College of
Veterinary Medicine

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October 15th, 2023

Tracy Egan
Executive Director
New York State Thoroughbred Breeding and Development Fund
One Broadway Center, Suite 601
Schenectady, NY 12305

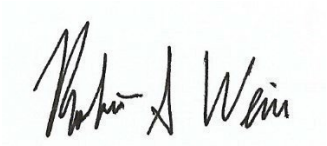
Dear Ms. Egan:

Enclosed is an electronic copy of the 2022 annual report for the Harry M. Zweig Memorial Fund for Equine Research, covering the award period of January 1, 2022 through December 31, 2022.

Included with the report are copies of the spring and fall issues of the Zweig News Capsule. Additional information about the Harry M. Zweig Memorial Fund for Equine Research can be found on the Zweig Memorial Fund public website at https://bit.ly/zweigfundcornell.

On behalf of Cornell University, we wish to extend our appreciation for your continued support of equine research.

Sincerely,



Robert S. Weiss, Ph.D. Professor of Molecular Genetics
Associate Dean for Research & Graduate Education

Cc: Lorin Warnick, D.V.M., Ph.D. '94, Austin O. Hooey Dean of Veterinary Medicine
Ms. Jill LaBoissiere, Comptroller, NYS Thoroughbred Breeding & Development Fund
Mr. Adam Lawrence, Registrar, NYS Thoroughbred Breeding & Development Fund

College of
Veterinary Medicine

&

The Harry M. Zweig Memorial Fund for Equine Research

2022 Annual Report

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SUMMARY REPORT

The 2022 Annual Report covering the period of January 1, 2022, through December 31, 2022, is provided herein.

For this reporting period, the Harry M. Zweig Memorial Fund for Equine Research Committee awarded funding for five of the five submitted projects. Two of the five projects were new, first-time submissions, one was a renewal, and two were revisions. The total amount allocated for new awards for calendar year 2022 was $356,638. This report includes the “lay summaries” for the public website (Appendix A). There were also four continuation awards approved for second year funding in the amount of $314,772, allocated at the 2021 annual meeting. The Fund further supported a resident grant submitted through the CVM Resident Research Grants Program in the amount of $9,997. The abstract is included with the lay summaries of the new awards.

The Zweig Family hosted the Annual Zweig Memorial Trot on July 7th, 2022, at the Vernon Downs Racetrack in New York. Additionally, on Wednesday, November 9, 2022, the Veterinary College hosted an in- person and Zoom seminar with scientific talks celebrating the collaboration between the Harry M. Zweig Memorial Fund for Equine Research and Cornell University College of Veterinary Medicine by showcasing faculty research to the College community and to the Zweig Committee.

The seminar can be viewed on the Zweig Virtual Presentations page.

RESEARCH AWARDS

CONTINUATIONS

Principal Investigator	Project Title	2022 Award
Felippe, Julia	Diagnostic markers in mares with placentitis	$79,352
Pigott, John	Multi-modal screening to identify Thoroughbred racehorses at increased risk for catastrophic injury of the metacarpophalangeal joint	$99,297
Todhunter, Rory	Genomics of Autopsy-Negative Sudden Cardiac Death in Racing Thoroughbreds	$49,672
Wagner, Bettina	Intranasal biomarkers of EHV-1 susceptibility and protection	$86,451
	SUBTOTAL:	**$314,772**

NEW AWARDS

Principal Investigator	Project Title	2022 Award
Antczak, Douglas	Factors Affecting Durability in Standardbred Racehorses	$65,827
Delco, Michelle	Synovial fluid extracellular vesicles in equine joint disease and therapy	$83,229
Diel de Amorim, Mariana	Inflammatory markers from endometrial swab/ cytobrush as a screening test for equine endometritis and endometrial fibrosis	$47,948
Reesink, Heidi	Equine joint sepsis and synovial fluid mucins	$67,973
Wagner, Bettina	Inflammatory biomarkers for prediction of breakdown injuries in horses	$91,661
	SUBTOTAL:	**$356,638**

NEW RESIDENT RESEARCH AWARDS

Principal Investigator	Project Title	2022 Award
Delco, Michelle	Investigation of Mitovesicles as a New Equine Orthobiologic	$9,997
		$681,407

