2012 Harry M. Zweig Memorial Fund for Equine Research Summary Report

The 2012 Annual Report covering the period of January 1, 2012 through December 31, 2012 is enclosed.

For this reporting period, The Harry M. Zweig Memorial Fund for Equine Research Committee granted approval of 5 of 14 submitted projects. Five were new studies. Three continuation awards were also approved. The total amount allocated for 2012 awards was $437,567. Copies of the investigators’ reports are provided.

Additionally, Cornell Hosted its fourth annual poster session celebrating the collaboration between the Harry M. Zweig Memorial Fund for Equine Research and Cornell University. Cornell’s faculty, students, and staff show cased their research to the research community and the Harry M. Zweig Memorial Fund for Equine Research Committee at the annual poster session on Thursday November 14, 2012.

2012 Harry M. Zweig Memorial Fund for Equine Research Awards

<table>
<thead>
<tr>
<th>CONTINUATION AWARDS</th>
<th>AWARD</th>
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<tbody>
<tr>
<td>Dorothy Ainsworth</td>
<td>$66,250</td>
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<tr>
<td>The Genetic Basis of Recurrent Laryngeal Neuropathy (RLN) in Thoroughbreds</td>
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<tr>
<td>Jon Cheetham</td>
<td>$11,736</td>
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<td>Diagnosis of Poor Performance in Racehorses</td>
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<td>Bettina Wagner</td>
<td>$62,733</td>
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<tr>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses</td>
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<table>
<thead>
<tr>
<th>NEW AWARDS</th>
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<tr>
<td>Lisa Fortier</td>
<td>$42,583</td>
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<tr>
<td>Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration (1 Year award)</td>
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Linda Mittel  Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector born) that Cause Fevers of Unknown Origin in Horses and in Horse-Associated Ticks in the Northeast, Mid-Atlantic, and Great Lakes Regions (1 Year award)  $54,744

Alan Nixon  Recruiting the Stem Cell Niche for Equine Cartilage Repair (1 Year award)  $62,580

Tracy Stokol  The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection (2 Year award)  $64,795

Bettina Wagner  A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (2 Year award)  $72,146

Interim & Completed 2011 Awards

Dr. Ainsworth’s project entitled “Genetic Basis of Recurrent Laryngeal Neuropathy (RLN) in Thoroughbreds” (2 Year). Dr. Ainsworth received an additional no cost extension through June 30, 2013. An interim report is included in this report.

Dr. Lisa Fortier’s project entitled “Determining Anti-Nociceptive and Matrix Restorative Mechanisms of Platelet Rich Plasma in Osteoarthritis” received a no cost extension through September 30, 2012. A final report is included in this report.

Dr. Robert Gilbert’s project entitled “Controlled Postponement of Ovulation by Progestagen Treatment” received a no cost extension December 31, 2011. A Final report is included in this report (Appendix B).
The Incentive Program enables the Fund to leverage its investment in Zweig-sponsored research by encouraging Veterinary College faculty to seek either additional or supplementary monies from external sponsors that base their award decisions on a process that involves informed scientific review. The external grant must be closely related to a Zweig project. Eligible sponsors include, but are not limited to, the Grayson Foundation, the NIH, the NSF, and the USDA’s National Research Initiative. Recipients provide an annual report on the use of these funds.

The following external grant awards resulted from Zweig funding:

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>External Award</th>
<th>Sponsor</th>
<th>Project Period</th>
<th>Awarded Amount</th>
<th>Incentive Award</th>
</tr>
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<tbody>
<tr>
<td>Dr. Lisa Fortier</td>
<td>Eqine IPS Cells and their Ability to Enhance Tendon Regeneration in-vivo</td>
<td>NIH-Mentored Research Scientist Award (K08)</td>
<td>5/1/12-4/30/13</td>
<td>$557,180</td>
<td>$5,000</td>
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<td>Lauren Schnabel</td>
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<td>Dr. Alan Nixon</td>
<td>Stem Cell Homing After IV Regional Limb Perfusion</td>
<td>Grayson-Jockey</td>
<td>04/01/12-03/31/14</td>
<td>$151,715</td>
<td>$5,000</td>
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<tr>
<td>Heidi Reesink</td>
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PUBLICATIONS

Publications resulting from awards from the Harry M. Zweig Memorial Fund for Equine Research during 2012 were:

1. Acquisition of a Novel Eleven Amino Acid Insertion Directly N-Terminal to a Tetrabasic Celvage Site Confers in Tracellular Clevage of an H7N7 Influenza Virus Hemagglutinin
   **Brian Hamilton, Xiangjie Sun, Changik Chung, Gary Whittaker**

2. Effects of Environmental Factors on Cribbing Activity by Horses
   **Lindsay Whisher, Mary Arum, Lisa Pina, Lucia Perez, Hollis Erb, Charles Houpt, Katherine Houpt** 2012

3. Motivation for Cribbing by Horses

4. Immunological Correlates of Vaccination and Infection for Equine Herpesvirus 1
   **Goodman, L, Wimer C, Dubovi E, Gold C, Wagner, B.** 2012
   [http://cdli.asm.org/content/19/2/235.abstract?sid=a950fa55-fb94-43c9-a3ef-9526b32ca0f2](http://cdli.asm.org/content/19/2/235.abstract?sid=a950fa55-fb94-43c9-a3ef-9526b32ca0f2)
5. Monoclonal Antibodies to Equine CD23 Identify the Low-Affinity Receptor for IgE on Subpopulations of IgM+ and IgG1+ in Horses
   Wagner B, Hillegas J, Babasyan S. 2012

6. Serological Responses and Clinical Outcome after Vaccination of Mares and Foals with Equine Herpesvirus Type 1 and 4 (EHV-1 and EHV-4) Vaccines
   Bresgen L, Wagner B, Osterrieder N, Damini A. 2012

7. Generation and Characterization of Monoclonal Antibodies to Equine NKp46
   Noronha LE, Harman RM Wagner, B, Antczak D. 2012

8. Progestin Treatment of Preovulatory Mares Fails to Delay Ovulation and May Impair Fertility

   Ortved KF, Nixon A, Mohammed H, Fortier L. 2012

10. Equine Herpes Myeloencephalopathy in 37% of Horses Experimentally Infected with Nurropathogenic Equid Herpesvirus Type 1 (EHV-1) while Tsting Metaphylactic use of RNA interference.
    Perkins G, Pusterla N, Benson C, Van de Walle G, Erb H, Osterrieder N.

11. Immunological Correlates of Vaccination and Infection for Equine Herpesvirus 1
    Goodman L, Wimer C, Dubovi E, Gold C, Wagner B. 2012

12. Comparison of Plasma and Peritoneal Indices of Hibrinolysis between Foals and Adult Horses With and Without Colic
    Watts A, Fubini S, Todhunter, R, Brooks M. 2012
    http://avmajournals.avma.org/doi/pdf/10.2460/ajvr.72.11.1535

13. Diagnostic Sensitivity of Subjective and Quantitative Laryngeal Ultrasonography for Recurrent Laryngeal Neuropathy in Horses
    Chalmers HJ, Yeager AE, Cheetham J, Ducharme N. 2012
At the 2007 Annual meeting, the Harry M. Zweig Committee approved the allocation of funds to help support a Cornell Clinical Fellow in Equine Health. Dr. Sophy Jesty was selected as Cornell’s 1st Clinical Fellow, and Dr. Sarah Pownder as the 2nd Clinical Fellow, supported in part by Zweig funds, both of whom are highly successful candidates. Cornell’s College of Veterinary Medicine’s two-year Clinical Fellows Program is the first in the country to address a growing shortage of academic veterinarians who conduct research on animal diseases and basic biology. The program is designed to help students meet the financial and time demands of qualifying for a position in veterinary academic medicine, which has traditionally required students to complete an M.S. or Ph.D. after they finish their doctorate in veterinary medicine (DVM). The two-year program, available to veterinarians who have completed a three-year residency, offers an annual salary of $65,000 plus benefits and an additional $15,000 per year to fund a research project.

OUTTREACH 2012

Patent updates for 2012
During 2012, Dr. Chang applied for patent 3080-10 “Novel Immunologenic Proteins of Leptospira” patent application number 13/459,791, filed 4/30/12.

In 2012 patent D-3080-07 – “Immunogenic Proteins of Leptospira” US patent 8,168,207, was issued to Dr. Chang on May 1, 2012.

Zweig News Capsules

There were two issues of the Zweig News Capsule published in 2012. Copies of these issues can be found in Appendix (F).

All Zweig News Capsules can be found at the Zweig Website at: and the latest one is attached (#55): http://www.vet.cornell.edu/zweig/

SUMMARY OF EXPENDITURES

The 2012 Summary of Allocations was presented and approved at the Zweig Committee Annual Meeting in November 2011 (Appendix C).

2013 ZWEIG PROGRAM

Seven projects were approved for funding, from a roster of 14 applications, at the Harry M. Zweig Memorial Fund annual November (2012) meeting. The list of projects funded for 2013 are shown in (Appendix D).
APPENDIX A

Progress & Final Reports Resulting from 2012 Funding

Dr. Ainsworth  The Genetic Basis of Recurrent Laryngeal Neuropathy (RLN) in Thoroughbreds

Dr. Cheetham  Diagnosis of Poor Performance in Racehorses

Dr. Fortier  Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration

Dr. Mittel  Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector born) that Cause Fevers of Unknown Origin in Horses and in Horse-Associated Ticks in the Northeast, Mid-Atlantic, and Great Lakes Regions

Dr. Stokol  The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection

Dr. Wagner  A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates

Dr. Wagner  Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses
Harry M. Zweig Memorial Fund
for Equine Research

2012 Progress Report

P.I.: Dr. Dorothy Ainsworth

Title: The Genetic Basis of Recurrent Laryngeal Neuropathy (RLN) in Thoroughbreds

Project Period: 1/1/11-12/31/13
Reporting Period: 1/1/12-12/31/12
Dr. Ainsworth received a no cost extension through June 30, 2013. A Progress report is attached.
PROJECT TITLE: The Genetic Basis of Recurrent Laryngeal Neuropathy (RLN) in Thoroughbreds

PRINCIPAL INVESTIGATOR(S): DM Ainsworth, NE Robinson, J Swinburne, RJ Todhunter, Z Zhang, J Stick, NG Ducharme.

A. Specific Aims: Aim 1: collect and archive DNA samples from 300 controls and 300 cases (grade 3 or 4 RLN-affected horses).

Aim 2: perform a Genome Wide Association Scan (GWAS) using the Equine SNP70 beadchip (Illumina) to identify chromosomal loci that are strongly associated with the disease.

B. Results:
Aim 1: Sample collection and DNA isolation from 600 horses has been completed. Comprehensive phenotype data (RLN grade, gender, coat color, height and weight) were available for 549 horses (288 cases and 261 controls), used in the GWAS analysis. DNA samples have been archived thanks to the assistance of personnel at the DNA bank (Drs. Todhunter, Castelhano and Ms. Liz Corey, Julie Jordan).

Aim 2: Genotyping and data analysis of 549 horses using the Equine SNP70 Beadchip was accomplished by our bioinformatics team: Drs. Adam Boyko, Samantha Brooks, Rory Todhunter and Joe Zhang.

