

## **Ontogeny of Uterine Gland Development in the Neonatal Dog**

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Ovine models suggest that administration of progesterone during neonatal life can permanently inhibit uterine gland development and cause infertility in the adult animal. Although extensive information is available on uterine gland development in other species, postnatal uterine development and uterine gland ontogeny in the dog has not been studied. The goal of this study was to evaluate neonatal canine uterine development and uterine gland ontogeny to identify the most efficacious window for neonatal/juvenile administration of progesterone as a contraceptive method in the dog. Neonatal canine uteri at 7, 14, 28, 42, and 56 days of age postnatal (n = 3 for all ages except 6 weeks, where n = 2) were evaluated by morphometric methods and Ki-67 immunohistochemistry (IHC) to evaluate proliferation in both epithelial and stromal compartments. Initiation of uterine gland development began around day 7, with epithelial cells beginning to bud off from the uterine luminal epithelium. Both luminal epithelial and stromal cells were proliferating rapidly, with a Ki-67 labeling index (LI) of  $36.8\% \pm 5.8\%$  and  $19.2\% \pm 4.9\%$ , respectively at day 7. At 14 days, glands were clearly identifiable, and proliferation of luminal, glandular and stromal cells remained active (LI=  $30.3\% \pm 4.8\%$ ,  $45.4\% \pm 3.7\%$  and  $14.4\% \pm 1.4\%$ , respectively). By day 28, glands had invaded further into the stroma, although proliferative activity had decreased in all cell compartments (LI=  $9.5\% \pm 0.6\%$ ,  $16.0\% \pm 1.5\%$  and  $2.2\% \pm 0.5\%$  in luminal, glandular and stromal cells respectively). By days 42 to 56, uterine gland development was more extensive than at day 28, although the amount of gland development was substantially less than seen in the dog at proestrous and estrous, indicating that substantial gland development takes place during the adult estrous cycle. Luminal, glandular, and stromal LI was minimal by day 42 (LI=  $1.9\% \pm 1.0\%$ ,  $2.7\% \pm .3\%$ , and  $0.2\% \pm 0.1\%$  respectively) indicating that the uterus is nearly mitotically quiescent by this age. In summary, luminal and stromal proliferation are maximal at day 7 and decrease to minimal levels by day 56. The onset of uterine gland ontogeny occurs at day 7, thus the optimal time frame of a progesterone induced-sterilization protocol would be from day 7 to day 42.

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## **A Combinatorial Genomic and Histopathology Approach to Studying the Effect of Surfactant Protein A on Fungal Growth and Pathogenesis**

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Inhalation of fungal spores is the means of contracting several fungal diseases of importance in humans and animals. Thousands of fungal spores are deposited onto the surfactant-lining layer of pulmonary alveoli during the course of daily life. The Surfactant Protein A (SP-A) is a calcium-dependent innate immunity protein that enhances the clearance of microbial pathogens from the lung by opsonization and membrane permeabilization. Little is known about SP-A/fungal interactions. A yeast deletion library was used to identify fungal factors that interact with SP-A. A library consisting of approximately 5,000 yeast deletion mutants was screened for their susceptibility to aggregation and killing by SP-A. Pools of yeast mutants were grown and replicated in 96-well microtiter plates and exposed to 50 microgram/ml SP-A in aggregation buffer. Yeast aggregation was scored by visual observation using an inverted light microscope. Aggregated yeast mutants were LIVE/DEAD stained to determine the extent of killing by SP-A. The homologs of these fungal targets will be identified by bioinformatics using genome sequences of pulmonary fungal pathogens. Immunohistochemistry was also performed to determine the relationship between SP-A and fungal infection. Paraffin-embedded lungs of birds and companion animals with known fungal infections were sectioned and stained for SP-A using a rabbit polyclonal antibody to determine SP-A levels. We hypothesize that fungal infection will decrease SP-A levels in the affected lungs and that deletion of fungal targets in pathogenic fungi will attenuate their virulence in the lungs.