PROGRESS IN 2022

PI	Project Title	Term Date	Report Type in Appendix B
Cheetham, Jonathan	Accelerating Recovery after Laryngeal Nerve Graft in Horses	12/31/22	Final
Delco, Michelle	Investigation of Mitovesicles as a new Equine Orthobiologic	06/30/23 NCE	Progress
Delco, Michelle	Synovial fluid extracellular vesicles in equine joint disease and therapy (Year 2)	12/31/22	Final
Diel de Amorim, Mariana	Inflammatory markers from endometrial swab/cytobrush as a screening test for equine endometritis and endometrial fibrosis	12/31/23 NCE	Progress
Felippe, Julia	Diagnostic markers in mares with placentitis	12/31/23 NCE	Progress
Perkins, Gillian	Equine gamma herpesviruses and equine gastric ulcer syndrome (EGUS) – is there a link?	6/30/22	Final
Pigott, John	Multi-modal screening to identify Thoroughbred racehorses at increased risk for catastrophic injury of the metacarpophalangeal joint	12/31/23 NCE	Progress
Reesink, Heidi	Unraveling lubricin signaling in equine joint injury	12/31/22	Final
Todhunter, Rory	Genomics of Autopsy–Negative Sudden Cardiac Death in Racing Thoroughbreds	12/31/23 NCE	Progress
Wagner, Bettina	Intranasal biomarkers of EHV-1 susceptibility and protection	12/31/23 NCE	Progress

* NCE = No Cost Extension

EXTERNAL FUNDING

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.

Douglas Antczak, June 2022 - $1,792
Zweig Award: 2020 Horse Genome Horse Genome Project Workshop at Cornell
($7,000 1/1/2020 – 12/31/2021)
USDA Conference Grant: 2022 International Horse Genome Project Workshop
($17,920 5/1/2022 – 9/30/2022)

PUBLICATIONS

Jager MC, Tomlinson JE, Henry CE, Fahey MJ, Van de Walle GR. Prevalence and pathology of equine parvovirus-hepatitis in racehorses from New York racetracks. Virol J. 2022 Nov 1; 19(1):175. doi: 10.1186/s12985-022-01901-3. PMID: 36320007; PMCID: PMC9628030.

Luedke LK, Ilevbare P, Noordwijk KJ, Palomino PM, McDonough SP, Palmer SE, Basran PS, Donnelly E, Reesink HL. Proximal sesamoid bone microdamage is localized to articular subchondral regions in Thoroughbred racehorses, with similar fracture toughness between fracture and controls. Vet Surg. 2022 Aug; 51(6):952-962. doi: 10.1111/vsu.13816. Epub 2022 Jun 7. PMID: 35672916.

Miller JL, Kanke M, Rauner G, Bakhle KM, Sethupathy P, Van de Walle GR. Comparative Analysis of microRNAs that Stratify in vitro Mammary stem and Progenitor Activity Reveals Functionality of Human miR-92b-3p. J Mammary Gland Biol Neoplasia. 2022 Dec; 27(3-4):253-269. doi: 10.1007/s10911-022-09525-7. Epub 2022 Oct 3. PMID: 36190643.

Rojas-Núñez I, Gomez AM, Selland EK, Oduol T, Wolf S, Palmer S, Mohammed HO. Levels of Serum Phosphorylated Neurofilament Heavy Subunit in Clinically Healthy Standardbred Horses. J Equine Vet Sci. 2022 Mar; 110:103861. doi: 10.1016/j.jevs.2021.103861. Epub 2021 Dec 31. PMID: 34979262.

Thomas MA, Fahey MJ, Pugliese BR, Irwin RM, Antonyak MA, Delco ML. Human mesenchymal stromal cells release functional mitochondria in extracellular vesicles. Front Bioeng Biotechnol. 2022 Aug 19; 10:870193. doi: 10.3389/fbioe.2022.870193. PMID: 36082164; PMCID: PMC9446449.

Van de Walle, G. The Potential of the Mesenchymal Stromal Cell Secretome in Equine Regenerative Medicine. 2022 Apr. In Tissue Engineering Part A; 28:S7-S7. 140 Huguenot Street, 3rd Fl, New Rochelle, NY 10801 USA: Mary Ann Liebert, Inc.

