2017 Research Day ABSTRACTS

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VETERINARY MEDICINE at ILLINOIS

Saving Silvery Salamanders

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The order Caudata, which includes salamanders and newts, is one of the most imperiled vertebrate groups due to habitat loss, overcollection, and disease. The silvery salamander *(Ambystoma platineum)* is state-endangered in Illinois. Despite the impact of disease on salamander conservation, and the presence of important salamander pathogens in Illinois, there are no published reports of health assessments or disease surveillance in *A. platineum.* The purpose of this research was to characterize the health and disease status of silvery salamanders in order to identify conservation threats. Adult silvery salamanders (N=84) were captured at 10 ponds during spring emergence in 2016. Metamorphs (N=14) were captured in June. Each salamander was weighed, examined, and assigned a body condition score (BCS) from 1-5. Swabs of the ventrum, feet, and oral cavity were collected for qPCR infectious disease testing. Physical examination parameters and qPCR pathogen detection were compared between sites using generalized linear models. Salamanders at Kickapoo State Park (KSP) were significantly heavier and had higher BCS than those at Middle Fork State Fish and Wildlife Area (MF, p=0.04). The number of salamanders with skin nodules (N=10) was highest in pond 75 at MF (p=0.004). The presence of skin nodules was significantly associated with testing qPCR positive for a frog virus 3-like ranavirus (p=0.02). This ranavirus was also detected during a mortality event in summer 2016 which killed over 80% of the silvery salamander larval population. This pilot study demonstrates that disease may pose a threat to silvery salamander conservation in Illinois, and underscores the need for continued health assessment in this species.

Circadian Disruption Affects Initial Learning but Not Cognitive Flexibility in an Automated Set-Shifting Task in Adult Long-Evans Rats

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Circadian disruption is caused by misalignment of innate rhythms to external cues such as light, sleep and food intake. Chronic circadian disruption negatively affects both physiology and cognition. We investigated the effects of circadian disruption on cognition in a rodent model. Adult Long-Evans rat were tested on an automated operant behavior task for 3 months under 12:12 h light: dark cycle, with testing occurring either 4h after lights-on or lights-off. This resulted in day-tested rats realigning their activity patterns to become diurnal, whereas night-tested rats remained nocturnal. Rats then transitioned to an automated set-shifting (SS) task to assess cognitive flexibility, the ability to adapt to changing situational demands. We hypothesized that circadian disruption would result in the day-tested rats being slower to adapt to task transitions as compared to the night-tested rats. Contrary to our hypothesis, night-tested rats took longer to reach criterion performance in the visual-cue detection stage of the SS task compared to day-tested rats. However, there were not differences between the two conditions in subsequent transitions to an egocentric-cue based phase or a reversal phase. We speculate that night-tested rats experienced a form of circadian disruption when they were exposed to ambient light during the testing procedure, and that this form of circadian disruption impaired initial task acquisition, but not actual cognitive flexibility, to a greater extent than testing during the day.

Hypoxia Inducible Factor 2 Alpha Regulates Uterine Stromal-Epithelial Crosstalk to Facilitate Embryo Invasion During Implantation

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Embryo implantation is a crucial step in establishment of successful pregnancy. At distinct steps of this process, the maternal endometrial and the embryonic trophoblast cells are in close communication with each other. Implantation is initiated when the receptive uterine luminal epithelium establishes a stable interaction with the embryo, which is followed by invasion of the embryo into the underlying endometrial stroma. In this study we show that the *Hypoxia inducible factor 2 alpha (Hif2α)* expressed in the sub-epithelial stroma surrounding the embryo, regulates the structural remodeling of the uterine luminal epithelium to facilitate embryo invasion. Using a conditional knockout mouse model we demonstrate that in the absence of Hif2α, the integrity of the luminal epithelium is maintained preventing the embryo from breaching the uterine wall and invade into the underlying stroma. We hypothesize that Hif2α regulates secretion of matrix remodeling enzymes at the site of embryo implantation. These enzymes degrade the extracellular matrix and disrupt the epithelial junctions allowing the embryo to invade into the underlying stroma. Thus this study will elucidate the mechanisms that trigger the process of embryo invasion. Although there are species variations in the extent to which the embryo invades into the maternal endometrium, many of the basic molecular mechanisms are conserved among species. Hence the knowledge gained from this study will be fundamental to mammalian reproductive biology.

G0s2 Represses Pi3k/Akt/Mtor Signaling and Increases Sensitivity to Pi3k/Mtor Pathway Inhibitors in Estrogen Receptor-Positive Breast Cancer Cells

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G0/G1 switch gene 2 (G0S2) is a direct retinoic acid target gene widely expressed in diverse organs and implicated in cancer based on frequent methylation-mediated silencing in diverse solid tumors. Our laboratory recently reported high levels of G0S2 expression in breast cancer, particularly estrogen receptor-positive (ER^+) breast cancer, that strongly correlates with survival, suggesting that G0S2 plays a role in breast cancer progression. However, the function(s) and mechanism(s) of G0S2 tumor suppression remain unclear. We hypothesize that G0S2 represses proliferation and oncogenic signaling in ER^+ breast cancer cells and promotes more effective responses to existing therapies targeting ER^+ breast cancer, leading to inhibition of recurrence. In order to determine potential mechanisms of G0S2 anti-oncogenic activity, we performed genome-wide expression analysis that revealed an enrichment of gene signatures related to PI3K/mTOR pathway activation in G0S2-null MEFs. G0S2-null MEFs also exhibited decreased sensitivity to PI3K/mTOR pathway inhibitors. Overexpression of G0S2 in human ER^+ breast cancer cells decreased basal mTOR signaling and sensitized the cells to pharmacologic mTOR pathway inhibitors. These findings indicate that G0S2 functions as a tumor suppressor in part by repressing PI3K/mTOR activity and may enhance therapeutic response to PI3K/mTOR inhibitors. Recent findings suggest that hyperactivation of PI3K/mTOR signaling promotes escape from hormone dependence in ER+ breast cancer. Our data implicates G0S2 in opposing this form of antiestrogen resistance, prompting further investigation of the potential role of G0S2 as an antineoplastic breast cancer target and biomarker for recurrence and therapy response.

Enrofloxacin Concentration in Aqueous Humor and Humor Vitreous of Fetus and Foals After Intravenous Administration in Pregnant Mares

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Enrofloxacin is an antibiotic widely used in veterinary medicine to treat infections against Gram-positive and Gram-negative pathogens. To date, it is not known if enrofloxacin reaches the aqueous humor and vitreous after crossing placental membranes, and whether it causes anatomical and/or histological abnormalities in fetus and foals when given in therapeutic concentrations to pregnant mares in final gestation. The goal of this study was to evaluate if enrofloxacin is able to cross ocular barriers during last stage of pregnancy and if it could cause ocular developmental alterations. Enrofloxacin was given to 08 health mares around 240 days of gestation, at doses of 5mg/kg (G1 - n=4) and 10mg/kg (G2 - n=4) intravenously every 24 hours for 15 days. Twenty-four hours after the last dose, parturition was induced with oxytocin. Fetus delivered alive were immediately euthanized with an intracardiac injection of sodium pentobarbital and sodium phenytoin. Next, both eyes were enucleated, morphometric analyzed, and submitted for histologic evaluation. Levels of enrofloxacin and ciprofloxacin in the aqueous humor and vitreous were measured by High Performance Liquid Chromatography (HPLC). Enrofloxacin was detected in the aqueous humor (G1 = 482 $\pm$ 282.2 ng/ml; G2 = 953.5 $\pm$ 670.8 ng/ml) and vitreous (G1 = 199.5 $\pm$ 38.3 ng/ml; G2 = 437.8 $\pm$ 22.3 ng/ml) of all individuals. No morphological, morphometric and histological abnormalities were detected on the fetuses' eyes. Enrofloxacin was able to cross ocular barriers and reach the aqueous humor and humor vitreous, however it did not cause any morphological and histological abnormalities in the fetuses' eyes.

Di(2-ethylhexyl) Phthalate and Its Metabolite Mono(2-ethylhexyl) Phthalate Do Not Affect Metabolic Enzymes in Neonatal Ovaries in the Mouse

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Di(2-ethylhexyl) phthalate (DEHP) is a chemical commonly used as a plasticizer in baby toys, food containers, carpeting, and upholstery. DEHP can be absorbed via ingestion, inhalation, and dermal contact and then enter multiple tissues in the body. Once absorbed, DEHP is metabolized into its bioactive metabolite, mono(2-ethylhexyl) phthalate (MEHP). Our previous studies indicate that exposure to MEHP, but not DEHP, accelerates primordial follicle recruitment in the mouse neonatal ovary, suggesting that MEHP, but not DEHP, is toxic to the neonatal ovary. This differing response may be due to the neonatal ovary lacking the metabolic enzymes required to convert DEHP to MEHP. Thus, the purpose of this study was to test the hypothesis that the neonatal ovary contains the metabolic enzymes required for the conversion of DEHP to MEHP. To test this hypothesis, ovaries from CD-1 mice (postnatal day 4) were cultured in media with either no additional chemicals (no treatment control), dimethylsulfoxide (DMSO; vehicle control), DEHP (0.2, 2, or 20 µg/mL), or MEHP (0.2, 2, or 20 µg/mL) (n=3/treatment group). After 6 days of culture, ovaries were subjected to qPCR to measure mRNA levels of the selected metabolic enzymes: isoamyl acetate-hydrolyzing esterase 1 homolog (Iah1), lipoprotein lipase (Lpl), alcohol dehydrogenase 1 (Adh1), and aldehyde dehydrogenase family 1, subfamily A1 (Aldh1a1). Further, some ovaries were subjected to immunohistochemistry to measure ALDH1A1. Interestingly, mRNA expression of all metabolic enzymes was measurable in the neonatal ovaries. Further, neither DMSO nor any of the tested levels of DEHP or MEHP affected metabolic enzyme expression (n=3; $p > 0.05$). Immunohistochemistry revealed no staining for ALDH1A1 in any of the treatment groups, indicating that the mRNA transcribed for this enzyme does not undergo translation into functional protein in the neonatal ovary. Collectively, these data show that the neonatal mouse ovary has detectable mRNA levels of the selected metabolic enzymes, but that the mRNA may not be translated into functional protein. Thus, it is possible that the neonatal ovary responds to MEHP, but not DEHP, due to a lack of metabolic enzymes required for metabolism of DEHP to MEHP. Supported by R56 ES 025147 (JAF), an Environmental Toxicology Training Grant T32 ES 007326 (CC), and an Environmental Toxicology Fellowship (PRH).

