2019 Research Day ABSTRACTS

Wednesday, April 24, 2019

ILLINOIS

College of Veterinary Medicine

Maternal and paternal inheritance of transgenerational effects of prenatal exposure to DEHP on male fertility

Radwa Barakat [1, 2], Po-Ching Patrick Lin[1] and CheMyong Jay Ko[1]

[1] Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL; [2] Department of Toxicology, Faculty of Veterinary Medicine, Benha University, Egypt.

Males and females are genetically different mainly in their sex chromosome complement, leading to sexual dimorphisms. Di-(2-ethylhexyl) phthalate (DEHP) is an endocrine-disrupting chemical and found in a variety of consumer products and is known to impact male reproduction in the current and future generations by altering epigenome. In this study, we tested an ongoing hypothesis that the impact of the exposure to DEHP is transmitted to future generations via both paternal and maternal lineages but a lineage-dependent manner. To test this hypothesis, pregnant CD-1 mice (F0) were orally dosed with vehicle or 20μg/kg/day DEHP from gestation day 11 until birth. F1 males and females were bred with unexposed proven breeders to produce F2 generation males and females, respectively. F2 males were bred with unexposed females to produce F3 males that are 'paternal lineage' males. F2 females were bred with unexposed males to produce 'F3 maternal lineage males. When these F3 males of different lineages reached the age of 15 months, the reproductive indices were compared between the lineages and with the vehicle controls. Compared to controls, paternal lineage F3 males had lower serum testosterone levels ($p=0.01$), fewer sperms in the cauda epididymis ($p=0.001$), and fewer motile sperms ($p=0.05$). Their testes exhibited a morphologically distinguishable degeneration such as sloughed germ cells, failure of spermiation, and abnormal residual bodies. The F3 maternal lineage males also had lower serum testosterone levels, fewer sperm in the cauda, and fewer motile sperms but at lesser impact than those in the paternal line. Interestingly, the transcriptomal analysis of the F3 generation Y-chromosomes on paternal lineage showed that 57.21% of the Y-chromosomal genes were significantly altered in their expression levels compared to those of control. Wheras, on maternal lineage males, Y-chromosomal genes was not different compared to those of control. Meanwhile, expression of X-chromosome genes was not different between paternal and materal lineage males. In conclusion, this study finds that prenatal exposure to DEHP causes severer transgenerational impact on the paternal lineage males than the maternal lineage males, in part due to an impact on the Y chromosome. [Supported by NIH1P20 ES01863/EPA, RD-83459301].

Conversion of Esr2 to Esr1 Expressing Cells During Development

Shah Tauseef Bashir, Po Ching Lin, Chan Jin Park, Sherry Qi Zhou, CheMyong Jay Ko
Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Most of the key functions of estradiol are mediated by its nuclear receptor alpha (ESR1) and beta (ESR2). The physiological outcomes mediated by these two receptors are usually opposite to each other, thereby maintaining a homeostatic equilibrium. In this study, we tested our hypothesis that a set of ESR2-expressing cells convert to ESR1-expressing cell in female reproductive organs during embryonic and neonatal stages of development. To this end, we created a transgenic mouse line where ESR1 expression was designed to be ablated in ESR2-lineage cells (*Esr1* $^{flox/flox}$ *Esr2* $^{iCre/WT}$; Esr2-Esr1KO). If the hypothesized conversion of ESR2- to ESR1-expression did not occur, Esr2-Esr1KO mice will not be different from the wild type counterparts. To our surprise, female Esr2-Esr1KO mice were infertile (0% vs. 100% in WT; $p < 0.0001$). ESR1 expression was reduced and CYP17A1 expression was increased in the ovaries, pointing towards Esr1 ablation in the endocrine theca cells. After exogenous gonadotropin stimulation, Esr2-Esr1KO ovulated less oocytes that wild type (WT) littermates (3.47 ± 1.46 vs. 16.2 ± 4.2 in WT, $p = 0.0008$). Therefore, we examined the pituitary and hypothalamus. On IHC analysis no difference in pituitary ESR1 expression was observed. However, reduced ESR1 immunoreactivity was observed in the AVPV. Based on the results, it seems that theca cells and kisspeptin neuron lineage undergoes ESR2 to ESR1 conversion or impacted by a conversion occurred elsewhere during development. The findings from this study may shed light on a novel mechanism that the action of estradiol exerted – same target (a cell lineage) but different biological impacts by changing its receptor from ESR2 to ESR1.

Hypoxia-mediated vesicular trafficking is critical for embryo implantation and establishment of pregnancy

Arpita Bhurke[1], Athilakshmi Kannan[1], Qiuyan Ma[2], Milan K. Bagchi[2] and Indrani C. Bagchi[1]
Department of Comparative Biosciences[1], College of Veterinary Medicine, University of Illinois Urbana-Champaign, IL 61802 and Molecular & Integrative Physiology[2], University of Illinois at Urbana-Champaign, Urbana, IL

Embryo implantation is a crucial step in establishment of successful pregnancy. During implantation the embryo attaches to the uterine luminal epithelium and subsequently invades into the underlying stroma. Eventually, an extensive vascular network is formed in the decidua to support the growing embryo prior to placentation. Interestingly, in many species, these events of early pregnancy occur in hypoxic environment. However, the mechanisms of maternal adaptation to hypoxia during early pregnancy remain unclear. In this study, we show that the transcription factor *Hypoxia-inducible factor 2 alpha (Hif2α)* is selectively induced in sub-epithelial stroma surrounding the embryo, and plays a crucial role in regulating maternal adaptation to hypoxia during early pregnancy. Using a conditional knockout mouse model we demonstrate that in the absence of HIF2α, the embryo attaches to the uterine epithelium but fail to breach through it, resulting in implantation failure. Gene expression profiling revealed that HIF2α regulates expression of factors involved in vesicular trafficking. Further studies showed that HIF2α controls trafficking of matrix metalloprotease 9, a protease involved in degradation of extracellular matrix and cellular remodeling. As pregnancy progresses, HIF2α-directed vesicular communication between stromal and endothelial cells promotes the development of the vascular network critical for the establishment of pregnancy. Collectively, our study provides novel insights into the molecular basis of hypoxic adaption that is critical for embryo implantation and establishment of pregnancy in humans and animals.

Prenatal Exposure to a Phthalate Mixture Alters Sex Steroid, Peptide, and Gonadotropin Hormones in the F1, F2, and F3 Generations of Female Mice

Emily Brehm, Changqing Zhou, Liying Gao, and Jodi A. Flaws
Department of Comparative Biosciences, University of Illinois, Urbana, IL

Phthalates are used in personal care products and plastic products. Previously, our laboratory developed a phthalate mixture that mimics human exposure and we found that prenatal exposure to this mixture impaired reproduction in multiple generations. The reasons for this effect were unknown, but could be due to effects on hormones. Thus, we tested the hypothesis that prenatal exposure to a phthalate mixture alters hormones in a multi- and transgenerational manner in female mice. Pregnant CD-1 dams were dosed with vehicle control (corn oil) or a phthalate mixture (20 µg/kg/day-500 mg/kg/day) daily from gestational day 10 to birth. F1 females born to these dams were used to generate the F2 generation by mating them with unexposed males and adult F2 females were used to generate the F3 generation by mating them with unexposed males. On postnatal days (PNDs) 1-60 and 13 months, females from each litter were euthanized and sera were collected to measure hormones. At PNDs 1-8, prenatal exposure to the phthalate mixture increased estradiol and progesterone levels (F1-F3). At PND 21, the mixture decreased estradiol and testosterone levels (F1). At PND 60, it altered estradiol (F1-F3), decreased progesterone (F1, F3), decreased testosterone (F1, F3), decreased follicle-stimulating hormone (FSH) (F1, F2), and increased luteinizing hormone levels (LH) (F2). At 13 months, the mixture decreased progesterone, testosterone, and inhibin B (F1, F2), but altered LH (F1, F3) and increased FSH levels (F1). These data suggest that prenatal exposure to a relevant phthalate mixture induces multi- and transgenerational effects on hormone levels in female mice. Supported by NIH P01 ES022848 and EPA RD-83459301.

Pharmacokinetics of a Modified, Compounded Theophylline Product in Dogs

Carrie Cavett[1], Jennifer Reinhart[1], Zhong Li[2] and Brendan McKiernan[1]
Department of Veterinary Clinical Medicine[1], Roy J. Carver Biotechnology Center[2], Univerity of Illinois, Urbana, Illinois, United States

Theophylline is a commonly used bronchodilator drug for treatment of chronic canine bronchitis, but no formulations validated in dogs are currently available. An oral, modified, compounded theophylline product (MCT), which could fulfill this need, is available through a USP-compliant, veterinary compounding pharmacy; however, its pharmacokinetic properties are unknown. Therefore, the aim of this study was to determine the pharmacokinetics of MCT and to recommend an appropriate dosing regimen for use in dogs. Plasma drug concentrations were measured in seven healthy, fed dogs after single doses of intravenous aminophylline (IVA, 8. 6 mg/kg theophylline equivalent) and oral MCT (10 mg/kg). Plasma concentrations were analyzed by liquid chromatography/tandem mass spectrometry. Administration of IVA best fit a 2-compartment model. The mean ±SD systemic bioavailability of the MCT was 96. 2 ±32. 9%. MCT mean ±SD time to maximum concentration, absorption time, and terminal half-life were 8. 85 ± 3. 63, 6. 95 ±3. 42, and 8. 67 ±1. 62 hours, respectively. Using a 12-hour dosing interval, significant drug accumulation is expected (accumulation ratio 1. 62 ±0. 18). Based on simulations of 10 mg/kg q 12 hr dosing, steady state plasma theophylline concentrations are expected to exceed the minimum therapeutic concentration commonly used in dogs (10 mg/mL) for 71. 7 ±35. 6% of the dosing interval. Overall, the MCT had high oral bioavailability in most dogs and a time-concentration profile suggesting twice daily dosing at 10 mg/kg may be an appropriate initial therapeutic dosing regimen. Follow up, multi-dose studies are indicated to evaluate plasma drug concentrations at steady state.

Sub-Chronic Exposure to Di(2-ethylhexyl) Phthalate and Diisononyl Phthalate During Adulthood Disrupts Hormone Levels and Has Long-Lasting Impacts on Female Fertility in Mice

Catheryne Chiang, Lily Lewis, Saniya Rattan, Emily Brehm, Liying Gao, Daryl D. Meling, Jodi A. Flaws
Department of Comparative Biosciences, University of Illinois, Urbana, IL

Di(2-ethylhexyl) phthalate (DEHP) is a known toxicant used in consumer products. Some manufacturers have replaced DEHP with diisononyl phthalate (DiNP). However, less is known about DiNP than DEHP. Thus, this study tested the hypothesis that adult exposure to DEHP or DiNP negatively affects female fertility. To test this hypothesis, adult female CD-1 mice (39-40 days) were orally dosed with vehicle control (corn oil), DEHP (20 µg/kg-200 mg/kg), or DiNP (20 µg/kg-200 mg/kg) for 10 days. Mice were euthanized in diestrus immediately following dosing or three or six months post-dosing. Sex steroid hormone levels were assayed. A separate cohort of mice were bred immediately post-dosing and at three and six months post-dosing. Immediately post-dosing, DEHP and DiNP decreased testosterone (20 mg/kg DEHP, 20 and 100 µg/kg and 200 mg/kg DiNP) and estradiol (20 µg/kg and 20 mg/kg DEHP, 100 µg/kg and 200 mg/kg DiNP) and increased progesterone levels (200 mg/kg DEHP and 20 mg/kg DiNP). At three months post-dosing, DiNP decreased testosterone (100 µg/kg), increased estradiol (200 mg/kg), and decreased progesterone levels (20 and 100 µg/kg). At six months post-dosing, DiNP decreased testosterone (100 µg/kg) and DEHP increased progesterone levels (200 µg/kg). At three months post-dosing, DEHP and DiNP decreased the ability of females to become pregnant (20 µg/kg). At six months post-dosing, DEHP increased pregnancy loss (200 mg/kg). These data show that short exposures to DEHP and a common DEHP replacement, DiNP, have long-lasting impacts on hormone levels and fertility for several months after exposure. Supported by R56 ES 025147 (JAF), R01 ES 028661 (JAF), Billie Field Fellowship (CC), and T32 ES 007326 (CC).