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## **Phenotypic Evaluation of a *Candida albicans* *pir1/pir1* Mutant and Analysis of PIR1 Allelic Variability in a Diverse Collection of *Candida albicans* Isolates**

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The opportunistic fungus *Candida albicans* causes various forms of disease including disseminated disease, most commonly associated with immunocompromised patients, and superficial, mucosal disease that can affect immunocompromised or normally healthy individuals. *C. albicans* PIR1 encodes a protein associated with beta-1,3-glucan in the *C. albicans* cell wall. Published studies suggested that Pir1 is required for proper cell wall architecture and that PIR1 is an essential gene. Our work suggested that Pir1 might contribute to adhesive interactions between *C. albicans* and mammalian cells. Contrary to literature predictions, we constructed a viable *pir1/pir1* mutant strain. The phenotype of the strain was compared to wild-type and reintegrant control strains for growth rate, cellular morphology, germ tube formation, sensitivity to compounds that interfere with polymerization of cell wall components, and adhesion to cultured vascular endothelial cells and freshly collected buccal epithelial cells. PIR1 allelic variation was also studied. Many genes encoding *C. albicans* cell wall proteins have considerable allelic variability, often arising from regions of repeated sequences. Based on differences between the PIR1 alleles from strain SC5314, we developed a PCR assay and assessed allelic variability in collections of diverse *C. albicans* isolates from humans and wildlife. Strains passaged for many generations in vitro or in vivo were used to assess stability of the repeated sequences within PIR1 alleles. The *C. albicans* cell wall provides fungal cell integrity and is a structure not present in mammalian host cells. As such, the cell wall is an important antifungal drug target and its study is significant.

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## **Genetic Characterization of Epizootic Hemorrhagic Disease Virus Using the Capsid (VP2) and Core (VP3) Protein Genes**

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Epizootic hemorrhagic disease virus (EHDV) is an insect-transmitted Orbivirus infecting ruminants and white-tailed deer. EHDV outbreaks occurred in 2007 in U.S. white-tailed deer populations, including those in Illinois. The purpose of this study was to determine the genotype of an EHDV isolate from the 2007 outbreak. EHDV contains 10 segments of double-stranded RNA that code for seven viral proteins and three non-structural proteins. Of these proteins, the major neutralizing antigen protein VP2 and the major structural protein VP3 were selected for genotyping. EHDV was grown in HeLa cells and the viral genomic RNA was purified. RT-PCR was used to reverse transcribe and amplify gene segments from VP2 and VP3. DNA sequences of the gene segments were compared to those from EHDV isolates available in GenBank. Comparison of VP2 gene segments representing positions 1318-1644 (327bp), 1716-2075 (360bp), and 2384-2873 (490bp) showed 97%, 95%, and 96% amino acid sequence identity, respectively, with EHDV sequences from an serotype-2 isolate from Alberta, Canada. Comparison of VP3 gene segment representing position 1654-2670 (1017bp) with an Alberta isolate showed 100% identity. These preliminary data suggest the 2007 outbreaks in the U.S. were most likely attributed to the EHDV serotype-2 strain common in North America. Completion of the VP2 and VP3 sequences will allow a more comprehensive analysis. Given the limited availability of EHDV sequences, information from this work will aid in identifying the source of future EHDV outbreaks.

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## **Regulation of Aromatase Expression in Mouse Uterus**

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Implantation is initiated when the embryo attaches to the uterine luminal epithelium during early pregnancy, triggering the transformation of uterine stromal cells to decidual cells in a process tightly coordinated by estrogen (E) and progesterone (P). Recent studies have shown that the intra-uterine biosynthesis of local E via induction of P450 aromatase is critical to sustain pregnancy in addition to the ovarian source of P. Plausible candidates

involved in regulating aromatase expression in the decidual uterus include members of the monomeric orphan nuclear receptor family, which are key aromatase transcription regulators in other E-producing tissues. Liver-receptor homolog-1 (LRH-1) and steroidogenic factor 1 (SF1) were the two major factors studied with an overall aim to identify the specific molecule expressed during decidualization. Uterine stromal cells from pregnant mice were isolated during days 3 -7 of pregnancy. RNA was isolated and subjected to cDNA preparation. Expression patterns of LRH-1 and SF1 were monitored via q-PCR analysis, and protein expression during pregnancy was identified via immunolocalization using an antibody against the specific protein. Expression of decidualization markers like alkaline phosphatase, prolactin-related peptide, and aromatase was also analyzed. A significant induction of LRH-1 in the isolated stromal cell population was observed, however, the expression of SF1 was not markedly induced. LRH-1 may be the transcription factor of interest that up-regulates aromatase expression in uterine stromal cells during implantation and decidualization. Future experiments should determine expression profiles in ovariectomized virgin mice to understand if the regulation of aromatase is specific to differentiating stromal cells.

