Research highlights from the Ontario Veterinary College summer leadership and research program

This study was conducted under the supervision of various professors in the Ontario Veterinary College, University of Guelph

Here we present research highlights from the Summer Leadership and Research Program (SLRP) at the Ontario Veterinary College. The SLRP offers BSc, DVM and other summer student researchers at OVC a stimulating slate of events from May to August. The program includes a Round Table series with veterinarians and scientists working in diverse and intriguing areas, field trips to cutting-edge research facilities and the Metro Toronto Zoo, and professional development workshops to prepare for conference-style sessions.

AROMATASE AND ESTROGEN RECEPTOR EXPRESSION IN EARLY EQUINE EMBRYOS

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During the early stages of pregnancy, the equine conceptus produces estrogens which are assumed to play a role in the interaction between the mare and the conceptus to promote the establishment and maintenance of pregnancy. Failure of this interaction can result in embryonic death, which is a significant economic burden to those in the horse breeding industry. It has been well established that extra-embryonic membranes such as the trophoblast and the hypoblast are responsible for the synthesis of estrogen; however, the vitality of estrogen synthesis by the embryo proper for embryogenesis and the establishment of pregnancy has only recently been demonstrated. The final step in the biosynthetic pathway of estrogen occurs via the enzyme aromatase, and sensitivity to estrogen requires the presence of estrogen receptors alpha and beta in the maternal and embryonic tissues. The objective of this study was to determine at what stage the equine embryo expresses aromatase activity and if the embryo proper contains estrogen receptors alpha and beta to utilize estrogens produced by the enzyme. Sixteen mares at the Arkell Research Station (Guelph, ON) were monitored for estrus, and by ultrasonography for follicular development, to allow artificial insemination and diagnosis of ovulation within ± 0.5 day. Conceptuses were collected by transcervical uterine lavage and the embryo proper was dissected. Immunohistochemical labelling was performed on sections of embryo proper from days: 27.5 (n=1), 26.5 (n=1), 25.5 (n=2), 24.5 (n=1), trophoblast, endometrium and placenta. Large amounts of aromatase were found in the trophoblast epithelium. This is consistent with a role for estrogen acting on endometrial estrogen receptors and being important in pregnancy maintenance. Aromatase and estrogen receptors are expressed in lower amounts in various embryonic tissues (heart muscle, kidney, mesenchyme of the genital ridge, and other locations) suggesting that estrogens are also important in organogenesis in these locations.

EFFECTS OF CAFFEINE ON THE LEVELS OF APP AND APP PROCESSING ENZYMES

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Beta amyloid (Aβ) plaques are neuronal pathologies common in both Alzheimer’s disease (AD) and Canine Cognitive Dysfunction Syndrome (CDS). Aβ is derived from the proteolytic cleavage of the larger transmembrane amyloid precursor protein (APP) via cleavage at its N-terminus by β-secretase (BACE1) followed by cleavage at its C-terminus by the γ-secretase complex. Cleavage of APP by one of three α-secretases (ADAM 9, 10 and 17) prevents the formation of Aβ. These two pathways are referred to as amyloidogenic and non-amyloidogenic, respectively. The purpose of this study was to examine the effects of caffeine, which has been reported to improve cognitive function, on the expression of APP and its processing enzymes. PC12 (rat adrenal pheochromocytoma) cells were treated with 100uM caffeine, 50ng/ml nerve growth factor (NGF) or NGF + caffeine for 24, 48 and 72 hours caffeine, and protein levels for APP and APP processing enzymes were assessed by western blot analysis. Caffeine in combination with NGF increased the expression of all isoforms of APP and BACE1 but not ADAM 17, suggesting caffeine increases the amyloidogenic
processing of APP. Promoter activity for ADAM17 and BACE1 was also assessed and was increased in cells treated with caffeine and NGF indicating that the increase in protein levels observed could be due to enhanced transcription. In contrast to protein and promoter analysis, treatment of PC12 cells with caffeine alone resulted in an increase in APP and ADAMs family mRNA levels. This study concludes that caffeine could influence APP processing.