Repurposing Exendin-4 to Restore Mucus Homeostasis in Chronically-Diseased Airways Infected by Pseudomonas Aeruginosa

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Airway mucus is a crucial component of the innate defense that forms a gel-like barrier covering the respiratory tracts. The mucus layer traps inhaled irritants, pathogens and particles, and prevents direct contact between potential antigens and airway epithelium. However, various chronic airway diseases, including cystic fibrosis (CF), COPD and ciliary dyskinesis are accompanied by mucus hypersecretion and failure in mucus clearance, which clog airways and create a thriving niche for microbial pathogens. The opportunistic respiratory bacterial pathogen *Pseudomonas aeruginosa* (PA) is an important pathogen of CF and COPD. *P. aeruginosa* is highly recalcitrant to antibiotics, which enables the pathogen to continuously stimulate and harm the airway epithelium. Pyocyanin (PCN) is a major redox-active toxin that generates ROS/RNS that overwhelm the antioxidant capacity of host cells. In this study, we show that PCN polarizes the airway epithelium toward a Th2 inflammatory response and causes goblet cell metaplasia by activating IL-13-STAT6-SPDEF, EGFR-PI3K/AKT and EGFR-RAS-RAF-MEK1/2-ERK1/2 signaling pathways. Activation of both STAT6 and EGFR signaling convergently facilitate the nuclear exclusion and degradation of FOXA2, which is a key transcriptional regulator of mucus homeostasis. Exendin-4 (EX-4) is a incretin mimetic approved by the FDA for type 2 diabetes patients. Ex-4 restores the nuclear localization of FOXA2, which in turn, inhibits the excessive expression of mucin biosynthesis genes *MUC5AC* and *MUC5B* induced by *P. aeruginosa* infection in mouse lungs, as well as in CF and COPD-diseased primary airway epithelial cells exposed to PCN. Mechanistically, we show that EX-4 activates the GLP1R-PPARγ that induce PTEN/PTP1B phosphotases, which dephosphorylate and inhibit key kinases within both STAT6 and EGFR signaling cascades. Our data demonstrate that Ex-4 is efficacious and an attractive adjunctive therapy against *P* infection in CF and COPD airways without exacerbating the burden of antibacterial resistance.

Peripheral Immune Activation Leads to Decreased Neurotrophic and White Matter Gene Expression in the Hippocampus of Intrauterine Growth Restricted and Appropriate for Gestational Age Piglets

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Intrauterine growth restriction (IUGR) affects 7.5 million infants worldwide each year. IUGR affects brain development, leading to aberrations in white matter (WM) and learning difficulties. IUGR infants are also more susceptible to infections. Immune activation leading to neuroinflammation can cause deviations in normal brain development if unchecked. We sought to examine neuroinflammation after intraperitoneal (i.p.) injection of *E. coli* lipopolysaccharide (LPS) in piglets born with IUGR. IUGR (≤ 0.9 kg at birth) and appropriate for gestational age (AGA; 1.3-1.6 kg) piglets were weaned at postnatal day 2, fed a commercial liquid milk replacer diet, and at postnatal day 14 injected i.p. with 5 μg/kg LPS or sterile saline. LPS led to an increased segmented neutrophil count ($p = 0.044$) and decreased monocyte count ($p = 0.044$) regardless of birth weight (BW). IUGR piglets had a lower lymphocyte count ($p = 0.021$) than AGA piglets, and LPS decreased the lymphocyte count ($p = 0.001$) both in AGA and IUGR piglets. Hippocampal gene expression was measured 4 h post LPS injection to assess neuroinflammation. IL-6 was elevated ($p = 0.019$) by LPS, but IUGR reduced expression of IL-6 in both LPS and control groups ($p = 0.037$). The i.p. LPS injection lead to decreased expression of brain derived neurotrophic factor (BDNF; $p = 0.001$), myelin basic protein (MBP; $p = 0.006$), and glutamic acid decarboxylase (GAD1; $p = 0.015$) with no effect of BW. These data show that LPS caused mild peripheral immune activation with suppression of lymphocyte populations in IUGR piglets. The susceptibility of IUGR infants to infections could lead to increased risk for behavioral disorders and disrupted WM development.

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Interferon-λ Induction During Influenza Infection in the Canine Epithelial Cell

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Interferons (IFNs) are a class of signaling proteins produced by host cells in response to the presence of pathogens. Some IFNs in epithelial cells act as the first line of defense against viral infections. During influenza infection, induction of IFNs results in cell-to-cell signaling producing antiviral proteins. It is clear human cells produce IFNs in response to influenza infections; however, much remains to be investigated regarding IFN production in canine cells. Recent emergence of two canine influenza viruses makes it critical to understand the innate immune response that occurs in canine respiratory epithelial cells. We investigated a type III IFN (IFN-λs) in the Madin-Darby canine kidney (MDCK) cell line by demonstrating the expression of the receptors IFN-λR1 and IL-10R2 and the genes IFN-λ1 and IFN-λ3. We extracted RNA from MDCK cells after 72 hours to produce cDNA. Following PCR for IFN-λR1 and IL-10R2 receptors (using previously published primers), we identified corresponding bands, confirming both receptors expression. We next stimulated MDCK cells with Poly(I:C) or infected with an H1N1 influenza virus. RNA was isolated from cells over various time points followed by generating cDNA. Using primers we designed for canine IFN-λ1 and IFN-λ3, we used qRT-PCR to measure relative expression of these IFNs compared to GAPDH. We found that under Poly(I:C) stimulation, there was upregulation of IFN-λ1 and IFN-λ3 in MDCK cells. Ongoing work will expand our understanding of cell response to influenza infections, including relative expression of IFN-λ in both MDCK cells and canine respiratory epithelial cells. This understanding will aid in development of novel diagnostic targets and therapeutic interventions.

Associations of Prenatal Exposure to Phthalates and Bisphenol A With Measures of Cognitive Function in 7.5-Month-Old Infants Participating in the Illinois Kids Development Study (I-KIDS)

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Phthalates and phenols are endocrine disruptors with potential neurodevelopmental toxicity. Exposure to these chemicals is ubiquitous due to their use in personal care and household products, plastics, and food containers. Few studies have assessed their impact on infant cognition. Looking behaviors were assessed in a subsample of full-term infants (>37 wks gestation; n=72) born to mothers in a prospective pregnancy cohort. Phenol and phthalate metabolites were measured in a pool of 5 urines collected across pregnancy; urinary concentrations were similar to other US pregnant populations. At 7.5 months, infants' visual recognition memory was tested. In familiarization trials, they saw 2 identical faces side-by-side; in test trials the familiar face was paired with a novel face. Exposure associations with average fixation time (processing speed), gaze shift rate between faces (visual attention) and novelty preference (recognition memory) were analyzed. Models were adjusted for infant age and sex, household income, maternal IQ and education, breastfeeding, and urine specific gravity. Urine monoethyl phthalate was associated with decreased novelty preference (β=-1.71%/interquartile range [IQR]); urine bisphenol A (BPA) was associated with longer fixation duration (β=0.158s/IQR). There were no associations with urinary di-(2-ethylhexyl)-phthalate metabolites, triclosan, or butylparaben. These findings suggest that prenatal exposure to BPA and diethyl phthalate may negatively impact infant cognition in this study population but need corroboration in a larger sample. Studies are underway to corroborate these findings in a large cohort, and to assess the impact of exposure to phthalates and phenols on other cognitive domains.

Effect of Feeding on the Pharmacokinetics of Oral Minocycline in Healthy Adult Horses

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The administration of minocycline in adult horses has grown in popularity and studies suggest superior oral bioavailability when compared to doxycycline. Unfortunately there is limited information regarding the impact that feeding may have on oral bioavailability of this antimicrobial in adult horses. Six healthy adult horses were administered intravenous (2.2mg/kg) and oral minocycline (4 mg/kg) under 2 separate feeding regimens using a Latin Square crossover design and a 7-day washout between treatments. For all horses feed was withheld the evening prior to oral drug administration. Fed horses then had access to hay at the time of drug administration. Fasted horses had access to hay delayed for 2 hours after drug administration. When comparing fasted to fed horses, mean ± SD bioavailability (fasted: 38.6% ± 4.6; fed: 15.7% ± 2.3), and maximum plasma concentration (Cmax) (fasted: 1.343 ± 0.418 µg/mL; fed: 0.281 ± 0.157 µg/mL) were both significantly greater in fasted horses ($P < 0.05$ both). Median (range) time (h) to maximum plasma concentration (Tmax) in fasted horses was 2.0 (1.5 – 3.5) and in fed horses was 5.0 (1.0 – 8.0) and was not significantly different between groups.In adult horses, delaying access to hay for 2 hours after oral minocycline administration improved drug absorption. This has important implications as optimizing antimicrobial plasma concentrations is critical for effective treatment of bacterial infections in adult horses. Delaying the feeding of hay after oral administration of minocycline improves bioavailability in adult horses.

Enrofloxacin Crosses the Equine Placenta Without Causing Lesions in Fetal Articular Surfaces and Growth Plates

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Enrofloxacin, a fluoroquinolone with broad-spectrum bactericidal activity, could be an excellent choice for resistant bacterial infections in pregnant mares. However, it is unknown if enrofloxacin crosses the equine placenta, and enrofloxacin is assumed to be toxic to the fetus based on *in vitro* studies. We hypothesized that enrofloxacin (EN) and its active metabolite (ciprofloxacin, CIP) cross the equine placenta without causing lesions in the fetus. Our objectives were to determine EN and CIP concentrations in plasma and fetal fluids when EN is administered to pregnant mares, and to evaluate the articular cartilage of fetal long bones from those mares. Healthy mares (~280d of gestation) were assigned to: control (n=3), therapeutic dose of EN (n=6, 5mg/kg), or double dose of EN (n=6, 10mg/kg). EN was administrated daily for 10d, and plasma was collected for 11d. Fetal fluid sampling was performed at d1, 5 and 11, and premature delivery was induced on d11. Fetal plasma was collected at delivery. EN and CIP were measured by LS-MS/MS. Long bone articular surfaces were examined macroscopically and histologically. Statistical analyses were performed using ANOVA with repeated measures and significance considered when $p<0.05$. EN and CIP reached minimal inhibitory concentrations for common pathogens in all fluids. CIP did not increase in plasma with increased EN dose, but allantoic fluid showed a 10 fold increase relative to maternal and fetal plasma concentrations. No macroscopic, cytological, or extracellular matrix lesions were found in fetal cartilage. These findings suggest that enrofloxacin crosses the equine placenta and does not induce cartilaginous lesions in the fetus at recommended doses in late pregnant mares.

