetes: A systematic review and meta-analysis

William Boyer, Robert Aug, Lindsay Toth, James Churilla, Eugene Fitzhugh

Kinesiology, Recreation and Sports Studies (Boyer, Toth, Fitzhugh), Plant Sciences (Aug), University of North Florida, Clinical and Applied Movement Sciences (Churilla)

Resistance training (RT) has been shown in previous meta-analyses to attenuate insulin resistance (IR) among adults with diabetes. However, the preventive effects in those without diabetes have yet to be systematically reviewed. Therefore, the purpose of this study was to systematically determine the relationship between RT and IR among adults without diabetes. We searched Medline through January 2016 and examined reference lists of retrieved articles. We excluded studies that were not conducted in humans, did not use validated methods to assess IR, used other modalities of physical activity (PA) that were not RT, included participants with diabetes diagnosis, or included a dietary component to the study design. Two separate investigators examined the literature. A standardized mean difference was used for effect size (ES). A random effects model was used for all analyses. Moderator analyses were conducted to determine the effect among males and females as well as those classified as overweight or obese. We identified a total of 17 studies with a total of 378 individual participants. A strong summary ES (-0.94, $P < .001$) was found. Following accounting for potential publication bias, the ES remained moderate and significant (-0.65, $P < .001$). Moderator analysis revealed a significant effect for males (-1.23, $P < .001$), females (-0.68, $P = .02$), and those classified as overweight or obese (-0.65, $P = .01$). These results indicate that RT independently affects IR among adults without diabetes. Efforts to attenuate or delay IR should emphasize the independent effects of RT and encourage inclusion in PA routines.

42. Demographic predictors of pertussis vaccine receipt in Tennessee in all age groups, 2014

Corinne Tandy

Comparative and Experimental Medicine

The purpose of this research was to identify demographic factors associated with pertussis (whooping cough) vaccination in Tennessee. Available literature concerning vaccine coverage in different demographic groups is limited, particularly at the state level, and analysis of all age groups is especially sparse. Data from the Behavioral Risk Factor Surveillance System (BRFSS), the National Immunization Survey-Teen (NIS-Teen), and the National Immunization Survey (NIS) were used to

identify demographic predictors of vaccine coverage in Tennessee for all infants, adolescents, and adults for the year 2014. Overall pertussis vaccine coverage was relatively high in infant and adolescent populations (95.2% and 83.5%, respectively) but very low in the adult population (15.4%). Logistic regression identified education level of the mother, income, age of the mother, Hispanic ethnicity, and number of influenza vaccines received as significant ($P < .05$) predictors of DTaP receipt in infants. Annual household income and number of influenza vaccines received were identified as significant predictors in adolescents for Tdap receipt. Multinomial logistic regression identified education, age, sex, race, receipt of influenza vaccine in the last 12 months, and receipt of pneumococcal vaccine ever as significant predictors of adult pertussis vaccination. These variables may be useful to state public health agencies in creating targeted efforts to improve vaccination coverage in Tennessee.

43. Spatial patterns of myocardial infarction death risks in Florida between 2000 and 2014

Evah Wangui Odoi, S. Robertson

Comparative and Experimental Medicine (Odoi), Florida Department of Health, Bureau of Chronic Disease Prevention, Tallahassee, FL (Robertson)

Identifying geographical areas with significantly high risks of myocardial infarction (MI) is crucial for the efficient targeting of control and prevention programs. The objective of this study was to investigate spatial patterns of MI mortality risks in Florida and to identify hotspots of MI mortality risks for the period 2000 to 2014. County-level mortality risk data was obtained from the Florida Department of Health. Spatial scan statistics were used to identify the approximate locations of clusters, and the spatial patterns were displayed in ArcGIS. Myocardial mortality risks decreased substantially throughout the state during the study period. However, geographical variations in mortality risks were seen, with a clear north to south gradient. Statistically significant clusters of high mortality risks were identified in the more rural, northern part of the state, while low mortality risk clusters were observed in the south. The high risk clusters in the north showed more dramatic decreases in mortality risks, with consequent reduction in disparities between the north and the south. However, the counties in the north still lagged behind those in the south by almost 15 years in as far as their mortality risks were concerned. Although MI mortality risks have decreased in Florida over the past 1.5 decades, significant disparities still exist. Efforts are needed to identify the determinants of these disparities, so as to reduce or eliminate them.

44. Intercanine distance as a statistical measure for bite mark analysis.

Alana Joy Ohana

Comparative and Experimental Medicine

The science of identification by bite mark analysis has recently been called into serious question. Human dentition is truly variable but often not unique. When animal bites are considered, a proper identification of the perpetrator is nearly impossible. Primary distortion (when the bite is made) and secondary distortion (during decomposition or healing) both further disrupt landmarks that might be used for identification. Using only ink marks and intercanine distance on live subjects, this study attempts to determine a maximum distortion possible for a variety of bite mark locations on skin. Lower arm, lower leg, and upper back were chosen because of the high instance of bites occurring in those locations. Though bite location likelihood does vary with age of the victim, these locations were relatively consistently high across age groups. Facial bites were common, but the face does not present continuous skin and therefore likely does not result in useful data. Facial bite examination is an option for future study. Caliper measurements of body fat will also be used to check for variation of distortion due to malleability of the area in question. It is hoped that this research will produce a numerical value relating the bite mark on the victim to the intercanine distance of the suspect (either human or animal) that will allow a rule-in or rule-out assessment. Also, it is hoped that a hospital

protocol can be developed so that bite mark victims are more likely to see justice and the perpetrators are accurately identified.

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University of Tennessee
Knoxville, TN

Inquiries:

Misty Bailey
mmcginn2@utk.edu