Study the role of hnRNP I in regulating Toll-like Receptor signaling in the intestine

Kristy Chin, Wesley Tung, Danielle Yee, Wenyan Mei

This project focuses on studying the function of hnRNP I, a protein known to be important for post-transcriptional regulation, in regulating toll-like receptor (TLR) signaling in the intestine. Specifically, we aim to determine if hnRNP I regulates TLR signaling in a Myeloid differentiation factor 88 (MyD88)-dependent manner. TLR signaling is known to play an important role in balancing the intestinal immunity and tolerance to gut microbes. Many of TLR signaling functions are mediated by MyD88, an adaptor protein of TLR signaling. Our previous studies demonstrate that hnRNP I suppresses the neonatal intestinal immunity through downregulation of the TLR signaling activity in the neonatal intestine. We found that this suppression is critical for formation of microbiota and prevention of colitis and colorectal cancer. To determine whether hnRNP I suppresses neonatal intestinal immunity through downregulating MyD88-mediated TLR signaling, we generated a double knock-out mouse model in which both hnRNP I and MyD88 are deficient in the intestinal epithelial cells. Through characterizing the intestinal defects in the hnRNP I and MyD88 double knockout mice, we found that hnRNP I regulates the neonatal intestinal immunity through mechanisms that are independent of MyD88.

Post-translational Modification of FOXA2 Regulates Mucus Hypersecretion Induced by the Redox Cycling Toxin Pyocyanin Secreted by *Pseudomonas aeruginosa*

Woosuk Choi and Gee W. Lau
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

Mucus layer is a slimy and viscous structure covering airway surfaces to protect lung from inhaled pollutants and pathogens. Airway mucus is composed of various mucins including MUC5AC and MUC5B, which are produced and secreted from goblet cells and submucosal glands. Despite its protective role, excessive mucus and failure in clearance causes airway obstruction, resulting in chronic bacterial infection commonly seen in chronic lung diseases including cystic fibrosis, chronic obstructive pulmonary disease, bronchiectasis and bronchitis. Previously, we and others have demonstrated that a common mechanism of mucus hypersecretion is caused by depletion of FOXA2, a key transcriptional regulator of mucus homeostasis. FOXA2 proteins displays many posttranslational modifications features important for regulation of insulin signaling. However, the importance of these FOXA2 post-translational modifications is unknown in mucin expression. In this study, we examine the role of FOXA2 acetylation and phosphorylation on nuclear-cytoplasmic shuttling and degradation, and how they modulate mucus production in human bronchial epithelial cells exposed to pyocyanin, a redox-cycling toxin secreted in abundant by *P. aeruginosa* and a major inducer of mucus hypersecretion in chronically diseased human lungs. In pyocyanin-exposed 16HBE cells, FOXA2 is phosphorylated at threonine 156 and translocated from the nucleus to the cytosol for ubiquitination and degradation. Phosphorylation-deficient (T156A) FOXA2 mutant is resistant to nuclear export, but does not alter mucin expression. Phosphorylated FOXA2 is susceptible for ubiquitination, whereas phosphorylation-deficient (T156A) mutant exhibits the lower level of ubiquitination than wild-type FOXA2. In addition, acetylation at lysine 259 regulates FOXA2 stability. Introduction of acetylation-mimicking (K259Q) mutation accumulates FOXA2 in the nucleus and inhibits mucin expression. Conversely, acetylation-deficient (K259R) FOXA2 mutant promotes mucin expression with lower amounts of nuclear retention. Acetylation-deficient (K259R) mutant shows higher level of ubiquitination than wild-type FOXA2 and acetylation-mimicking (K259Q) mutant. Collectively, these data indicate that acetylation and phosphorylation modulate localization and stability of FOXA2, which impact mucin expression in pyocyanin-exposed bronchial epithelial cells.

Upregulation of the hedgehog pathway and associated anti-apoptotic factors can be inhibited by itraconazole in canine osteosarcoma cell lines

Dana R. Connell, Holly C. Pondenis, Anne M. Barger, Timothy M. Fan
Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61802

Introduction: Osteosarcoma (OS) is the most common primary bone cancer in canines, yet therapies for pet dogs with metastatic OS remain clinically ineffective. With the advent of precision medicine, strong scientific and clinical impetus exists to further investigate druggable molecular perturbations that might contribute to canine OS pathology. Dysregulated activity of the hedgehog (HH) pathway has been identified in diverse cancers in people and animals, and leads to overexpression of anti-apoptotic proteins including Bcl-2. Itraconazole is an antifungal agent that can inhibit Smoothened (SMO), a crucial G protein-coupled receptor located on the primary cilium, which initiates HH pathway signaling. We hypothesize that components of the HH pathway are overexpressed in canine OS cell lines with concurrent Bcl-2 overexpression relative to non-malignant osteoblasts. Additionally, itraconazole would inhibit HH pathway signaling by promoting SMO relocation off the primary cilium.

Methods: RNA transcript and protein expressions of SMO and Bcl-2 were characterized in 4 canine OS cell lines and normal canine osteoblast cultures. The IC50 concentrations of itraconazole in OS cell lines were determined, and itraconazole's effects on the HH pathway signaling partners were evaluated at RNA and protein levels.

Results: OS cell lines overexpress SMO and Bcl-2 relative to normal osteoblasts. The IC50 of itraconazole in OS cells ranged from 490-790 nM. Itraconazole effectively perturbs protein expressions of the HH signaling pathway and downstream targets.

Conclusions: SMO and Bcl-2 are overexpressed in OS cells and itraconazole can disrupt the HH signaling pathway at biologically-relevant concentrations, warranting further investigation of itraconazole as an adjuvant therapy for OS.

Age and Sex Related Differences in Hematologic and Biochemical Parameters in Iditarod Sled Dogs

Sara Connolly[1], Stuart Nelson[2], Tabitha Jones[2], Julia Kahn[3], Peter Constable[1]
1. Department of Pathobiology, University of Illinois, Urbana, Illinois
2. Iditarod Trail Committee, Wasilla, Alaska
3. Veterinary Specialty Center, Buffalo Grove, Illinois

Review of hematology and serum biochemistry data are a key part in the evaluation of health in our veterinary patients. Changes in these values can occur as a response to normal physiological adaptations including age, sex and pregnancy. Knowledge of the deviations that can occur secondary to normal physiological adaptations is important to prevent the misdiagnosis of disease. We hypothesized that the age related changes in Iditarod Sled Dogs will be unique to those previously noted in Beagles due to the Sled Dogs' status as an elite athlete. As part of a pre-race screening program, hematology and serum biochemistry testing is performed on all dogs that may participate in the Iditarod Trail Sled Dog race within one month of the beginning of the race. This protocol has allowed for collection of a large hematology and biochemistry data set from elite endurance trained male and female dogs with a wide age range. Mixed models analysis was applied to the data from 3,417 individual dogs examined from 2015 - 2018, with dogs nested within team to account for team specific diet, environment and management factors. The analysis indicated significant ($p<0.01$) main effects of age on calcium, glucose, WBC count, phosphorus, MCV and platelets. Significant main effects of sex were noted for platelets, SUN, creatinine, total protein, albumin, globulins, ALT and bilirubin. Further investigation into the physiologic cause(s) of these age and sex related changes will allow for a better understanding of changes or differences in metabolism and performance with age and sex in canine athletes and possibly the general canine population.

Identifying the role of novel tumor suppressor gene G0S2 in breast cancer

Andrea Corbet, Christina Yim, Ratnakar Singh, Ema Khan, Emmanuel Bikorimana, Jennifer Rodriquez, Sarah Freemantle, and Michael Spinella
Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

The G0/G1 switch gene 2, or G0S2, is a direct target of retinoic acid (RA) and has been shown to be silenced via methylation in a variety of solid tumors. Our lab has demonstrated that G0S2 expression is decreased in patients with breast cancer and that this correlates with an increased rate of relapse. We have also shown that G0S2 null fibroblasts are more prone to oncogenic transformation compared to wild-type cells and have increased PI3k/mTOR and MYC activity. Our hypothesis is that G0S2 has a tumor suppressive role in normal breast cells and that loss of G0S2 may lead to a more cancer prone state. Our first aim is to determine whether G0S2 plays a tumor suppressive role in both human and murine mammary epithelial cells. G0S2 knock-down and over-expressed lines are being engineered in normal, immortalized and transformed cells. The cells will be used in proliferation/transformation assays and xenograph studies. Our second aim is to determine if G0S2 plays a tumor suppressive role in a transgenic mouse model. We have begun to cross G0S2 -/- mice with transgenic MMTV-PYMT mice that develop spontaneous mammary tumors to assess whether G0S2 loss decreases tumor latency or causes in increase in tumor aggressiveness. We are also conducting proximity labeling experiments coupled with mass spectrometry to gain an understanding of the mechanistic roles of G0S2. We believe these findings will further elucidate the potential role of G0S2 as a tumor suppression in breast cancer.

The use of SP-10 protein as an acrosome marker in stallion seminiferous tubules

Anamaria Cruz, Derek Sullivan, Karen Doty, Rex Hess, Igor F. Canisso, Prabhakara P. Reddi

Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana, Illinois

Testicular dysfunction is a major cause of poor reproductive performance in stallions, leading to losses to the horse industry. Diagnosis of declining testicular function requires employment of proper methods to evaluate the progression of spermatogenesis. Previous studies in mice and men have shown the intra-acrosomal antigen SP-10 as a useful marker for round spermatids and spermatozoa. The goal of this study was to evaluate the usefulness of the SP-10 antibody in the staging of the stallion seminiferous epithelium using immunohistochemistry. Adult stallion (n=4) testis cross sections were treated with an in-house guinea pig polyclonal antibody raised against the mouse SP-10 antigen. The Zeiss Axiovert 200M Widefield microscope, the Axiocam 506 Color camera, and ZenPro software were used to capture images. Two hundred tubule cross sections were observed per horse. We have found the SP-10 antibody labeling to be highly specific for the acrosome and were able to follow the progression of acrosome development. Based on the acrosome staining pattern we were able to identify 16 separate steps of spermatids in the horse. High degree of demarcation in acrosome morphology in turn helped us to identify the distinct sets of male germs found in association with spermatids at various developmental time points within testis cross sections. Thus, we were able to identify 12 stages of the cycle of seminiferous epithelium in the stallion. Previous studies found only 8 stages in the horse, likely owing to the poor resolution of acrosome morphology offered by the H&E and PAS staining. Because of the superior demarcation of the acrosome by the SP-10 antibody, we were able to discern 16 steps of spermatid development and 12 stages in the horse seminiferous epithelium. Unlike the rodent which typically shows one stage per tubule cross section, we observed multiple stages within a cross section (72% of the time) in the horse. In this regard, the stallion exhibited similarities to the human seminiferous epithelium wherein multiple stages are found in a given cross section. Overall, we have found that the SP-10 antibody is an ideal reagent for staging of the horse seminiferous epithelium. Future studies will explore its utility in understanding declining fertility in stallions.