1. Performance of the beadchip was confirmed by using coat color and gender identification. Of the 65,157 SNP markers on the Equine SNP70 Beadchip, 7,593 SNPs had missingness >5% and 9,956 had minor allele frequencies <1% causing them to be excluded. 16 additional SNPs were excluded due to significant frequency differences between males and females; 168 SNPs were excluded due to significant departures from Hardy-Weinberg expectation (P < 1 x 10^-4). The final data set contained 51,149 SNPs. Genotyping rates of 92-99% per individual (mean =99.6%) were achieved.

2. Association mapping of body size: GWAS was performed on height using gender as covariates. Regardless of whether sex/neuter covariates were included in the model, all tests yielded a significant association signal at the LCORL gene (equine chromosome 3, ECA3), known to be associated with growth and size in TB horses. A linear model consisting of age, neuter and LCORL allele count explained 25% of the variation of height, with LCORL alone accounting for 18% of the variation. (Note that association tests were performed with GEMMA using a centered relatedness matrix to control population stratification and running a standard Linear Mixed Model (LMM) with P-values computed using a Wald test).

3. Association mapping of RLN: Correcting for population structure in the full data set, the strongest association with RLN was at the LCORL gene on ECA3. (Figure 1, page 2). A second marginal peak occurred on the X chromosome and a tertiary peak occurred on ECA18. Treating RLN score (1…4) as a quantitative trait yielded the same three QTL signals (P=4.5 x 10^-9, 7.4 x10^-6, 8.8 x 10^-6). Using sex and neuter as covariates further increased the significance of the LCORL signal at position (P=3.6 x 10^-10). Even after accounting for sex and neutering, body size had a significant effect
on RLN. After accounting for gender, a four-inch increase in height increases the probability of being RLN by 29%.

**Figure 1.** In the Manhattan plot on the left, each circle depicts an allele frequency test at a unique SNP. The X-axis is the chromosome location, the Y-axis is the SNP P-value for its association with RLN. Three SNPs on ECA3 exceed stringent Bonferroni cutoff (redline), two additional SNPs are located on ECA18 and the X chromosome. The Quantile: Quantile plot on the right depicts the expected distribution of association P-values (X-axis) from a random normal distribution compared to the observed P-values (Y-axis). Deviation from the solid red line (upper right) shows true disease variants.

**C. Conclusions and significance:** Our data suggest that the gene(s) associated with RLN are located near the LCORL gene on ECA3, with potential contributions to this complex trait by genes located on the X chromosome and on ECA18. In our current Zweig (2013), we are interrogating these loci using an across-breed genotyping strategy (investigating RLN in Standardbred horses).

Once we have identified the genes involved in RLN, we hope to market a commercially available SNP chip that can be used to blood test foals at-risk for developing RLN (and thus require surgical intervention for successful racing careers). Perhaps more importantly, horses passing on susceptibility loci can be targeted for removal from the breeding pool. Finally, once the genes involved in abnormal laryngeal function are identified, novel therapies will emerge for which CU will be at the cutting edge.

**D. Publications**


Harry M. Zweig Memorial Fund for Equine Research

2012 Annual Report

P.I.: Dr. Lisa Fortier

Title: Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration

Project Period: 1/1/11-12/31/13
Reporting Period: 1/1/12-12/31/12
PROJECT TITLE: Identification of the Optimal biologic to Enhance Endogenous stem cell recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration.

PRINCIPAL INVESTIGATOR(S): Lisa A. Fortier

A. Progress Report
In the original proposal, there were four specific aims that were expected to be performed in parallel. In general, each aim focuses on a different cell type while testing all biologics simultaneously for chemoattraction of the cell type in question. No aim is dependent on the completion or success of another.

Specific Aim 1 is designed to determine the migratory pattern and distance that chondrocytes travel in response to chemoattractants released from platelet rich plasma, autogenous fibrinogen, bone marrow aspirate, or bone marrow aspirate concentrate. These biologics were chosen because they have known anabolic and mitogenic effects on equine musculoskeletal tissues and stem cells derived from bone marrow or adipose tissue. Using a microfluidics device created by our Co-I for similar cell migration experiments, all biologics can be simultaneously tested on one cell type in a competitive fashion. The device allows for quantification of the number of cells and the migratory distance that cells travel toward the biologic/putative chemoattractant. The migratory pattern of cells is also monitored.

Specific Aim 2 will be similar to Aim 1, but will test the migratory response of tenocytes to the biologics.

Specific Aim 3 will focus on bone marrow-derived mesenchymal stem cells as the cell type.

Specific Aim 4 will assess migration of adipose-derived stem cells to biologics. An N=8 will performed in each aim. Each N will consist of cells and biologics derived from a different horse. For each N, three replicates using three devices will be performed.

The expectation is that knowledge gained though the studies outlined in this proposal will provide immediately relevant and clinically applicable methods of regenerative therapies for musculoskeletal tissue injuries by use of simple, autogenous biologics that will recruit endogenous cells to the site of injury. Harnessing the body’s own reparative abilities will result in more rapid application of treatments that are universally available to aid horses in their return to athletic performance.

B. Studies & Results
We have made considerable progress toward completion of Aims 1 and 3. Our preliminary data on n=5 samples from different horses indicates that both chondrocytes and mesenchymal stem cells preferentially migrate to biologics that contain the highest concentration of leucocytes – platelet rich plasma type B and bone marrow concentrate. Because we are using low passage cells to maintain phenotype, we are ipso facto using a heterogenous population of cells. Added biological variability comes not only from each animal’s cells, but from the biologics themselves that are used as chemoattractants. Therefore, we anticipate that a minimum of n=8 from each group will be required for statistical analyses. The results of our preliminary studies using mesenchymal stem cells has been presented at a recent Gordon Research Conference (Appendix 1). The work was presented by Brooke Wilson who is performing the experiments as her project during completion of her Veterinary Student Training in Biomedical Research Program. Brooke will also obtain a M.S. for this work. Enthusiasm from the audience for this approach was much like ours – that one’s own biological material can be used to recruit stem
cells and that this microfluidics device can measure not only the number of cells that are migrating, but the exact distance that each cell is moving toward a chemoattractant. This is different compared to standard migration assays that can only count the number of cells migrating and not the distance.

Our greatest impedance to completion of the experiments has been a limited supply of microfluidics devices from our collaborator Dr. Mao. We were initially probably naïve in how many devices Dr. Mao’s group could provide for these studies. This has been resolved by working with Dr. Mao to transfer the knowledge necessary to physically make the devices here on the Cornell-Ithaca Campus.

C. Significance

The work of the studies in this proposal are important for the clinical realization of stem cell therapy. The concept is that through use of the patients’ own natural biologically-derived solution, that stem cells can be recruited to the site of injury to facilitate tissue repair.

D. Plans

There are no modifications to the original proposal plan. In Year 2, we will obtain data from additional chondrocyte and mesenchymal stem cell experiments and perform the full complement of parallel experiments using tenocytes and synoviocytes to gain the information needed to understand how each injured cell type behaves with respect to migration toward biological products commonly used in regenerative therapy.
2012 Progress Report

P.I.: Dr. Jonathan Cheetham

Title: Diagnosis of Poor Performance in Racehorses

Project Period: 1/1/11-2/28/13
Report Period: 1/1/12-12/31/12

Dr. Cheetham was granted a no cost extension through February 28, 2013. A progress report is provided (a final report will be included next year).
Diagnosis of Poor Performance in Racehorses

Principal Investigator(s): PI: Jonathan Cheetham

Specific Aims and Findings:

(Aim 1) Validate a novel technique for determining cross-sectional area of the CAD muscle using transesophageal ultrasound. We have validated two in vivo techniques (transesophageal ultrasound (TEU) and computed tomography (CT)) for determining muscle geometry based on known geometry in ex vivo larynges. This study was performed in twenty four specimens. Briefly, for each specimen, the thickness of the mid-body of the Cricoarytenoid Dorsalis muscle (CAD) was determined under light sedation using transesophageal ultrasound; the volume and mid-body cross-section of the CAD muscle was determined following computed tomography using Mirmics™ with Livewire™. Our results indicate that cross-sectional area and muscle volume correlate very closely (R² = 0.70 and 0.77, respectively) with actual (ex vivo) muscle volume and weight and that muscle thickness based on transesophageal ultrasound has a fair correlation (table 1, figure 1).

<table>
<thead>
<tr>
<th>Displacement</th>
<th>Displaced Weight (g)</th>
<th>Displaced Volume (mm³)</th>
<th>CT Reconstruction Volume (mm³)</th>
<th>CTMid-body Cross-Sectional Area (mm²)</th>
<th>TEU Thickness (mm)</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>10.85</td>
<td>1033.3</td>
<td>1063.7</td>
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<td>Standard Deviation</td>
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<td>445.1</td>
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<td>0.42</td>
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Table 1. Relationship between TEU, CT and ex vivo measures. Slope, coefficient of variation (R²) and y-intercept value. Figure 1. Relationship between: mid-body cross-sectional area; muscle volume based on CT reconstruction and midbody muscle thickness based on transesophageal assessment (all standardized on y-axis) against actual muscle volume ex vivo (x-axis).

Significance: These data demonstrate the ability of CT reconstruction to accurately determine muscle geometry in anesthetized horses. This is a crucial tool for investigating the response of the CAD muscle in novel treatments such as nerve muscle pedicle graft and a number of tissue engineering techniques. Muscle geometry correlates closely with the ability of the muscle to generate force. Our ability to measure both muscle geometry and laryngeal function (at treadmill exercise) was highlighted as a novel strength in a recent NIH-R21 critique. The data also show that TEU provides a fair estimate of muscle geometry when compared to ex vivo geometry. This was a key step in developing TEU as a tool for the early diagnosis of RLN (aim 3). In addition, based on this work an early collaboration has recently developed between the PI and Professor Martin Birchall, National Ear Nose and Throat Hospital, London.
who is interesting in adapting the TEU technique to human patients and using it to predict the return of laryngeal function following nerve injury.

**(Aim 2)** **Determine the relationship between laryngeal position and the presence or absence of DDSP at exercise in a clinical population presenting for poor performance.** To date, we have complete data for 59 horses. We have identified two discrete populations of horses based on laryngohyoid position (based on radiographic evaluation) that correspond closely with the presence of absence of DDSP at exercise (Figure 2). Horses with DDSP at exercise have a laryngohyoid position (all three reference points) that is more dorsal and more rostral than horses without DDSP.

![Figure 2. Relationship between three markers of laryngohyoid position and the presence or absence of DDSP at exercise.](image)

**Significance:** Multivariate analysis of these data demonstrates that we use this reference system to determine which horses have DDSP and which do not with 80% accuracy. This finding is extremely novel. It will allow us to screen horses for potential DDSP by a simple laryngeal radiograph. This will provide valuable information for trainers and owners considering referral for exercising endoscopy (treadmill or wireless examination). It also provides some clues towards the etiology of this common yet incompletely understood condition. We are continuing to collate data and are developing statistical tools (principle component and cluster analysis) with which to analyze the complete data set.