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## **Determining the Dosing Regimen of PAC-1-II-9 for Treatment of Murine Lymphoma**

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Caspase-3 is a critical executioner enzyme in apoptosis that exists intracellularly as the proenzyme procaspase-3. In cancer cells, abnormal expression of proteins in apoptotic pathways prevents the activation of procaspase-3 to caspase-3, thus preventing apoptosis. Since procaspase-3 is often upregulated in cancer, it represents a viable and cancer-specific target for chemotherapeutics. Recently, the small molecule PAC-1 was shown to activate procaspase-3 to caspase-3, thereby inducing apoptosis in vitro. Studies in mice showed that PAC-1 inhibits tumor growth, but causes neurotoxicity. A sulfonamide derivative of PAC-1 (PAC-1-II-9) was synthesized which induces apoptosis in vitro without producing neurotoxicity in vivo at predicted therapeutic concentrations. Prior to testing PAC-1-II-9 in treatment of murine lymphoma a proper dosing regimen must be established. To

determine the importance of drug concentration versus exposure time for cytotoxicity, IC50s of PAC-1-II-9 were completed using a sulforhodamine B assay for times ranging from 1 to 72 hours and compared to those of PAC-1, doxorubicin, and paclitaxel. Results showed that PAC-1-II-9 was dependent upon both exposure time and concentration for cytotoxicity. The pharmacokinetics of 150 mg/kg subcutaneous injections of PAC-1-II-9 maintained a therapeutic concentration for two hours. Blood samples were taken at time points between 10 minutes and 1 day post-injection and analyzed via liquid chromatography-mass spectrometry. Determining the dependence of PAC-1-II-9 on exposure time and concentration, and determining its pharmacokinetics in vivo are important precursors to testing the efficacy of PAC-1-II-9 against cancer in vivo.

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## **The Effects of the Antifibrotic Drugs Halofuginone and Trichostatin A on Leiomyoma Smooth Muscle Cells of Aged Laying Hens**

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Uterine leiomyomas (fibroids) are the leading cause of hysterectomies for women in the United States. These benign tumors occur in women during their reproductive years and are characterized by increased smooth muscle cell (SMC) proliferation and extracellular matrix (ECM) production. We recently observed that aged laying hens, *Gallus domesticus*, spontaneously develop fibroid-like polyps on their oviducts similar to human uterine leiomyomas. We used primary SMC cultures derived from hen fibroids as a potential in vitro model to test novel, alternative therapeutic compounds as treatments for human uterine fibroids. Two antifibrotic drugs, Halofuginone and Trichostatin A, were tested on hen leiomyoma cells for their effects on cell proliferation, apoptosis, and production of the ECM proteins collagen type I and type III. We hypothesized that the anti-fibrotic drugs Halofuginone and Trichostatin A inhibit cell proliferation and collagen production while increasing apoptosis in chicken leiomyoma cells. Time-course and dose-response studies assessed effects of both drugs on proliferation using thymidine uptake assays and manual cell counting. Changes in collagen type I, type III, and TGF-beta 1 production were analyzed using real-time PCR, while effects on apoptosis were assessed by immunoblotting using an anti-poly (ADP-ribose) Polymerase-1 (PARP) antibody. Initial results showed that both Halofuginone and Trichostatin A inhibited proliferation of chicken leiomyoma

cells. We anticipate that both Halofuginone and Trichostatin A significantly inhibit growth of chicken leiomyoma SMCs, reinforcing the concept of using aged laying hens as a model species for further research on uterine leiomyomas in women.

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## **The Effect of Female Presence on Risk-Taking Behavior of Male Sticklebacks**

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Reproduction is often associated with increased predation risk, considering that the attributes used to attract females may also heighten conspicuousness to predators. Additionally, males will often engage in riskier behavior in the presence of a female and her cues. Using Three-Spine Sticklebacks, we investigated whether males behave more boldly toward a predator in the presence of a female compared to her absence. Because males might engage in risk-taking behavior in the presence of conspecifics due to risk dilution with increased density, we tested the focal male in the presence of male to ensure that changes in boldness correlated specifically to female presence. Males were placed in individual tanks containing materials used for constructing nests, the completion of which indicated his availability for testing. Once ready, a predator model was placed in the tank of the focal male, while a jar, empty or containing a stimulus fish, was placed in an adjacent tank. An opaque screen allowed the focal male and stimulus fish to interact with each other, but prevented the stimulus fish from seeing the predator. We predict that in the presence of a female, males will react more boldly to the predator by increasing the number of risk-taking behaviors. Increases in risky behavior can inform males of the potential threat of predation, indicate high fitness to the female, or be a spillover effect from courtship activity. Further research can determine the purpose and benefit, if any, of female induced increased risk-taking behavior in stickleback males.