Wang Z, Chivu AG, Choate LA, Rice EJ, Miller DC, Chu T, Chou SP, Kingsley NB, Petersen JL, Finno CJ, Bellone RR, Antczak DF, Lis JT, Danko CG. Prediction of histone post-translational modification patterns based on nascent transcription data. Nat Genet. 2022 Mar;54(3):295-305. doi: 10.1038/s41588-022-01026-x. Epub 2022 Mar 10. PMID: 35273399; PMCID: PMC9444190.

PATENTS

There were 0 applications filed and 0 patents issued related to Zweig Memorial Fund funding for calendar year 2022.

Michelle Delco ’98, D.V.M. ’02, Ph.D. ’16
Harry M. Zweig Assistant Research Professor in Equine Health
2022-2024

Michelle Delco has been named the Harry M. Zweig Assistant Professor in Equine Health In recognition of her dedication to extending the healthy lifespan of horses and improving their quality of life.

The professorship is a three-year, endowed position for a junior faculty member who shows great promise for advancing equine research. It can be instrumental in helping junior faculty secure funding and develop high-level publications necessary for long-term success. Delco has received grants previously from the Zweig Memorial Fund to support individual research projects. She has also received support from the NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases, the American College of Veterinary Surgeons, Cornell Stem Cell Program, EndoCellutions and Orthopaedic Research Society.

In recognition of her dedication to extending the healthy lifespan of horses and improving their quality of life, Michelle Delco ’98, D.V.M. ’02, Ph.D. ’16, “Dr. Delco is an accomplished and highly valued member of our college community,” said Dr. Susan Fubini, senior associate dean for academic affairs and professor in large animal surgery. “Her research program is on a tremendous upward trajectory, and its focus on equine joint disease and osteoarthritis aligns well with the objectives of the Zweig Memorial Fund.”

Delco is a board-certified large animal surgeon and assistant research professor in the Department of Clinical Sciences whose time spent in clinical practice treating equine athletes for sports injuries has motivated her to discover new ways to treat and prevent osteoarthritis.

“We’ve domesticated this amazing species. Horses have worked alongside humans since the beginning of civilization,” Delco said. “It’s our job to keep them sound and healthy.”

After receiving her bachelor’s and D.V.M. degrees from Cornell, Delco completed internship training at Rood & Riddle Equine Hospital in Lexington, Kentucky, followed by residency training at the University of California at Davis. She then served in a faculty position at Kansas State University before working for a number of years in private practice in the Pacific Northwest.

“I did a lot of work on sport horses with complicated lameness issues,” Delco said. “I got more and more frustrated diagnosing career-ending orthopedic injuries without effective treatment options to offer clients and their horses.”

This spurred her return to Cornell in 2012, where she studied post-traumatic arthritis in the lab of Lisa Fortier, Ph.D. ’98, the James Law Professor of Surgery. Dr. Robert Weiss, associate dean for research and graduate education, noted “With superb training as a veterinary clinician scientist, Dr. Delco is extremely well-positioned to conduct rigorous, cutting-edge biomedical research and then translate those findings for the benefit of animal health.”

“I wanted to know what we were missing,” Delco says. “Why weren’t we making progress treating this disease?” She consequently did her Ph.D. research on the function of mitochondria, the energy

generating powerhouses of cells, and their links to joint injury. Now a faculty member at Cornell with a lab of her own, Delco has developed an innovative niche in the area of mitochondrial biology within the fields of osteoarthritis and regenerative medicine. In particular, she is exploring new research questions that build on the concepts of mitochondrial dysfunction as a driver of osteoarthritis and enhancement of mitochondrial function as a new therapeutic strategy.

Delco's goal is to prevent chronic joint pain and dysfunction in both horses and humans. "For decades, lifespan has been steadily increasing — largely thanks to scientific discoveries in human and veterinary medicine," Delco said. She notes, however, that health span — the number of high-quality years lived — has not similarly increased. "We're developing new approaches to stop joint degeneration after injury. Whereas arthritis in human athletes can be career-ending and painful, for equine athletes, it can be life-threatening. Our newest research (funded by Zweig) goes beyond prevention; our goal is to develop new regenerative therapies. Orthopedic tissues heal very poorly after injury, in part because they lack blood supply to provide nutrients and oxygen. By focusing on mitochondrial function, we hope to enhance the energy these tissues have available for repair and healing".