**(Aim 3)** **Determine the relationship between cross-sectional area of the CAD muscle (transesophageal ultrasound), and laryngeal function at exercise** Here we determine the relationship between the mid-body thickness of the left and right CAD muscle and laryngeal function at exercise. To date we have gathered data on n=59 horses with a spectrum of naturally occurring disease from normal function (grade A); 4 horses with partial collapse (grade B) and 7 horses with complete collapse (C). We have observed a decrease in the mid-body thickness of the left CAD muscle as exercising laryngeal grade worsens (figure 3). We also observe an unexpected increase in right CAD thickness in partially affected horses.

![Figure 3. Relationship between mid-body thickness of the left and right CAD muscle and laryngeal function at exercise.](image)

**Significance:** This is a very novel observation which suggests that compensation hypertrophy may be occurring. When these data are complete we will determine the sensitivity and specificity of CAD thickness for determining laryngeal function. This would be a very valuable tool for trainers.
considering a treadmill examination; would aid decision making for marginal surgical candidates and would provide preliminary data for a planned study designed to predict future disease in 2 year olds.

Publications: Two manuscripts have resulted from this work. The first represents the data for aims 1 and 3 together and is in review. The second reflects aim three and is currently in the final stages of review prior to submission. This work has also provided training for two large animal surgery residents (Alanna Zantigh and Mike Maher) and a biomedical engineering undergraduate student (Melissa Kenny). This work stimulated the Melissa’s research interest and she is now a graduate student at Wake Forest, VA.
Dr. Fortier was granted a no cost extension through September 30, 2012.

The results of the study are in the form of a manuscript. It has been submitted to the American Journal of Sports Medicine and is in revision. Once accepted, the final version will be reported on next year.
Harry M. Zweig Memorial Fund  
for Equine Research

2012 Progress Report

P.I.: Dr. Linda Mittel

Title: Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector born) that Cause Fevers of Unknown Origin in Horses and in Horse-Associated Ticks in the Northeast, Mid-Atlantic, and Great Lakes Regions

Project Period: 1/1/12-12/31/13
Reporting Period: 1/1/12-12/31/12
Dr. Mittel was granted a no cost extension through December 31, 2013. A Progress report is provided (a final report will be included next year).
2012 HARRY M. ZWEIG MEMORIAL PROGRESS REPORT

PROJECT TITLE: Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector borne) that Cause Fevers of Unknown Origin in Horses in the Northeast, Mid-Atlantic, and Great Lakes areas of the US

PRINCIPAL INVESTIGATOR(S): Dr. Linda Mittel with Dr. Amy Glaser

A. Progress Report

Specific Aims: 1). Identify the presence of potential tick-transmitted (Anaplasma sp., Babesia sp., Borrelia sp., Ehrlichia sp., Rickettsia sp., or other non-respiratory bacterial infections (Leptospira, Bartonella sp., Neorickettsia sp.) in horses with FUO and controls from the same premise, in clinical practices in the North-East, Mid-Atlantic, and Great Lakes areas of the United States.

We have received 132 samples from all the areas of study. Although we were hoping to receive multiple samples from the same practices some practices sent a lower number than initially was suggested. We had been contacted by other practices that were interested in the study and due to lower number of samples received from the targeted practices, we accepted these additional samples from similar geographical areas. We also received samples without controls. We contacted the DVMs when possible and had a control animal sample sent even if not taken the same day as the initial sample. We identified 11 Anaplasma phagocytophilium positive samples and Neorickettsia risticii in 2 samples out of 132 cases.

2) Determine the correlation between the presence of identified agents with fever in the study subjects. We have not completed the complete study to determine this correlation.

3) Identify ticks and determine the prevalence of tick-associated pathogens in ticks in the same environment as the samples and control horses and determine the association between tick pathogen load and the likelihood of causing fever in horses from the participating locations.

We received 11 ticks. One tick was positive for Borrelia burgdorferi and 5 ticks positive for Anaplasma.

B. Summary

The collection of clinical samples for this project and testing for Borrelia burgdorferi and Anaplasma phagocytophilum in clinical samples is completed. Because of concerns regarding the potential lack of sensitivity for direct detection of Bartonella species peripheral blood samples, samples from all EDTA and SPS sterile blood culture tubes submitted for cases and controls were cultured for 4 weeks in two bacterial growth medias used to propagate Bartonella sp. Samples were obtained from the cultures weekly for 4 weeks and frozen for later analysis.

Analysis of the collected samples for the detection additional pathogens listed above is in progress. Individual broad-based PCR amplification targeting Borrelia species, Rickettsia species, Bartonella species and Leptospira species has been performed for 95 of the clinical samples. Additionally, Bartonella species PCR has been performed on first week and final week cultures. Initial assessment of amplification products by high resolution melt curve analysis indicated that there are differences between individual animals in the products produced. The identification of the amplified products will be resolved via sequence analysis. All amplification reactions for all targets will be pooled for each individual animal to create a barcoded sequencing library representing all products amplified. Because we will have one opportunity to
resolve all of the sequence amplified for an individual sample, we are being very careful in the construction of the components of the library pool. Because of this, the elements of the library are taking longer than anticipated to complete.

**C. Significance**

*Horses with FUOs that we sampled appear to have an additional agent causing the fever due to the few number of positive Anaplasma and Potomac horse fevers found. It is expected that we will find additional agents with the broad based PCR testing that we are doing that may be responsible for the fevers.*

**D. Publications and other grant submissions**

Pending completion of the proposed study.
Harry M. Zweig Memorial Fund for Equine Research

2012 Annual Report

P.I.: Dr. Tracy Stokol

Title: The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection

Project Period: 1/1/12-12/31/13
Reporting Period 1/1/12-12/31/12
PROJECT TITLE: The Role of Platelets in the Pathogenesis of Equid herpes virus type-1 Infection

PRINCIPAL INVESTIGATOR(S): Dr. Tracy Stokol

Specific Aims:

The aims have not been modified. However, it should be noted that we only began this project in early April when Dr. Yeo, the Postdoctoral Associate on the grant, was able to work on the grant (due to a different funding source for his salary before this time). We plan to ask for a no-cost extension in order to complete the work within the proposed time frame.

Studies & Results

Aim 1: Direct activation of equine platelets by EHV-1: Here we proposed to test whether EHV-1 bound to equine platelets, causing them to become activated, as shown by flow cytometric analysis for P selectin expression and phosphatiidylserine (PS) exposure on platelet surfaces.

We have spent most of our efforts on this Aim. We have found that EHV-1 strains, Ab4 and RacL11 (neuropathogenic and abortigenic strains, respectively) both induce platelet activation within 10 minutes of incubation with platelets at a multiplicity of infection (MOI) of 5. Activation is characterized by robust expression of P selectin (a marker of alpha granule secretion or the platelet release reaction, Figure 1A) and weak exposure of PS (not shown). Activation was dependent on the viral MOI (Figure 1B, only Ab4 shown). Viral-induced P selectin expression is dependent on thrombin, because it was inhibited by a specific thrombin inhibitor, hirudin (not shown). P selectin expression also requires exogenous calcium. Preliminary performed RT-PCR for viral glycoprotein 2 have and shown that this viral gene product can be amplified from platelets, but not from controls (not shown), suggesting that the virus is binding to or being internalized by platelets. These results still need to be verified by additional experiments. Dr. Osterrieder, our collaborator in Germany, is sending us a viral strain that expresses RFP in its capsid, and we plan to use this fluorescent virus for verifying viral binding to platelets with flow cytometry.
Aim 2: Indirect activation of equine platelets by EHV-1-infected monocytes: Here we proposed to determine if EHV-1-infected monocytes activate platelets.

We have not begun this technically more difficult aim.

Aim 3: Indirect activation of equine platelets by EHV-1-infected endothelial cells: Here we proposed to determine if platelets adhere to EHV-1-infected endothelial cells in a microfluidic device.

We have cultured primary equine carotid artery endothelial cells (CAEC) obtained from a horse in our microfluidic device. The CAEC grew within the device, but the particular cell line formed tubes in culture dishes and within the device. When we infused 6 um beads into the channel, fluid flow was restricted to one side of the channel (Figure 2). We are currently amplifying CAEC from another horse and will culture them within the device to determine if this problem is recapitulated. A single layer of CAEC is required to permit us to flow platelets over the cells. We do not see this problem with bovine CAEC and are hoping that the problem is unique to the one horse. These cells are challenging to work with because they grow slowly and, as a primary cell line, cannot be passaged more than 2-3 times, limiting the number of experiments that can be performed. If the problem is not horse-specific, we will vary channel dimensions and shear rates at which the cells are cultured in the channel to attempt to get a confluent CAEC lining within the channel.

**Significance**

We are excited to discover that EHV-1 activates platelets, by inducing P selectin expression and some PS exposure. This finding is of relevance because platelets play a crucial role in thrombosis, the cause of abortion and myeloencephalopathy due to EHV-1. If platelets are involved in the pathogenesis of these EHV-1-associated disease syndromes, administration of platelet inhibitors, e.g. clopidogrel, may prove useful in the treatment of infected horses.

Aim 1: There are now many more questions to answer regarding the mechanism of platelet activation in EHV-1. These are some (but not all) of the planned experiments: 1) Time course of incubation of virus with platelets: To determine if PS exposure increases with longer times; 2) Confirm binding of EHV-1 to platelets by using a mutant EHV-1 with a fluorescent capsid from Dr. Osterrieder; 3) Verification of preliminary RT-PCR results; 4) Elucidate mechanisms of calcium dependence of viral induced P selectin exposure; 5) Determine if platelet activation is unique to EHV-1 by testing EHV-4. Many of these additional experiments were not originally planned but are necessary to explain our exciting findings.

Aim 2: This more technically challenging Aim will be addressed when we have more understanding of the mechanisms responsible for direct viral activation of platelets (in Aim 1).

Aim 3: We are hoping to overcome difficulties with growing primary CAEC in the microfluidic device. If we cannot grow a confluent layer, we may infuse EHV-1-infected platelets (which are activated) over bovine CAEC. Although the use of a heterologous system is not ideal, at least it will allow us to answer whether viral-activated platelets show increased binding to endothelial cells, which would predispose to thrombosis and potentially inflammation.
Harry M. Zweig Memorial Fund
for Equine Research

2012 Annual Report

P.I.: Dr. Bettina Wagner

Title: A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates

Project Period: 1/1/12-12/31/13
Reporting Period: 1/1/12-12/31/12
A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates

Bettina Wagner & Gillian Perkins

Specific Aims: If the aims have not been modified, state this. If they have been modified, give the revised aims and the reason for the modification. Please note that substantial changes must be reviewed by the Associate Dean for Research & Graduate Education as directed by the Harry M. Zweig Memorial Fund postaward guidelines.

The specific aims have not been modified.

In this project, we investigate the hypothesis that antigen-specific activation of basophils and production of the cytokine interleukin-4 by activated basophils is a major pathway to induce antibody production in foals after birth. The hypothesis is tested by two specific aims.

In Aim1, we propose to generate antibody/EHV-1 antigen complexes that mediate activation of basophils in vitro. Five EHV-1 antigens previously known to induce antibody responses were planned to be expressed and tested. Basophils were isolated, loaded with antibody and activated with EHV-1 antigens. Basophil activation was evaluated by cytokine induction. In this aim, we also proposed the development of a sensitive EHV-1 antigen-specific assay with the goal to improve the evaluation of EHV-1 antibody responses in horses.