Associations of Prenatal Exposure to Triclosan and Benzophenone-3 with Visual Recognition Memory in 7.5-Month-Old Infants

Kelsey L.C. Dzwilewski[1,2], Mariya Yanovskaya[2,3], Andrea Aguiar[2,4], Antonia M. Calafat[5], Xiaoyun Ye[5], Susan A. Korrick[6,7], Susan L. Schantz[2,4]

[1]Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL
[2]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL
[3]School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL
[4]Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL
[5]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA
[6]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA
[7]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA

Triclosan (TCS) is used in personal care products as an antimicrobial agent and benzophenone-3 (BP3) is used in personal care products and plastics as an ultraviolet absorber. Due to their endocrine disrupting properties, prenatal exposure to these chemicals may affect brain development, but little is known about their effect on infant cognition. Prenatal exposure to TCS and BP3 and visual recognition memory (measured by novelty preference) were assessed in 177 7.5-month-old infants participating in a prospective study in Illinois, USA. Study mothers were mostly white (86%), with 88% having at least a college education and 75% a household income above $60,000. In a pool of five first morning urine samples collected across pregnancy, we quantified (median[IQR]) TCS (14.1[63.1] µg/L) and BP3 (118.6[273.0] µg/L). Infrared eye tracking recorded infant looking time at a trial with two identical faces followed by trials in which the familiar face was paired with a novel face. General linear models were used to assess associations of each exposure biomarker (adjusted for urine specific gravity) with novelty preference (percent time looking at the novel face) adjusted for infant age, gestational age, birth weight, sex, assessment condition, household income, and maternal IQ and education. There were no associations of TCS or BP3 with novelty preference and no sex differences in associations. Each IQR increase in TCS was associated with a 0.10% decrease (95% CI: -0.49,0.29) in novelty preference, while each IQR increase in BP3 was associated with a 0.04% increase (95% CI: -0.38,0.47). Generalized additive models showed no evidence of nonlinear associations of either TCS or BP3 with novelty preference. This preliminary analysis found no evidence associating maternal prenatal urinary TCS or BP3 with infants' recognition memory at 7.5 months, but the results need to be confirmed in a larger sample. Acknowledgements: NIEHS ES007326, ES022848, ES028607, OD023272, USEPA RD83543401

Analysis of Treatment and Monitoring Protocols of Cytosine Arabinoside in Canine Patient

Mary Kate E. Feldner, Kari D. Foss, Devon W. Hague, David J. Schaeffer
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Cytosine arabinoside (CA) is a chemotherapeutic used as an immunosuppressant or anti-neoplastic agent within veterinary medicine. It is particularly useful in the treatment of diseases of the central nervous system based on its ability to cross the blood brain barrier. Side effects such as myelosuppression, GI disturbance, and hepatotoxicity have been reported. Therefore, it is critical to monitor patients that receive CA. The purpose of this study is to collect and compare data about CA treatment protocols in veterinary medicine. Diplomates and candidates of the American College of Veterinary Internal Medicine (ACVIM) were contacted and asked to fill out a 26-question online survey about the administration, monitoring, and side effects in dogs being treated with CA. Compiled survey data were compared statistically to similar data extracted from records of dogs treated with CA at the University of Illinois Veterinary Teaching Hospital. Results from the survey indicate a significant difference in the dose utilized in treatment protocols ($p < 0.01$). Despite variation in dosage, results show a majority of veterinarians perform similar monitoring procedures prior to the first dose of CA. However, after completing the first dose, monitoring procedures varied significantly ($p < 0.01$). Analysis of both the retrospective and survey data suggest that intravenous administration is associated with a higher risk for the development of side effects. Based on these results, there is variation in CA treatment protocols amongst veterinarians. Additionally, the route of administration may affect the side effects that are observed. Additional research is required to further investigate how route of administration can alter patient outcomes.

The Role of Dzip1 During the Development of Primordial Germ Cells

Jia Fu, Hyojeong Hwang, Aurora Turgeon, Zhigang Jin, Jing Yang
Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, United States.

In animals like *Drosophila*, *C. elegans*, zebrafish and *Xenopus*, the primordial germ cells (PGC), the precursors of the gametes, are specified through the inheritance of germ plasm. Early PGC development is regulated by the genetic program coded by unique maternal factors in the germ plasm. Currently, the molecular mechanisms by which germ plasm components regulate early PGC development are poorly understood. Previous studies identified Dzip1 as a binding partner of Dazl (Deleted in Azoospermia-like), an RNA binding protein essential for germ cells development. We recently discovered that Dzip1 (Daz-interacting protein1) is a novel components of germ plasm. Results from our loss of function analysis reveal that Dzip1 regulates the first wave of PGC proliferation. Consistently, we found disrupting the interaction between Dzip1 and Dazl results in a significant reduction in the number of PGC. Taken together, we conclude that Dzip1 is essential for germ line development and likely functions by controlling the activity of Dazl.

Iodoacetic Acid Inhibits Follicle Growth and Alters Expression of Genes that Regulate Apoptosis and the Cell Cycle in Mouse Ovarian Follicles

Andressa Gonsioroski[1], Daryl Meling[1], Liying Gao[1], Michael Plewa[2], and Jodi Flaws[1]
Department of Comparative Biosciences[1], and the Safe Global Water Institute[2], University of Illinois, Urbana, IL

The reaction between disinfectants and organic matter in water generates water disinfection by-products (DBPs). Iodoacetic acid (IAA), an unregulated DBP, is a reproductive toxicant but its effects on the ovary are not well known. This study was designed to determine whether IAA exposure affects ovarian follicle growth and expression of genes that regulate apoptosis and the cell cycle. Ovaries were collected from CD-1 mice. Antral follicles were dissected from the ovaries and placed individually in culture plates. The follicles were treated with either 0.075% dimethyl sulfoxide (DMSO; vehicle control) or IAA (2-15 μM). The follicles were cultured for 96 h and follicle growth was measured every 24 h. After culture, follicles were snap-frozen at -80˚C until RNA extraction. RNA was extracted, reverse transcribed, and subjected to quantitative polymerase chain reaction (qPCR) to analyze expression of apoptosis regulators (*Bax* and *Bcl2*) and cell cycle regulators (*Ccna2*, *Ccne1*, *Ccnb1*, *Ccnd2*, *Cdk4* and *Cdkn1a*). IAA exposure significantly decreased follicle growth compared to controls, beginning at 72 h and continuing through 96 h of culture. Further, IAA exposure decreased expression of the cell cycle regulators *Ccnd2* and *Ccna2* and it decreased expression of the anti-apoptotic factor *Bcl2*. In addition, IAA exposure increased expression of the pro-apoptotic factor *Bax* and the cell cycle regulator *Cdk4* and it increased expression of the cell cycle inhibitor *Cdkn1a*. Collectively, these data show that IAA exposure inhibits follicle growth and upregulates pro-apoptotic factors and cell cycle inhibitors, whereas it downregulates anti-apoptotic factors and some cell cycle regulators.
Supported by NIH R21 ES028963 and NIH T32 ES007326.

Controlled clinical trial using terbinafine nebulization in wild Lake Erie watersnakes with Ophidiomycosis

Ellen Haynes, Matthew C. Allender
Wildlife Epidemiology Lab, Department of Comparative Biosciences, University of Illinois College of Veterinary Medicine, Urbana, IL

Ophidiomycosis (snake fungal disease) is an infectious disease caused by the fungus *Ophidiomyces ophiodiicola*. It has been documented in over 30 species of wild snakes in North America and Europe and clinical signs include thickened scales, crusts, and ulcers on the head and body. Disease impacts occur at both the population and individual levels. To reduce the impacts on individual snakes, we are conducting a controlled clinical trial using nebulization with the antifungal drug terbinafine. We hypothesized that terbinafine nebulization would produce clinical and molecular resolution of disease. Twenty-five wild-caught Lake Erie watersnakes with Ophidiomycosis (typical lesions present and qPCR positive for *O. ophiodiicola*) were divided into control and treatment groups using a matched-pairs experimental design. Snakes in the treatment group were nebulized with a 2mg/mL solution of terbinafine in 0.9% saline for 30 minutes daily for 30 days, while the control group received 0.9% saline. Skin lesions were mapped and photographed weekly and qPCR was repeated after 30 days of treatment. Snakes received multiple courses of treatment if they were persistently qPCR positive. Terbinafine nebulization resulted in significant reduction in fungal copy number compared to the control group and to pre-treatment levels. In both groups, some snakes showed improvement in their clinical signs, while others showed progressive disease. This study indicates that terbinafine nebulization is a promising treatment for Ophidiomycosis. However, some snakes may require several months of nebulization to achieve significant improvement and the treatment may not result in complete resolution of disease in all cases.

Novel functions of the ubiquitin-independent proteasome system in regulating *Xenopus* germline development

Hyojeong Hwang[1], Zhigang Jin[1, 2], Vishnu Vardhan Krishnamurthy[3], Anumita Saha[4], Peter Klein[4], Benjamin Garcia[4], Wenyan Mei[1], Mary Lou King[5], Kai Zhang[3], and Jing Yang[1]

[1]Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[2]College of Chemistry and Life Sciences, Zhejiang Normal University, China

[3]Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, USA.

[4]Smilow Center for Translational Research, Perelman School of Medicine, University of Pennsylvanis, Philadelphia, PA, USA

[5]Department of Cell Biology, University of Miami Miller School of Medicine, Miami, FL, USA

The proper primordial germ cell (PGC) development is important for animal reproduction. In *Xenopus*, since early PGC development initiates before the beginning of zygotic gene transcription, maternal factors including RNAs and proteins are critical for driving PGC development. These maternal factors are regulated by post-transcriptional, translational modification, and protein turnover systems. Here, we report that the ubiquitin-independent proteasome system regulates the expression of *Xenopus* Dnd1, which is the most critical regulator of vertebrate germline determinant. We found that the expression of Dnd1 is low in the oocyte, but increased dramatically after fertilization. This expression pattern is mediated by ubiquitin-independent proteasome pathway through an evolutionarily conserved degron. Interestingly, we found that RNAs coding for proteasome components, including PA28α, PA28β, PA28γ, and PA200, the 20S proteasome core particle, and the 19S regulatory particle, were dynamically relocalized to the animal hemisphere during oocyte maturation. Ectopic expression of PA200 in the vegetal pole causes a significant decrease in the numbers of PGCs. These results indicate that separation of the ubiquitin-independent proteasome system from the germ plasm during meiotic oocyte maturation is extremely important for proper PGC development. In conclusion, these results suggest dynamic control of the protein homeostasis machinery is critically important for vertebrate PGC development.

Micro-mechanics of Meiotic Chromosomes in Prophase 1 of Meiosis

Pranathi Karumanchi[1], John Marko[2] and Ronald Biggs[2], Huanyu (Joe) Qiao[1]
Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois, USA

Meiosis involves two divisions to form four haploid daughter gamete cells from a diploid parent cell. In both mitosis and meiosis, chromatin needs to condense tightly enough so they can be separated during cell division. Chromosome condensation is also essential for homologous chromosome alignment in prophase 1 of meiosis. However, the higher levels of meiotic chromosome condensation are still poorly understood. One way to understand the architecture of meiotic chromosomes is by studying their mechanical properties. We adopted a micromanipulation technique to measure the elasticity of mouse meiotic chromosomes. Similar to mitotic chromosomes, we found meiotic chromosomes can be dissolved by non-specific nuclease MNase but not by 6-base cutter restriction enzyme PvuII and proteases, which indicates that meiotic chromosomes form gel-like network structure during higher level condensation. Different to mitotic chromosomes, the 4-base cutter AluI disassembles meiotic chromosomes faster than the mitotic ones. Moreover, prophase I meiotic chromosomes from spermatocytes are approximately ten times stiffer than mitotic chromosomes. Both results indicate that prophase I chromosomes of meiosis have a different structure compared to mitotic chromosomes. To find the resources of this additional strength, we measured the elasticity of meiotic chromosomes from *Sycp1*$^{-/-}$ mutant mice to see if transverse filaments SYCP1 cause the increase of chromosome stiffness. We didn't observe the difference of chromosome stiffness between wild type and *Sycp1*$^{-/-}$ mutants, which suggests that meiosis-specific protein SYCP1 does not increase the stiffness of meiotic chromosomes. In the future, we would like to determine if other meiosis-specific factors cause the ten-fold increase in strength of meiotic chromosomes compared to mitotic chromosomes.