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We have identified two strains of mice, BALB/cJ and 129S1/SvImJ, that are resistant to *Yersinia pestis*, the pathogen responsible for plague; however, the mechanism of resistance has not yet been elucidated. In comparison to susceptible C57BL/6J mice, the resistant substrains have markedly decreased bacterial burdens in the spleen early after infection. Nevertheless, spleens from all infected mouse strains exhibit high numbers of infiltrating neutrophils which are known to play a general role in controlling bacterial growth in the early stages of infection. To assess their potential contribution to resistance, peritoneal neutrophils from different strains of mice were co-incubated for varying times with *Y. pestis* D27 and samples were plated to determine bacterial growth. Here we report that neutrophils from resistant mice exhibited an enhanced capacity to control the growth of *Y. pestis* when it is cultured at 23°C but not when cultured at 37°C. Two important virulence factors, an antiphagocytic capsule and a Type III Secretion System, are induced by this change in temperature as *Y. pestis* adapts from the flea vector to the mammalian host following a flea bite. Two *Y. pestis* mutants, each lacking one of these virulence factors, are currently being utilized to investigate their role in bacterial evasion of neutrophils from resistant mice. Initial results support the idea that neutrophils play an important role in controlling *Y. pestis* growth in the early stages of infection.

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## **Alterations in Hepatic mRNA Expression of CMO-I/CMO-II in Mice Fed Carotenoid-Containing Diets**

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Epidemiological studies suggest that increased intake of tomato products and higher blood levels of the carotenoid, lycopene, reduce the risk of development or progression of certain cancers. 15,15'-carotenoid monooxygenase (CMO-I) and 9',10'-carotenoid monooxygenase (CMO-II) are the principal cleavage enzymes for carotenoids. CMO-I is recognized as the primary enzyme responsible for converting provitamin A carotenoids to vitamin A. Theoretically, CMO-II eccentrically cleaves lycopene into apo-10'-lycopenal and other lycopenoids. Previous research has shown alterations in the bioaccumulation of carotenoids in CMO-I and CMO-II knockout (KO) mice when fed either a lycopene or a beta-carotene containing diet, suggesting



alteration in the expression of CMO-I/CMO-II. To measure alteration in expression, we fed either a lycopene diet (100 mg lyc/kg diet in beadlet form), a placebo beadlet diet, a 10% tomato power diet, or an AIN-93G based diet to CMO-I KO, CMO-II KO, and wild-type (WT) mice for 30 days. Because carotenoids are stored and found in high concentrations in the liver, and the liver is the primary storage site for beta-carotene conversion to vitamin A, we collected liver tissue at sacrifice. Real-time PCR was used to measure the relative abundance of CMO-I and CMO-II mRNA. Preliminary data suggest that CMO-I is down-regulated in CMO-II KO and WT mice fed lycopene and CMO-II is down-regulated in WT mice fed lycopene. These changes are important because carotenoid cleavage enzymes are essential for the production of bioactive carotenoid metabolites that can combat chronic diseases.

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## **Microbial Ecology and Environmental Quality in Egg-Laying Facilities**

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Laying-hen housing currently exists in production agriculture as caged or cage-free systems. While both categories are commonly found in practice, there still remains inadequate information regarding the comparisons and consequences of alternative housing schemes. Issues of particular importance are those that affect food safety and human health as poultry products may become rapidly contaminated by environmental bacteria and pathogens. We hypothesize that management strategy differences impact the diversity of microorganisms within different housing operations and their associated environmental variables. Samples of air, litter, water, surfaces, eggs, and feces were collected at different types of laying-hen facilities in Illinois and Indiana. Environmental variables such as temperature, relative humidity, carbon dioxide, and atmospheric ammonia levels were measured simultaneously. We then performed DNA extraction followed by automated ribosomal intergenic space analysis (ARISA) to identify the microbial community composition of each sample. Detection of zoonotic pathogens *Salmonella* and *Campylobacter* within the laying-hen environment was completed via selective enrichment of fecal, egg, and dust samples. Characterization of microbial populations in the laying-hen environment and identification of the environmental factors that influence community

structure may provide key insight into the control and prevention of the contamination of poultry meat and eggs.<

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## **Evaluating the Potential for the Als2 Cell-Surface Glycoprotein to Function in *Candida albicans* Cell Growth and Division**