Current results: We have expressed four of the five EHV-1 antigens (gB, gC, gD, gG). Expression of the fifth antigen (gp2) is still ongoing. The first two expressed antigens (gC and gD) have been administered to the neonatal foals in June 2012 (see Aim 2). They were also used to perform in vitro experiments to further test the underlying principle of this project. Basophils were enriched from peripheral blood, IgE was loaded on the cells by o/n incubation and stimulation with antigen was performed to induce IL-4 secretion from basophils (Figure 1). This confirmed that high concentrations of B-cell stimulatory IL-4 can be induced from basophils in an antigen-specific way and by using our ‘immune tools’. We determined the optimal IgE and EHV-1 antigen concentration and continued with the in vivo experiment (aim 2).

Figure 1: In vitro binding of IgE to basohils (A) and stimulation of IL-4 secretion from basophils after IgE cross-linking

(B). Different concentrations of IgE and antigen for IgE crosslinking were tested to determine optimal concentrations for the in vivo experiment in aim 2.

The gC and gD antigens were used to develop a multiplex assay for detection of EHV-1 antibodies in serum. Previously tested serum samples with known SN-values ranging from <2 to 768 were used to validate the fluorescent bead-based multiplex assay. These samples were also used to optimize serum and antibody concentrations for the EHV-1 multiplex assay. SN-titers and multiplex results highly correlated (Figure 2). The multiplex assay allows for a more accurate and fully quantitative determination of antibodies to EHV-1 and can also be modified, e.g. for the analysis of equine immunoglobulin isotypes.
Figure 2: Correlation of SN-titers and EHV-1 multiplex results in equine serum. The multiplex assay uses recombinant gC and gD antigens of EHV-1 to simultaneously measure antibodies to both surface glycoproteins. Each of the EHV-1 antigens is coupled to a specific fluorescent color-coded bead to distinguish between the individual assays. We used 58 pretested equine sera with SN-values between <2 to 768 to validate the multiplex assay. Sera are diluted at 1:400. The multiplex assay results are reported as median fluorescence intensities (MFI). Spearman rank correlations for both the gC and gD assays are 0.87.

Next steps: We will continue to express gp2 antigen and will add the remaining three assays to the multiplex assay. The final assay will be used to evaluate all serum and nasal secretion samples obtained from foals enrolled in Aim 2. Since the foals were treated after birth with EHV-1 gC and gD, the assay will also provide a valuable tool to distinguish antibodies induced by this treatment compared to those induced by infection. Because of the assay’s excellent performance, it can be used for advanced and more rapid detection of EHV-1 antibodies. It is very likely that the assay will be offered through the AHDC in the future.

Aim 2 was to test the potential of a novel neonatal vaccination strategy in foals directly after birth. We gave IgE to foals from EHV-1 naïve mares within the first 6 hours after birth. At day 2, foals obtained EHV-1 antigen to induce antibody production. After weaning, protective immunity will be evaluated by challenge infection with EHV-1. Clinical signs, viral loads and EHV-1 specific humoral and cellular immunity will be evaluated.

Current results: We perform the first part of this Aim in foals that were born this summer (June 2012) at Cornell. Their dams were imported to Cornell in February 2012 and came from Iceland. Iceland is free of EHV-1. The SPF-status of the horses was maintained by isolated housing at Cornell. Consequently, the foals did not obtain EHV-1 specific antibodies from their dams via colostrum. This allowed us to determine the foal’s endogenous EHV-1 immune response in the absence of maternal EHV-1 specific immunity. Fifteen foals were placed in 3 groups. Group 1 received IgE orally during the first 3 hours after birth and EHV-1 antigen on day 2 of life. Group 2 received EHV-1 antigen on day 2 but no IgE. Group 3 was not treated. Blood and nasal swab samples were/are constantly taken from the foals. IL-4 expression by neonatal foal basophils was confirmed after birth. Preliminary data (SN-titers) also indicate that antibody responses were initiated in foal group 1, i.e. by oral IgE treatment followed by EHV-1 antigen stimulation on day 2. The SN-titer in group 1 foals that received IgE and antigen was significantly higher than the SN-titers of the other two foal groups (Figure 3).

Figure 3: SN-titer comparison in neonatal foal groups between day 2 (before EHV-1 antigen treatment) and day 5 after birth. IgE was administered to group 1 directly after birth. EHV-1 antigen was given to groups 1 and 2 on day 2 of life. Serum for SN-titer determination was taken before EHV-1 antigen injection on day 2 and on day 5 after birth. SN-titers on day 5 are expressed as fold-differences compared to the SN-value of each respective foal on day 2 (set as 1/dotted line).

Next steps: The experimental infection study with EHV-1 (NY03 strain) is planned for December 2012 to test if the initiation of the B-cell response at birth provides a priming
effect on the foal’s immune system that results in protection from clinical signs, reduces EHV-1 shedding and increases immunity after challenge with the virus. We also continue to measure immune parameters in all foals and evaluate the experimental data obtained thus far. In particular, the samples (sera and nasal secretion) will also be measured using the EHV-1 multiplex assay described in Aim1. Cellular immunity is monthly evaluated using a cytokine secretion assay and by flow cytometry.

**B. Studies & Results**

Describe the studies directed toward specific aims during the current budget year and the positive and negative results obtained. If applicable, address any changes to the innovative potential of the project. If technical problems were encountered in carrying out this project, describe how your approach was modified.

**Positive results:** The principles of EHV-1 specific IL-4 induction in neonatal basophils could be confirmed. Preliminary data suggest that IgE and EHV-1 antigen administration to neonatal foals initiated an EHV-1 specific antibody response.

**Negative results:** Antigen expression of three EHV-1 antigens got delayed for three antigens. Two of them are by now expressed. The third antigen (gp2) seems to be a little more challenging but expression is ongoing. The delay in expression did not result in major changes of the experiment. We used the first two recombinant antigens (gC and gD) for treatment of the neonatal foals which is sufficient to perform the experiments and to obtain proof-of-principle for the proposed novel vaccination strategy in neonates.

The Innovative potential of this project is very high. Current vaccines (EHV-1 and all other commercial vaccines for horse) are recommended to be used at 5-6 months of age because they simply do not induce any immune responses in younger foals. An alternative mechanism to induce adaptive immunity early in life and to develop a respective vaccination strategy has a very high potential not only to improve protection against EHV-1 in horses but also for neonatal vaccination in general.

Thus far, modifications to the approach were not made. Major technical problems were not encountered.

**C. Significance**

During the past 9 months of this project, we obtained the first proof-of-principle that our novel strategy of inducing EHV-1 specific IL-4 responses in basophils may indeed induce adaptive immunity in neonatal foals. If the preliminary findings can be confirmed and protection from disease can be shown by our treatment strategy, this is a completely novel finding. It provides a mechanism and strategy to improve neonatal vaccination and immunity that has not been considered elsewhere.

See also B. Innovative potential for potential impact.

**D. Plans**

Summarize plans to address the Specific Aims during the next year of support. Include any important modifications to the original plans.

This is included in section A at the end of each aim (‘next steps’). The project will be continued as planned and outlined in the main proposal.
P.I.: Dr. Bettina Wagner

Title: Innate Immune Mechanisms and T-cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses

Project Period: 1/1/11-12/31/12
Reporting Period: 1/1/12-12/31/12
PROJECT TITLE: Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses

PRINCIPAL INVESTIGATOR(S): Dr. Bettina Wagner, Dr. Gillian Perkins

SUMMARY/DESCRIPTION OF THE PROJECT:

Equine herpesvirus type 1 (EHV-1) outbreaks continue to occur in the US despite the wide use of vaccination. Although disease outbreaks have been rapidly detected and were professionally managed to prevent wider distribution of disease, a more effective EHV-1 vaccine is needed to reduce the impact of this virus on equine health and economic losses in the equine industry. The long-term goal of our group is to identify specific EHV-1 genes as new targets for vaccines that improve adaptive cellular immunity in horses. Adaptive cellular immunity, and in particular T-cell immunity, is considered to provide protection against EHV-1. Current vaccines were shown to induce robust humoral immunity (antibody responses) but fail or are of low efficacy in providing cellular adaptive immunity. Existing vaccines clearly helped to decrease respiratory disease and abortion storms in horses. However, they largely failed in preventing the repeated severe neurological outbreaks we have faced in the US during the past few years.

In the project preceding this proposal, we investigated immune modulating effects of different EHV-1 genes on equine peripheral blood cells in vitro. In collaboration with Dr. Osterrieder’s group in Berlin, we designed deletion mutants of EHV-1 that were lacking specific viral genes (e.g. ORF1/2, UL49.5, or ICP0). The EHV-1 deletion mutants were then cultured with equine blood cells to evaluate their in vitro effects on innate cellular immunity and EHV-1 specific T-cell stimulation. All three genes significantly modified cellular immune responses to EHV-1 and induced less ‘danger signals’ (type I interferon and chemokine responses) compared to the wild type EHV-1 strain. High induction of adaptive T-cell immunity was observed for the ORF1/2 deletion mutant. ORF1/2 genes of EHV-1 are expected to alter antigen presentation. T-cell initiation depends on the antigen-presentation process. Therefore, we developed the hypothesis that the deletion of the ORF1/2 genes from the EHV-1 virus is likely to improve T-cell immunity, protection against EHV-1 and severe disease induced by current EHV-1 strains.

In this project, we propose to analyze in vivo whether the ORF1/2 deletion mutant indeed induces increased adaptive cellular immunity and protection against EHV-1 in horses (Aim 1). We will also continue to analyze the underlying immune principles that lead to the observed immune activation by EHV-1 deletion mutants with focus on ORF1/2 (Aim 2). To perform this project, our team of investigators includes EHV-1 experts from various disciplines: Dr. Perkins (Large Animal Internist, Co-PI) will be responsible for all clinical aspects of this project. As collaborators on this project, Drs. Osterrieder (Virologist and world-leading EHV-1 expert) and Dr. Van der Walle (Virologist and well established young investigator in EHV-1 research) will contribute with expertise, specific tools and methods to the virologic parts of the project. Dr. Wagner (Immunologist, PI) will oversee the project and will be responsible for all immunologic aspects of the study. Dr. Van der Walle will join the Baker Institute faculty as an Assistant Professor in January 2013. Dr. Wagner’s and Dr. Osterrieder’s group have worked collaborative on EHV-1 research during the past five year, i.e. while Dr. Osterrieder was still at Cornell and since he is in Berlin, Germany.

To perform Aim 1 of this project, we will use horses from our SPF-herd originating from Iceland. This herd is currently kept at Cornell and provides a unique group of horses that have not been previously exposed to EHV-1. EHV-1 and many other common US horse pathogens do not exist in Iceland. The herd is kept under specific restrictions (access only for project associated personnel, no contact to other horses, etc.) at Cornell to maintain the EHV-1
negative status. The EHV-1 status (humoral and cellular immunity) of the animals is constantly monitored.