Nuclear Localization Signal of Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid (N) Protein is Essential for NF-κB activation

Hanzhong Ke and Dongwan Yoo
Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL.

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent for PRRS which is one of the most economically significant diseases in the swine industry worldwide. One of the mechanisms that PRRSV causes respiratory disease is the onset of inflammation at the site of infection. PRRSV activates NF-κB during infection and the nucleocapsid (N) protein of PRRSV has been identified as the activator. PRRSV N directly binds to protein inhibitor of activated STAT1 (PIAS1), which is a negative regulator of NF-κB by binding to RelA (p65) thereby preventing NF-κB from binding to κB sites. We showed the interaction of N and PIAS1 as the mechanism for N-mediated NF-κB activation. The region between 37 and 72 amino acids of N was identified as the binding domain to PIAS1, and this region overlapped the nuclear localization signal (NLS) of N. The NLS knockout mutant N protein did not translocate to the nucleus and lost the NF-κB activation as shown by reduced NF-κB reporter activities, reduced p65 phosphorylation, and reduced expression of IL-1β, IL-6, IL-8, and TNF-α genes. NLS knockout mutant N protein did not bind to PIAS1 in the nucleus. The NLS knockout mutant PRRSV was rescued by the reverse genetics and the phenotype of this virus was characterized. In conclusion, the NLS of N protein is essential for N binding to PIAS1 and for activation of NF-κB.

A new super-resolution tool for neuron imaging: Confocal Reflection Microscopy

Yee Ming Khaw[1], Makoto Inoue[1,2]
Neuroscience Program[1], Department of Comparative Biosciences[2], Urbana-Champaign, Illinois

Golgi-Cox staining is an established method used to visualize neurons in great morphological detail in brain or spinal cord slice preparation. Aside from using the conventional technique of viewing Golgi-Cox stained tissue in 2D under the light microscope, 3D visualization techniques have been recently developed to capture the 3D structural integrity of Golgi-Cox stained neurons using the confocal microscope's reflection modality. This microscopic technique allows the simultaneous acquisition of Golgi-Cox reflective metallic signals and fluorescent signals present in prepared slice samples. However, the current 3D resolution of Golgi-Cox confocal reflection is inadequate to perform detailed analysis regarding fine morphological details of neurons. Here, we report a super-resolution achievement of the Golgi-Cox confocal reflection technique by minimizing the pinhole size of the confocal micrsocope. This super-resolution confocal reflection technique results in 45% improvement in resolution and allows object distinction with superior clarifty. We show that the achievement of super-resolution in Golgi-Cox 3D imaging attains an accurate quantitative data of neuron morphology such as dendritic spine density and spine morphological classification. Thus, we introduce the Golgi-Cox confocal super-resolution imaging tool as a means to investigate detailed neuron morphology in the central nervous system. The super-resolution imaging tool can be used to extend new knowledge into real life applications to improve the health of animals and people.

Generation of Type I Interferon Suppression-negative and NF-κB Activation-negative PRRSV and Characterization of Their Phenotypes in Cells

Jineui Kim, and Dongwan Yoo
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Porcine reproductive and respiratory syndrome virus (PRRSV) has the ability to suppress the production of type I interferons (IFNs-α/β) for its survival in a host and predispose to secondary infections by activating NF-κB signaling, leading to porcine respiratory disease complex (PRDC). Previously, a mutation in the SAP (SAF-A/B, Acinus, and PIAS) motif in non-structural protein (nsp) 1β of PRRSV was shown to confer IFN suppression-negative, and subsequently, the nuclear localization signal (NLS) in the viral nucleocapsid (N) protein was identified to overlap NF-κB activation. We hypothesized that simultaneous mutations in the both nsp1β and N proteins would render type I IFNs suppression-negative and NF-κB activation-negative. Such viruses may be effective vaccine candidates for PRDC. By extralong inverse PCR using full-length infectious clones, six specific mutant constructs were generated. The constructs represented wild type PRRSV (pCMV-S-P129), three mutants of type I IFN suppression-negative PRRSV (P1β-Δ126, P1β-Δ135, P1β-Δ126/135), NF-κB activation-negative PRRSV (PN-ΔNLS), and three mutants of type I INF suppression-negative and NF-κB activation-negative PRRSV (PD-Δ126-NLS, PD-Δ135-NLS, PD-Δ126/135-NLS). By the reverse genetics, progeny viruses were successfully rescued from the mutated clones. The mutant PRRSV were characterized for their growth kinetics, plaque morphology, type I IFN suppression, NF-κB activation, and production of proinflammatory cytokine during infection in cells. The mutant viruses are anticipated to be attenuated and reversion-negative to wild-type, and to relieve the severity of respiratory disease that may be caused by co-infection of PRRSV and other swine pathogens.

Endocannabinoid Dysregulation in Multiple Sclerosis

Justin Kim[1], Andrew Steelman[2], Aditi Das[1,3]
[1]Division of Nutritional Sciences, [2]Department of Animal Sciences,[3]Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana IL

Cannabis-derived cannabinoids including Δ^9-tetrahydrocannabinol and cannabidiol mitigate symptoms of Multiple Sclerosis, a chronic inflammatory and neurodegenerative disease. Specifically, their anti-inflammatory and immunomodulatory properties are attributed to their interactions with cannabinoid receptors. The human body contains endogenous cannabinoids (endocannabinoids) that elicit similar effects as the plant-derived molecules. Notably, endocannabinoids including anandamide and 2-arachidonoylglycerol decrease inflammation and promote immune homeostasis to alleviate symptoms and disease progression of Multiple Sclerosis. We recently discovered a novel class of omega-3 lipid metabolites generated by Cytochrome P450 epoxygenases that are anti-inflammatory and activate cannabinoid receptors with higher potency than anandamide. We investigate how these omega-6 and novel omega-3 lipid metabolites are dysregulated in relevant animal model of Multiple Sclerosis, EAE. Through detailed immunohistochemistry and cytokine analysis, we further understand how changes in immune homeostasis may be caused by endocannabinoid dysregulation. Lastly, as dietary DHA suppresses EAE, we further explore the therapeutic potential of endocannabinoid-epoxides in promoting health and reducing severity of Multiple Sclerosis. These results provide better insight into how endocannabinoid dysregulation occurs in Multiple Sclerosis and indicate the potential of omega-3 derived endocannabinoid-epoxides as a therapeutic treatment in autoimmune diseases.

Controlled expression of the Marek's disease virus (MDV) conserved herpesvirus protein kinase (CHPK) using a dihydrofolate reductase (DHFR) instability domain

Andrea Krieter, Nagendra Prabhu Ponnuraj and Keith W. Jarosinski
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

The orthologues of the protein kinase encoded by Marek's disease virus (MDV) UL13 are conserved among the *Herpesviridae* and are called conserved herpesvirus protein kinases (CHPKs). We have previously shown that MDV CHPK is essential for host-to-host transmission of MDV in chickens, but we currently do not know what stage of infection it is required. MDV infection begins through inhalation of infectious virus via the respiratory route and spreads to feather follicle epithelial (FFE) cells of the skins where it is shed from infected chickens. We hypothesize that MDV CHPK plays a role in both initiation of infection in the naïve host and release of infectious virus from infected chickens. Since there are no antibodies against MDV UL13 available, our first step in addressing these hypotheses was to create epitope tagged UL13 proteins in the context of the viral genome (vUL13c3×Flag), as well as a kinase mutant (vUL13K170Mc3×Flag). We tested the ability of these viruses to replicate in chickens and determined both vUL13c3×Flag and vUL13K170Mc3×Flag replicated similar to the parental virus using qPCR assays of viral genomes in the blood of infected chickens. Consistent with previous studies, the kinase mutant virus was unable to transmit to naïve contact chickens, while both parental and vUL13c3×Flag transmitted with similar efficiency. We are currently developing a system in which we selectivity express UL13 using the dihydrofolate reductase (DHFR) domain that targets the protein for degradation. Treatment of cells with trimethoprim (TMP) is expected to stabilize UL13; thereby, we can selectively express UL13 in the presence of TMP or degrade UL13 in the absence of TMP. We are testing this system *in vitro*.

Comparison of cerebellomedullary and lumbar cerebrospinal fluid analysis in dogs with neurological disease

Rachel Lampe, Kari Foss, Samantha Vitale, Devon Hague, Anne Barger
University of Illinois, Urbana, IL

Cerebrospinal fluid (CSF) is commonly analyzed in dogs with neurological disease to help categorize the underlying disease present. Consensus dictates that the CSF should be collected caudal to the lesion, however there is little evidence at this time to justify this. This study aims to evaluate the differences between CSF collected from the cerebromedullary (CM) and lumbar cisterna in dogs with neurologic disease. Dogs undergoing MRI for investigation of neurological disease were prospectively enrolled in the study. CSF was collected from the CM and lumbar cisterna in all patients. The total protein (TP), red blood cell count (RBC), and total nucleated cell counts (TNCC) were analyzed within 30 minutes of collection. Thirty-one paired samples were collected. Lumbar taps had a significantly higher value of TNCC ($p < 0.005$), RBC ($p < 0.0001$), and TP ($p < 0.0001$) compared to CM taps. The pathologist interpretation differed between sites in 67% of total cases (21/31). When grouped by localization, 52% (9/17) of brains, 87.5% (7/8) of cervical myelopathies, and 83% (5/6) of thoracolumbar myelopathies resulted in different pathologist interpretation between the two sites. Four cases localized to the brain or cervical spine resulted in only one of the taps being abnormal. None of the thoracolumbar localizations had pleocytosis in the CM taps, compared to 4/6 of the lumbar taps. These results suggest that CSF analysis results differ between CM and lumbar taps within the same dog. In thoracolumbar cases, CM taps may lead to false negatives and not be representative of the underlying disease process. In dogs that localize to the brain or cervical spine, there may be clinical benefit in collecting fluid from both locations.

A Retrospective Study of Prognostic indicators for Survival in 955 Eastern Grey Squirrels (*Sciurus carolinensis)*

Ivana Levy[1], Matthew Allender[1,3], Stephanie Principati[3], Julia Whittington[2,3], Sarah Reich[2,3], Krista Keller[1,3]
Wildlife Epidemiology Laboratory[1], College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL
Wildlife Medical Clinic[2], College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL
Department of Veterinary Clinical Medicine[3], College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

The eastern grey squirrel (EGS), *Sciurus carolinensis*, is a tree squirrel native to the eastern United States that commonly presents to wildlife facilities. The purpose of this study was to determine prognostic indicators for survival in young or orphaned EGS in order to maximize their survival and ensure appropriate allocation of limited resources at wildlife medical clinics and rehabilitation centers. Data was collected from a total of 955 EGS from 2012-2018 who fit the inclusion criteria at the University of Illinois Wildlife Medical Clinic. Squirrels were identified as survivors (surviving, transferred, or released within 72 hours of presentation) or non-survivors (euthanized or died within 72 hours of presentation). Presenting weight, body system abnormalities, method of feeding, and singleton versus group presentation were categorically recorded for each case. The outcome status (survivor vs. non-survivor) was modeled using logistic regression models fitted using the glm. Squirrels that had any sort of body system abnormality ($p<0.0001$) including neurological signs ($p=0.0006$), respiratory signs ($p<0.0001$), and diarrhea ($p=0.003$) were more likely to be non-survivors. Animals that presented within Dec-May were more likely to be non-survivors than those who presented from June-Nov ($p=0.009$). Therefore, survival status was negatively impacted by any type of body system abnormality as well as time of the year EGS presented. Based on the results of this study, triage care for EGS with these findings should be aggressive for a greater chance of survival. Further research is necessary to determine therapeutic methods that can be utilized to minimize mortality in this species.