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The fungus *Candida albicans* causes disseminated and mucosal disease. Risk factors for disseminated candidiasis are often associated with clinical settings. Oropharyngeal candidiasis is one form of mucosal disease and is strongly associated with progression from HIV infection to AIDS. The *C. albicans* genome encodes several gene families, some of which are associated with pathogenesis. Proteins in the Als (agglutinin-like sequence) family are most commonly considered as adhesins. Recently, a different role for Als1 was described. Deletion of ALS1 from the *C. albicans* genome results in cells that are smaller than those of a wild-type control strain; reintegration of ALS1 restores the wild-type phenotype. The purpose of this work is to expand on observations regarding Als1 and cell size and to determine if Als2 also plays a role in cell growth and division. ALS1 was overexpressed in two *C. albicans* backgrounds. We hypothesized that Als1 production at levels above those in the wild-type strain would result in cells larger than wild-type. Less is known about Als2 than other Als proteins because the ALS2 open reading frame and ALS2 locus have proven difficult to manipulate. ALS2 experiments involved developing standard growth conditions in a rich and a minimal growth medium, comparing growth characteristics of the mutant and control strains, and measuring ALS2 transcription over the course of culture growth. These studies provide new insights into the function of proteins within a gene family, and contribute to a greater understanding of the biology of an important fungal pathogen.

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## **Development of an Assay for the Detection and Quantification of Cephapirin and Ceftiofur in Goat Milk**

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Cephapirin and ceftiofur are both cephalosporin antibiotics that inhibit bacterial cell wall synthesis. Cephapirin, a first generation cephalosporin, is predominantly active against gram positive organisms, while the third generation ceftiofur has a broader spectrum covering both gram positive and gram negative bacteria. These antibiotics are commonly used on and off label in dairy animals for the treatment of mastitis. The FDA has set a maximum residue limit of 20 ng/ml for cephapirin and 100 ng/ml for ceftiofur in milk. In the present study, we developed and validated an LC-MS method for the detection and quantification of these two antibiotics in goat milk. The simple extraction procedure involved centrifugation of the samples for defatting, precipitation of the proteins with acetonitrile and passage of the samples through Amicon Ultra 4 filters (Millipore Corporation, Temecula, CA). Following extraction, 30 µl of the filtrate was injected into the LC-MS system. The extraction efficiency ranged from 54-72% for cephapirin and 40-51% for ceftiofur. The within and between assay accuracies for cephapirin were 80-92% and 91-101%, and for ceftiofur were 96-106% and 88-95%, respectively. The precision of the method (% CV) for both compounds was equal to or less than 15%. This validated method can be used to determine the pharmacokinetics of cephapirin and ceftiofur following intramammary infusions in goats, and also to accurately determine their withdrawal times in milk samples.

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## **The Effect of the Spatial Organization of Vegetation on the Productivity of Culex Mosquito Larvae in Catch Basins in an Area of High West Nile Virus Infection**

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Selected residential areas southwest of Chicago have historically been hotspots for St. Louis Encephalitis and West Nile Virus, both mosquito-borne diseases. *Culex* species mosquitoes are the primary transmitters of West Nile Virus and are known to use the stagnant water found in the sumps of stormwater catch basins to deposit their eggs. Previous studies have found that some catch basins are significantly more productive than others, but analyses across multiple dimensions of the basins and their neighborhoods have not clarified a causal mechanism. We hypothesized that the type and spatial organization of vegetation surrounding catch basins may influence the productivity of *Culex* within basins. To test these hypotheses, we collected data for mosquito both productivity and vegetation. We measured mosquito productivity by sampling weekly for number of larvae and pupae present in sixty catch basins that have had high levels of *Culex* larvae in previous study years. For vegetation data, we surveyed the species and number of trees with a height greater than three meters within the study areas. Additionally, the number, type, height and area of shrubs and plants less than three meters in height were surveyed in regions within a 75-foot radius of each basin. Water depth and quality were also monitored within the basins in an effort to assess the effect of the surrounding vegetation on the microhabitat within the basins. The mosquito productivity and vegetation data will be analyzed and the results will add to the current understanding of mosquito-borne disease management.