Delco is also keen to bring this scholarly expertise to her role as a surgeon at the Cornell Equine Hospital, where she works on the orthopedic surgery service. She's especially interested in minimally invasive surgery techniques like arthroscopy in standing horses. In both the hospital and her research lab, Delco collaborates with a team of skilled surgical residents, students, technicians and researchers. "Beyond her many research accomplishments, Dr. Delco also is active in teaching and mentoring, and her passion for helping to develop young scientists is an asset to all of us," Fubini said. "There's such a richness that comes from a diverse group of people working together," Delco said. "For example, my research group is a collection of talented and engaged people, all at different stages of their training, who are excited about science and progress. They inspire me. I feel lucky to work with such a great team."

(Cordova)

CORNELL CLINICAL FELLOW IN EQUINE HEALTH

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 first Clinical Fellow, followed by Dr. Sarah Pownder, and more recently Dr. Joy Thomlinson. Supported in part by Zweig funds, all have been highly successful. 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.

There was no Clinical Fellow appointed for 2022.

APPENDIX A
Lay Summaries for New Awards

Antczak, Doug	Factors Affecting Durability in Standardbred Racehorses
Delco, Michelle	Investigation of Mitovesicles as a New Equine Orthobiologic (Resident Research Grant)
Delco, Michelle	Synovial fluid extracellular vesicles in equine joint disease and therapy
Diel de Amorim, Mariana	Inflammatory markers from endometrial swab/cytobrush as a screening test for equine endometritis and endometrial fibrosis
Reesink, Heidi	Equine joint sepsis and synovial fluid mucins
Wagner, Bettina	Inflammatory biomarkers for prediction of breakdown injuries in horses

Principal Investigator:	Dr. Doug Antczak
Title:	Factors Affecting Durability in Standardbred Racehorses
Project Period:	1/1/22 – 12/31/23

LAY SUMMARY

Introduction: Durability in racehorses is a consequence of many non-hereditary factors and hereditary factors. Hereditary factors may include psychological factors such as enthusiasm for training, competitive nature and physical factors related to soundness. Catastrophic injuries during racing or in training are genuine tragedies for the racehorses, riders or drivers, trainers, and owners involved. Previous investigations have implicated many factors that may contribute to such injuries, including fitness, training regimes, track surfaces, prior health status of the horses involved, use of illegal drugs, and genetics. With so many variables and relatively few affected horses, it has been difficult to dissect the genetic component from environmental factors. Here we would focus on the other end of the spectrum and examine racehorses that have demonstrated durability and longevity in their careers. In this revised Zweig proposal we would focus on Standardbreds. This breed is important in New York state. Data from the NYS Gaming Commission database shows that approximately 30% of the racing associated horse fatalities over the past five years in New York were in Standardbreds. On the other hand, there are also many Standardbred horses, primarily geldings, that have long and successful racing careers that extend up to 10 years of age.

Hypothesis: We propose that horses with extended successful racing careers have a sound genetic foundation that is coupled with excellent care and training. We **hypothesize** that it will be possible to identify genetic signatures correlated with longevity of racing career / durability. Recently published data from other investigators support this hypothesis.

Specific Aims

We would use genome wide technology to test groups of durable Standardbreds with long and successful racing careers and compare them with control Standardbreds selected from the general breed population. The goal is to determine if there are genetic regions associated with durability and longevity.

Aim 1) Using a Genome Wide Association Study (GWAS) and the 670k Equine Single Nucleotide Polymorphism (SNP) array, we would compare three groups of horses:

1) 100 aged Standardbreds with long racing careers and competitive race records;
2) 100 aged Standardbreds with long racing careers but relatively weak earnings records;
3) 100 or more control Standardbreds selected from previous studies.

Aim 2) We would obtain 20 x coverage whole genome sequence from 10 selected durable and 10 control Standardbreds. This will enable fine-scale comparison of genomic regions identified in Aim 1

We have assembled a strong interdisciplinary team of investigators from Cornell and other universities, and we have secured commitments of support and cooperation from the US Trotting Association.