The immediate goal of this project is to provide proof-of-principle for the efficacy of a new vaccine candidate (Ab4ΔORF1/2) for EHV-1. Based on our accumulated immunological data from in vitro studies during the past few years, the Ab4ΔORF1/2 deletion mutant is a very promising EHV-1 vaccine candidate with a clear potential to lead to protection against neurological disease (myeloencephalopathy) and, in the long-term, an overall better protection against EHV-1 in the horse population.

This project is a continuation proposal. Our project on innate and adaptive immunity to EHV-1 has been funded by the Zweig Memorial Fund for the past three years. It resulted in 4 peer-reviewed publications and 4 presentations at conferences. Additional manuscripts are in preparation. We submitted an EHV-1 proposal twice to the NIH (not funded). We plan to submit a proposal extending this project (focusing on the other deletion mutants) to USDA/NIFA at the end of this year for potential funding in summer 2013 (or 2014 if re-submitted). The NIFA call for proposal is scheduled for September this year.
APPENDIX B

Final/Annual Reports Resulting from 2011 Funding
Harry M. Zweig Memorial Fund for Equine Research

2011 Final Report

P.I.: Dr. Robert Gilbert

Title: Controlled Postponement of Ovulation by Progestagen Treatment

Project Period: 1/1/10-12/31/11
Reporting Period: 1/1/11-12/31/11
Dr. Gilbert received a no cost extension through December 31, 2011. A final report is provided.
PROJECT TITLE: Controlled postponement of ovulation by progestagen treatment

PRINCIPAL INVESTIGATOR: Robert O. Gilbert

Summary:
The overall goal of this research was to postpone ovulation in mares in a predictable way by using exogenous progestagen administration during the preovulatory period. To achieve optimal fertility in mares, mating or insemination should occur close to (ideally, immediately before) ovulation of the mare. Means of controlling ovulation time are therefore desirable to effectively manage breeding mares by allowing ovulation to occur in proximity to availability of popular stallions or shipped semen. Some strategies allow for advancement of ovulation time (e.g. administration of human chorionic gonadotrophin [hCG]), but there is currently no means of delaying imminent ovulation. Such a tool would be valuable in breeding management of thoroughbred horses as well as breeds that allow artificial insemination with shipped semen. This project tested the ability of two progestagens, oral administration of altrenogest (Regumate®) or an intravaginal progesterone-releasing device (CIDR, Pfizer) to delay ovulation and the effects of treatment on estrus behavior and fertility.

Specifically, the research tested the following hypotheses. Administration of progestagens would 1. retard follicular growth; 2. delay ovulation; 3. allow normal estrus behavior; 4. be followed by normal development of a functional corpus luteum, and 5. would not depress pregnancy rates markedly.

To achieve this, we followed 12 cyclic mares during the natural breeding season by daily transrectal ultrasonography and teasing. Once mares were in estrus and with a dominant follicle of 35 mm or greater in diameter, they were allocated in random order to two cycles of each of: no treatment (control), oral altrenogest at 0.044 mg/kg/d for two days, or application of a CIDR for two days. Estrus behavior, follicular growth rate and time of ovulation were determined and recorded. Mares were bred with fresh diluted semen from a single stallion (500 million progressively motile sperm) every 48 hours, beginning at detection of a 35 mm follicle for control cycles and starting the day after completion of the progestagen treatment in treatment cycles. Cervical tone and uterine fluid accumulation as well as vaginal inflammation were also recorded. Pregnancy was determined by transrectal ultrasonography 12 days after ovulation. Daily blood samples will be assayed for progesterone concentration from ovulation until Day 12 after ovulation.

This allowed comparison of estrus behavior, follicular growth rate, time of ovulation, luteal function, and pregnancy rate with the two treatments in comparison to the control group, and allow an assessment of whether this represents a viable strategy for commercial horse breeding.

Specific Aims and Findings:
Specific Aims:
The specific aims of this project were to measure the effect of each of two exogenous progestagens (oral altrenogest or intravaginal application of a CIDR) on:
1. Time to ovulation
2. Follicular growth rate (and size of preovulatory follicle)
3. Estrus behavior
4. Postovulatory luteal function
5. Pregnancy rate
Findings:
That experiment sought to evaluate use of progestagens, specifically intravaginal CIDR® (EZI-BREED®, Pfizer) or oral altrenogest (Regumate®), for controlled postponement of ovulation in mares. Mares were randomly assigned to treatment groups when the dominant follicle reached 35 mm in diameter, were treated for 2 days (control group received no treatment), and then are bred every 48 h until ovulation. Signs of estrus and ultrasonographic findings were recorded.

Neither altrenogest nor the CIDR delayed ovulation (Figure 1) and the size of the ovulatory follicle was not affected by treatment (Figure 2).

Treatment had no effect on rate of follicular growth. Figure 3 shows daily follicular size from the day of detection of a 35 mm follicle. Figure 4 shows the same data corrected for the day of ovulation.

Both forms of progestin treatment had prompt and dramatic effects on estrous behavior – effectively abolishing estrous behavior within 24 h.

Response to the stallion returned to the control level after cessation of treatment (Figure 5). Similarly, both forms of treatment mediated reduction in endometrial edema (assessed
ultrasonographically) beginning at 24 h for the CIDR and 48 h for altrenogest. Edema returned to normal after cessation of treatment (Figure 6).

**Figure 5.** Daily teasing score (mean ± SEM) after Gorecka et al., 2005. Both forms of progestin treatment reduced teasing score ($P < 0.0001$).

**Figure 6.** Median daily edema score. CIDR reduced edema after 24 and 48 h of treatment ($P < 0.001$) and altrenogest only after 48 h ($P = 0.008$).

In addition to having no effect on time of ovulation, altrenogest treatment tended to reduce the chance of pregnancy (Figure 7; $P = 0.09$). We knew when planning this trial that only a major effect on fertility would be identifiable, which makes this finding quite important. Use of progestins to postpone ovulation should be vigorously discouraged, both because it lacks efficacy and because it threatens successful establishment of pregnancy. This means that practitioners still lack an effective method of postponement of ovulation.

This does not mean that there are no practically useful findings to this trial. Mares tolerated the CIDR very well. This may mean that CIDR represents a reasonable choice for temporary (and prompt) repression of signs of estrus, a finding which may be useful in management of show horses or management/diagnosis of horses with estrus cycle-related behavioral problems.

**Figure 7.** Per cycle pregnancy rate by treatment (61%, 54% and 36%, respectively; $P = 0.09$ for altrenogest.)

Luteal function was not altered by treatment. Examined by multilevel mixed effects linear regression with mare as a random variable, neither altrenogest ($P = 0.725$) nor CIDR ($P = 0.874$)
altered progesterone concentrations. There was also no effect of cycle number (i.e. stage of season; \( P = 0.671 \)). The interaction between treatment and cycle day was also not significant (\( P > 0.5 \)). Of course, cycle day itself was highly significant (\( P < 0.0001 \)).

This study also enabled us to determine progesterone concentrations brought about in horses by use of CIDRs. These values are shown in Figure 9.

Additional findings were (1) that altrenogest treatment increased the likelihood that the mare would accumulate intrauterine fluid in the periovulatory period, another negative consequence, and (2) the observation that progesterone concentrations were higher in pregnant than in non-pregnant mares on Day 5 after ovulation, regardless of treatment.

**Significance:**
Progesterone supplementation, already used by some practitioners for postponement of ovulation, should be actively discouraged. In addition to being ineffective, it depresses pregnancy rates. The mechanism of disruption of fertility is not mediated by impaired function of the corpus luteum. CIDRs will maintain progesterone concentrations of greater than 3 ng/ml for at least 2 days in horses. Horses tolerate CIDRs well.

**Publications:**

**Listing of grant applications and their status resulting from Zweig funding:**
APPENDIX C

SUMMARY OF 2012 EXPENDITURES

2012 Research Awards $437,567
2013 Public Relations and Administrative Budget $25,600
2012 Incentive Awards $10,000

Total Expenditures: $473,167
# APPENDIX D
## 2013 Harry M. Zweig Memorial Fund for Equine Research Awards

<table>
<thead>
<tr>
<th>CONTINUATION</th>
<th>ANNUAL AWARD</th>
</tr>
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<tr>
<td>Lisa Fortier: Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue (Year 2)</td>
<td>$50,302</td>
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<tr>
<td>Tracy Stokol: The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection (Year 2)</td>
<td>$69,003</td>
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<tr>
<td>Bettina Wagner: A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (Year 2)</td>
<td>$68,875</td>
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Sub-Total: $188,180

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<tr>
<th>NEW</th>
<th>ANNUAL AWARD</th>
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<tr>
<td>Dorothy Ainsworth: Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN) (1 Year award)</td>
<td>$45,828</td>
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<tr>
<td>Douglas Antczak: T-Cell Mediated Immunity and Vaccine Development in Horses (2 Year award)</td>
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<tr>
<td>Lisa Fortier: Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging (1 Year award)</td>
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<td>Thomas Divers: Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease) (2 Year award)</td>
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<td>Norman Ducharme: An Exploratory Study into the Practical Application of a Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway (1 Year award)</td>
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<td>Alan Nixon: Osteoarthritis Control Through Combinatorial Stem Cell Therapy (1 Year award)</td>
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<td>Bettina Wagner: Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naive Horses&quot; (2 Year award)</td>
<td>$85,030</td>
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Sub-Total: $471,394

TOTAL: $659,574
APPENDIX E

POSTER SESSION PRESENTATIONS
November 14, 2012
Cornell University College of Veterinary Medicine
Equine researchers present to Zweig Fund committee

Faculty, graduate students, and researchers presented on the College's many equine-related research projects in a series of posters and lectures on November 14, 2012, at the Veterinary Education Center. Members of the committee administering the Harry M. Zweig Memorial Fund for Equine Research attended the event to see the success of currently funded projects and to learn about new research going on at the College.

Speakers included Lisa Fortier, Associate Professor, Large Animal Surgery, Department of Clinical Sciences, Bettina Wagner, Associate Professor - Harry M. Zweig Professor (2009-2011), Department of Population Medicine & Diagnostic Sciences, with Gillian Perkins, Senior Lecturer, Large Animal Internal Medicine, Department Clinical Sciences, Margaret Brosnahan, Post Graduate DVM, Department of Clinical Sciences, and Soon Hon Cheong, Assistant Professor, Section of Theriogenology -Department of Clinical Sciences, with their respective lectures on “Optimizing Biologics”, “Horses from Iceland: A Model to Study EHV-1 Specific Immunity and More”, “The Equine Endometrial Cup Reaction: 100 Years of Discovery”, and “Patterns of Uterine Fungal Susceptibility to Therapy.” A reception followed outside the lecture hall, where faculty, students and staff mingled with the Zweig Committee to talk about the research presented at the lecture and posters on display.

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine industry. In 1979, by amendment to the pari-mutuel racing and wagering law, the New York State legislature created the Harry M. Zweig Memorial Fund for Equine Research to promote equine research at the Cornell University College of Veterinary Medicine. The Harry M. Zweig Committee was established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians. The Fund contributes a percentage of its revenue to support a variety of equine-related research. The Fund is proud to support the Harry M. Zweig Memorial Fund for Equine Research. This first-rate research helps to provide protection and preventative planning for the equine industry, which in turn helps to ensure a healthy and positive future for the horse racing industry.