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### **Auditory Function in Rats Exposed to a PCB and PBDE Mixture During Development**

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Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are chemically similar substances that are present in the environment and bioaccumulate in the food chain. PCBs were a component of dielectric fluids in transformers and capacitors. These compounds are no longer manufactured since their toxicity is known; however, they are very stable and still present in the food chain. PBDEs are currently used as flame

retardants in many household products. Their toxicity is not as well-studied, but they have a similar chemical structure to PCBs. Previous studies concluded that rats developmentally exposed to an environmentally relevant PCB mixture have hearing deficits. We hypothesized that due to the similarity in structure to PCBs, PBDE exposure during gestation and lactation will also cause hearing deficits, and that a combination of PCBs and PBDEs will result in an additive effect on auditory function. In this study, we examined auditory function in rats exposed to PCBs and/or PBDEs during gestation and lactation. Dams were exposed to 3 mg/kg/day or 6 mg/kg/day of PCBs, 5.7 mg/kg/day or 11.4 mg/kg/day of PBDEs, or a combination of 3 mg/kg/day PCBs + 5.7 mg/kg/day PBDEs or 6 mg/kg/day PCBs + 11.4 mg/kg/day PBDEs orally. Auditory function was assessed when the pups reached adulthood using two methods: auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs), which evaluate the integrity of the outer hair cells of the cochlea. These data will provide important information on the ability of two widespread environmental contaminants to cause hearing loss following developmental exposure.

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## **A Potential Novel Cancer Treatment: Evaluation of Myxoma Virus Oncolysis in Feline Cancer Cells**

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Although there is a high incidence of cancer in dogs and cats, treatment options for canine and feline cancer patients lag behind treatments available for humans. We investigated the effectiveness of myxoma virus-induced oncolysis in two feline cancer cell cultures as a potential cancer treatment. Single- and multi-step growth curves quantified myxoma virus replication and spread in tumor cells. Flow cytometry assays assessed the extent of virus-induced cell death. The level of phosphorylated Akt was detected by immunoblot to determine if Akt activation (which inhibits apoptosis) was necessary for myxoma virus infection. Each cancer cell line studied demonstrated differences in susceptibility to viral infection and cellular lysis. Feline metastatic carcinoma cells (Stude) showed significant cytopathic effects 24 h post infection (hpi), whereas, at 72 hpi there was minimal cell lysis in feline squamous cell carcinoma cells (SCCF1). At 24 hpi, peak virus titers were reached, but Stude had a three-fold higher virus load compared to SCCF1. Compared to fully permissive rabbit kidney cells, feline cancer cell

cultures produced far fewer infectious virus particles. Even though there was production of viral proteins (shown by viral expression of red fluorescent protein) there was little viral spread in feline cancer cells. We will collect additional feline tumor cells to assess whether feline sarcoma cells are more permissive to infection than carcinoma cells. As companion animals live longer, the prevalence of cancer is increasing. Studies investigating novel cancer treatments are essential for advancing the standard of care in cancer Patients.

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### **Prevalence of *Leptospira* in Wildlife and Watersheds: a Measure of Ecosystem Health**

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Leptospirosis is a waterborne bacterial disease. Wildlife and domestic animals can serve as reservoirs for multiple pathogenic serovars. Urban sprawl has increased wildlife interactions with humans and domestic animals. Therefore, assessments of the prevalence and incidence of leptospirosis in wildlife and leptospire in watersheds can be used as an initial evaluation of pathogen distribution and potential risk of disease transmission to humans, wildlife, and domestic animals. Serum samples from raccoons, opossums, and feral cats from a local natural area were analyzed using a Microscopic Agglutination Test (MAT) for the presence of antibodies against 7 pathogenic *Leptospira* serovars (*Leptospira interrogans* serovars Autumnalis, Bratislava, Canicola, Icterohaemorrhagiae, Pomona; *Leptospira kirschneri* serovar Grippotyphosa; *Leptospira borgpetersenii* serovar Hardjo). MAT titers greater than 1:25 were considered positive; titers higher than 1:800 suggested an active or recent infection. Titers were not detected in serum from feral cats (prevalence = 0%, n = 9). Of 51 opossums, 29 (57%) were positive, 3 with evidence of active or recent infection. Forty-four raccoons (45.4% of n = 99) had positive titers of which 13 were 1:800 or greater. Watersheds most likely used by the sampled animals were collected and analyzed by real-time PCR using primers that recognize the *Leptospira* genus. Nine of 17 water samples were *Leptospira*-positive. When analyzed by chi-square test, the watersheds were not significantly related to the MAT titers.

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## **The Effects of Detomidine Sedation During Pulmonary Function Testing in Horses**

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**Objective**—To evaluate the bronchodilatory effects of detomidine sedation on airway responsiveness in horses undergoing pulmonary function testing.

**Animals**—6 adult horses (1 gelding, 1 stallion, 4 mares).

**Procedures**—Airway responsiveness using flowmetric plethysmography and histamine bronchoprovocation will be determined without sedation and after administration of low dose (LD = 0.005 mg/kg) and high dose (HD = 0.01 mg/kg) detomidine with a 72 hour minimum washout between tests. PC35 (provocative concentration of histamine needed to increase flow by 35%) will be used as a measure of airway responsiveness.