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Effectiveness of Four Commercial Culture Plate Systems to Diagnosis Related-Mastitis Pathogens in Dairy Cows

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A precise on-site diagnosis of mastitis cases is critical for targeting an appropriated antimicrobial therapy. However, a major constrain for implementation of on-farm culture plates is their uncertain accuracy when compared to laboratorial microbiological analyses. Therefore, the purpose of the present study was to evaluate the effectiveness of four commercial culture plates (Accumast, Minnesota Easy System, SSGN and SSGNC Quad plates) for the identification of pathogens associated with clinical mastitis in dairy cows. Milk samples from the quarter of cows with clinical mastitis were collected aseptically and processed at the University of Illinois. Aliquots of the samples were also analyzed by two laboratories and culture results with the same diagnosis were used as gold standard (GS). Accuracy (Ac), Sensitivity (Se), Specificity (Sp) and Cohen's kappa coefficient (*k*) of plate tests were determined based on the GS culture of 211 milk samples. Accumast had greater Ac, Se and Sp (89.6%, 97.6% and 84.5%, respectively) than the other plate tests (Ac ≤ 79.1%, Se ≤ 88.9%, Sp ≤ 79%). A substantial agreement (*k* = 0.79) was detected between Accumast with GS, while the inter-rater agreement of Minnesota, SSGN and SSGNC with GS was denoted moderate (*k* ≤ 0.55). All plates correctly identified ≥ 84.9% of milk samples with no bacterial growth. Accumast was more effective to diagnosis *E. coli* than SSGN and SSGNC (Ac = 77.8%, 6.3% and 54.2%, respectively). Only Accumast properly diagnosed *S. aureus* (Ac = 66.7% and Se = 100%). Also, Accumast had greater Ac for *Streptococcus sp.* (Ac = 55%) than the reaming plate systems (Ac ≤ 38.9%). Accumast was the most efficient plate test to diagnose mastitis-related pathogens in dairy cattle.

Transgenerational Effects of Di-(2-Ethylhexyl) Phthalate on Behavior and Hippocampal Gene Expression in Male and Female Mice

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Phthalates are plasticizers used in PVC plastic products, including food packaging and cosmetics. Di-(2-ethylhexyl) phthalate (DEHP), one of the most widely used phthalates, acts as an endocrine disruptor and exposure has been shown to alter behavior. Evidence indicates that transgenerational DEHP exposure alters social behavior and pituitary gene expression. However, the extent of these transgenerational effects remains unknown. Here, we assessed the effects of transgenerational DEHP exposure on anxiety, spatial memory, and brain gene expression. We modeled transgenerational exposure by orally dosing pregnant CD-1 mice daily with oil control or DEHP (20 or 200 µg/kg/day; 500 or 750 mg/kg/day) from gestational day 10.5-birth to produce the F1 generation. Females from the F1 and then F2 generations were bred to the F3 generation, yielding the first unexposed generation. The elevated plus maze and Morris water maze were used to assess anxiety and spatial memory, respectively, in F3 adults. Following behavior assessments, we collected brains and isolated hippocampal samples for qPCR analyses. We selected two genes relevant to anxiety-like behavior, glucocorticoid receptor (GR) and estrogen receptor 2, and one gene relevant to epigenetic maintenance, DNA methyltransferase 3a (Dnmt3a). Behavior tests revealed that transgenerational DEHP exposure decreases anxiety-like behavior in females but not males. DEHP exposure did not affect spatial memory. Preliminary data indicate that DEHP exposure modifies GR and Dnmt3a expression in the hippocampus of males but not females. Together, these data add to the growing body of evidence showing that transgenerational DEHP exposure can modify behavior and brain gene expression.

Mice Null for Usp18 Develop Multi-Organ Autoinflammation

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USP18 (ubiquitin-specific protease 18) is a dual function protein. It removes ubiquitin-like modifier interferon stimulated gene 15 (ISG15) from conjugated proteins and it inhibits the activity of the interferon receptor by interacting with IFNAR2. We previously reported that USP18 null mice develop leiomyosarcomas that recapitulated key features of clinical leiomyosarcomas. Necropsy examination of mice, prior to tumor development, revealed immune infilatration of multiple tissues including brown adipose, pancreas, salivary glands and skin. We are currently characterizing the immune infiltrates using immunohistochemistry to determine the relative abundance of different types of cells; T cells (CD3+), macrophages (Mac-2+), and B cells (CD45R+). Chronic inflammation in these tissues results in extensive fibrosis visible using collagen specific stains, Sirius red and Masson's trichrome. Immunohistochemistry to identify activated pancreatic stellate cells (PSCs) is also underway as these are associated with fibrosis of the pancreas. Clinical studies have shown that patients with autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis, have an increased risk for certain tumor types. Because these USP18 null mice develop autoinflammation prior to sarcoma onset we hypothesize that USP18 inactivation provides a link between autoinflammation and tumorigenesis and may reveal a pathway that could be pharmacologically targeted. This could reduce both the severity of the autoinflammation and prevent tumor formation. Future work aims to determine which aspect of USP18 activity is responsible for the autoinflammation described here and how we can pharmacologically modify this.

Identification and Characterization of Herpesvirus Genes Essential for Transmission Using a Natural Animal Model; Marek's Disease Virus in Chickens

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Herpesviruses are an evolutionary success, having been discovered throughout a huge spectrum of vertebrates. Most herpesviruses are closely associated with a single host species in nature. This close relationship indicates that the viruses have evolved with their host over the millennia. Based on sequence comparisons, the mammalian and avian herpesviruses were descended from a common ancestor. Mammalian herpesviruses populate all three subfamilies, *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*, while all avian herpesviruses have been classified as members of the *Alphaherpesvirinae*. Marek's disease virus (MDV) is an avian herpesvirus that causes immune suppression, neurological lesions, and lymphomas in chickens. It has been estimated that the economic impact of Marek's disease on the poultry industry costs $US 1-2 billion annually; however, very little is known about how MDV spreads within the chicken population. We have identified two conserved herpesvirus proteins that are essential for transmission of MDV - namely, UL44 or glycoprotein C (gC) that is conserved among the *Alphaherpesvirinae* subfamily; and UL13, the conserved herpesvirus protein kinase (CHPK) conserved among all members of the *Herpesviridae* family. The major focus of my laboratory is to understand the role these viral proteins play during transmission of herpesviruses using molecular tools and natural virus:host models to define the complex interactions required to facilitate dissemination of herpesviruses in a population. Our long-term goal is develop vaccines or therapies that target spread of herpesviruses within a population.

Ability of Milk pH to Predict Increased Somatic Cell Count at Dry-Off in Dairy Cattle

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Milk pH is increased in lactating dairy cattle with subclinical mastitis (SCM). Milk pH testing may therefore provide an economic, rapid, and practical method for diagnosing SCM in the field. Our objective was to evaluate the clinical utility of measuring milk pH using a PICCOLO plus® pH meter, Multistix® 10 SG Reagent Strips for Urinalysis (Multistix® strips), and pH Hydrion® paper as on-farm screening methods for diagnosing SCM in dairy cattle at dry-off. Quarter foremilk samples were collected from 117 dairy cows at dry-off. Quarter somatic cell count (SCC) was measured using a Delaval® cell counter with SCC≥200,000 cells/ml as the reference method for SCM. Milk pH was measured using the pH meter, Multistix® strips, and pH Hydrion® paper. Spearman correlation coefficient, area under the receiver operating curve (AUC), and kappa coefficient (κ) were calculated. $P<0.05$ was considered significant. Compared to the reference method, Multistix® strips had the highest association (r_s=0.61) with Delaval® SCC, and at the optimal cut-point (pH≥7.0), AUC=0.77 and κ=0.42. The pH meter (r_s=0.52) was inferior to Multistix® strips, and at the optimal cut-point (pH≥6.69), AUC=0.74 and κ=0.34. pH Hydrion® paper performed poorly at an optimal cut-point of ≥6.2 (r_s=0.36; AUC=0.59, κ=0.12). We conclude that milk pH does not provide a clinically useful primary method for diagnosing SCM at dry off in dairy cattle. Combining milk pH with other diagnostic tests may improve the clinical utility of milk pH to detect quarters with increased SCM.

Clinical Utility of Four Point-of-Care Enzymatic Tests in Predicting Somatic Cell Count in Dairy Cattle

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Inflammation of the mammary gland increases the activity of more than 20 enzymes in the glandular secretion. Our primary objective was to characterize the ability of three enzymes in quarter milk samples, catalase, lactate dehydrogenase (LDH), and esterase, to predict the presence of a high somatic cell count (SCC) and intramammary infection (IMI) in lactating dairy cattle. Quarter foremilk samples were collected from 117 dairy cows at dry-off and 109 fresh cows within 4-7days post calving. A Delaval® cell counter was used as the reference method to measure quarter SCC, with IMI being defined as SCC≥200,000 cells/ml. Milk catalase activity was measured using the Accutest Uriscreen® test. Milk LDH activity was measured using Udder-Check® strips. Milk esterase activity was measured using Multistix® and Serim PeriScreen® strips. Spearman correlation coefficient, area under the receiver operating curve (AUC), and kappa coefficient (κ) were calculated. $P<0.05$ was considered significant. Compared to the reference method, Udder-Check® strips had the strongest association (r_s=0.79) with IMI, and at the optimal cut-point score≥1, AUC=0.88 and κ=0.73. The Accutest Uriscreen® test had the second highest association (r_s=0.72) with IMI, and at the optimal cut-point foam height≥4 mm, AUC=0.85 and κ=0.54. Serim PeriScreen® strips had the third highest association (r_s=0.69), and at optimal cut-point score≥1, AUC=0.72 and κ=0.42. Multistix® strips had the lowest association (r_s=0.45; AUC=0.70, κ=0.19) at an optimal cut-point score≥1. We conclude that Udder-Check® strips and the Accutest Uriscreen® test may provide clinically useful on-farm enzymatic screening tests for predicting the presence of IMI.