The Harry M. Zweig committee was established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians. The committee administering the fund always includes the chairman of the New York State Racing and Wagering Board or his designee, the dean of the College of Veterinary Medicine at Cornell or his designee, a member or the executive director of the Agriculture and New York State Horse Breeding Development Fund, a member or the executive director of the New York State Thoroughbred Breeding and Development Fund, and at least five New York State breeders, owners, trainers, or veterinarians in equine practice. Dean Michael Kotlikoff currently serves on the committee, representing the College and its many researchers who have received the Fund’s support for research projects advancing equine health and athleticism.
Dr. Harry M. Zweig Memorial
MARKET SHARE
Aug. 26, 2012  1:52.1  Purse: $350,000
Driver: Tim Tetrick  Trainer: Linda Toscano
Owner: Richard S. Gutnick, T L P Stable & William J. Augustine
Welcome to the 4th Annual Poster Session of the Harry M. Zweig Memorial Fund for Equine Research, highlighting equine research supported by the Fund.

The posters have been created by faculty, graduate students and residents, most of whom have been a recipient of an award from the Fund.

We would like you to enjoy looking at these posters and feel free to ask questions. We would also like to thank the presenters for taking the time to help us celebrate many years of support from the Harry M. Zweig Memorial Fund for Equine Research.

Thank you for your attendance.

Dr. Joel D. Baines, Associate Dean for Research and Graduate Education
Laura Mathews, Zweig Secretary
College of Veterinary Medicine
And
The Harry M. Zweig
Memorial Fund for
Equine Research

Poster Session
Wednesday, November 14, 2012
3:00pm-6:00pm
Veterinary Education Center
Welcome to the 4th Annual Zweig Poster Session and talks in support of equine research going on at the College, and to recognize the support of the Zweig Fund and the successful collaboration between Cornell’s College of Veterinary Medicine and the Harry M. Zweig Memorial Fund for Equine Research.

The posters have been created by faculty, graduate students and residents who have been a recipient of an award from the Fund, or are working on equine related research, and featuring speakers from Cornell faculty members.

We would like you to enjoy looking at these posters and please feel free to ask questions. We would also like to thank the presenters for taking the time to join us in celebration of the Harry M. Zweig Memorial Fund for Equine Research.

Thank you for your participation.

Dr. Joel D. Baines, Associate Dean -Research and Graduate Education

Laura A. Mathews, Secretary for the Zweig Committee
Like other domestic mammals, the horse has significant variation in body size; this is seen when one compares the very smallest breeds such as the American Miniature to draft breeds like the Percheron. Although the genetics of size is extremely complicated in naturally reproducing species such as humans, we and others have recently shown that size genetics is highly simplified in domestic mammals like dogs. With this in mind, we sought to identify patterns of genetic control that may be conserved among domestic mammals. We identified five quantitative trait loci for horse body size in a pair of genome-wide association scans using both Thoroughbreds and extreme-sized breeds. With the goal of fine-mapping these loci to identify causal variants for size, in the present work we have whole-genome sequenced two horses (American Miniature and Percheron), discovered millions of putative sequence variants, and validated a subset of them. We find that these variants are experimentally validated at a high rate, and we also find several plausible candidate variants.
Equid herpesvirus type 1 activates equine platelets

Tracy Stokol, Deborah Burnett, Wee Ming Yeo

Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, 14853.

Presenting Author: Wee Ming Yeo

Thrombosis of spinal and placental vessels results in the clinical syndromes of equid herpesvirus type 1 (EHV-1) myeloencephalopathy and abortion. The mechanisms by which EHV-1 causes thrombosis in some infected horses are unknown. Platelets are integral to the production of both normal fibrin clots and pathologic thrombi. Activated platelets release stored coagulation factors, express phosphatidylserine (PS) on their membranes, and shed membrane-derived microparticles (MP), all of which promote thrombus formation. We hypothesize that EHV-1 directly causes platelet activation. Platelet-rich plasma (PRP) was obtained by low speed centrifugation of platelet-leukocyte-rich plasma derived from 3.2% citrate-anticoagulated equine blood. PRP was incubated for 10 minutes with two EHV-1 strains (abortifacient RacL11 and neuropathogenic Ab4) at multiplicities of infection (MOI) of 1 and 5. Virus was purified from rabbit kidney (RK) cell lysates by sucrose gradient centrifugation. Thrombin (0.015 U/mL) and convulxin (0.5 ug/mL)-stimulated platelets and vehicle or mock-infected RK lysate-treated platelets were positive and negative controls, respectively. Platelet activation was assessed by membrane P-selectin (an alpha granule protein) and PS expression using flow cytometry. We found that both EHV-1 strains activated platelets in a MOI-dependent fashion, with P-selectin and PS expression occurring in an average of 44-96% and 3-28% of platelets, respectively. P-selectin expression required exogenous calcium and was inhibited by hirudin, a thrombin antagonist. Preliminary results with qPCR analysis using primers for viral glycoprotein B suggest that EHV-1 binds to platelets. Our results indicate that EHV-1 activates equine platelets, likely through direct binding. Exposure of platelets to EHV-1 in vivo may activate platelets, which could contribute to the thrombus formation that occurs in some infected horses.
Common Variable Immunodeficiency (CVID) in horses is characterized by a late-onset depletion of B cells, decreased serum IgM and IgG concentrations, and recurrent bacterial infections. The phenotype of equine CVID is similar to that of CVID-affected humans who lack B cells. In the horse patients, B cells fail to develop in the bone marrow. The index equine CVID case was diagnosed 12 years ago, and CVID accounts for approximately 10% of the samples submitted to the Equine Immunology Laboratory for immunological testing. Our objective here is to investigate the extent of B cell differentiation in the bone marrow of affected horses. Quantitative RT-PCR was used to measure mRNA expression of genes and transcription factors known to be essential for successful B cell differentiation and survival. Transcriptome sequencing was undertaken to obtain a comprehensive view of gene expression in the bone marrow. MethylSeq was performed to compare the genome-wide methylation status between CVID and healthy horses. Analysis of bone marrow mRNA expression from CVID horses revealed a significant decrease in E2A expression and a drastic decrease in PAX5 expression with both qRT-PCR and transcriptome methods. Differential methylation of the PAX5 gene body was identified by MethylSeq in bone marrow from CVID patients. In conclusion, the block in B cell hematopoiesis in the bone marrow of CVID patients occurs at the transition from pre-pro B cells to pro-B cells, and may be due to a difference in the epigenetic status of key B cell genes.
The purpose of this work was to compare PRP: Arthrex ACP Double Syringe System and high molecular weight hyaluronan or HA on expression of anabolic and catabolic genes as well as inflammatory mediators in OA cartilage and synoviocytes. The hypothesis was that both ACP and PRP would decrease catabolism and increase anabolism in comparisons to controls, but the magnitude of effect would be greater with ACP in comparison to HA. Human knee cartilage and synovial membrane was procured from patients undergoing total knee arthroplasty and ACP was generated from blood donors using the Arthrex Double Syringe System.

Cartilage and synoviocytes were treated with ACP, HA, or culture media for 96 hours. The results obtained revealed that pain relief after PRP or HA could be through a decrease in TGF-a expression, which is a mediator of acute inflammation and an activator of MMPs. PRP can also decrease pain and inflammation through an effect on MMP-13 by increasing production of hyluronan synthase. HA did not provide this benefit. There were minimal effects on cartilage matrix metabolism by both PRP or HA but matrix molecule expression can be increased or decreased depending on stage of OA.
Culture Conditions Supportive of B Cell Differentiation from Equine CD34+ Hematopoietic Stem Cells.

Ute Schwab, Rebecca Tallmadge, Mary Beth Matychak, and Julia Felippe.
Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Presenting Author: Ute Schwab, Ph.d.

The development of an in vitro system for the generation of B cells is critical for our studies on common variable immunodeficiency (CVID), a late-onset condition that impairs B cell development in the bone marrow (BM). The B cell development from hematopoietic stem cells (HSCs) is initiated by niches of BM stromal cells that supply specific factors and cytokines, and regulated by transcription factors and epigenetic modifications. We investigated how equine B cells differentiate in vitro from CD34+ HSCs in the presence or absence of MS-5 murine stromal cells. When equine CD34+ HSCs were cultured on MS-5 cells for 4 weeks, fewer (<50.7% CD19+ cells) B cells were generated than in the absence of stromal cells but in the presence of their pre-conditioned medium and cytokines (>77% CD19+ cells). The addition of different cytokine combinations and a higher number of HSCs seeded at the beginning of coculture did not result in an increase in B cell precursors. However, stromal cell pre-conditioned media with additional cytokines stimulated B lymphocyte development. Using an adhesive slide assay, monoclonal antibodies and immunocytofluorescence microscopy, we characterized the generated equine B cell precursors and distinguished B cell developmental stages. Our future experiments aim to sort the cells from each developmental stage for subsequent quantitative analysis of B cell differentiation gene expression and epigenetic studies. Our goal is to apply the same system to support B cell differentiation from HSCs harvested from horses with CVID. While rescuing their B cell development in vitro, we hope to identify the mechanism that impairs B cell production in vivo.
A Regenerative Medicine Approach to Reconstruction of the Equine Larynx

Marta Cercone, DVM. Ph.D., Bernard Grevemeyer, DVM., Dipl. ECVS, Lewis Bogdanovic, DVM., Stephen Canton, Caroline Seitz, B.S., Guy St. Jean, DVM, Dipl. ACVS, Norm G. Ducharme, DVM, Dipl. ACVS, Bryan N. Brown, Ph.D.

Présentations Author: Marta Cercone

The upper airway of horses must be able to resist large fluctuations in airway and wall pressure during exercise. Pharyngeal and laryngeal collapses at exercise are common causes of airway obstruction in horses. Treatment of those conditions is well established and relatively successful if the laryngeal cartilages are normal. However, when the epiglottic or arytenoid cartilages are deformed due to an inflammatory process, the treatments options and success are limited.

A regenerative medicine approach to reconstruction may provide the capability to stabilize pathologic laryngeal structures. Decellularization of intact laryngeal tissues has recently been described (Baiguera et al, 2011).

Hypothesis

Equine laryngeal cartilages are suitable for decellularization process to obtain extracellular matrix (ECM) scaffolds to be implanted in pathologic subjects so to improve constructive remodeling response of diseased native cartilages.

Objectives

The aims of this preliminary study were:
- to evaluate the efficacy of a decellularization approach in obtaining bioengineered scaffolds from equine laryngeal cartilages.
- to characterize the biocompatibility of the decellularized laryngeal samples in a pre-clinical evaluation model.
- to evaluate the scaffold materials as upper airway implants in an equine clinical pilot study, exploring the technical feasibility to implant decellularized cartilage in a high motion and non-sterile environment.

Materials and Methods

All animal procedures complied with the guidelines provided by the Institutional Animal Care and Use Committee.

Part I: Equine laryngeal cartilages, harvested from adult horses (3-5 years old) immediately following euthanasia, were frozen, thawed, and decellularized using the method described by Remlinger et al (2010). Following decellularization, the samples were lyophilized and sterilized. Decellularization was assessed qualitatively by histology performed on scaffolds samples (H&E stains) and quantitatively by determining DNA content by PicoGreen assay.