**Results**—Six horses completed testing without sedation and with LD detomidine. Nonsedated horses moved more frequently during testing and variation in response to sedation was noted. Baseline flow values were higher in nonsedated horses. PC35 with and without sedation did not differ for three horses. PC35 significantly decreased in two horses (8.94 mg/ml vs 2 mg/ml; 16.28 mg/ml vs 5.54 mg/ml) and increased in one horse (3.18 mg/ml vs 27.67 mg/ml) after sedation.

**Conclusions and Clinical Relevance**—Increased PC35 values after sedation suggests a bronchodilatory effect of detomidine. One horse demonstrated an increase in PC35 after sedation, however decreased PC35 in two horses suggests factors other than bronchodilation may influence test outcome after sedation. Many of the observed differences may be attributable to increased movement and agitation in nonsedated horses, or individual differences in response to sedation. Further evaluation of the potential bronchodilatory effects of detomidine sedation on airway responsiveness is needed before its use can be recommended in a clinical setting.

Student support: Morris Animal Foundation

## **Investigating Phospholipase D2 Expressions in Canine Osteosarcoma**

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**Introduction:** In dogs with osteosarcoma (OS), a limited number of poor prognostic factors have been consistently identified including elevations in serum bone alkaline phosphatase (bALP). Despite its accepted value for prognostication, it remains poorly understood why elevated bALP is associated with more aggressive OS biology. Under physiologic conditions, the release of bALP into circulation is regulated through the enzyme phospholipase D2 (PLD2), which promotes and regulates cancer cell motility, cytoskeletal rearrangement, proliferation, and survival. As such, elevated bALP in dogs with OS may be a surrogate biomarker of increased PLD2 activity originating from a sub-population of metastatic malignant osteoblasts.

**Methods:** Clonally-derived OS cell lines of variant biologic aggressiveness derived from 3 different species (human, canine, and murine) will be utilized to assess differences in PLD2 expressions. Gene transcription of PLD2 will be assessed with real-time polymerase chain reaction. Protein translation of PLD2 will be determined by western blot analysis using the human U937 cell lysate as a positive control.

**Results:** For gene transcription, PLD2 expressions were not statistically different between clonally-derived variants of known differing metastatic capacity (non-metastatic versus highly-metastatic). For western blot analysis, PLD2 protein is identified for all cell lineages regardless of metastatic capacity.

**Conclusions:** PLD2 is expressed by OS cell lines from various species. No obvious differences in gene expression and protein translation of PLD2 were identified. However, differences in enzymatic activity may be present between clonally-derived variants requires further investigation and may be the underlying reason for why bALP concentrations are elevated in dogs with more aggressive OS.

Student support: Morris Animal Foundation

## **Effect of Tendon-Derived Progenitor Cells on Extracellular Matrix Production and Collagen Fiber Alignment in a Collagenase-Induced Model of Tendinitis in Horses**

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Tendon injuries are a common cause of lameness in performance horses. Stem cell-based treatments for tendon injuries show promise, but are not widely used or universally successful. Furthermore, no studies thus far have evaluated the effects of cell injections on fibre alignment or extracellular matrix production, which is responsible for tissue mechanical strength. We

hypothesize that autogenous tendon-derived progenitor cells promote tendon healing with improved tissue architecture and fibre alignment in a collagenase-induced tendinitis model in horses. Collagenase-induced tendinitis was created in the superficial digital flexor (SDF) tendons of the forelimbs of eight clinically normal horses. Subsequently, a randomly chosen forelimb was injected with 10 million tendon-derived cells, and saline was injected into the contralateral forelimb serving as a control. The horses were euthanized 12 weeks post treatment, and the SDF tendons of both forelimbs and a normal hind limb were harvested for histologic evaluation, which included picro-sirius red staining for fibre alignment and quantitative estimation of collagen and toluidine blue staining for the estimation of proteoglycan using image analysis software. The results obtained from this study will help determine effects of cell-based therapies for the clinical treatment of tendinitis. In addition, these results could revolutionize tendinitis treatments by promising a healthier, stronger tendon and quicker healing of the injured tendon.

Student support: Pfizer, Inc.