Comparison of Six On-Farm Tests to Estimate Somatic Cell Count at Dry-Off in Dairy Cattle

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Somatic cell count (SCC) is the most commonly used method to monitor udder health in dairy cattle. Our objective was to evaluate the clinical performance of different cow-side tests (California Mastitis Test {CMT}, Somaticell® test and three PortaSCC® tests) for diagnosing subclinical intramammary infection (IMI) at dry-off. Quarter foremilk samples were collected from 117 dairy cows at dry-off. Quarter SCC was measured using Delaval® cell counter (reference method; IMI=SCC>200,000 cells/ml), CMT, Somaticell®, and three PortaSCC® tests. Spearman correlation coefficient, area under the receiver operating curve (AUC), and kappa coefficient (κ) were calculated. P<0.05 was considered significant. Compared to the reference method, CMT had the highest correlation (r_s=0.89), and at optimal cut-point (score ≥ trace), AUC=0.88 and κ=0.77. PortaSCC® color test was the second best performing (r_s=0.82), but required 45 minutes to produce result, and at optimal cut-point (50,098 SCC/ml), AUC=0.91 and κ=0.74. PortaSCC® quick test was the third best performing test (r_s=0.80), and at optimal cut-point (54,482 SCC/ml), AUC=0.88 and κ=0.62. PortaSCC® reader (r_s=0.68) was inferior to PortaSCC® color test, and at optimal cut-point (73,419 SCC/ml), AUC=0.82 and κ=0.50. The Somaticell® test run on milk at 37 °C didn't perform well (r_s=0.44; AUC=0.68, κ=0.24) with an optimal cut-point of 123,864 SCC/ml; however, it is recommended to be run using milk at 1-4 °C. We conclude that the CMT provides the most accurate, practical, and least cost on-farm screening test to predict IMI at dry-off. However, because the CMT is a semi-quantitative test, we recommend using the PortaSCC color test® if a more quantitative on-farm screening test is required.

Simultaneous Effects of Weather and Landscape on Mosquito Vector Abundance in Suburban, Chicago, Illinois

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The local abundance of *Culex* vector mosquitoes is critically important for the transmission of the West Nile virus (WNV). Temporal and spatial differences in abundance are affected by both the weather and landscape features simultaneously. However, there are limited studies that takes account of weather and landscape variables together while analyzing the mosquito abundance data. The main objective of this study is to understand how the weather and landscape simultaneously affect the *Culex* abundance in a region with significant WNV activity in suburban, Chicago, Illinois from 2009 to 2012. A multilevel modeling approach with trap sites, year and weeks of mosquito collection as random effects were used to model the *Culex* abundance data. The results indicated that weeks of mosquito collection and location of traps significantly affected the number of *Culex* captured. The higher average temperature of the same week, total precipitation one week before, average humidity two weeks before, traps being closer to water bodies and having less number of catch basins around 100 m distance from the trap sites were associated with higher *Culex* abundance. Maximum wind speed of the same week and three weeks before were associated with lower *Culex* abundance. This study highlights that local weather conditions contributed more compared to landscape features in the local abundance of *Culex* mosquitoes.

Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid Protein Activates NF-κB Through Binding to PIAS1

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Porcine reproductive and respiratory syndrome virus (PRRSV) triggers the onset of inflammation during infection, and various pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8, and TNFα have been shown to be upregulated in PRRSV-infected porcine alveolar macrophages (PAMs), suggesting the activation of NF-κB pathway. In this present study, we show that in cells expressing the viral nucleocapsid (N) protein, the p65 subunit of NF-κB was increasingly phosphorylated and translocated to the nucleus, resulting in the activation of NF-κB. This data demonstrates PRRSV N is the viral component activating the NF-κB signaling pathway. Further studies show that the N protein bound directly to PIAS1 (protein inhibitor of activated STAT1). Since PIAS1 binds to the Rel A (p65) subunit of NF-kB and prevents NF-κB activity, PIAS1 is considered a negative regulator of NF-kB. In cells expressing PRRSV N protein, N was found to bind to PIAS1, such that p65 was released from PIAS1. Further studies mapped the N-terminal region of PIAS1 for binding to N and prevented the translocation of p65 to the nucleus. This data confirms the preferable binding of PRRSV N with PIAS1. A series of deletions and truncations of PRRSV N were constructed to determine the domain responsible for PIAS1 interaction, and the region between 37 and 72 of N protein was identified as the PIAS1 binding domain. This domain was shown to activate the NF-κB signaling, demonstrating the correlation between N-PIAS1 interaction and NF-κB activation. Taken together, we report a novel strategy of PRRSV for NF-κB activation through the binding of viral N protein to PIAS1.

Loss of Type I Interferon Suppression Function in Nsp1β Confers Attenuation of Porcine Reproductive and Respiratory Syndrome Virus in Pigs

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Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses the type I interferon (IFN) production during infection. PRRSV nsp1β has been identified as one of the most potent IFN antagonists, and subsequently a SAF-A/B, Acinus, and PIAS (SAP) motif of 126-LQRRLQVNGL-135 has been identified in nsp1β as the functional motif for IFN suppression. Mutational studies of the SAP motif identified L126, R128, R129, L130, and L135 as critical amino acids for IFN antagonistic function. Two SAP mutant viruses, vL126A and vL135A, were generated by reverse genetics. These mutants retained the infectivity but lost the function of IFN suppression in cells. To determine the pathogenic role of IFN-suppression-negative PRRSV, 40 piglets were allotted to four groups and each group was infected intramuscularly with vL126A, vL135A, placebo, or wild type (WT) PRRSV. Pigs infected with vL126A or vL135A exhibited less severe clinical signs with lower and shorter durations of viremia when compared to those of pigs infected with WT PRRSV. The levels of PRRSV-specific antibody remained comparable in all infected groups. The neutralizing antibody titers were also higher in vL126A- or vL135A-infected pigs than those of control pigs. When examined for persisting viruses in the tonsils, two of 10 pigs in the vL135A-infection group were PRRSV-negative. Reversion to wild-type sequence was observed in some pigs, and the revertants regained the ability to suppress the IFN production, indicating strong selection pressure in the IFN suppression Taken together, our data demonstrate that the IFN antagonism mediated by nsp1β contributes to viral virulence in pigs, and the loss of function confers viral attenuation.

Deciphering Host-Parasite Molecular Networks Mediating Resistance/Susceptibility to *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that infects a wide range of mammals. Rats, like humans, develop a subclinical chronic infection, but vary in their susceptibilities to *T. gondii* depending on the rat strain. Compared to the *T. gondii*-susceptible Brown Norway (BN) rat, the Lewis (LEW) rat is extremely resistant to *T. gondii*. Thus, these two rat strains are ideal models for dissecting host molecular mechanisms that are important for host resistance of *T. gondii* infection. We performed global transcriptome analysis of the LEW versus BN rat, with or without *T. gondii* infection, in order to unravel the molecular factors directing the robust protective early innate immune response in LEW rat. RNA-sequencing analysis of mRNA transcripts from freshly isolated peritoneal cells showed that 4901 genes were differentially expressed (FDR p-values < 0.05) between the LEW and BN rats at 24 hours post-infection. Among the differentially expressed genes were some innate immunity-associated genes (IAGs) that we found to be upregulated in the *T. gondii*-infected LEW rat. We engineered BN rat and human cell lines for inducible over-expression of the candidate LEW rat IAGs. We found that over-expression of some of the IAGs conferred resistance to *T. gondii* infection by inhibiting intracellular parasite growth. Together, our findings indicate that upregulation of selected IAGs contribute to the robust refractoriness of the LEW rat to *T. gondii*. Elucidation of protective innate immune responses to *T. gondii* infection would be beneficial in developing strategies for designing new effective therapies and vaccines against *T. gondii*.

Investigations Into the Function of *Candida albicans* Pir Proteins

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The cell wall of the opportunistic fungal pathogen *Candida albicans* maintains cell structure and serves as the point of interaction between the fungal cell and its environment. In the context of host-pathogen interactions, the fungal cell wall is an outstanding drug target because of its unique structure relative to the mammalian host. Among the proteins that comprise the *C. albicans* cell wall are those encoded by the *PIR* (proteins with internal repeats) genes. Pir proteins are best characterized in *Saccharomyces cerevisiae*, but full understanding of Pir function is compromised by various circumstances. For example, *S. cerevisiae* encodes multiple *PIR* loci, but a null mutant has yet to be constructed due to the discovery of an additional *PIR* locus that was more recently recognized in the *S. cerevisiae* genome sequence. In *C. albicans*, Pir characterization was hindered by a report that suggested deletion of *PIR1* was a lethal event. Work in our laboratory demonstrated viable *pir1/pir1* cells, but multiple *PIR1* allelic variants that may display phenotypic diversity. The goal of this study is to construct a *C. albicans* null mutant by deletion of both the *PIR1* and *PIR32* loci. This strain will provide the necessary background for functional studies of the variants of Pir1 and Pir32. We anticipate that Pir proteins are required for wild-type cell wall organization and composition. Analysis of the Pir proteins will lead to a better understanding of *C. albicans* cell wall structure and its importance as an antifungal drug target.

The Effects of Estrogenic Components of Licorice Root on a Hippocampus-Sensitive Task

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Many dietary supplements contain estrogenic compounds, the efficacy and safety of which are poorly understood. This study investigated the efficacy of components of licorice root to alter performance on a hippocampus-sensitive metric change in object location (MCOL) task. We investigated isoliquiritigenin (ISL), *Glycyrrhiza glabra* root power (LRP), and a methanol extract of *Glycyrrhiza glabra* root (LRE). We also explored whether a high fat diet (HFD) would impair performance on this task and whether the botanicals could mitigate any negative effects. Young adult Long-Evans female rats were ovariectomized (OVX) and exposed to either a HFD or a LFD for five weeks prior to testing. A subset of rats on each diet were exposed to ISL, LRP or LRE at a concentration of 0.075%, 5% or .5% respectively of the diet for three weeks prior to testing. Estradiol improves performance on the MCOL task and thus was included as a positive control. Rats in the estradiol group were injected subcutaneously 48 and 24 hours prior to testing with 45 µg/kg of estradiol. In the MCOL task, rats were allowed to explore two objects in a chamber while object exploration time was recorded for three 5-min trials with a 3-min inter-trial interval in the rat's home cage. For the fourth 5-min trial, the objects were moved closer together and exploration time was again recorded. An increase in object exploration time in the final trial suggests that the rat detected the change in object locations. Estradiol, ISL and LRE all led to significantly better performance on the task. Diet had no effect on its own and did not interact with botanical exposure.