**VARIATION IN IMMUNE RESPONSE PHENOTYPE OF PIGS TREATED WITH HEAT-KILLED ESCHERICHIA COLI IN A NEONATAL MODEL OF ALLERGY**

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Immune response (IR) of pigs varies by litter and individual such that ratios of type-1 and type-2 IR differ. Genetics and the environment contribute to this variation. Vaccination response of neonates is often type-2 biased; this can lead to inappropriate responses to type-1 infection, predisposition to allergic disease, and reduced health and productivity. The purpose of this experiment was to determine the effect of daily pre-treatments with *Escherichia coli* delivered intramuscularly (IM) to neonatal pigs on allergic sensitization to the egg white protein ovomucoid (Ovm). Food allergy was used as a monitor of type 1 and type 2 bias. It was hypothesized that piglets pre-treated with *E. coli* in the first seven days of life would have reduced type-2 IR phenotypes, resulting in a decreased frequency of clinical signs of allergy upon oral challenge with egg white. Three litters of 12 Yorkshire piglets were used. Half of each litter was pre-treated with *E. coli*, the other half with phosphate buffered saline. Piglets were sensitized to Ovm by intraperitoneal injection; sensitization was monitored through skin testing. Blood was collected on days 14 and 45 to quantify T-regulatory lymphocytes (T-reg) and to detect serum antibody activity. Piglets were orally challenged on day 46 with egg white and clinical signs of allergy were scored. Severity of skin test reactivity and clinical signs on day 46 were correlated. Groups were similar in expression of allergy. There was more Ovm-specific antibody activity in the *E. coli* treated group associated with IgG, IgE, and IgG1, while there was no difference in Ovm-specific IgG2 antibody activity. There was no treatment effect on the percentage of T-reg on day 45 and no difference between T-reg percentages in allergic versus clinically tolerant piglets. Contrary to the hypothesis, treatment with *E. coli* increased type-2 bias.

**USING THE THROMBOELASTOGRAPH (TEG) VELOCITY CURVE TO CHARACTERIZE BLOOD CLOT FORMATION IN DOGS WITH IMMUNE-MEDIATED HEMOLYTIC ANEMIA (IMHA)**

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Immune mediated hemolytic anemia (IMHA) is an autoimmune disease characterized by premature red blood cell destruction. Hemostatic abnormalities such as thromboembolism are a major cause of death. The thromboelastograph (TEG) is a global hemostasis assay utilizing whole blood that provides an evaluation of the entire hemostatic system (clot initiation, clot formation and fibrinolysis) in the form of a TEG tracing. TEG may detect hemostatic aberrations when traditional plasma based tests fail to do so. The TEG tracing converts to a velocity curve (Vcurve), providing additional variables on clot formation, kinetics, strength and thrombus generation. In one assay, TEG may provide similar information usually determined by several traditional blood clotting tests. The objective of this study was to develop Vcurve reference values for normal healthy dogs and compare Vcurve values in dogs with IMHA to normal dogs. 41 normal adult dogs of various breeds and sexes and 17 dogs with IMHA were utilized to gather data; blood was collected from IMHA dogs on the day of admission prior to treatment. Citrated unactivated blood samples were analyzed for all dogs. Readings began after 340μL of blood was added to a cup containing 20μL of calcium chloride. A TEG was performed and tracings were then converted to a Vcurve. Variables evaluated were thrombus generation (TG), maximum rate of thrombus generation (MRTG) and time to maximum rate of thrombus generation (TMRTG). Results: The mean TG from the normal dogs was 644.3mm/min, while the mean TG from IMHA dogs was significantly higher at 964.5mm/min (p<0.0001). A significant difference was also found with MRTG; IMHA dogs had 29.135 mm/min, compared to normal dogs at 8.7 mm/min (p<0.0001). There was no significant difference between mean TMRTG for normal dogs (7.0 min) and IMHA dogs (5.5 min) (p=0.2215). Conclusion: IMHA dogs generated more clot strength and at a faster rate. This may suggest a hypercoagulable state. The Vcurve variables TG and MRTG could be examined as prognostic indicators for risk of mortality in future IMHA dogs.
THE EFFECT OF PACKED RED BLOOD CELL AGE ON MORTALITY, TRANSFUSION REACTIONS, AND POST-TRANSFUSION COMPLICATIONS