Part II: Samples were surgically implanted subcutaneously in the neck of ten donkeys, and harvested 4 weeks later. The animal clinical status was evaluated daily, including documentation of the length, width, and height of any postsurgical swelling around the implantation site. The histopathological appearance of the tissue as assessed by multiple stains (H&E stain, Safranin O, Verhoeff’s stain, and Masson Trichrome) was graded.

Part III: In a subsequent pilot study the decellularized scaffold material was placed into three separate sites within the larynx of one horse: the body of each arytenoid cartilage and the rostral half of the epiglottis. A decellularized porcine urinary bladder matrix (UBM) was placed to cover the surface of the scaffolds. Clinical status was evaluated daily while video laryngoscopy was performed once a week starting from day 1, to document the laryngeal function and the macroscopic appearance of the implantation sites during the healing process. Laryngeal ultrasound was performed bilaterally to assess the arytenoids cartilages.

At 28 days after surgery, implants were removed and fixed in formaldehyde 10% solution for histo-pathologic evaluation. Hematoxylin and eosin, Safranin O, and Verhoeff’s elastic stain were used to examine overall tissue structure, maintenance of glycosaminoglycan content, and elastin organization, respectively. All results were compared to native tissues as a control.
**Results**

√ Through the decellularization process the samples maintained their ultrastructural and 3-dimensional architecture but epithelial and glandular cells were removed from the laryngeal matrix and only few chondrocytes were still visible in the samples; on average there was a 88% reduction in dsDNA content.

√ The samples implanted subcutaneously in the donkey were minimally degraded by one month post implantation, no foreign body response or fibrotic encapsulation was detected.

√ The horse recovered uneventfully from surgery. Endoscopic assessment at rest revealed that the respiratory and protective functions of the laryngeal structures were preserved, despite the moderate swelling of the structures due to the initial inflammatory response. Overtime the swelling decreased and the UBM remodeled and retracted, being replaced by healthy mucosal layer.

√ On histology, the implants placed in the equine upper airway were replaced by dense connective tissue, newly growth vascular and glandular structures, and an epithelial layer covering the sites. No histologic sign of bacterial infection was detected.

**Discussion**

The results of the present study demonstrate the feasibility of a scaffold based regenerative medicine approach to reconstruction of the equine upper airway. These preliminary results of implantation are promising, however further studies investigating alternative decellularization protocols, long-term integration, and potential formation of new cartilage are needed.

**References**


To prevent the deadly neurologic form of equine herpesvirus type I (EHV-1), recombinant vaccines are necessary to circumvent viral immune evasion while preserving neuro-antigenicity. Several viral proteins are thought to play a role in circumventing the horse’s immune defenses. Mutations of three of these proteins in a complete infectious neuropathogenic EHV-1 clone were assessed for their contribution to immune modulation in ex vivo virus re-stimulated equine PBMC. An ORF1/2 (homolog of pUL56) mutant of neuropathogenic strain Ab4 produced significantly different responses in cells from naïve horses and horses with pre-existing immunity from repeated infections. Induction of Type I and II interferons as well as both pro- and anti-inflammatory cytokines were substantially reduced compared to the parental strain. Another viral clone lacking the UL49.5 protein was reduced in type I interferon induction. A mutant of infected cell protein 0 (ICP0) had varying profiles between previously exposed and naïve horses; lack of this protein was also a significant inhibitor to viral growth in vitro. These results suggest that EHV-1ΔORF1/2, alone or possibly in combination with other mutations, may provide a strong vaccine candidate.
Accumulation of TAR DNA-Binding Protein in Equine Motor Neuron Disease: A Possible Oxidative Stress Marker

Iqbal El-Assaad, Koji Yasuda; Thomas J. Divers; Hussni Mohammed. Dept. of Population Medicine and Diagnostic Sciences and Department of Clinical Sciences, College of Veterinary Medicine; Weill Cornell Medical College in Qatar, Cornell University

Presenting Authors: Korana Stipetic and Hussni Mohammed

Equine motor neuron disease (EMND) is a neurodegenerative disorder of unknown etiology affecting horses worldwide. Studies have demonstrated abnormal aggregates of Trans-Active Response DNA Binding Protein of 43 kDa (TDP-43) in the central nervous system (CNS) of several neurodegenerative conditions in humans including Amyotrophic Lateral Sclerosis (ALS). Although the role of these proteins in the pathogenesis of ALS is not fully understood, it is accepted that these proteins are ubiquinated, accumulate in the neuronal cytoplasm and hence, may play role in the risk of the disease. We carried out a study to examine whether horses afflicted with EMND express the TDP-43 in CNS. Detection of presence of TDP-43 protein in the CNS was analyzed by immunohistochemical staining using rabbit anti-human TARDBP (TDP-43) polyclonal antibody. Formalin fixed neuronal tissues from medulla, cervical, and lumbar spinal cord were harvested from EMND and from control horses. Sections were assigned randomly to TDP-43 treated or rabbit anti-IgG as control.

Nuclear staining of TDP-43 was detected in one of the neural tissues of 75% of EMND-positive and 0 of 0% of control horses in the central nervous system (medulla, and/or cervical spinal cord and/or lumbar spinal cord). TDP-43 antibody was detected in the nucleus of EMND horses and no cytoplasmic staining was noted. As in ALS, there was no pattern of age clustering associated with the detection of TDP-43.

This is the first report on the staining of TDP-43 in neuronal tissues of horses and suggests that TDP-43 may play a role in the pathogenesis of EMND. Identification of a marker(s) that is commonly found in neurodegenerative diseases of man and animals may shed light in understanding the mechanism and treatment of these diseases.
APPENDIX F

ZWEIG NEWS CAPSULES
Keeping blood free to flow

Even in their mothers’ wombs unborn foals need proper blood flow to survive and grow. A clot cutting off blood to the wrong place can spell disaster or death.

That’s exactly what happens when the infectious disease equid herpes virus-1 (EHV-1) causes abortions and adult neurological disease. Infected horses can form clots in blood vessels feeding the placenta or spinal cord.

No one yet knows why horses with EHV-1 get clots, but clinical pathologist Dr. Tracy Stokol plans to find out by investigating platelets as potential culprits. Platelets are involved in normal blood clotting, which stops bleeding after an injury. Following injuries, platelets start attaching to blood vessels, become activated, and stick together, helping a clot to form. But this same process that closes off wounds to stop blood flowing where it shouldn’t can also form clots that stop blood from flowing where it should.

“My theory is that EHV-1 is somehow activating platelets to start forming clots and encouraging them to grow,” said Dr. Stokol. “The question is how: is it through direct contact between the virus and platelets, indirect contact with virus-infected cells releasing fragments that turn platelets on, or some combination?”

Using flow cytometry, Dr. Stokol’s lab tested whether certain neurologic and abortion-causing strains of EHV-1 directly bind to and activate equine platelets. Preliminary data suggest they do. Yet questions remain: how does EHV-1 activate platelets? Can we prevent this from happening? Can cells infected with EHV-1 activate platelets that haven’t been exposed to the virus?

A technique novel to veterinary medicine will help Dr. Stokol and postdoctoral associate Dr. Wee Ming Yeo determine whether platelets are activated by EHV-1-infected cells that make up the inner lining of blood vessels (endothelial cells). Using a microfluidic device her lab made in 2010 with help from bioengineer Dr. Michael Shuler, Dr. Stokol made a life-sized model (0.1mm thick) that mimics equine endothelium using living horse cells. By infecting the model cells with EHV-1 and infusing platelets over the cells, her lab can watch the platelets’ interactions with infected endothelium in real-time using digital video microscopy, then analyze the recordings.

“This device lets us examine what’s happening in a life-like environment,” said Dr. Stokol. “If we can show platelets are the missing link bridging EHV-1 infections to the clots that cause EHV-1-related abortions and neuropathy, we’ll have found a new target for therapies. There are several commercially available platelet inhibitors, such as clopidogrel and aspirin, that could easily be tested for their ability to prevent platelet activation after EHV-1 infection. If effective, these medicines could potentially help change the outlook for infected horses and their young.”

Funded by the Zweig Memorial Fund for Equine Research
Long-time Zweig member Dr. John Jagar ’74 retires

After a fruitful career as an equine veterinarian, Dr. John Jagar ’74 is retiring in Fall 2012 to Beaufort, S.C. Dr. Jagar joined the Harry M. Zweig Memorial Fund Committee in 1994 and has been one of its longest-standing members.

“The Zweig Committee has held a special place for me,” said Dr. Jagar. “It is humbling to realize that, in a small way, I aided the process that provides significant funding to so many brilliant people at Cornell in equine research. I am in awe that Dr. Zweig had the foresight to establish this fund and of the benefits it continues to accrue for the horse and mankind.”

Originally from Atlanta, Ga., Dr. Jagar first came to N.Y. to earn a DVM from Cornell’s College of Veterinary Medicine. He earned a master’s degree in large-animal surgery at Auburn University, and later returned to N.Y. in 1980 as a resident veterinarian at a breeding farm. In 1984, he and two partners opened the Millbrook Equine Veterinary Clinic, P.C. in the Hudson Valley, hub of N.Y.’s horse breeding industry, where he has practiced since.

At its annual November meeting, the Zweig Committee acknowledged Dr. Jagar’s many talents and contributions to the committee and the fund, applauding his dedication, expertise, and commitment, and adding that he would be sorely missed.

New N.Y. Breeding Fund Executive Director

Mr. Mike Mullaney will take the reins as Executive Director of the Agriculture and New York State Horse Breeding Development Fund, having been appointed unanimously by the fund’s board in September 2012.

His duties will include promoting the breeding of Standardbred horses bred in New York; helping organize the New York Sire Stakes harness racing program; and overseeing administration of the Excelsior, State Fair, and County Fair harness races. He will also coordinate distribution of the Fund’s contributions to the Harry M. Zweig Fund for Equine Research, New York’s 4-H program, and county agricultural societies.

“I’m proud to promote and enhance New York’s already-stellar Standardbred breeding and racing programs and further the Fund’s mission,” said Mr. Mullaney. “The Fund plays a vital role in supporting New York’s agricultural economy, and I am eager to do my part to help our breeding program grow even further.”

The Agriculture & New York State Horse Breeding Development Fund administers the New York Sire Stakes, the country’s oldest harness racing program. Established in 1965, the Fund receives capital from the pari-mutuel handle at the licensed tracks across N.Y. to fund purse money for harness racing in the state.

Mr. Mullaney will take the reins as Executive Director of the Agriculture and New York State Horse Breeding Development Fund, having been appointed unanimously by the fund’s board in September 2012.

Over the last year Mr. Mullaney has served as General Manager of Arizona’s Yavapai County Farm & Agriculture Association. Prior to that, he was Director of Media Relations for Gulfstream Park in Hallandale Beach, Fla., for 10 years.