Proper Exiting of Competence State Benefits *Streptococcus Pneumoniae* D39 Acute Infection

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Streptococcus pneumoniae (pneumococcus) is a commensal of respiratory tract that is capable of causing serious infections, and its competence system is known to facilitate the spread of antibiotic resistance through the uptake of new DNA from co-colonizing bacteria in nasopharynx, as well as contribute to invasive diseases. The DNA processing protein A (DprA) protein of pneumococcus regulates physiological exit from the competent state. Previously, we and others have shown that Δ*dprA* mutant fails to exit competent state, with reduced virulence in both mouse pneumonia and bacteremia models, and we hypothesized that inability in exiting from the competent state attenuates virulence of Δ*dprA*. Previous studies by us and others have shown that after the activation of competent state by the competence stimulating pheromone peptide (CSP), competent pneumococcus undergo lysis (allolysis) *in vitro*, by upregulating the expression of cell wall hydrolases. Interestingly, we found that, although exposure to CSP causes a slight growth delay, it does not induce more allolysis in Δ*dprA* than the parental strain wide-type D39. However, Δ*dprA* growth can be delayed by a lower concentration of CSP than D39. Moreover, Δ*dprA* is more sensitive to cell lysis triggered by detergents. Because the expression of allolysis genes are regulated by both basal and competence-specific promoters, mutants that only retain the basal promoter are being constructed and will be used to determine whether competence-induced allolysis contributes to virulence attenuation in Δ*dprA* during lung and blood infection in mice. Unveiling the regulatory role of DprA will increase the understanding of the evolutionary purpose of competence system in pneumococcal virulence.

Investigating the Pharmacokinetics, Tolerability, and Anti-Cancer Activity of the Novel Drug Isobutyl-Deoxynyboquinone (IB-DNQ) in Feline Oral Squamous Cell Carcinoma

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Feline oral squamous cell carcinoma (FOSCC) is a common oral tumor in cats. Current treatments are ineffective, justifying the discovery of new therapies. NQO1 is a detoxifying enzyme overexpressed in many human tumors and serves as a preferential target for treatment. Isobutyl-deoxynyboquinone (IB-DNQ) is a substrate for NQO1, resulting in reactive oxygen species (ROS) generation and cell death. We have generated data identifying NQO1 expression in FOSCC cell lines and 40 spontaneous FOSCC tumors, have characterized cytotoxic effects of IB-DNQ in vitro, and have characterized the in vivo pharmacokinetics, tolerability, and anti-cancer activity in healthy cats and pet cats with OSCC. To characterize the pharmacokinetics of IB-DNQ, four healthy cats were treated with at five different regimens; 0.5 mg/kg IV, 1.0 mg/kg IV, 2.0 mg/kg IV, 1.0 mg/kg oral capsule, and 1.0 mg/kg oral gavage. Results then generated the next phase consisting of treating five tumor-bearing cats at 1.0 mg/kg IV every two weeks for three total treatments. Tumor response to treatment was objectively quantified with serial CT scans. NQO1 expression was assessed by IHC in tumor-bearing cats. In healthy cats, IB-DNQ was tolerable at all dosages with minimal toxicity and generated reproducible pharmacokinetic profiles; an optimal dosage of 1.0 mg/kg IV was identified for clinical evaluation. In tumor-bearing cats, repeated administration of IB-DNQ (1.0 mg/kg IV) was tolerable and exerted anticancer activity correlating with tumoral NQO1 expressions. IB-DNQ demonstrates anti-cancer effects in cats with spontaneous FOSCC and is well-tolerated warranting future evaluation in prospective clinical setting.

Cross-Sectional Study to Determine Factors Associated with Risk of Dengue Infection in Dehiwala and Ratmalana Divisions of Colombo, Sri Lanka

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The number of dengue cases in Sri Lanka has increased over the last 16 years, from 5,203 in 2000 to 55,150 in 2016. Nearly 51% of these cases come from the Western Province consisting of Colombo, Gampaha and Kalutara districts, with Colombo being the most affected.

The aim of this study was to determine factors associated with dengue risk.

A cross-sectional survey was conducted from May-September 2015 in the Dehiwala and Ratmalana divisions of Colombo. Eligible households had at least one person previously diagnosed with a dengue infection during 2013 (n=1,312). Participants were then selected by choosing every 10th case from the household list. Topics covered by the survey included info on the participant household and neighborhood as well as awareness of dengue threat and precautions. The study area was categorized into high, medium, and low risk areas using dengue rates from 2010-2014, and ordinal logistic regression was used to study associations between survey responses and risk level in the household's location.

The 131 survey participants were categorized into Low (n=30), Medium (n=45), and High (n=56) risk areas. Factors found to be significantly associated with high risk areas were age of dengue (OR=0.971, $p < 0.05$), house structure (residing in a high rise/flat; OR=11.518, $p < 0.05$), and socio-economic status of neighborhood (OR=0.289, $p < 0.05$).

Dengue is a serious concern within Sri Lanka that continues to worsen. Due to the lack of a vaccine or treatment beyond supportive care, surveillance and control are the best methods of reducing infection rates. This study provided further evidence on which risk factors governments should consider to determine appropriate target areas for surveillance and control.

Evaluation of in Vitro Chemosensitivity of Feline Injection Site-Associated Sarcoma Cell Lines to Carboplatin

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Feline injection site-associated sarcoma (FISAS) is a highly invasive cancer of cats. Surgical excision must be aggressive as local recurrence rates are high. Implantable carboplatin-impregnated calcium sulfate hemihydrate (CI-CSH) beads release carboplatin at concentrations greater than that achieved in plasma, however, the concentration necessary for tumor cell death has not been previously characterized. Thus, the objective of this study is to determine the 50% inhibitory concentration (IC-50) of carboplatin against feline injection site sarcoma cell lines. A second objective is to determine if IC_{50} values are within the range of concentrations that elute from commercially available CI-CSH beads. Cells from 5 FISAS tumor cell lines were seeded in cell culture plates. Cells were treated with concentrations of carboplatin ranging from 5 to 450 µM. Viable cells were assessed using a bioreductive fluorometric assay at 24, 48, and 72 hours after treatment. An apoptosis analysis was performed on cells after treatment with carboplatin for each time point (24, 48, 72 hours). Flow cytometry was performed and the relative percentages of viable, apoptotic and late apoptotic/necrotic cells were reported. Carboplatin exerted dose-dependent and time-dependent effects on the viability of FISAS cells. The IC_{50} values were within the range of carboplatin concentrations that elute from CI-CSH beads. Concentrations of carboplatin that elute from CI-CSH beads are sufficient to result in 50% growth inhibition of FISAS cells *in vitro*. Based on this data, local FISAS tumor control could be achieved by implantation of CI-CSH beads following radical excision of the primary tumor or by implantation without tumor resection.

Ultrasonographic Determination of Longissimus Dorsi Muscle Thickness and Intramuscular Fat Percentage in Periparturient Dairy Cattle

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Several studies have examined the rate of backfat mobilization in periparturient dairy cattle, but the rate of intramuscular fat (IMF) mobilization relative to backfat mobilization and muscle tissue mobilization does not appear to have been studied. Our objective was therefore to characterize the changes in IMF relative to changes in muscle thickness and backfat thickness (BFT) in the physiologic response to negative energy balance in periparturient dairy cattle. One hundred and six periparturient Holstein-Friesian cattle (34 primiparous, 72 multiparous) at -3 and +28 days relative to parturition were examined. A 5 MHz linear ultrasound probe was used to measure BFT and the maximum thickness and IMF percentage (determined using a thresholding-segmentation method) of the longissimus dorsi muscle in the thoracic region (LD-thoracic). Spearman's correlation coefficient (r_s) and mixed models analysis were used for statistical analysis. $P<0.05$ was declared significant. The mean decrease in BFT over the 31 day period was similar for primiparous (-46%) and multiparous cows (-45%). The mean decrease in IMF of the LD-thoracic muscle was similar for primiparous (-22%) and multiparous cows (-30%). Likewise, the mean decrease in LD-thoracic muscle thickness was similar for primiparous (18%) and multiparous cows (21%). The decrease in IMF was not associated with BFT decrease ($r_s=0.03$), but was associated with the decrease in LD-thoracic muscle thickness ($r_s=0.59$, $P=0.0011$). We conclude that ultrasonographic measurement of the LD-thoracic muscle thickness and IMF complements ultrasonographic measurement of BFT when quantifying negative energy balance in periparturient dairy cattle.

Clinical Utility of Measuring Plasma Fructosamine Concentration During Early Lactation in Dairy Cattle

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Fructosamine (FRA) is widely used as a long term hyperglycemic biomarker in humans and dogs, but the clinical usefulness of FRA as a hypoglycemic biomarker is uncertain. Our objective was to evaluate the clinical utility of FRA in quantifying the magnitude of hypoglycemia and negative energy balance (NEB) during early lactation in dairy cattle. Plasma samples were collected weekly from 106 Holstein-Friesian cattle (34 primiparous, 72 multiparous) on days 4-34 postpartum. Plasma concentrations of glucose ([gluc]), β-hydroxybutyrate ([BHB]), total protein ([PP]), and other clinicopathologic indices of energy status were determined. Backfat thickness and longissimus dorsi muscle thickness (LDT) were measured ultrasonographically. Plasma FRA concentration ([FRA]) was measured at approximately 28 days postpartum. Associations between [FRA] and study variables were evaluated using Spearman's rho and stepwise linear regression. $P<0.05$ was considered significant. A positive association was detected between plasma [FRA] and mean plasma [gluc] for days 4-28 postpartum (r_s=+0.34, p=0.0016), and between plasma [FRA] and LDT (r_s=+0.31, p=0.0039). Plasma [FRA] was negatively associated with mean plasma [BHB] for days 4-28 postpartum (r_s=-0.31, p=0.019). Stepwise linear regression identified a positive association between plasma [FRA] and mean [PP] for days 4-28 postpartum (r_s=+0.24, p=0.015). After correcting plasma [FRA] for [PP], there was only a marginal improvement in the association between plasma [FRA] and mean plasma [gluc] (r_s=+0.37, p=0.0009). We conclude that plasma [FRA] is not sufficiently sensitive or specific to provide a clinically useful method for quantifying the magnitude of hypoglycemia or NEB in early lactation.

The Microbial Killing Capacity of Gaseous and Aqueous Ozone on Different Surfaces Contaminated with Cattle Feces

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A high reactivity and leaving no harmful residues make ozone an effective disinfectant for farm biosecurity improvement and prevention of infectious diseases. Our objective was therefore to evaluate the microbial killing capacity of gaseous and aqueous ozone on rubber and plastic surfaces contaminated with cattle feces. Six rubber and plastic strips (10 X 5 cm) were contaminated with different dilutions of fresh cow feces. Four strips were exposed to gaseous and aqueous ozone with concentration of 1.0, 2.0, 4.0, and 9.5 ppm for 2, 4, 8, and 10 minutes. Two strips (positive control) were held in upright position for the same corresponding time of exposure. The strips were then washed with buffered peptone water and 1.0 ml of washed solution was plated on 3M™ Petrifilm™ rapid aerobic count plate (RAC) and the number of colonies were counted automatically using 3M™ Petrifilm™ Plate Reader. Student's t-test or Mann-Whitney Rank Sum Test based on the Shapiro-Wilk test for normality were used to evaluate the killing capacity of ozone. $P<0.05$ was considered significant. On smooth surface, plastic, ozone in water at 4.0 ppm or greater reduced bacterial load below detectable limit within 2 minutes. Gaseous ozone at 9.5 ppm for 10 minutes reduced ($P = 0.004$) bacterial load by about 6 logs. On complex surfaces, rubber, both gaseous and aqueous ozone at up to 9.5 ppm were unable to significantly ($P = 0.351$, $P = 0.201$, respectively) reduce the bacterial load. We conclude that the ozonized water on smooth surfaces, provides an effective method for controlling most of the microbial fecal-borne diseases in dairy industry. However, on complex surface, ozone alone is not an adequate means of controlling bacterial populations.