Relevance to equine health and the racing industry: Understanding the basis for durability in racehorses is important, whether that outcome is determined primarily by genetics or environment (early husbandry, nutrition, training regimes, veterinary care, etc.). Currently, there is very little information about why some horses display an “Iron Horse” phenotype. This study will contribute to knowledge of horses that have long and successful racing careers. That information may be important in the management of all racehorses and thus contribute to equine welfare.

Principal Investigator:	Dr. Michelle L. Delco (Resident Research Grant)
Title:	Investigation of Mitovesicles as a New Equine Orthobiologic
Project Period:	1/1/22 – 12/31/22

ABSTRACT

Osteoarthritis (OA) is a major cause of equine lameness, which affects the athletic capacity and welfare of horses across breeds and disciplines. There are no therapies available to the equine practitioner to manipulate the joint environment to slow the destructive process of OA. Though regenerative therapies including mesenchymal stromal cells (MSCs) are often employed with the intent of enhancing the inherent poor healing capacity of articular cartilage, the mechanisms by which MSCs improve clinical symptoms have yet to be elucidated. Further, disadvantages include regulatory burdens, cost, and time delay associated with MSC therapy. Therefore, alternative cell-free regenerative therapies are currently being explored, including extracellular vesicles (EVs).

EVs are small membrane-bound particles that facilitate cell-cell communication. Derived from many cell types, EVs contain diverse cargos, including whole organelles. Recent evidence suggests that intact mitochondria can be transferred from MSCs to injured cells by EVs, or mitovesicles (mitoEVs). This intercellular mitochondrial transfer has been shown to improve recipient cell survival and healing in other tissues but has not been investigated in cartilage. Our group has successfully isolated and characterized mitoEVs produced by MSCs. Furthermore, we have shown that MSCs can donate healthy mitochondria to injured chondrocytes. However, the effects of isolated mitoEVs on chondrocytes have not yet been studied. We hypothesize that isolated mitoEVs will be taken up by stressed chondrocytes, improving their respiratory capacity. Furthermore, we hypothesize that mitoEVs are abundant in equine blood products, which would provide a culture-free source of mitoEVs.

Therefore, our broad objective is to investigate mitoEVs as a potential cell-free and culture-free regenerative therapy. Our specific project aims are 1) to investigate the effects of mitoEVs on chondrocytes and 2) to investigate non-cultured sources of mitoEVs.

We will collect blood and bone marrow from healthy adult horses to obtain plasma, platelet rich plasma (PRP), bone marrow aspirate concentrate (BMAC), and the cell-conditioned media (CCM) of cultured MSCs. Using filtration, mitoEVs will be isolated from each source. To investigate the effect of mitoEVs on chondrocytes (Aim 1) we will culture equine chondrocytes in a validated OA model and treat them with mitoEVs harvested from CCM. Microrespirometry will be used to assess the ability of mitoEVs to rescue chrondrocyte mitochondrial function. To investigate non-cultured sources of mitoEVs (Aim 2), we will use flow cytometry to characterize and compare the subpopulations of mitoEVs obtained from non-cultured blood products (plasma, PRP, BMAC), and further, to compare these to subpopulations from cell-culture (CCM).

Principal Investigator:	Dr. Michelle L. Delco
Title:	Synovial fluid extracellular vesicles in equine joint disease and therapy
Project Period:	1/1/22 – 12/31/22

LAY SUMMARY

Background: Cartilage provides near-frictionless joint surfaces and cushioning to protect underlying bone. Even mild cartilage damage can impair its ability to dissipate loads, exposing the underlying bone to repeated micro-trauma, which can ultimately lead to fracture. Further, cartilage has little or no ability to heal after injury. Therefore, "regenerative therapies", including mesenchymal stromal cells (also known as stem cells or MSCs) are commonly used to promote cartilage healing after joint trauma in equine and human athletes. However, there are important drawbacks to using cells treatments, including the time and expense required to grow stem cells in the lab, adverse reactions, and the lack of uniformity within a pool of cells, which may lead to unpredictable results. Therefore, cell-free regenerative therapies, including extracellular vesicles (EVs) are gaining attention in human orthopedic medicine, but have not yet been investigated for horses.