Comparison of Axillary and Inguinal Temperature to Rectal Temperature in Healthy Guinea Pigs (*Cavia porcellus*)

Ivana Levy[1], Matthew Allender[1,2], Caitlin Hemby[1], Krista Keller[1,2]
Wildlife Epidemiology Laboratory[1], College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL
Department of Veterinary Clinical Medicine[2], College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Core body temperature is an essential health parameter. Temperature aberrations can indicate several infectious or inflammatory disorders, influence clinical management decisions, and serve as a prognostic indicator for patient recovery in other species. Historically, rectal temperature measurements have been utilized in companion animals, however there is a growing interest in less-invasive methods including auricular and axillary measurements to determine core body temperature. In this study, temperature measurements (axillary (AT), inguinal (IT), and rectal (RT)) were performed concurrently in 19 healthy guinea pigs (12 female, 7 male). Paired sample t-test was utilized to evaluate differences in mean body temperature measurements between each body site. Agreement analysis was performed using a one-sample t-test on the difference between each paired body site. There was poor agreement in body temperature measurements between the body sites with IT (mean = 100.6 ºF, 38.1 ºC) significantly lower than both AT (mean = 101.3 ºF, 38.5 ºC; $p = 0.003$) and RT (mean = 102.1 ºF, 38.9 ºC; $p < 0.0001$), and AT was significantly lower than RT ($p = 0.003$) These results indicate that AT, IT, and RT cannot be used interchangeably to represent core body temperature Therefore, RT should remain the gold standard to measure core body temperature in guinea pigs and be utilized with proper handling techniques to minimize stress in clinical practice.

Unraveling the Spatiotemporal Induction of Competence System during Pneumonia-derived Sepsis by *Streptococcus pneumoniae*

Jingjun Lin and Gee W. Lau

Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

The human respiratory pathogen *Streptococcus pneumoniae* (pneumococcus) causes multiple mild to fatal infections. Pneumococcus is long recognized for its ability to undergo a short burst (~15 min) of natural competence for genetic transformation during *in vitro* growth, which allows exogenous DNA uptake and incorporation into the genome. We and others have reported that the competence regulon is important for virulence, and for horizontal gene transfer between pneumococcal strains during host colonization and infection. However, the spatiotemporal induction of competence during host infection and its importance remain UNKNOWN. Here, we constructed a competence-specific luciferase reporter strain to track competence induction in a mouse model of pneumonic sepsis. At an infectious dose of 10^7 CFU, the competence was naturally-initiated between 27-30 hr post-infection (hpi) in 12/15 mice. In 7/15 mice, infection quickly progressed to death (~ 34-44 hpi), with persistent increase in competence induction until death. For the remainder 5/15 mice that died between 59-70 hpi, their competence dropped at 39 hpi but resumed before approaching death. All mice reaching endpoint (12/15) developed pneumonic sepsis with systemic spread of pneumococcus. Collectively, these results suggest that pneumococcal competence is initiated only after successful establishment of host infection. Additionally, in stark contrast to the short duration of natural and artificially-induced competence *in vitro*, the competence induction during infection is prolonged and persistent. Unveiling the *in vivo* competence induction will further reveal the contribution of pneumococcal competence to virulence and paving the way for non-antibiotic based therapy.

Increasing tick surveillance in Illinois through collaboration between, governmental, academic, and citizen scientists

Lee Ann Lyons, Marilyn O'Hara Ruiz, Rebecca Smith
Department of Pathobiology, College of Veterinary Medicine, University of Illinois

Between 1990 and 2013, the number of reported cases from the four most common tick-borne diseases (TBDs) in humans increased ten-fold in Illinois. Previously, IL has lacked a tick surveillance program leading to insufficient information on how, where, and why people are exposed to ticks. The aim of this research it to address these gaps. Three surveillance strategies were utilized to gather information about the ticks of public health concern: 1) IL Tick Inventory Collaboration Network (I-TICK); 2) systematic collection; 3) special collections. I-TICK kits were used by people within IL who work outside on a regular basis. They provide data on how many ticks they find on themselves during the day and return ticks to network hubs. Systematic collection was performed by researchers every two weeks in predetermined field locations. Special collections were targeted collections in locations where particular TBDs are of concern. Ticks from all three methods (2282 total) were collected, quantified, and identified in 2018. Of collected ticks, 906 were from I-TICK, 172 from systematic collections in 3 counties, and 1204 were from a special collection. Four species of ticks were identified: *Amblyomma americanum* (1484); *Amblyomma maculatum* (39), *Dermacentor variabilis* (696); *Ixodes scapularis* (63). Ticks were collected in 54/102 (52.9%) IL counties. There were 52 counties for which tick species status (established or reported) changed. We hypothesize, from these findings, that ticks of public health concern are established within every county of the state and *A. maculatum* is established within multiple counties. While TBDs have been relatively rare in the past, we believe that more people within IL are being exposed closer to home.

The Effects of Phthalate Metabolites on Expression of Genes Important for Sex Steroid Hormone Synthesis in Antral Follicles

Daryl D. Meling, Jason R. Szumski, Genoa R. Warner, Liying Gao, Andressa V. Gonsioroski, Saniya Rattan, and Jodi A. Flaws
Department of Comparative Biosciences, University of Illinois, Urbana, IL

Phthalates are used as solvents and plasticizers in a wide variety of consumer products, which leads to exposure through ingestion, inhalation, and dermal contact. Little is known about whether the phthalate metabolites are ovarian toxicants. Thus, this study tested the hypothesis that phthalate metabolites influence the expression of genes involved in the synthesis of sex steroid hormones by mouse antral follicles *in vitro*. The selected metabolite mixture was based on concentrations in urine of pregnant women in the I-Kids study in Illinois; it included 36% MEP, 19% MEHP, 15% MBP, 10% MiNP, 10% MiBP, and 8% MBzP. Antral follicles from adult CD-1 mice (32-42 days) were cultured in groups of 10-12 follicles for 96 hours with vehicle control (DMSO) or metabolite mixtures (0.1-500 μg/ml). Growth of follicles in culture was monitored every 24 hours. Following culture, total RNA was extracted and reverse transcribed. Real-time PCR was then performed for *Star*, *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, *Hsd17b1*, and *Cyp19a1*, which are required for sex steroid hormone synthesis. Follicle media were tested for accumulation of hormones in this pathway with ELISAs. The higher doses of phthalate mixture inhibited follicle growth compared to controls. Some of the treatments led to decreased expression of *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, *Hsd17b1*, and *Cyp19a1* compared to controls. Corresponding to differential dose responses for changes in gene expression, changes in hormone levels followed different patterns. The 500 μg/ml mixture decreased estradiol 10-fold compared to control. Further, the mixture at 100 μg/ml increased testosterone 4-fold and the mixture at 500 μg/ml increased testosterone over 10-fold compared to controls. The mixture at 100 μg/ml also increased progesterone over 10-fold and the mixture at 500 μg/ml increased progesterone over 2-fold compared to controls. Collectively, these data suggest that the phthalate metabolite mixture impacts ovarian expression of key genes and hormones in the sex steroid hormone synthesis pathway.

Dynamic Changes of TDP-43 from Germ Cell Development to Sperm Formation

Lyndzi Miller, Prabhakara Reddi
Department of Comparative Biosciences, University of Illinois College of Veterinary Medicine, Urbana-Champaign, Illinois, Unites States of America

TAR DNA-binding protein of 43 kDa (TDP-43) is a ubiquitously expressed and evolutionarily conserved protein. It was first discovered as a transcriptional repressor of human immunodeficiency virus type-1 (HIV-1). TDP-43 has several functions which include DNA/RNA binding, gene transcription, mRNA splicing, stability, transposon silencing, and micro RNA biogenesis (1). Using genetic mouse models, we demonstrated that TDP-43 is essential for spermatogenesis and male fertility. We also showed that sperm from infertile men contain aberrant forms of TDP-43 (2). One in six couples worldwide struggle with infertility with male factor accounting for half the cases, highlighting the importance of better understanding the mechanisms regulating spermatogenesis. We are specifically working on understanding the role of TDP-43 in male germ cell differentiation and fertilization. The goal of the present study is to evaluate the dynamic changes of TDP-43 expression in the testis and sperm. CD1 and B6-background mouse testes and sperm were collected and immunoblots were performed. We observed identical patterns of TDP-43 immunoreactivity between the CD1 and B6-background mice (n=2). The testis samples showed TDP-43 bands, in order of prominence, at 43 kDa (the expected size for a 414aa protein) and ~80 kDa. In contrast, the sperm samples displayed a prominent TDP-43 band at ~55 kDa. We find this difference interesting and predict that protein dimerization and/or post-translational modifications account for the 55 and 80kD TDP-43 bands. Future experiments will attempt to resolve this issue. We hypothesize that dynamic changes in TDP-43 are reflective of the different functional requirement of this protein in germ cells versus sperm.

One RING to Rule Them All: RNF212 Regulates The Size of The Ovarian Follicle Pool

Supipi Mirihagalle[1], Jared H. Fong[2], Manali Sapre[2], Neil Hunter[2], Huanyu (Joe) Qiao[1,2]

1Department of Comparative Biosciences, University of Illinois, Urbana-Champaign, Illinois, USA
2Department of Microbiology & Molecular Genetics, University of California, Davis, Davis, California, USA

The size of oocyte pool is very important for fertility for woman, because it is a good characteristic of reproductive lifespan. Ovary control eliminates oocyte with exceeding DNA damage in meiosis. This process is regulated by RNF212, a SUMO ligase, which is essential for crossover. We used RNF 212, SPO11, MSH4 mutant mouse and radiation to investigate the function of RNF 212. With the result, we found RNF 212 could promote the apoptosis of oocytes with defect. Since SPO11 and MSH4 mutation could cause oocyte death, mutation of RNF 212 restoring oocyte pools means RNF 212 has pre-apoptotic function. The increased number of oocyte in RNF 212 mutant after radiation also proves that RNF 212 induces apoptosis in post-natal ovaries. It also leads to oocytes death of mouse having synapsis and recombination defect.

Prenatal Exposure to Di-(2-ethylhexyl) Phthalate and High-fat Diet Synergistically Disrupts Mouse Fetal Oogenesis and Affects Folliculogenesis

Supipi Mirihagalle, Tianming You, Lois Suh, Chintan Patel, Liying Gao, Saniya Rattan, and Huanyu Qiao
Department of Comparative Biosciences, University of Illinois, Urbana-Champaign, IL

Di-(2-ethylhexyl) phthalate (DEHP) is a chemical that is widely used as a plasticizer. Exposure to DEHP has been shown to alter ovarian function in humans. Additionally, foods high in fat content, regularly found in the western diet, have been shown to be another potential disruptor of fetal ovarian function. Due to DEHP's lipophilicity, high-fat foods can be easily contaminated with DEHP. Therefore, exposure to DEHP and a high-fat diet are both health concerns, especially in pregnant women, and the effects of these exposures on fetal oocyte quality and quantity should be elucidated. In this study, we hypothesized that exposure to DEHP at an environmentally relevant level (20 μg/kg body weight/day) and high-fat diet can disrupt oogenesis and folliculogenesis in F1 female mice. Dams were fed with a high-fat diet (45 kcal% fat) or a control diet (10 kcal% fat) one week before mating and during pregnancy and lactation. The pregnant mice were dosed with DEHP (20 μg/kg body weight/day) or vehicle control from E10.5 to litter birth. We discovered that prenatal exposure to DEHP + high-fat diet increases the incidence of asynapsis during meiotic prophase I and affects preantral follicle development of F1 females at PND 21. Thus, simultaneous consumption of a high-fat diet and exposure to DEHP during pregnancy is a health concern.