Evaluation of the Analytical Performance of the Multistix 10 SG® Urine Dipstick for Measuring Urine Specific Gravity in Dairy Cattle

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Urine specific gravity (U_{sg}) is considered the most common method to assess urine concentration and hydration status in the dairy industry. The Multistix 10 SG® urine dipstick may provide a convenient method for measuring U_{sg} in dairy cattle as it is minimally affected by urinary glucose or protein concentration. Our objective was therefore to evaluate the analytical performance of the Multistix 10 SG® dipstick for measuring U_{sg} in dairy cattle. Urine samples were collected weekly on days 4-34 postpartum from 106 periparturient Holstein-Friesian cattle. Urine specific gravity was measured using an optical refractometer ($U_{sg\text{-}refractometer}$, reference method) and Multistix 10 SG® dipstick ($U_{sg\text{-}dipstick}$). Urine pH was measured using a pH meter that incorporated a glass electrode (reference method) and Multistix 10 SG® dipstick. Associations between $U_{sg\text{-}dipstick}$ and study variables were evaluated using Spearman's rho and stepwise linear regression. $P<0.05$ was considered significant. A weak positive association was detected between $U_{sg\text{-}dipstick}$ and $U_{sg\text{-}refractometer}$ (r_s=+0.11, p=0.0003). A moderate negative association was detected between $U_{sg\text{-}dipstick}$ and urine pH measured by dipstick (r_s=-0.64, p=0.0001) and urine protein-dipstick (r_s=-0.43, p=0.0001). Stepwise linear regression identified a significant impact of urine pH ≥ 6.0 on the measured value of $U_{sg\text{-}dipstick}$ (p=0.0001). We conclude that the Multistix 10 SG® urine dipstick is not sufficiently accurate to provide a clinically useful method for measuring urine concentration in dairy cattle at urine pH ≥ 6.0. We do not recommend the use of the Multistix 10 SG® urine dipstick for estimating U_{sg} in lactating dairy cattle because they typically have an alkaline urine pH.

Using MALDI-TOF MS to Identify the Infecting Leptospira Serovar

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Leptospirosis, a re-emerging zoonotic disease, is an increasing cause of morbidity and mortality. In dogs, leptospirosis can cause a broad spectrum of disease and dogs chronically infected may serve as an ongoing source of infection. Four serovars associated with canine disease are represented in commercial vaccines. As over 250 pathogenic serovars are recognized, it is important to identify circulating serovars to ensure vaccine efficacy. It is also critical for determination of outbreak containment measures. However, serovar-specific PCR assays are lacking and serology results often reflect cross-reactions. We propose that matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used for serovar discrimination. We hypothesize that MALDI has sufficient discriminatory power to detect serovar identification patterns. We are developing a MALDI protocol using paired canine urine and serum samples to determine seroprevalence and level of agreement between MALDI and Microscopic Agglutination Test results. We have created a Main Spectrum Profile for each serovar we plan to test, and have combined these into a Leptospira MALDI Biotyper reference library. Preliminary specificity testing has been promising. Successful MALDI protein peak discrimination of serovars will require concentration of Leptospira in urine samples to sufficient numbers. Our efforts are centered on concentration methods and increasing protocol sensitivity. We plan to expand the study to include wildlife hosts. Our goal is to produce a rapid and inexpensive method to diagnose leptospirosis cases, monitor trends in seroprevalence, identify outbreaks, and that can ultimately improve vaccine design and efficacy.

Chronic Exposure to Dietary Levels of Genistein Affects Fertility and Pregnancy Outcomes in Female Mice

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Genistein is a phytoestrogen that people are exposed to through consumption of soy and soy-based products. Previously, we found that genistein decreases estradiol levels and inhibits antral follicle growth *in vitro*. Proper hormone production and follicle growth are key factors for normal fertility. Therefore, we hypothesized that genistein negatively affects female fertility and pregnancy outcomes. To test this hypothesis, we dosed female CD-1 mice (35 days) with 0, 300, 500, or 1000 ppm genistein for 30, 60, 150, and 240 days. The selected doses of genistein result in serum genistein levels similar to those observed in humans after genistein exposure (1-3 μM). At the end of the dosing periods, the females were paired with a mate, and placed on the control diet. To determine if genistein exposure affected fertility, we measured mating rate, pregnancy rate, fertility rate, gestation time, and parturition time. To determine if preconception exposure to genistein affected pregnancy outcomes, we examined pup mortality, litter size, average pup weight, and maternal behavior. After 30 days of dosing, genistein exposure decreased gestation time. After 60 days of dosing, genistein exposure increased gestation time, decreased litter size, increased average pup weight, and increased pup mortality. After 150 days of dosing, genistein exposure increased parturition time, pup mortality, and poor maternal behavior. After 240 days of dosing, genistein exposure decreased fertility rates, increased pup mortality, and increased poor maternal behavior. Collectively, our results show that chronic exposure to dietary levels of genistein negatively affects gestation and parturition time, and increases pup mortality and poor maternal behavior.

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Prenatal Exposure to Di-(2-ethylhexyl) Phthalate Impairs Puberty in Female Mice

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Di-(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer in products such as vinyl flooring, plastic food containers, and children's toys. Prenatal exposure to DEHP causes reproductive toxicity in subsequent generations of male mice, but its effects on subsequent generations of female mice is unknown. Therefore, we tested the hypothesis that prenatal exposure to DEHP impairs puberty in the F1, F2, and F3 generations of female mice. Pregnant CD-1 dams were orally dosed with tocopherol-stripped corn oil (vehicle control) or DEHP (20 and 200 µg/kg/day, and 500 and 750 mg/kg/day) daily from gestational day 10.5 until birth (n=7-28 dams/treatment). Pups born to the exposed dams were labeled the F1 generation. F1 females were mated with untreated males to produce the F2 generation. F2 females were mated with untreated males to produce the F3 generation. Starting on postnatal day 21, female mice were weighed and monitored for the onset of puberty, timing of first estrous, and estrous cyclicity. In the F1 generation, prenatal exposure to DEHP did not impact body weight, age of vaginal opening, age of first estrus, or estrous cyclicity compared to controls. In the F2 generation, prenatal exposure to DEHP at 500 mg/kg/day decreased the age of first estrus, increased time spent in estrus, and decreased time spent in diestrus compared to controls ($p \leq 0.05$). In the F3 generation prenatal DEHP exposure at 20 µg/kg/day, 500 and 750 mg/kg/day decreased age at vaginal opening compared to controls ($p \leq 0.05$). Collectively, these data indicate that prenatal exposure to DEHP accelerates puberty and alters estrous cyclicity in multiple generations of mice. Supported by NIH P01 ES022848, EPA RD-83459301, and T32 ES007326.

Enhancing Chelonian Health Data Through a Prospective Cohort Study for Better Population Management

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Captive and free-range chelonian populations are at risk of rapid die-offs from emerging infectious diseases. Specifically, frog virus-3-like virus (FV3), a ranavirus in the family Iridoviridae, infects multiple classes of ectothermic vertebrates and has shown the potential for high morbidity and mortality among turtle and tortoise species. Effective disease management in a free-range setting is limited by the lack of epidemiological studies. Currently, chelonian health is evaluated by parameters such as hematology, pathogen presence, and clinical pathology. However, no distinct prospective studies exist observing these parameters within individuals over time. To address this deficit, we are following Eastern box turtles (*Terrapene carolina carolina*) via radiotelemetry in central Illinois. At Kickapoo State Park, a known ranavirus outbreak site, a total of 22 turtles were affixed with radiotransmitters and iButtons to obtain location and temperature, respectively. Four times per week from May-November 2016, the turtles were located and environmental data were obtained. Every other week, blood samples were drawn from the subcarapacial sinus and oral/cloacal swabs were obtained for laboratory diagnostics. The turtles will be observed and sampled over the entire 2017 active season and the beginning of 2018. The proposed research aims to investigate movement, pathogen detection, and environmental characteristics for the subjects using repeated measures. The observed patterns of chelonian movement and interactions will enable better population protection in response to ranavirus disease.

Circadian Disruption Impacts Cognition in a Rodent Model

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Circadian disruption is caused by misalignment of internal rhythms and external stimuli, such as exposure to light, untimely meals, and an altered sleep-wake cycle, causing physiological and behavioral changes. Studying these behavioral changes provides us a better understanding of the deleterious effects of working beyond regular hours and exposure to light at night. The present study is aimed at determining the effect of two kinds of circadian disruption on attention and impulsive behavior in a rodent model using a 5-choice serial reaction time task (5-CSRTT). Adult Long-Evans rats were maintained on a 12h:12h light dark cycle and were tested under three circadian conditions: 4h after the lights were turned off with no exposure to light (control condition), 4h after the lights were turned off with exposure to a pulse of light at the time of testing (a model of light-at-night), and 4h after the lights were turned on (a model of shift work). We hypothesized that the rats tested during their dark-phase with no light pulse would perform better than the rats tested under the two different models of circadian disruption. Our preliminary results reveal that rats tested under dark-phase with no light pulse were more attentive than the rats tested during the light-phase and the dark-phase with a pulse of light, the two models of circadian disruption, while the 3 conditions did result in differences in the extent of impulsive behavior on the task. Our findings demonstrate that different forms of circadian disruption can affect attention in a similar manner.