EVs are small, spherical structures that are released from many cell types. EVs are wrapped in cell membrane, and contain diverse cargoes, which are loaded for export by their cells of origin. These cargos include proteins, DNA, and even whole mitochondria (so-called 'mitovesicles'). EV are messengers; they play a critical role in cell-cell communication. Cargos are delivered to, then taken up by target cells, affecting a wide array of functions in the recipient cell. The role of EVs in disease processes has long been recognized. More recently, stem cell-derived EVs have been investigated for their anti-inflammatory and pro-healing properties in human medicine. However little information is available regarding EVs in veterinary medicine. Furthermore, mitovesicles have not been investigated as possible regenerative orthobiologics.

Recent work by our group revealed that mitochondrial dysfunction is one of the very earliest responses of cartilage to overloading. Mitochondria are best known as the "powerhouses" of cells, because these organelles produce the energy required for normal tissue function and repair. Targeted therapies that improve mitochondrial function may be the key to stimulating repair mechanisms in poorly-healing tissues like cartilage. Our group also made the exciting discovery that MSCs can rescue injured cartilage cells by donating healthy mitochondria. Our findings also indicate that stem cells may deliver these life-saving mitochondria inside mitovesicles (mtEVs).

Our goal is to is to perform foundational investigations of **mitochondria-containing extracellular vesicles (mitovesicles, mtEVs)** as a new potential regenerative joint therapy in horses. We are also interested in examining EVs released into joint fluid after joint injury.

Hypothesis: Our overarching **hypotheses** are that EVs containing healthy mitochondria, such as those derived from stem cells, can rescue injured cells and promote tissue repair. Furthermore, EVs containing dysfunctional mitochondria are released into synovial fluid after joint injury, representing a potential early marker of disease. Finally, unhealthy mitovesicles can enhance cartilage repair mechanisms by stimulating the release of healthy mitovesicles by MSCs.

Strategy: In **Aim 1,** we will determine if mtEVs isolated from MSCs can improve mitochondrial function, prevent cell death and stimulate pro-healing mechanisms in injured chondrocytes. In **Aim 2**, we will characterize EVs from joint fluid of horses with and without injury, to determine if there is a difference in function of mitochondrial cargo within mtEVs between healthy and injured joints. Finally, we will

investigate if there is crosstalk between these two processes; we will determine if dysfunctional mtEVs can enhance healthy mtEV release by MSCs.

Relevance to equine health and racing: Understanding the role of mtEVs in the events after joint injury may lead to new diagnostic tests and help identify horses requiring targeted therapy or modified training programs. These studies will also provide a foundation for developing MSC mtEVs as a new cell-free regenerative therapy in horses. These studies will provide data in direct support of a larger NIH grant proposal.

Principal Investigator:	Dr. Mariana Diel de Amorim
Title:	Inflammatory markers from endometrial swab/cytobrush as a screening test for equine endometritis and endometrial fibrosis
Project Period:	1/1/22-12/31/22

LAY SUMMARY

Inflammation and fibrosis of the uterine lining (endometrium) is the most common cause of infertility in the mare. This inflammation of the uterine lining is known as endometritis. Equine endometritis is a prevalent condition in equine practice and has been rated one of the three most common conditions that the horse veterinarian encounters in daily practice.

Endometritis often poses as a challenge to the practitioner, as many mares that have a subclinical or a chronic form of the disease commonly will not have any clinical signs or detectable abnormalities. But, despite good breeding practices, those mares fail to get pregnant. Common diagnostic tests for endometritis include endometrial cytology, culture, and normally left as a last resort, an endometrial biopsy. Non-invasive diagnostic tests such as uterine cytology and culture are performed when mares are in heat prior to breeding as screening tools or in suspected cases. However, they lack sensitivity. Endometrial cytology and culture are commonly performed through uterine swab or cytobrush since those are easier and faster to perform in a field setting. However, a subset of mares that are suspected to have subclinical disease, or that are considered “problem mares” due to poor pregnancy outcome or other subtle abnormalities that the veterinarian may have identified during a routine reproductive examination, may benefit from a low-volume uterine lavage (LVL) as a form to obtain sample for cytology and culture. Low-volume lavage is more sensitive than endometrial swab and cytobrush, but it is more cumbersome as the uterus needs to be infused with sterile fluid through a catheter, and the uterine fluid that is recovered needs to be processed in the lab by centrifugation so the sediment of the fluid can then be used for a cytology and culture. Regardless of the method utilized, endometrial cytology and culture sensitivity range between 17 to 33% when compared to endometrial biopsy, the gold standard, but increases slightly to 42% when both tests are performed in combination.