Transmission of waterborne fish and plant pathogens in aquaponics and their control with physical disinfection and filtration: a systematized review

Jameson Mori and Rebecca L. Smith
Department of Pathobiology, UIUC College of Veterinary Medicine

The objective of this systematized review was to collect and analyze information about the waterborne spread of fish and plant pathogens through aquaponics systems, as well as investigate physical disinfection or filtration methods used to control transmission. Information gathered from comparable aquaculture and hydroponics systems was also considered for its applicability to aquaponics, and a bias assessment of accepted literature was conducted. One-hundred and forty sources were included in the review, with 85 from aquaculture systems, 55 from hydroponics, and 0 from aquaponics. Transmission was studied using cohabitation of naïve and infected organisms or direct inoculation of the water. Pathogen control methods included ultraviolet (UV) irradiation, blue light-emitting diodes, media filtration, membrane filtration, heat, sonication, and three methods that fell outside these categories. Water quality and flow rate were identified as important factors influencing disinfection efficacy, along with parameters specific to each disinfection method. The lack of studies on pathogen transmission and control in aquaponics systems, paired with the risks associated with a disease outbreak, make this an important, yet neglected, area of research.

GM-CSF$^+$ Tc17 cells are necessary for Vaccine-Induced Immunity and attenuation of pathology against Lethal Fungal Pneumonia

Srinivasu Mudalagiriyappa and Som G. Nanjappa
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL-61802

Fungal infections in CD4$^+$ T cell immunocompromised patients are on an upsurge in recent years. The use of vaccines is critical to prevent such infections, yet no licensed vaccines available. The design of rational vaccines requires identifying the correlates and understanding the mechanisms of vaccine-immunity. Previously, using an experimental fungal vaccine, we have shown that CD8$^+$ T cells can compensate for the lack of CD4$^+$ T cells. Vaccine-immunity required the cytokines produced by fungal-specific CD8$^+$ T cells, where IL-17A (Tc17 cells) played a dominant role complemented by Type-I cytokines (IFNγ, GM-CSF, TNF). However, the role of multi-cytokine producing cells is not understood during fungal infections. Here, we investigated the generation and functions of GM-CSF$^+$ Tc17 cells for fungal-vaccine immunity and immunopathology. We found that the cytokines, IL-1 and IL-23, are essential for the formation of GM-CSF$^+$ Tc17 cells, former for their differentiation, whereas, the latter for their numbers. Interestingly, IL-23 was not required for GM-CSF$^+$ Tc17 survival but for the recall responses on a per-cell basis. Our challenge studies showed that vaccinated mice required CD8$^+$ T-cell intrinsic IL-17A for immunity that was enhanced in the presence of GM-CSF leading to less pathology. On the contrary, unvaccinated mice showed the dependency primarily on GM-CSF over the IL-17A. Collectively, our data suggest that GM-CSF$^+$ Tc17 cells are essential for vaccine-immunity during lethal fungal infections; IL-1 and IL-23 distinctly maintain their phenotype during the expansion phase, the recall responses and for attenuation of pathology. Our studies have a significant impact on the design of vaccines against lethal fungal infections.

Method Comparison of the Nova Prime Plus VET, Nova pHOx Ultra, and Beckman Coulter AU680 Chemistry Analyzers

Courtney Nelson[1], Sara Connolly[2], and Peter Constable[2]
1. Department of Pathobiology, University of Illinois, Urbana, IL
2. Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL

Blood gas analyzers are routinely used to measure biochemical values in a rapid manner when evaluating patients in emergency situations. However, the gold standard for measuring biochemical values is the use of reference laboratory analyzers, which require a larger sample volume and more time to yield results. Method comparison studies are performed to determine whether measurements obtained from new analyzers (Nova Prime Plus VET and pHOx Ultra) using direct methodology are comparable with those obtained from an established method (Beckman Coulter AU680) that uses indirect methodology involving sample dilution. The hypothesis for this study was that good agreement existed between the three analyzers. Three hundred and fifty serum samples were collected from a convenience sample of dogs running before and after the 2018 Iditarod Trail Sled Dog race in Alaska and measured by the three analyzers. Data acquired using the blood gas analyzers (Prime Plus VET, pHOx Ultra) for 8 analytes (sodium, potassium, calcium, magnesium, chloride, glucose, creatinine and urea nitrogen) were plotted on the y axis and compared to results obtained using the reference method (AU680) that were plotted on the x-axis. The plots of the preliminary data were visually evaluated and Pearson's correlation coefficient (*r*) was calculated. The *r* values obtained were excellent (r = 0.932 to 0.989) for serum glucose and urea nitrogen concentrations, but r <0.90 for the remaining 6 analytes, primarily because the range of values was not large relative to the mean value. Ordinary linear regression should not be used for method comparison analysis when r <0.99. Accordingly, methods comparison will be performed on the final data set using Passing-Bablok regression and Bland-Altman plots, and the bias of the new analyzers compared to accepted criteria for the analytes to determine if the methods are interchangeable. This analysis is ongoing. The results of this study highlight the need for individual instrument reference ranges and that patient results obtained from different instruments may not be interchangeable.

Evaluating a Newly Created MALDI-TOF MS Biotyper *Leptospira* Library

Jennifer Nickolyn-Martin and Carol Maddox
Department of Pathobiology, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL

Leptospirosis, an increasingly important public and veterinary health concern, can be caused by over 300 pathogenic *Leptospira* serovars. Given the proclivity for certain associations between these serovars and reservoir hosts, typing of clinical isolates to the serovar-level is key to understanding the epidemiology of this disease. With an economical and rapid tool for serovar typing still lacking, Matrix-Assisted Laser Desorption Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) was explored as a potential method for serovar identification from PCR-positive canine urine samples. Original serovar Main Spectrum Profiles (MSPs), created using the recommended guidelines, were unable to consistently differentiate between serovars. New MSPs, using alternative creation parameters, were constructed and tested for serovar specificity. The best-performing MSP for each serovar was then used to create a *Leptospira* reference library. We hypothesized that a reference library consisting of optimized MSPs would allow MALDI to identify *Leptospira* isolates to the serovar-level. Serovar stock cultures, adjusted to 50 %T with UHPLC-grade H_2O, were used to test the library in a blind-coded trial using real-time classification software. Trial results yielded correct identification of all seven serovars with almost 100% specificity. These results demonstrated that the MALDI platform can be used for serovar-level identification in a diagnostically valuable time-frame by optimizing the creation parameters used to generate custom MSPs. An additional blind trial is planned which will test serovar cultures diluted in canine urine, the leading sample type submitted for leptospirosis testing.

ESR1 to ESR2 conversion in the ovarian granulosa cell lineage

Chan Jin Park, Po-Ching Lin, Sherry Qi Zhou, Shah Tauseef Bashir, Radwa Barakat, CheMyong Jay Ko
Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Two nuclear estrogen receptors (ESR1 and ESR2) are simultaneously but exclusively expressed in different cells in an organ or a tissue. In response to stimulation by estrogens, cells expressing ESR1 and those expressing ESR2 activate signaling pathways that often lead to physiologically opposite outcomes. An erroneous expression of ESRs therefore results in a pathological condition such as reproductive cancers. In the adult ovary, ESR1 is expressed in the epithelium, interstitial cells, and theca cells, whereas ESR2 is expressed in granulosa cells (GCs). In this study, we tested the hypothesis that ovarian GC lineage undergoes a change from ESR1- to ESR2-expressing cells during perinatal gonad development. To test the hypothesis, we created a transgenic mouse in which *Esr2* expression was to be ablated in the ESR1-lineage cells (*Esr1-Esr2*KO). Immunolocalization of ESR2 showed that while adult wild type ovary showed a robust ESR2 expression in the GCs, no ESR2 proteins were seen in that of the *Esr1-Esr2*KO. Single-cell transcriptome profiling of perinatal ovaries revealed that *Lgr5*+ ovarian stem cells expressing *Esr1* were differentiated into pre-GCs followed by losing *Esr1* expression and began to express *Esr2* later. Gene network analysis indicated that TGFβ might play a role as an inducer of the conversion ESR1 to ESR2 expression in the GC lineage. Indeed, treatment of TGFβ1 to cultured embryonic gonads significantly decreased *Esr1* (0.6-fold, $P<0.05$) but increased *Esr2* expression (13-fold, $P<0.05$). Taken together, these results indicate that extracellular TGFβ signal induces the conversion of ESRs expression in the ovarian GC lineage. It will be interesting to see if TGFβ can induce such conversion in other cells.

MSUC-scRNAseq Mapping Uncover Essential Meiotic Genes

Yiheng Peng, Joe Qiao

Department of Comparative Biosciences

College of Veterinary Medicine

University of Illinois, Urbana-Champaign

Urbana, IL , U.S.

Meiosis, a cell-division process that produces sperm and eggs, is crucial for human reproduction. Meiotic defects can cause male and female infertility. Two coordinated meiotic events at the beginning of prophase I are homologous chromosome pairing and SPO11-induced DNA double-strand break (DSB) formation. During pairing, homologous chromosomes search their partners based on DNA sequence homology, which is facilitated by DSB repair. Paired chromosomes remain transcriptionally active in meiosis. In contrast, the unpaired chromosomes or chromosomal regions, like X and Y chromosome pair, are transcriptionally silenced. This silencing phenomenon is called Meiotic Silencing of Unpaired Chromatin (MSUC). Meiotic Sex Chromosome Inactivation (MSCI) during spermatogenesis is induced by unpaired sex chromosomes and a special case of MSUC. X and Y chromosomes fail to pair completely in normal spermatocytes because they have different length and lack DNA sequence homology. Male-specific checkpoint(s) in prophase I are related to MSCI because many “killer” genes on X and Y chromosomes need to be silenced via MSCI. If MSCI fails in some meiotic mutants like *Spo11*$^{-/-}$, those killer genes will function as checkpoint factors to eliminate the defective spermatocytes. In the absence of SPO11, there are no DSBs and no homology search assisted by DSB repair. Thus, in *Spo11*$^{-/-}$ spermatocytes, the homologous chromosomes cannot pair up and then MSUC can happen to any unpaired chromosomes, including autosomes. Germ-cell death will also be induced if autosomal genes important for spermatogenesis are silenced. Based on the two types of lethal mechanisms, we ***hypothesize*** that several meiotic checkpoints are present in *Spo11*$^{-/-}$ spermatocytes due to the failure of MSCI and autosomal MSUC.

Because of the randomness of MSUC, the routine RNAseq methods cannot be used to identify the key genes of those checkpoints. Whereas, Single-Cell RNAseq (scRNAseq) technology provides an opportunity to utilize the randomness. Thus, the combination of scRNAseq and MSUC generates an innovative mapping approach to identify novel meiotic genes. Using this MSUC-scRNAseq mapping approach, we treat apoptosis as a “phenotype”. Both activation of toxic sex-linked genes and inactivation of meiotic house-keeping autosomal genes can induce apoptosis, indicating those genes are critical to meiosis. The dying spermatocytes can be distinguished by their expression of apoptosis-related genes, such as *Bax* and *Bcl2*. By using Loupe Cell Browser and UCSC Genome Browser, we can track the silenced regions in each dying spermatocyte. Although the silenced regions are different in each cell, the overlapping MSUC regions from the dying spermatocyte clusters could help us narrow down the candidate toxic and house-keeping genes. The common upregulated and downregulated genes within the same cell cluster can also help us clarify the different apoptotic pathways induced by expressing toxic genes or repressing the house-keeping genes.