DHA Endocannabinoid Epoxides are Anti-Tumorigenic and Anti-Migratory in Human Osteosarcoma

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Endocannabinoids (EDC) are molecules that bind to cannabinoid receptors and are involved in regulation of various physiological processes and maintaining homeostasis. ω-6 EDC anandamide (AEA) is an endogenous molecule that reduces angiogenesis and induces apoptosis in various cancers. While most studies have been performed with ω-6 EDCs and their effects on cancer, studies on ω-3 EDCs are sparse. With growing interest in links between dietary consumption of ω-3 fatty acids and cancer, there is a strong interest to study the role of ω-3 EDCs and their metabolites on cancer and related physiological effects. Recently, our laboratory discovered a new class of molecules ω-3 endocannabinoid epoxides that are anti-inflammatory and implicated in reducing pain. In this study, we investigate whether docosahexaenoic acid derived endocannabinoid epoxides are anti-tumorigenic and anti-migratory in addition to being anti-inflammatory. Using cell titer blue assay, we identified regiosiomers of these molecules that reduce cell metabolism, followed by evaluation of their pro-apoptotic and anti-migratory properties using annexin-V binding and wound healing assay respectively. We have identified ω-3 endocannabinoid epoxide derivatives that show pro-apoptotic and anti-migratory effect in osteosarcoma (OS) cell lines. However, these molecules are labile to soluble epoxide hydrolase (sEH) and fatty acid amide hydrolase (FAAH). Thus, our current goal is to make stable derivatives of these molecules, using organic synthesis, to reduce hydrolytic lability and for in vivo studies in tumor models of OS. The long-term goal is to evaluate the capacity of ω-3 endocannabinoid epoxide derivatives as anti-cancer and anti-bone pain therapeutics.

Systematic Optimization of Therapy for Canine Intracranial Neoplasia Using Pac-1, a Novel and Potent Procaspase-3 Activator

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Canine central nervous system tumors can develop deeply within the brain parenchyma, precluding surgical resection and limiting therapeutic options. PAC-1 is a novel, blood-brain barrier penetrant, pro-apoptotic small molecule activator of procaspase-3 (PC-3), with orphan drug status for the treatment of human glioblastoma multiforme. PC-3 is frequently overexpressed in malignantly transformed tissues, providing an opportunity to selectively induce apoptosis in cancer cells with dysregulated upstream apoptotic circuitry. This study evaluates the *in vitro* activity of PAC-1 against a panel of brain tumor cells, and the feasibility of combining PAC-1 with conventional therapies in dogs with spontaneous brain cancer. Immunohistochemical characterization of PC-3 was performed in 21 normal canine brains and more than 450 canine and human intracranial neoplasms. Murine, canine, and human glioma cell lines were evaluated for PC-3 expression and *in vitro* sensitivity to PAC-1 and radiation. Dogs with spontaneously-arising glioma and meningioma were treated with oral PAC-1 in combination with conventional therapies to determine tolerability, with serial clinical and MRI outcome assessments. PC-3 is overexpressed in canine intracranial neoplasms and high-grade human astrocytomas relative to normal brain tissues. Immortalized glioma cell lines show *in vitro* sensitivity to PAC-1 and radiation monotherapies at biologically relevant exposures. 4 dogs received PAC-1 combinatorial therapies and achieved objective responses (1 CR, 3 PR, 2 SD). Investigation of therapeutic approaches that combine PAC-1 with radiation therapy and/or temozolomide will further elucidate its therapeutic potential in murine models and canine patients.

Past Use of Arsenic and Associated Health Risks: A Riskscape Study

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Arsenic in several forms has been used extensively as a pesticide, including for killing mosquitoes and ticks. It is a public health concern because arsenic has been linked to several forms of cancer, skin lesions, and cardiovascular disease. In Florida, residual levels of arsenic and other chemicals from various arsenic-contaminated can lead to poor environmental quality in rural and urban residential areas. Former sites of contamination have since been abandoned or have been developed into playgrounds, nursing homes, subdivisions, and industrial workplaces. Many of these places are locations where people reside or frequently visited, and the increased concentration of arsenic in the soil may be a risk to human health, especially children. The *objective* of this study is to examine sources of arsenic and use data synthesis to map the risk of arsenic in Florida from multiple sources, and compare the distribution of potential arsenic exposure to surveillance data from drinking water, contaminated sites of concern and diagnoses of cancer of bladder, skin, and lung to assess demographic vulnerability. Geographic Information System (GIS) and statistical methods were used to calculate risk levels and assess the correlation between arsenic sources and four types of contaminated sites as well as water quality violations. Results indicate that most low risk counties could be found in many northern counties and some central counties that were part of the everglades. Moderate risk counties were among the entire state with many states bordering coastlines and state-lines. High-risk counties could be found in the panhandles as well as in central Florida where mining practices are common and in highly populated southern Florida. Correlations could be found in between these sources of arsenic and specific contaminated sites.

Biomechanical Evaluation of a Modified Laryngoplasty Using a Toggle Technique For Equine Arytenoid Cartilage Stabilization

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The gold standard surgical treatment for recurrent laryngeal neuropathy in the horse is the prosthetic laryngoplasty. It has been reported that 95% of horses experience post-operative loss of arytenoid abduction. We developed a technique that uses a titanium toggle to anchor the suture to the arytenoid cartilage, which would eliminate failure at the muscular process of the arytenoid cartilage. Therefore, we evaluated and compared the biomechanical strength and resistance to cyclical loading of the standard and modified laryngoplasty techniques in ex-vivo constructs. Laryngoplasty constructs were tested in either monotonic or cyclic loading (10,000 cycles), and the maximal load at failure, mode of failure, and loss of arytenoid abduction (evaluated by Dixon grade and change in rima glottidis area) were compared between techniques. The modified laryngoplasty constructs had higher ($P<0.001$) tensile strength at failure (191 N) than the standard laryngoplasty constructs (91 N). None of the modified laryngoplasty constructs failed at the muscular process, while this was the most frequent site of failure with the standard laryngoplasty constructs. The modified laryngoplasty constructs maintained a greater grade of arytenoid abduction ($P<0.001$) than the standard laryngoplasty constructs during cyclic loading. In addition, the modified laryngoplasty constructs showed less loss of cross-sectional area over 10,000 cycles compared to the standard laryngoplasty ($P<0.001$). Results demonstrate that the modified laryngoplasty outperforms the standard laryngoplasty in both cyclic and monotonic loading. In vivo testing of the modified technique is warranted to determine if post-operative loss of arytenoid abduction can be eliminated.

Optical Coherence Tomography for Surgical Margin Assessment: The Cutting Edge for Feline Injection Site Sarcoma

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Introduction: Surgery is the mainstay of treatment for feline injection-site sarcoma and complete histologic margins has been associated with decreased tumor recurrence rates and improved outcome. Currently surgical margins are assessed by post-operative histopathology, which assesses < 1% of margins and takes days. Despite complete histologic margins tumor regrowth occurs in 14-42% cats. Optical coherence tomography (OCT) is an alternative solution for intraoperative assessment of surgical margins allowing visualization of the microstructure over a larger surface area of the surgical margins in seconds. The aim of this preliminary study was to assess intraoperative OCT imaging in dogs and cats with soft tissue sarcomas to assess the optical tissue properties of sarcomas and their delineation from normal tissues.

Materials and Methods: Dogs and cats with soft tissue sarcomas underwent excision of their tumors and 4 areas of interest were imaged with a commercial OCT imaging system (Envisu C2300, Bioptigen) under IACUC approved protocols. Histology sections from these areas were taken for correlation with OCT images.

Results: Different normal tissues types and delineation between these tissues and tumor were seen throughout the imaged specimens. Specifically, increased and heterogenous scattering intensity helped to identify tumor areas while muscle tissue appeared more homogenously lower scattering. Adipose tissue had a bubble like appearance with low scattering reflecting visualization of mature adipocytes.

Discussion: Surgical margin imaging results show that OCT has potential for showing the demarcation between tumor and other normal tissues including muscle, fat, blood vessels and other connective tissues.

Prenatal Thyroid Hormone Insufficiency Diminishes Short-Term Object Recognition Memory In Long-Evans Rats

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Children whose mothers are severely hypothyroid during pregnancy exhibit learning and memory deficits. Fortunately, overt maternal hypothyroidism is usually recognized and treated. Yet, many women who are subclinically hypothyroid (SCH) are asymptomatic and are therefore never diagnosed or treated. The prevalence of SCH is thought to be higher in pregnant women than in the general population, but little is known of the effects of maternal SCH on the neurobehavioral outcomes of children. To model maternal SCH, we exposed pregnant rat dams to a low dose of propylthiouracil (3 ppm in drinking water) from gestation day 6 through postnatal day 14, and pups were cross-fostered at postnatal day 2, resulting in four treatment groups: control, prenatal exposure, postnatal exposure, and perinatal exposure. Rats were tested on the novel-object recognition paradigm at 36-38 weeks of age to examine effects of maternal SCH on short-term memory. Rats were allowed to investigate two identical objects, and then, one hour later, they were allowed to explore one of the prior objects and one novel object for 3 minutes in order to assess short-term object recognition memory. Prenatal exposure resulted in decreased time and decreased percent time exploring the novel object, decreased total time exploring both objects, and fewer entries to the novel object in the first minute of exploration. These results demonstrate that maternal hypothyroidism during the prenatal period, but not during the postnatal period, negatively affects short-term object recognition memory. Now that we have narrowed the sensitive period for the effect of thyroid hormone on development of short-term memory, we will begin exploring underlying mechanisms.

Nerve Growth Factor-B Improves Corpus Luteum Function and Enhances Conceptus Development in Cattle

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Nerve Growth Factor-β (NGF) is present in seminal plasma of bulls and has a luteotrophic effect in heifers. The objective of this study was to determine if purified bull NGF would improve corpus luteum function and enhance conceptus development. Our hypothesis was that systemic administration of NGF to cows at artificial insemination (AI) would increase progesterone (P) production and systemic markers of conceptus development that included pregnancy-specific protein B (PSPB) concentrations and expression of interferon-stimulated genes (ISGs). Cows were assigned to CONT (n=30) or NGF (n=30) groups and synchronized using a 7-day Co-Synch + CIDR program. At d 0 (AI), NGF cows received 296 µg purified bovine NGF IM. Blood samples were collected for quantification of plasma P (d 0-19) and PSPB (d 24) using ELISA. Peripheral blood leukocytes were harvested at d 19 for measuring expression of ISGs using qPCR. Pregnancy detection was performed with ultrasound at d 28. Statistical analyses were performed using ANOVA with repeated measures in SAS. NGF cows had higher plasma P concentrations at d 10-19 (p=0.04). Pregnancy rates at d 28 were 75% in NGF cows and 59% in CONT cows (p=0.13). PSPB concentrations were higher in pregnant NGF vs CONT cows (p<0.05) at d 24. Fold-change expression of interferon-stimulated genes ISG15 and MX2 were also higher in pregnant NGF vs CONT cows (p<0.05) at d 19. Collectively, these results demonstrate that NGF administration at AI improved corpus luteum function and subsequently enhanced markers of early conceptus development. Future studies are warranted to investigate whether NGF can be used to decrease the incidence of early embryonic death and improve reproductive efficiency of cattle.