“Throughout his career, Mike Mullaney has demonstrated a great ability to see the big picture and bring forth positive results,” said Darrel J. Aubertine, Commissioner of the Department of Agriculture and Markets and member of the Board of Trustees. “I congratulate him on this well-deserved appointment and look forward to working with him on behalf of New York’s agriculture and horse racing industries.”
N.Y. Equine Health Seminar 2012

Six researchers from Cornell’s College of Veterinary Medicine trekked to Saratoga Springs, N.Y. on August 18, 2012 to talk with horse breeders, owners, trainers, and other equine stakeholders from across N.Y. about advances in equine health procedures and practices. Discussions during the day-long event included overviews of clinical services Cornell can provide to the equine community, ways to make horses safer on the farm and the track, and best practices for preventing equine disease.

Talks and speakers for the seminar included:

- “Upper Airway Dynamic Diagnosis” (Dr. Norm Ducharme)
- “Stem Cells for Tendonitis” (Dr. Lisa Fortier)
- “Lyme Disease in Horses” (Dr. Bettina Wagner)
- “Equine Protozoal Myelitis: Diagnosis and Treatment” (Dr. Thomas Divers)
- “Breeding Success after a Racing Career” (Drs. Robert Gilbert and Jennifer Sones)
- “Bio-security and Strangles” (Dr. Thomas Divers)

Zweig Committee member Ms. Jean Brown coordinated the event hosted by the Harness Horse Breeders of New York State, of which she is a board member. Fellow members Mrs. Anna Zweig and her son, Brian, attended.

“Much of the Zweig-supported research contributed to the topics,” said Ms. Brown. “It was a great outreach opportunity to share information and bridge the gap between clinicians and equine-related communities in N.Y.”

This event was sponsored by The Agriculture and New York State Horse Breeding Development Fund.

Horses at home photo series

Many members of the Zweig Fund Committee enjoy equine companions at home as well as at work. The “Horses at home” photo series will run over several issues of the Zweig News Capsule featuring images of committee members with their horses.

Above: Ms. Jean Brown with Neat, her 2012 yearling.
Horses at home

Zweig Committee member Ms. Patricia Wehle tends her yearling stud colt, Timothy. They are watched by the horse's namesake: Mr. Timothy McCormick (right.) Mr. McCormick was CEO of the hospital system in Rochester that Ms. Wehle chaired for six years. She named the colt in his honor.

Above: Cornell staff and faculty joined Mrs. Anna Zweig in the Winner's Circle for the 2012 Harry M. Zweig Memorial Trot at Vernon Downs on Sunday, August 26.
Soothing fraying nerves

The Harry M. Zweig Memorial Fund committee has selected equine surgeon Dr. Jon Cheetham as the first Zweig Research Scientist, a new position for junior faculty at Cornell’s College of Veterinary Medicine who show promise and productivity in equine research.

Awarded in December 2012, the yearlong position will fund Cheetham’s research into diagnosing and treating recurrent laryngeal paralysis, commonly known as roaring. Horses with this condition have developed weakened airway nerves and muscles and cannot breathe fully during exercise. Unfound and untreated, it can quickly end a horse’s career.

Cornell’s animal hospital is currently using the fruit of Cheetam’s past roaring research to help patients. Clinics now use the trans-esophageal ultrasound, a technique Cheetham developed to evaluate the geometry of horse’s airway muscles during rest and exercise. This technique allows clinicians to find roaring in young horses early, giving them a chance to recover.

Now Cheetham has turned his focus to improving the odds for that chance. His current project focuses on reimagining the nerve graft, a technique that takes a healthy nerve from neck muscles and puts it onto a damaged nerve to stimulate neglected muscles and help them recover.

“Nerve grafting has been around awhile, but it’s slow,” said Cheetham. “Currently you directly implant a healthy nerve into atrophied muscle, but only part of the muscle heals and it takes a long time. We’re working to develop a new, faster, better nerve graft method.”

With strong preexisting data supporting the concept, Cheetham will use his time as Zweig Research Scientist to develop a new method of enhancing nerve grafts. He has started pioneering research into extracellular matrix scaffolds, an area that has not been well explored.

“Our goal is to develop a way to manipulate the micro environment at a nerve repair site to promote healing,” said Cheetham. “I’m delighted to have this opportunity from the Zweig Fund to help expand healing options in horses suffering from roaring.”
2013 Research Awards

New
$134,000 to Dr. Thomas Divers with Dr. Bud Tennant for “Etiology and Prevention of Equine Serum Hepatitis (Theiler's Disease)”

$46,412 to Dr. Lisa Fortier for “Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging”

$80,147 to Dr. Alan Nixon for “Osteoarthritis Control Through Combinatorial Stem Cell Therapy”

Revised / Renewed
$45,828 to Dr. Dorothy Ainsworth with Dr. Samantha Brooks for “Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN)”

$100,000 to Dr. Douglas Antczak with Dr. Rebecca Tallmadge and Dr. Nikolaus Osterrieder for “T-Cell Mediated Immunity and Vaccine Development in Horses”

$96,977 to Dr. Norman Ducharme with Brian Brown for “An Exploratory Study into the Practical Application of Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway”

$173,529 to Dr. Bettina Wagner with Dr. Gillian Perkins for “Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses”

Continued
$50,302 to Dr. Lisa Fortier for “Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration”

$69,003 to Dr. Tracy Stokol for “The Role of Platelets in the Pathogenesis of Equid Herpes Virus type-1 Infection”

$68,875 to Dr. Bettina Wagner for “A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates”

Zweig Memorial Trot 2013
New Dean for Research and Graduate Education

Please welcome Dr. Joel Baines as the College’s Associate Dean for Research and Graduate Education.

With the title comes new responsibilities for the James Law Professor of Virology. As the associate dean, he oversees the doctoral program at the College of Veterinary Medicine; the Veterinary Investigators’ Program for veterinary students seeking research experiences; and several advanced training programs for clinicians, including the Clinical Fellows Program. He also directs efforts to help scientists secure external funding for research.

His most ambitious goal, though, has the potential to unite the worlds of basic and applied science in a manner that will advance the health and well-being of animals and people.

“The College is in a unique position to conduct research that benefits all species,” said Baines, who is leading an effort to strengthen the clinical research programs at the College. “We are at the beginning of a new era in veterinary medicine, an era in which disease mechanisms can be used to explain the physical manifestations of the condition. This level of understanding can lead to novel therapies.”

Baines, a Professor in the Department of Microbiology and Immunology at the College, joined the department in 1993. He received a BS in Microbiology from Kansas State University in 1979 and a VMD from the University of Pennsylvania in 1983. He then received a PhD from Cornell University in 1988, where he studied the molecular virology of feline coronaviruses.

He obtained postdoctoral training at the University of Chicago, where he studied the molecular virology of herpes simplex virus replication. In addition to his duties as associate dean, Dr. Baines will continue his research program with continual funding from the National Institutes of Health since 1995.

2013 Equine Seminar

On Sunday, August 25, at 2:00pm at Vernon Downs, researchers from Cornell’s College of Veterinary Medicine will offer the following talks. Registration is at 1:30pm.

- “How to Protect Your Horse from Infectious Disease, at Home and at the Track,”
  Dr. Linda A. Mittel
- “Platelets and Herpes Virus Type-1 Infection,”
  Dr. Tracy Stokol
- “Diagnosis of Poor Performance in Racehorses”
  Dr. Jonathan Cheetham
- “Equine Hepatitis Virus Discovery and its Potential Importance to Equine Health,”
  Dr. Thomas Divers; Dr. Bud Tennant
- “Alternative Sources of Equine Mesenchymal Stem Cells”
  Dr. Gerlinde Van de Walle
Zweig Committee welcomes two new members

Dr. Janet Durso ’89

A veterinarian in Middletown, N.Y., Dr. Janet Durso joined the Zweig Committee in 2013. Her obsession with horses began when she was a little girl growing up in suburban Connecticut, where she fell in love with all things horses, working at local riding stables just to be near them.

After completing her undergraduate studies at Cornell University, she joined the polo team and remained in Ithaca to attend Cornell’s College of Veterinary Medicine and complete an internship in large animal surgery.

Durso has been an equine practitioner in the lower Hudson Valley since 1990, and started her own practice in 1994. Her husband is Ray Schnittker, a standardbred trainer, driver, and owner whose outstanding career wins include Deweycheatumnhowe (2008 Hambletonian winner), One More Laugh (2010 Meadowlands Pace winner), and Check Me Out (2012 Zweig Trot winner as well as 2011 and 2012 Trotting Filly of the Year.) They have several standardbred brood mares and stallion shares, as well as recent partnerships in some thoroughbred racehorses.

She worked as a part-time on-duty emergency veterinarian at the Meadowlands for 11 years and ran a bustling pleasure horse practice for many years, but has recently concentrated her practice on the family’s large racing stable. She is a member of the board of directors of the Goshen Historic Track and still finds time to play arena polo at Gardnertown Farms in Newburgh, N.Y.

Dr. Gabriel Cook ’92

Dr. Gabriel Cook joined the Zweig Committee in 2013. He graduated from Vassar College in 1986 and Cornell University College of Veterinary Medicine in 1992, then took an internship at the Rood and Riddle Equine Hospital in Lexington, Ky.

He completed a large animal residency at North Carolina State University in 1996 and subsequently served as a staff surgeon at a private practice in the San Francisco Bay area for two years. He is presently a partner at New England Equine Practice in Patterson, N.Y., where he has worked since 1998.

Dr. Cook’s clinical interests include medical and surgical lameness diagnosis and management; abdominal surgery; arthroscopy; laparoscopy; and critical care.
Dr. F. Richard Lesser ’81 has stepped down from the Harry M. Zweig Memorial Fund for Equine Research Committee to pursue a vocation in ordained ministry. Joining in 2004, he was a major proponent of the Zweig Fund’s mission to support research advancing the well-being of race horses. He offered expertise, heartfelt discussion, and sound judgment in helping administer the fund.

After a successful career as an equine veterinarian, Lesser retired in Fall 2012. He and his wife, Marilyn Schmidt ’78, founded the Equine Clinic at Oaken Croft in Ravena, NY. She died suddenly in 2005, and Lesser raised their three children on the family farm. In 2013 he was given the Lifetime Achievement Award by the Capital District Hunter Jumper Council, an award last presented in 2006 to Marilyn.

Lesser is dedicated to the betterment of equine practice. While managing an ambulatory and in-house equine clinic employing many veterinarians, interns, and support staff, he has also served as speaker, teacher, and advisor for equine and veterinary practices around the country. For years he engaged in the American Association of Equine Practitioners, serving on several committees and chairing the National Commission on Veterinary Economics Task Force and the Task Force on Equine Veterinary Technicians.

He spoke on equine issues to people around the country, taught equine health at SUNY Cobleskill, and chaired Veterinary Management Group VII, a group of 21 veterinary practices dedicated to bettering equine practice. He served as co-facilitator of Veterinary Management Group XX, a group of academic equine hospitals, including Cornell, dedicated to maximizing university clinics’ missions.

In May 2013 Lesser earned a Master in Divinity degree and will enter the Blessed John XXIII National Seminary as a student this Fall. We wish him well on his latest journey!
Many members of the Harry M. Zweig Fund Committee enjoy equine companions at home as well as work. This photo series runs over several Zweig News Capsule issues featuring Committee members with their horses.

Left:

Mr. Paul Kelly with “You Want Me,” a two-year-old Sire Stakes trotting filly champion for New York State in 2012.