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Storage of packed red blood cells (pRBCs) prior to transfusion is associated with changes that negatively impact the post-transfusion viability of red cells. In humans, the transfusion of pRBCs stored for long periods of time can increase the risk of systemic inflammation, immunosuppression, organ injury, and death. This study investigated the association between the storage age of canine pRBCs and mortality, transfusion reactions, and post-transfusion-complications in dogs. Records were obtained for dogs receiving pRBC transfusions at the Ontario Veterinary College Health Sciences Center (OVC-HSC) between June 2008 and May 2011. Records were retrospectively reviewed for patient and transfusion information, outcome, transfusion reactions (i.e., fever, hemolysis, anaphylaxis, delayed reactions, volume overload), and post-transfusion complications (i.e., acute kidney injury, pneumonia, new infection, acute lung injury). 202 dogs (98 males, 104 females) received pRBC transfusions. pRBC age did not significantly affect outcome, transfusion reactions, or post-transfusion complications. These results are consistent with some human studies that suggest a decrease in pRBC storage time is not warranted. There was a significant association between the number of pRBC units transfused and non-survival (p<0.003), which was still present in a multivariate analysis accounting for severity of illness (p=0.005). Prospective randomized studies are needed to further assess the effect of blood product storage on veterinary patient outcome.

ASSESSMENT OF MITOCOCHONDRIAL FUNCTION DURING BOVINE PREIMPLANTATION EMBRYOGENESIS

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Embryo health evaluation is one of the most important tasks in reproductive biotechnology; yet, a reliable, noninvasive predictor of embryo viability still remains elusive. Studies in our lab have shown that embryos with faster cleavage (FC) rates have a higher developmental potential and differ in their metabolomic profile from slow cleaving (SC) embryos. Since mitochondria are essential energy-producing organelles for embryonic development and the predominant site for metabolic reactions within the cell, we propose that mitochondrial function between fast and slow growing embryos differs. Therefore, the objective of the present study was to assess the expression of mitochondria-related genes [ATP synthase (ATP5A1); Cytochrome c oxidase (COX17)] in bovine preimplantation embryos of different cleavage rates. Embryos were produced in vitro using standard IVF procedures and collected at timed stages of development according to a standard embryo developmental timeline. Using real-time RT-PCR we demonstrated that relative gene expression for ATP5A1 and COX17 genes were higher in SC embryos during the first few cleavage stages (2-cell & 4-cell), while the same genes were expressed at a higher level at 8-cell stage in the FC embryos. While ATP5A1 gene is required for oxidative metabolism, cytochrome c oxidase catalyzes the electron transfer from reduced cytochrome c to oxygen. Upregulation of both these genes in SC embryos reflects higher demand for aerobic respiration in the developmentally challenged embryos. The high-energy demand observed in FC embryos at 8-cell stage can be attributed to the kick-start of the embryonic transcriptional machinery. In FC embryos, high expressions of COX17 transcripts were observed at the blastocyst stage, suggesting increased respiratory role during blastocyst formation. Altogether, our results provide evidence that differences in the mitochondrial function exist between SC and FC embryos and future studies will focus on elucidating the differences in the mitochondrial number and membrane potential between SC and FC embryos.

CONSTITUTIVE VARIATION IN HEPATIC EXPRESSION OF PORCINE INNATE IMMUNE GENES

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Infectious diseases are a major economic problem in the pig industry, causing direct losses due to death and indirect losses related to impaired growth performance due to subclinical disease and costs associated with therapeutics and prevention strategies. Deficiencies in innate immune components can be caused by both environmental and genetic factors, and may reduce resistance to infectious disease. Single nucleotide polymorphisms (SNPs) in some innate immune proteins causing impaired expression and/or function has been identified in many species including humans and pigs. This study aims to identify SNPs in the promoter regions of porcine innate immune genes associated with impaired gene expression. Liver samples of 1003 healthy pigs were collected from a southern Ontario processing facility. 96 pigs were selected for microarray analysis (Agilent Porcine Gene Expression microarray) of hepatic gene expression based on genotyping results for 19
previously identified innate immune SNPs to ensure genetic heterogeneity. The innate immune genes mannann-binding lectin 2 (MBL2) and secretoglobin were among the 15 genes with the highest variability in expression, based on gene expression ratios. Gene expression of MBL2 as assessed by microarray was consistent with previous data generated by qPCR. For many of the most variable innate immune genes there is a small subset of animals with a marked drop in gene expression, as observed previously for MBL2, potentially due to SNPS within the promoter regions of these genes. Sequencing of these promoter regions (currently underway for secretoglobin 1A1/uteroglobin) should identify promoter polymorphisms that impact expression levels and decrease innate resistance to common infectious diseases of swine. The discovery of these SNPS may lead to the development of a more complete genetic selection panel leading to increased disease resistance which will increase production, promote animal health and welfare, and decrease the use of antimicrobials and thus help reduce antimicrobial resistance.