Research support: American Quarter Horse Foundation

### **Temporal Evaluation of Collagen Fibre Alignment, Extracellular Matrix Production in a Collagenase Model of Tendinitis in Horses**

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Tendinitis is a common cause of breakdown injuries in equine athletes and accounts for up to 30% of all racing injuries. The purpose of this project is to evaluate the temporal and spatial effects of tendon-derived progenitor cells on tendon healing in a collagenase model of tendinitis. Our hypothesis is that the progenitor cell-treated tendon will have an improved fibre alignment and increasing extracellular matrix production over time with reference to normal tendon. Autogenous tendon-derived progenitor cells were isolated from the lateral digital extensor tendon using a differential adherence technique. Collagenase-induced tendonitis was created in the superficial digital flexor tendon by injecting 2000 units of collagenase at 3, 4, 6, and 8 weeks prior to euthanasia. Two weeks post injury, all 8 horses were injected with 10 million tendon-derived progenitor cells into two sites of maximal injury. The opposite normal control tendon and the collagenase injured tendon were collected for analysis 1, 2, 4, and 6 weeks (n=2) after treatment with progenitor cells. Tendons harvested were subjected for histologic evaluation of collagen, proteoglycan content and collagen fibre alignment. The aim of this study is to determine the progression of tendon-derived progenitor cells in the healing

process of tendinitis. Results of this study are important to assess the viability of cells over time and its ability to fuse to native tenocytes, and also its effect on the healing process to evaluate the usefulness of cell-based therapy for clinical treatment of tendinitis.

Student support: Pfizer, Inc.

Research support: American Quarter Horse Foundation

## **Mono-OH Methoxychlor (Mono-OH) and Steroidogenesis in the Mouse Antral Follicle**

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Methoxychlor (MXC) is an organochlorine pesticide that reduces fertility in female rodents by causing ovarian atrophy, decreasing antral follicle numbers and increasing follicular atresia. MXC is readily metabolized in the body, and previous studies have shown the metabolite mono-OH to be more ovotoxic than the parent compound. MXC exposure at 10-100 $\mu$ g/mL decreases the production of estradiol, a steroid hormone that is essential for normal ovarian function. However, the effects of mono-OH on estradiol levels were unknown. Thus, this work tested the hypothesis that mono-OH exposure decreases production of sex steroid hormones in the estradiol biosynthesis pathway. To test this hypothesis, antral follicles were isolated from 39-day-old CD-1 cycling adult mouse ovaries and cultured in supplemented  $\alpha$ -minimum essential media. Follicles were either untreated, exposed to dimethylsulfoxide (DMSO; vehicle), or exposed to mono-OH MXC (0.1-10 $\mu$ g/mL) for 96 hrs. After culture, the media was collected and estradiol levels were measured using enzyme-linked immunosorbent assays. The results indicate that mono-OH significantly decreases estradiol levels at the 10 $\mu$ g/mL dose when compared to DMSO treated control groups (control = 3009.72  $\pm$  744.99 ng/mL; mono-OH 0.1 $\mu$ g/mL = 1679.66  $\pm$  461.99 ng/mL; mono-OH 1 $\mu$ g/mL = 1752.72  $\pm$  532.41 ng/mL; mono-OH 10 $\mu$ g/mL = 45.89  $\pm$  33.83 ng/mL; n = 11; p  $\leq$  0.05). Collectively, these data suggest that mono-OH exposure significantly decreases the production of the steroid hormone estradiol by mouse antral follicles. This reduction in levels of steroid hormones could lead to mono-OH MXC induced ovotoxicity.

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## **Biological Characteristics of Catch Basins Affect Mosquito Larvae Productivity, the Influence of Vector Habitat on the Potential for West Nile Virus Transmission.**

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Catch basins in suburban areas provide an ideal habitat for *Culex pipiens* and *Culex restuans* mosquitoes, potential vectors of West Nile virus (WNV), to deposit their eggs. Basins vary in larvae productivity, but the reasons for these differences are not clear. The goal of this study was to determine the characteristics of productive catch basins in an area southwest of Chicago, a hot spot for WNV. We hypothesized that catch basins with higher levels of organic nutrients, and less variation in water level and temperature, would be more productive. Sensors were placed in 60 catch basins in the study area to record changes in temperature, light level, and water level. Basin water pH was measured and the water was tested for organic enrichment through ammonia, phosphate, and nitrate concentrations. Larvae and pupae were collected from the catch basins on a weekly basis, quantified by basin, and identified to the species level. The number of larvae and pupae were compared between basins and within the same basins over time and were compared with basin characteristics. We used SPSS for statistical analysis and ESRI ArcGIS to process and map spatial data. The results of this study provide insight into biological characteristics that increase the abundance of *Culex* mosquitoes. This information will provide guidance for more efficient, effective and targeted mosquito control and, therefore, potentially decrease disease transmission.

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