The interpretation of the inflammation (cut off for number of neutrophils) on endometrial cytology may vary according to studies and clinician experience, making it difficult for equine practitioners to have a good objective screening test to make timely decisions on whether to breed or to potentially treat the mare in that estrous cycle. Furthermore, chronic inflammation and fibrosis can only be detected through an endometrial biopsy as chronic inflammation is normally detected in the deeper layer of the endometrium that the endometrial swab and cytobrush are unable to get. Endometrial biopsy is invasive, is not always an option to obtain in the same breeding cycle, is more costly, and has a long turnaround time; but the endometrial biopsy gives a prognosis of a mare to carry a foal to term (Kenney and Doig system)[1] as it is not only able to detect acute and chronic inflammation but also degree of fibrosis and tissue degeneration. This diagnostic test is used as the last resort after the mare has failed to get pregnant for several cycles. Therefore, fast, reliable and less invasive methods to detect mares with fibrosis (worse endometrial biopsy grading) and with acute and/or chronic inflammation is desirable by the practitioners and will have significant economic impact for the equine industry.

Pro- and anti-inflammatory molecules regulate the amount of inflammation and will help coordinate a response against an infection or pathogen. Many inflammatory cytokines have been previously investigated in mares with endometritis or in studies that compared mares classified as susceptible or resistant to endometritis based on experimental models of how they respond to infusions of bacterial or dead sperm in the uterus. Even though such studies have given insight into the pathophysiology of endometritis, most have focused on the gene expression levels and have used experimental models.

Few studies investigated cytokine protein concentrations, and those studies have used less accurate molecular techniques or non-horse specific antibodies. Hence, data and repeatability varied among studies.

A novel group of inflammatory markers in horses include C-C motif chemokine ligands (CCL) CCL2, CCL3, CCL5, and CCL11 along with TNF-α, IFN-γ, IL-1β, IL-4, IL-10, IL-17A, and soluble CD14 (sCD14)16,17,28. Those inflammatory markers have been validated in different equine diseases, such as neonatal septicemia, recurrent airway obstruction, and horses infected with equine herpes virus by the Wagner Lab. Chemokines, TNF- α and IL-1 β act as pro inflammatory molecules and immune activators, whereas IL-10 is an anti-inflammatory cytokine. IFN-γ, IL-4 and IL-17A play important regulatory roles in both the innate and adaptive immune system, and CCL2 was shown to be increased in mare's biopsy post uterine inoculation of E.coli. Besides the aforementioned equine diseases, our research group has investigated this novel group of inflammatory markers in the low-volume uterine lavage of healthy mares, and mares with acute and chronic endometritis in a naturally occurring disease. We identified 4 out of those 11 inflammatory markers to be elevated in mares with chronic endometritis. These inflammatory molecules present the potential of becoming additional diagnostic markers for endometritis. However, the low-volume lavage (250 ml) may have a dilution effect on some of the inflammatory markers and it requires more time to collect and process the samples. Therefore, our broad objective is to identify diagnostic markers for both acute and chronic inflammation in the uterine samples of mares through fast and minimally invasive uterine sampling.

Principal Investigator:	Dr. Heidi Reesink
Title:	Equine joint sepsis and synovial fluid mucins
Project Period:	1/1/22-12/31/23

LAY SUMMARY

THE RESEARCH PROBLEM

Equine Synovial Sepsis (Joint Infection)

Synovial sepsis most commonly affects joints, but these infections can also involve other synovial fluid-filled structures, including tendon sheaths and bursae. Joint and other synovial fluid infections are challenging to treat all species; however, synovial sepsis carries a poorer prognosis in horses as compared to humans and many other domestic veterinary species. The reduced prognosis for resolving joint infections in horses can be attributed to several factors, including the necessity for most horses to be sound as performance animals, the propensity for horses to develop support limb laminitis and other complications associated with protracted lameness, and economic considerations. In addition, successful therapy can be complicated by the inability to culture bacteria from synovial fluid in order to guide appropriate antibiotic selection.