**EXTRAORDINARY MAN, EXCEPTIONAL TIMES: HOW AN OVC VETERINARY PATHOLOGIST BECAME THE ‘34TH PATRIOT’ OF THE KOREAN INDEPENDENCE MOVEMENT**

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Francis William Schofield (1889-1970) is a man remembered on two continents. Born in England, Schofield immigrated to Canada in 1907 and graduated from the Ontario Veterinary College (OVC) in 1910. After joining the OVC faculty for a brief time, he moved to Korea with his wife in 1916 to teach bacteriology and hygiene as a medical missionary at Severance Medical College in Seoul. While there, he became actively involved in the 1919 March First Movement seeking Korean independence, until attention from the Japanese government and his wife’s deteriorating health necessitated a return to Canada in 1920. For the next three decades, Schofield taught at the OVC and carried out extensive research, most notably discovering that a hemorrhagic disease in cattle was caused by mouldy sweet clover, leading to the eventual identification of dicumarol. Upon retirement, he returned to Korea for the remainder of his life. Both in his lifetime and posthumously, he was the recipient of numerous awards and honours, both academic and cultural, and remains the only foreigner to be buried in the Patriots’ Section of the Korean National Cemetery. This project sought to uncover the factors contributing to his successes in both Canada and Korea. Research methods included accessing primary source materials at the University of Guelph Library Archives and the CAV Barker Museum, surveying secondary literature to understand the context of Dr. Schofield’s time, and conducting oral history interviews with students and colleagues of the late Dr. Schofield. Ultimately, it was determined that Schofield’s successes and legacy, while undoubtedly the result of his dedication and indefatigable spirit, were influenced by contemporary events and must be viewed within the global context of his time. He lived and worked at a pivotal time in history, marked by wars, depression, and changing philosophies toward colonization, and through his responses to these developments left an international legacy as a veterinarian, teacher, medical missionary and humanitarian.

**EFFECTS OF DEXAMETHASONE ON ISOLATION FREQUENCY OF EQUINE CORD BLOOD MSCS, EXPANSION KINETICS, AND CHONDROGENIC POTENCY**

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Previous research in our lab has shown that equine multipotent mesenchymal stromal cells (MSCs) isolated from equine cord blood (eCB) may have superior chondrogenic potency compared to adipose and bone marrow-derived equine MSCs. The role of dexamethasone on initial MSC precursor frequency, cell expansion kinetics and subsequent differentiation potential is unknown. The hypothesis of this study is that exposure to dexamethasone in the culture medium until the first passage preselects for MSCs with enhanced chondrogenic potency. Twelve pony mares were naturally bred, observed during foaling and cord blood collected. The blood was erythrocyte depleted prior to seeding: 50mL of whole blood was included in each treatment group and cultured in DMEM-low glucose medium containing 1% each L-glutamine and penicillin/streptomycin, 30% fetal bovine serum, and either 0 M (no dexamethasone), 10⁻⁶ M, 10⁻⁷ M or 10⁻⁸ M dexamethasone. Umbilical venous blood was successfully collected from 7 of 12 foals without complications. Initial colonies were noted between day 7 and 12. First passage occurred between day 7 and 15. The population doubling time between passage 2 and 3 range from 1.2 to 5.1 days, average 2.53 days. In vitro differentiation towards the adipogenic, osteogenic cell fates was performed on 3 cell cultures, each from the 0 M (no dexamethasone) group. Oil Red O and Alizarin Red S staining, respectively, demonstrated successful adipogenic and osteogenic differentiation. Analysis of the tri-lineage differentiation is ongoing at time of abstract submission.