Ancestral Exposure to Di(2-ethylhexyl) Phthalate Disrupts Gene Expression and Pathways Critical for Mouse Ovarian Function in Three Generations

Saniya Rattan, Hannah K. Beers, Emily Brehm, and Jodi A. Flaws
Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer found in polyvinyl chloride products that leaches from products, exposing humans and animals. Previously, we showed that prenatal DEHP exposure disrupts the ovary in three generations, but little is known about the transgenerational mechanism causing ovarian dysgenesis. Therefore, we investigated how prenatal and ancestral DEHP exposure alters ovarian gene expression in three generations. Pregnant CD-1 mice (F0 generation) were dosed with corn oil or DEHP (20 µg – 750 mg/kg/day) daily from gestation day 10.5 until birth. Pups born to the dams were considered the F1 generation. F1 females were mated with untreated males to obtain the F2 generation, and F2 females were used to produce the F3 generation. On postnatal day 21, female pups from each generation were euthanized and ovaries were subjected to qPCR to quantify mRNA expression of the phosphoinositide 3-kinase (PI3K) pathway, cell cycle regulators, steroid hormone receptors, and steroidogenic enzymes. In the F1 generation, prenatal exposure to DEHP dysregulated mRNA expression of cell cycle regulators and steroid hormone receptors. In the F2 generation, prenatal exposure to DEHP dysregulated mRNA expression of PI3K factors and steroidogenic enzymes. In the F3 generation, ancestral exposure to DEHP decreased mRNA expression of PI3K factors, cell cycle regulators, and steroid hormone receptors. Collectively, these data indicate that prenatal and ancestral DEHP exposure cause multigenerational and transgenerational changes in gene expression of pathways critical for normal ovarian functions.
Supported by NIH P01 ES022848, EPA RD-83459301, T32 ES007326, F31 ES030467, and the Billie A. Field Fellowship.

Normal Ocular Parameters and Ocular Surface Microbiome in Healthy Adult Bearded Dragons (*Pogona vitticeps)*

Elyse Salpeter, Krista Keller, Md Tauqeer Alam, Bianca Martins, and Kathryn Fleming
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA

Bearded dragons (*Pogona vitticeps*) are increasing in popularity as household pets, resulting in amplified demand for species-specific medical knowledge. To date, normal ocular parameters and microbiome characterization have not been fully determined for this group of lizards, making ocular disease detection more difficult. The goal of this study was to establish normal ocular parameters and ocular surface microbiome in healthy adult bearded dragons. Ten individual animals received a full ophthalmic examination including slit-lamp biomicroscopy, indirect fundoscopy, and measurement of palpebral length, tear production (PPTT), tonometry (IOP), corneal touch threshold (CTT), and central corneal thickness (CCT). Conjunctival swab samples were obtained for microbiome analysis and bacterial and fungal profiles established using DNA extraction, amplification by Fluidigm Access Array, and next-generation sequencing. Examination revealed palpebral margins lined by small spicules, the presence of a nictitating membrane, a circular pupil surrounded by iridal vasculature in an inverted “U” shape, anangiotic retina, absent tapetum, and a dark conus papillaris. Mean palpebral length was 6.3 ± 0.8 mm, mean PPTT was 4.9 ± 1.4 mm/30s, mean IOP was 5.0 ± 2.3 mmHg, mean CCT was 250.4 ± 8.0 µm, and mean CTT was 0.8 ± 0.35gr/mm^2. Tear production was significantly higher OD compared to OS (P=0.02) and IOP significantly higher in males than females (p=0.005). Fungal organisms were rarely detected in conjunctival swabs and largely unclassified. Common bacteria identified included *Proteobacteria* spp. and *Actinobacteria* spp. This is the first report of normal ophthalmic characteristics in bearded dragons.

Structural and Cellular Effects of Different Preservation Methods and Freeze-thaw Procedure in the Equine Cornea

Rachel Neto[1], Elyse Salpeter[2], Kelli Zimmerman[2], Herbert Whiteley[1], Bianca Martins[2]
[1]Department of Pathobiology and [2]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign

Corneal diseases are common sight threatening and painful conditions in horses. Keratoplasty ("corneal transplant") treatment is often indicated for such conditions and has optical, therapeutic and structural advantages over other techniques. Frozen corneal banks allow medium to long-term storage of healthy donor tissues, however little is known regarding effects of the freeze-thaw process on equine corneas at different storage temperatures and implications on graft quality. The aim of this study is to investigate and compare the structural and cellular effects of cryopreservation at -20° C and -80° C, at short, intermediate and long-term intervals, and of hypothermic media storage (short term), on the equine corneas. Corneas from healthy horse donors were harvested, and placed in either Eusol at 4° C for 2 weeks, or assigned to storage at -20° C or -80° C for 2-3 weeks (short), 3 months (intermediate), or >6 months (long-term). After thawing, corneas were assessed for transparency, thickness, cellularity, and epithelial or stromal changes, and compared to fresh corneas. Corneas stored in -80° C had similar transparency, cellularity and stromal structure to fresh corneas, regardless of storage time, and had superior epithelial and stromal integrity compared to those in the -20° C and Eusol groups. Stromal changes (irregularity, vacuoles) were mostly observed in corneas stored at -20° C for <3 months. Corneal haze was positively correlated to epithelial and stromal changes. In summary, corneal cryopreservation at -80° C yields overall superior transparency, and epithelial and stromal quality at all timeframes compared to storage in –20° C and Eusol.

Examining arsenic levels from surface soil and drinking water sources and it contribution to the occurrence of low birth weight infants in Escambia and Santa Rosa counties, Florida

Maya Scott, Marilyn O'Hara Ruiz, and Rebecca L. Smith
Department of Pathobiology, University of Illinois Urbana-Champaign, Urbana, IL

Arsenic is a special public health concern because of its widespread distribution and high toxicity, even when doses are small. Chronic exposure to arsenic results in cancer, low birth weight (LBW), and childbirth complications. The objective of this study is to determine the spatial patterns of arsenic and examine the relationship between the distribution of arsenic concentrations in surface soil, drinking water sources, and LBW in infants. Approximately 137 surface soil samples were collected across the two counties. Private well data was obtained from the Florida Department of Environmental Protection, geocoded by address, and untested wells omitted. To measure LBW, we obtained data on birth weight, child and maternal demographic information, and residential location data from the Bureau of Vital Statistics at the Florida Department of Health for the years 2005, 2010, and 2015. We used multivariate statistical approaches to analyze and compare variability of LBW as a factor of arsenic levels in soil and water. The models control for individual risk factors and socio-economic characteristics of the population. These health outcome models assess the degree to which arsenic levels at the local level contribute to the risk of LBW.

Purification of Cell-Free Marek's Disease Virion *In Vitro* and *In Vivo*

Yung-Tien Tien, Nagendra Prabhu Ponnuraj, and Keith W. Jarosinski
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

Marek's disease (MD) is a tumorigenic viral disease in chickens caused by *Gallid alphaherpesvirus* 2, better known as Marek's disease virus (MDV). MDV can cause mortality up to 100%. It is well established that MDV is strictly cell-associated *in vitro,* which is also the method that vaccines are prepared for the poultry industry. Because of this, cell culture and liquid nitrogen storage is required, which is costly and inconvenient for many producers, and improper handling of vaccines can lead to ineffective vaccination. Therefore, our long-term goal is to develop an *in vitro* cell culture system in which cell-free vaccines can be produced. According to previous literature, the only cell type that produces cell-free infectious MDV particles is in the feather follicle epithelium (FFE) of infected chickens. Here, we compared the relative amount and infectivity of virions purified using a gradient Ficoll® method comparing virions from infected-tissue culture cells (*in vitro*) or -FFE cells (*in vivo*). Consistent with previous literature, only virions from *in vivo* samples were infectious when inoculated onto chicken kidney cells, while infectivity was not apparent in *in vitro* samples, even though the amount of particles collected after Ficoll® purification were similar. Using electron microscopy to compare the ultrastructure of virions between *in vitro* and *in vivo* samples, there was no obvious difference between the two samples. Next, we will use a global proteomics approach to identify differences in the protein make up of *in vitro* and *in vivo* virion samples, with the goal of identifying proteins required for infectivity that are lacking in the *in vitro* virions. Our future goal is to develop a system to supply these proteins *in vitro* to generate infectious cell-free MD vaccines.

Study the age-specific roles of hnRNP I in intestinal homeostasis

Wesley Tung, Kristy Chin, Danielle Yee, Wenyan Mei

The intestinal epithelium plays an important role in mediation of host-microbe responses. An inability to properly maintain the integrity of the intestinal epithelium is associated with various gastrointestinal inflammatory diseases and colorectal cancer. Investigating the mechanisms that govern intestinal epithelial homeostasis is critical for elucidating the etiology behind these diseases. Previously, our lab group discovered that hnRNP I, an RNA binding protein, is crucial for regulating neonatal intestinal immune tolerance and preventing colitis and colorectal cancer through downregulation of the Toll-like signaling pathway, a pathway that is fundamentally important for regulating the intestinal innate immunity. However, it is unclear whether hnRNP I plays a direct role in preventing intestinal tumorigenesis or indirectly prevent cancer through the control of intestinal immunity. To determine the specific role of hnRNP I in these processes, we have generated a tamoxifen-inducible, intestinal epithelial cell-specific hnRNP I knockout mice model using the Cre-Lox recombinase technology. We show here that deletion of hnRNP I in the intestinal epithelial cells of adult mice results in severe weight loss and death. We further show that hnRNP I ablation leads to loss of intestinal stem cells and impaired intestinal regeneration. Our results reveal a critical role of hnRNP I in maintaining adult intestinal epithelial homeostasis through the control of intestinal stem cells.

Assessing Ultra-Fine-Scale Factors to Improve Human West Nile Virus Disease Models

Johnny Uelmen[1], Surendra Karki[1], Patrick Irwin[2], Rebecca Smith[1]
[1]*University of Illinois at Urbana-Champaign, Department of Pathobiology, 2522 Veterinary Medicine Basic Sciences Building, 2001 South Lincoln Avenue, Urbana, IL, 61802, USA*
[2]*Northwest Mosquito Abatement District, 147 West Hintz Road, Wheeling, IL, 60090, USA*

Since 1999, West Nile virus (WNV) has moved rapidly across the United States, resulting in thousands of human cases. Both the number of cases and rate of mosquito infection (MIR) vary across time and space and are related to numerous abiotic and biotic forces, ranging from differences in microclimates to socio-demographic factors. Because the interactions among these multiple factors affect the locally variable risk of WNV illness, it has been especially difficult to model human disease risk across multiple spatial and temporal scales. Cook County, comprising the city of Chicago and surrounding suburbs, is among the areas hardest hit by WNV in the United States. Despite active mosquito control efforts, there is consistent WNV risk annually, resulting in more than 350 confirmed WNV human cases and 12 deaths in the past 5 years in Cook County alone. Starting from the existing Cook and DuPage Counties model (Karki 2019), additional data processing and field collections tripled the number of key covariates to test in our model. Additionally, we narrowed our focus to the thirty most high and low risk study areas in the Northwest Mosquito Abatement District (NWMAD), an enclave ¼ the size of the previous study area. Contrary to expectation, multivariate statistical approaches revealed that the ultra-fine-scale model resulted in fewer explanatory variables to improve upon the already well-fit model. These results suggest human WNV illness may be associated with fewer, but increasingly critical, key variables at local scales. These results provide a positive outlook for predicting WNV human risk in very small, targeted locations.

Marek's disease alphaherpesvirus (MDV) RLORF4 is not required for interindividual spread

Widaliz Vega-Rodriguez, Nagendra Prabhu Ponnuraj, Keith W. Jarosinski
Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Marek's disease virus (MDV) is an oncogenic alphaherpesvirus that causes Marek's disease (MD) in chickens. RLORF4 is a MDV-specific gene located in the repeat long (RL) regions of the genome and is directly involved in attenuation. Former studies have also shown that expression of glycoprotein C (gC) is diminished following attenuation, which coincides with the systematic deletion of RLORF4 from the MDV genome, suggesting these two events may be linked. Unfortunately, the original studies in which RLORF4 was deleted utilized an infectious bacterial artificial chromosome (BAC) clone that lacked gC expression, due to frame-shift mutation, and did not spread among chickens. Here, we utilized a BAC clone in which gC expression was restored and shown to be able to spread from chicken-to-chicken, and tested our hypothesis that RLORF4 is important for expression of MDV gC and subsequently, transmission. Using the RLORF4 null virus showed that gC expression was unaltered both *in vitro* and *in vivo* and this virus was able to efficiently transmit from chicken to chicken. Therefore, we can conclude that RLORF4 does not regulate gC expression and is not required for transmission, as previously thought.