Cholinergic Drugs Influence Impulsivity and Attention on the 5-Choice Serial Reaction Time Task (5-CSRTT) in a Rodent Model

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In a prior study we determined that circadian disruption increases impulsive behavior on the 5-choice serial reaction time task, which is a behavior task that assesses impulsivity and attention. Acetylcholine (ACh) neurotransmission is important for circadian rhythmicity and attention, while impulsivity is modulated by dopamine signaling. We postulate that the relationship between circadian disruption and impulsive behavior is mediated by ACh-modulation of dopamine release in the prefrontal cortex of the brain. To investigate this relationship, we are performing a pharmacologic study in which we will use cholinergic and dopaminergic drug challenges to examine the roles of both neurotransmitter systems. The first part of this study involved training adult Long-Evans rats on the 5-CSRTT and then administering 3 different cholinergic drugs to gauge the effects on behavior. Nicotine, a nicotinic ACh receptor (nAChR) agonist, caused a dose-dependent increase in impulsivity, but did not affect attention at the doses we administered. Mecamylamine, a general nAChR antagonist, caused dose-dependent decreases in impulsivity and attention. The third drug, DHBE, is an antagonist of nAChRs containing α4ß2 subunits, which is one subtype of nicotinic receptors implicated in the release of dopamine in the prefrontal cortex. DHBE did not have significant effects on attention or impulsivity at the doses administered. Our results suggest that ACh can affect both impulsivity and attention through nAChR activation, but the effects may not be mediated through nicotinic receptors containing α4ß2 subunits. An alternate explanation is that nAChRs may compensate for the role of α4ß2 nAChRs in impulsivity and attention when α4ß2 nAChRs are blocked. In subsequent phases of the study we will administer dopaminergic drugs and combinations of cholinergic and dopaminergic drugs to better understand the relationship between the two neurotransmitter systems.

Normal Values of Intraocular Pressure, Central Corneal Thickness and Conjunctival Microflora in Adult White-Tailed Deer (Odocoileus Virginianus)

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Knowledge of normal ophthalmic parameters is essential for diagnosis and treatment of ocular diseases. White-tailed deer have recently gained popularity, however little is known of their ocular features. This study's goals were to determine reference values for intraocular pressure (IOP) and central corneal thickness (CCT), and to describe the normal ocular surface microbiota. Following sedation, IOPs on eight adult white-tailed deer (16 eyes) were measured using rebound (TonoVet®) and applanation (TonoPen®) tonometry. CCT was measured by ultrasound pachymetry. Culture swabs were obtained from the lower conjunctival fornix and submitted for culture and susceptibility. Statistical analysis compared differences between eyes for IOP and CCT (ANOVA) and between tonometers (T test). Overall mean IOP were 16.19 ±2.72mmHg and 12.81 ±1.91mmHg for applanation and rebound tonometry, respectively. There was no significant differences in IOP between eyes for Tonopen (p=0.79) or Tonovet (p=0.1). The difference between tonometers was statistically significant (p=0.00023). Overall mean CCT was 747.78 ± 43.88µm (range 684 to 919µm) with no significant difference between eyes (p=0.79). The most prevalent microorganisms cultured and identified were: *Staphylococcus* sp., *Bacillus* sp., *Corynebacterium* sp., *Streptomyces* sp. and *Enterococcus* sp. One single sample recovered a fungal organism (*Scedosporium* sp.). *Staphylococcus sp*. was resistant to bacitracin and polymyxin B, *Corynebacterium* sp. to ciprofloxacin, polymyxin B and tobramycin, and *Bacillus* sp. to cefazolin. The study determined the normal parameters of IOP, CCT and ocular surface microbiota in white-tailed deer, which is essential for proper diagnosis and therapy of ocular diseases.

Preliminary Evaluation of Anti-Angiogenic Properties of Equine Amniotic Membrane Homogenate in Tears of Dogs With Vascularized Ulcerative Keratitis

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Various ocular surface diseases can promote corneal neovascularization, which may lead to consequent visual impairment. Amniotic membrane has angiostatic activities that appear to inhibit corneal neovascularization. This study aims to evaluate the angiostatic effect of equine amniotic membrane homogenate (EAMH) in tears of dogs with vascularized corneal ulcers. Tear samples were collected using capillary tubes from both affected and contralateral eyes of 30 dogs with vascularized corneal ulcers, and from 25 healthy dogs. Ten EAMs were obtained, processed into homogenates and submitted to enzyme-linked immunoassays (ELISA) for quantification of vascular endothelial growth factor (VEGF) and pigmented epithelium derived factor (PEDF). Tear samples were pooled accordingly: untreated tears (G1), tears with buffer (G2), tears with 0.21mg/ml EAMH (G3), tears with 0.42mg/ml EAMH (G4), contralateral tears (G5), and normal tears (G6). Each group was submitted to Western blot (WB) and ELISA for quantification of canine VEGF. The mean concentration of equine PEDF and VEGF in EAMH were 72.34 ± 7.07ng/ml and 0.658 ±0.07ng/ml, respectively (ratio 110:1). Final ELISA concentrations of canine VEGF were 12.26pg/ml in G1, 6.36pg/ml in G2, 6.14pg/ml in G3, 5.0pg/ml in G4, 10.84pg/ml in G5, and 1.54pg/ml in G6. On WB analysis, detection of VEGF was decreased in all groups in comparison to G1. The use of 0.42mg/ml EAMH decreased the concentration of VEGF by 21.4% in comparison to same volume dilution with buffer (G2). This study demonstrated that EAMHs maintains a high concentration of PEDF and is potentially able to neutralize VEGF in-vitro. EAMH has beneficial properties to inhibit neovascularization, and should be further investigated.

Porcine Epidemic Diarrhea Virus Blocks IRF1-Mediated Type III Interferon Production in the Intestinal Epithelial Cells for Innate Immune Evasion

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Porcine epidemic diarrhea (PED) is a highly contagious acute enteric disease characterized by vomiting, watery diarrhea, and severe dehydration accompanied with a high mortality in suckling piglets. PED emerged in the US in 2013 and became endemic, posing significant economic concerns. PEDV infects epithelial cells of the small intestine *in vivo*. Type III interferon (IFN-λ) plays a key role to maintain the antiviral state of the mucosal epithelial surface in the gut, and in turn enteric viruses may have evolved to evade the type III IFN responses during infection. To study the innate immune evasion of PEDV from the type III IFN response, we first developed a pig intestinal epithelial cell line (PIEC-DQ) to support efficient PEDV infection. PEDV appeared to suppress the type III IFN production in these cells when stimulated with a double-stranded RNA analog. The recombinant IFN-λ1 and IFN-λ3 potently suppressed the PEDV infection over time in a dose-dependent manner, indicating the type III IFNs contain a potent anti-PEDV activity. We found that PEDV blocked the IFN-λ1 promoter activation by interfering the activation of both IRF1 and NF-κB. PEDV did not alter the expression of IRF1, but instead inhibited its nuclear translocation. Peroxisomes are innate antiviral signaling platforms crucial for activation of IRF1-mediated IFN-λ production, and we further found that peroxisomes were decreased in number in PEDV-infected cells. Our study for the first time provides the evidence that PEDV evades the IRF1-mediated type III IFN responses in the intestinal epithelial cells. Our finding may facilitate to design a novel approach to the control of intestinal viral infections.

Phthalate Mixture Exposure Reduces Antral Follicle Growth and Hormone Production in Mouse Ovaries

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Phthalates are used in building materials, medical devices, and personal care products. Most studies on phthalates have focused on single phthalates, but it is important to study mixtures of phthalates because humans are exposed to such mixtures daily. Thus, we test the hypothesis that exposure to an environmentally relevant phthalate mixture decreases ovarian antral follicle growth and hormone production. Antral follicles from adult CD-1 mice were cultured with vehicle control or phthalate mixture (1-500 µg/ml; n=6 cultures, 6-12 follicles/treatment/culture) for 96 hours. The mixture was based on the composition of phthalates detected in women and included 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. During culture, antral follicle diameters were measured every 24 hours to monitor growth. After culture, media and follicles were subjected to measurements of sex steroid hormones and atresia, respectively. The phthalate mixture (100 and 500 µg/ml) decreased antral follicle growth and androstenedione (10, 100, and 500 µg/ml), testosterone (10 and 500 µg/ml), estradiol (10, 100, and 500 µg/ml), and estrone (100 and 500 µg/ml) levels compared to control ($p<0.01$). The mixture (10, 100, and 500 µg/ml) also induced oocyte fragmentation. These data suggest that exposure to a phthalate mixture significantly inhibits antral follicle growth, reduces several sex steroid hormone levels, and induces oocyte fragmentation. Further, these data suggest that the phthalate mixture adversely affects female reproduction. NIH P01 ES022848, EPA RD-83459301, and an Environmental Toxicology Fellowship.

Uterine Microbiome, Antibiotic Resistance Genes and Virulence Factors of Metritic Treated Cows That Cure or Failed to Cure From Metritis

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Metritis is a major postpartum disease in dairy cows causing vast economic losses due to reduced milk production, impaired fertility, and costs with treatments. Antibiotics are the main therapeutic option. However, ~35% of the cows fail to cure of the disease after its use. Herein, we used whole genome shotgun sequencing (WGS) to shed light of uterine microbiome, antimicrobial resistance gene (ARG), and virulence factors gene (VFG) profiles of 24 cows that cured or failed to cure of metritis after treatment with ceftiofur or ampicillin. Uterine swab samples for each cow were collected at the time of metritis diagnosis (d1) and 5 days later (d6) one day after treatments finished. Half of the cows (12/24) cured after the 5-day treatment (7 from ampicillin and 5 from ceftiofur). Our WGS revealed that over time (from d1 to d6) the mean relative abundance of the genera Bacteroides, Prevotella, Alistipes, Fusobacterium, and Tannerella were reduced ($P < 0.01$), whereas Porphyromonas was increased ($P < 0.01$) independent of treatment or cure status ($P > 0.05$). Antibiotic treatment independent of treatment type decreased VFGs abundance ($P < 0.01$), but increased ARGs abundance ($P < 0.01$). The resistome of metritic cows was dominated by Tetracycline resistance genes, but beta-lactam ARGs such as CMY-2 were not changed by treatment or time ($P > 0.05$). A higher mean relative abundance and presence VFGs for Streptococcus spp., Mycoplasma pneumoniae, and Vibrio cholerae was identified suggesting these bacteria and VFGs may be liked to metritis pathogenesis. In conclusion, antibiotics treatment over time (from d1 to d6) independent of type and ability to cure altered uterine microbiome, reduced VFGs abundance and increased ARGs abundance.


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