The role of alphaherpesvirus conserved glycoprotein C (gC) in host-to-host transmission

Widaliz Vega-Rodriguez, Nagendra Prabhu Ponnuraj, Keith W. Jarosinski
Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Members of the family *Herpesviridae*, in particular the *Alphaherpesvirinae* subfamily that infect mammalian, avian and reptilian species, continuously cause problems affecting human and food-animal species. Previous research in our laboratory showed that the alphaherpesvirus conserved glycoprotein C (gC), encoded by UL44, is essential for transmission of Marek's disease alphaherpesvirus (MDV) in chickens. MDV infected T cells that circulate to the periphery, transfer virus to feather follicle epithelial (FFE) cells, where virus is shed from the chicken in dander into the environment, a process that completes the virus life cycle through transmission to naïve chickens. We hypothesize that gC of other alphaherpesviruses are also required for host-to-host transmission and direct host-specificity. To test our hypotheses, we use a host-to-host transmission model where chickens are experimentally infected with MDV by inoculation and the ability of the virus to infect naïve contact chickens through natural infection is determined. To evaluate different alphaherpesvirus gC proteins in host-to-host transmission, we removed MDV gC and replaced it with the UL44 gene encoding the different alphaherpesvirus gC proteins. Chickens were inoculated with the recombinant viruses and housed with naïve birds in order to test the ability of these viruses to transmit from chicken-to-chicken. Our results showed that turkey alphaherpesvirus gC (HVT gC) was able to facilitate transmission of MDV, while chicken gC from infectious laryngotracheitis virus (ILTV) could not. These results will strongly suggest that gC is not a major factor in host specific transmission of alphaherpesviruses.

Comparison of Serum Trace Nutrient Concentration in Dogs with Idiopathic Epilepsy Compared to Healthy Dogs.

Samantha Vitale[1], Devon Hague[1], Maria Cattai de Godoy[2], Laura Selmic[3], Kari Foss[1]
1. Department of Veterinary Clinical Medicine, University of Illinois Urbana Champaign, Urbana, IL. 2. Department of Animal Sciences, University of Illinois Urbana Champaign, Urbana, IL. 3. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Idiopathic epilepsy is the most common cause of seizures in dogs, reportedly affecting 0.5-5.7% of the population. There have been several investigations regarding the serum concentrations of trace nutrients, including copper, selenium, zinc, manganese, and iron in human epileptics and animal models. However, no research of this nature is available in dogs with naturally occurring epilepsy. This is a prospective study, designed to compare the serum concentrations of several trace nutrients in non-epileptic dogs to dogs with epilepsy. Blood samples were collected from 50 healthy control dogs and 92 dogs (95 samples) with idiopathic epilepsy. The epileptic patients were further subdivided into three groups (controlled: n=29, uncontrolled: n=43, and untreated: n=23). Serum was evaluated for concentrations of copper, selenium, zinc, cobalt, manganese, molybdenum, and iron using inductively coupled plasma mass spectroscopy. Significantly higher levels of copper were found in both controlled and uncontrolled epileptics ($p<0.0001$) compared to normal or untreated dogs. Untreated epileptics had significantly higher iron concentrations than any of the other three groups ($p=0.04$). Uncontrolled epileptics had significantly higher manganese levels ($p=0.007$) than any of the other three groups. Both controlled and uncontrolled epileptics had significantly higher molybdenum levels than untreated and normal groups ($p=0.01$). Finally, uncontrolled and controlled epileptics had significantly higher levels of selenium ($p=0.0003$) than normal dogs, and uncontrolled epileptics had higher levels of zinc ($p=0.0002$) than normal and untreated dogs. The significant difference in the serum concentrations of several trace nutrients (manganese, selenium, and zinc) may suggest a role for these nutrients in the pathophysiology and/or treatment of epilepsy. Additionally, these results suggest that anticonvulsant therapy may affect copper and molybdenum metabolism.

Ovarian Metabolism of an Environmentally Relevant Phthalate Mixture

Genoa R. Warner[1], Zhong Li[2], Madeline L. Houde[1], Cassandra E. Atkinson[1], Daryl D. Meling[1], Catheryne Chiang[1], Jodi A. Flaws[1]

[1]Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[2]Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Phthalates are synthetic chemicals found in consumer products with widespread human exposure. Evidence suggests that metabolites mediate the toxic effects of phthalates and that medical exposure can lead to unmetabolized phthalates reaching organs such as the ovary. Thus, the ovary may be bioactivating phthalates in-house, increasing the concentration of toxic metabolites in a highly sensitive organ. This study investigated the ability of neonatal and adult mouse ovaries to metabolize an environmentally relevant phthalate mixture. Whole neonatal ovaries (postnatal day 4) and adult antral follicles from CD-1 mice were cultured in media treated with dimethyl sulfoxide (vehicle control) or phthalate mixture (0.1–100 µg/mL) composed of 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate, which is representative of exposure of pregnant women in Illinois. After four days of culture, media were subjected to liquid chromatography mass spectrometry to measure the amounts of monoester metabolites. Metabolites for five of the phthalates were detected in the media for both culture types. Ovaries and follicles were collected to measure gene and protein expression of enzymes required for phthalate metabolism, lipoprotein lipase (LPL) and aldehyde dehydrogenase family 1, subfamily A1 (ALDH1A1). Neonatal ovaries predominantly expressed LPL, whereas adult follicles expressed high levels of ALDH1A1. These data demonstrate that neonatal and adult ovaries are capable of metabolizing low doses of phthalates and suggest that metabolic capacity differs for follicles at different stages of development.

Cytochrome P450-specific interactions with phytocannabinoids reveal insights on *Cannabis* toxicity

Austin Weigle[1], Aditi Das[2]
[1]Department of Chemistry, [2]Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL

Phytocannabinoids (pCBs) are a structurally diverse set of bioactive phytochemicals comprising the controversial *Cannabis* mixture. Aside from Δ^9-tetrahydrocannabinol and cannabidiol, the molecular details and comparative pharmacology surrounding pCB interactions remain largely uncharacterized. While pCBs exert their physiological effects through the cannabinoid receptors 1 and 2, cytochrome P450 (CYP) enzymes serve as another basis to probe molecular interactions. CYP-mediated metabolism of pCBs can act as a biotransformative pathway by changing their pharmacology. We show that 5 of the 6 predominant pCBs found within *Cannabis* noncompetitively inhibit the generation of cardioprotective and anti-inflammatory epoxyeicosatrienoic acids (EETs) by human heart CYP2J2. These phytocannabinoids act as better CYP2J2 substrates than the EETs, suggesting a need for endogenous activities to be halted for mitigation of potential phytocannabinoid toxicity through direct metabolism. We expand upon these CYP-specific findings by showing that human and canine CYP3A orthologs produce a similar THC metabolite. We also highlight how cannabidiol is capable of inactivating CYP3A4 and evaluate its potential as a mechanism-based inactivating agent. Our *in vitro* work carries the potential to define the molecular details and consequences resulting from *Cannabis* consumption.

Revealing effects of N,N-Diethyl-meta-toluamide (DEET) on early meiotic events in cultured mouse spermatocytes by detecting synapsis defects and DNA damage.

Yiding Xu, Ning Liu, Huanyu (Joe) Qiao
Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL

N,*N*-Diethyl-*meta*-toluamide (DEET) is the most commonly used insect repellent, which is effectively against invertebrates such as mosquitoes, biting flies, honey bees, and ticks. DEET functions as a mosquito repellent by activating an olfactory receptor neuron in special antennal sensilla of mosquitoes. Although it has been used for more than 70 years by millions of people worldwide, only a few studies on toxicology of DEET is reported. Meiosis is a special cell division that produces four haploid cells with half a genome. Meiotic synapsis and homologous recombination are key events in early meiotic process, because the defects in these two process might lead to meiotic cell apoptosis and reproductive deficiency. In this study, using in vitro testis culture system and immunostaining method, we are able to reveal the toxicological effects of DEET on early meiotic events in mouse spermatocytes. Testis from male mouse at 5 days postpartum were *in-vitro* cultured for 7 days. The cultured testis were then treated with 100μM, 500μM, and 1000μM DEET respectively to go through meiotic process. Immunostaining the chromosome spreads with the antibodies against components of synaptonemal complex (**SC**), SYCP1 and SYCP3 proteins, are used to identify synapsis defects while immunostaining of gH2AX, which is a biomarker for DSBs, can be used to visualize abnormal processes of DNA damage repair. Results from this study will uncover the adverse effects of DEET on reproductive system and widen our knowledge on DEET toxicology.

Characterizing the role of hnRNP I in regulating sertoli cell-dependent Spermatogenesis

Danielle Yee, Wenyan Mei

Spermatogenesis is the biological process by which sperm is generated, and is required for male fertility. Mouse spermatogenesis progresses in a topographically coordinated manner called the spermatogenic wave, of which there are multiple. In the first wave of development, gonocytes develop directly into type A spermatogonia, which then differentiate into mature sperm cells. In subsequent waves, gonocytes develop into spermatogonial stem cells, which then differentiate into mature sperm cells. During spermatogenesis, the progression of germ cells to spermatozoa is facilitated by sertoli cells located in the seminiferous tubule. Using the Cre-loxP technology, our lab generated a knockout mouse model in which a gene called hnRNP I is deleted in mouse sertoli cells. Our preliminary studies have shown that deletion of hnRNP I in sertoli cells causes male infertility in mice, including a disrupted first wave of spermatogenesis and subsequent spermatogenesis waves. We performed histological analysis to check for defects in spermatogenesis, and found that the cytoskeleton in the seminiferous tubules are impaired, and the sertoli cells have detached from the basement membrane. Our findings indicate that hnRNP I plays an important role in regulating spermatogenesis through the control of the sertoli cell function .

Role of TDP-43 in Sertoli cell function

Helena Zomer and Prabhakara Reddi
Department of Comparative Biosciences, University of Illinois, Urbana-Champaign, Illinois

Sertoli cells play a key role in spermatogenesis by offering physical and nutritional support to the male germ cells throughout differentiation. Sertoli functions include the engulfment and clearance of dead germ cells from the seminiferous tubules, the formation of the blood-testis barrier by tight junctions (TJ) and the attachment of germ cells by ectoplasmic specializations (ES). However, the proteins involved and the mechanisms that regulate the Sertoli-germ cell interactions are not well understood. The TAR DNA binding Protein of 43 kD (TDP-43) is a ubiquitously expressed transcription factor and RNA-binding protein with major human health relevance. Our laboratory developed a conditional knockout (cKO) mouse that lacks TDP-43 in Sertoli cells and found that it causes male infertility. Sloughing of round spermatids was observed, indicating loss of contact with the Sertoli cells. Thus, we hypothesize that TDP-43 regulates TJ and ES formation and/or maintenance and that it may also be involved in the clearance of dead germ cells. We are performing immunostaining to determine the expression of occludin and N-cadherin, key proteins of TJ and ES, respectively, both in histological samples and in primary cultures of Sertoli cells obtained from wild type and TDP-43 cKO mice. We are also using Sertoli cell (TM4) cultures with TDP-43 knockdown as an additional model. Finally, we are using Oil Red O lipid staining to characterize the phagocytic function of Sertoli cells. By these means, we aim to understand how TDP-43 regulates Sertoli function and its impact on spermatogenesis and fertility.

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