Antibiotic Resistant and Multi-Drug Resistant (MDR) Bacteria

In humans and other domestic veterinary species, rates of antimicrobial resistance and multi-drug resistance (MDR) are increasing, and the armamentarium of antibiotics available to veterinarians is becoming more restrictive in an attempt to preserve antibiotic efficacy for difficult-to-treat human infections. In a study evaluating antibiotic resistance in equine fecal *Escherichia coli* (*E. coli*) isolates from northern England, *E. coli* of equine fecal origin were commonly resistant to antibiotics used in human and veterinary medicine, and rates of MDR were significantly higher in hospital samples as compared to livery samples (48% of hospital isolates; 12% of livery isolates)[1]. Two recent studies have reported on antimicrobial susceptibility patterns of bacterial isolates cultured from equine synovial fluid samples with suspected synovitis in Canada[2] and the United Kingdom (UK)[3]; however, analyses were restricted to geographic regions with unique antimicrobial prescription practices and antimicrobial stewardship policies as compared to the US. The most recent report on the epidemiology of equine synovial sepsis in the US was published in 1992[4] and, although bacterial isolates were reported, data on antimicrobial susceptibility patterns was not provided. Because of the potentially life- threatening nature of equine joint infections and the challenges associated with identifying the causative organism and antibiotic susceptibility, reserved antibiotic classes are more commonly used to treat joint and orthopedic infections in horses in the United States. The UK was one of the earliest countries to adopt antimicrobial stewardship policies for equine practitioners to encourage responsible antimicrobial usage, and the British Equine Veterinary Association has published guidelines for responsible antimicrobial use in horses[5]. Antimicrobial stewardship involves the judicious use of antimicrobials balanced against the requirement to treat the presenting clinical condition[6], and the concept of antimicrobial stewardship in equine practice has been gaining traction in other countries[7]. Rather than eliminating certain classes of antimicrobials for use in equine practice all together, the availability of faster and more accurate techniques for identification and sensitivity testing of bacterial pathogens could be an alternative strategy to promote antimicrobial stewardship. Antibiograms are an essential component of antimicrobial stewardship and should ideally be used to guide empiric antimicrobial used decisions. In addition, the development of novel agents to combat bacterial infection is a critical need in both human and veterinary medicine, underscoring the importance of a fundamental mechanistic understanding of bacterial pathogens and host defenses in synovial sepsis.

Challenge: Culturing Bacteria from Synovial Fluid and Choosing an Appropriate Antibiotic

In an ideal scenario, veterinary practitioners would be able to culture bacteria and obtain antimicrobial susceptibility information to guide proper antibiotic selection in every case of synovial sepsis. However, it can be quite challenging to culture bacteria from synovial fluid as compared to other body fluids, with the equine literature reporting the prevalence of positive cultures ranging from 25% (96/379) up to 79%

(71/90) using specialized enrichment protocols4,8. Culture-positive rates for the two most recent studies evaluating equine synovial sepsis were only 31% (114/379) and 49% (34/70)2,3. One explanation for the low positive culture rates is that synovial fluid has intrinsic antimicrobial properties, which have been attributed to constituent molecules, including proteins, hyaluronic acid and other small peptide molecules, though no consensus exists. A 2010 study reported an increased likelihood of positive culture when equine synovial fluid samples were submitted in blood culture 4 flasks with automated enrichment as compared to conventional culture media8. Digestion of proteins in synovial fluid prior to experimental bacterial inoculation may increase the ability to culture bacteria from synovial fluid; however, non-specific protease digestion will also kill bacteria. Mucins and mucinlike proteins are glycoproteins which undergo a special type of sugar modification—*O*-linked glycosylation—which is unique to mammals.

Because bacteria cannot produce mucin proteins with *O*-glycosylation, treatment of synovial fluid with mucin-degrading enzymes, called mucinases, will degrade synovial fluid mucins without harming bacteria. Mucins line every epithelial surface of the body, thereby protecting these surfaces from pathologic bacterial invasion. Interestingly, our data suggests that lubricin is the predominant *O*-glycosylated protein in synovial fluid, and evidence is accumulating to suggest that lubricin may serve many of the same functions in synovial fluid as mucins do in other organs. Lubricin prevents bacterial adhesion to tissue surfaces9, and our preliminary data suggests that lubricin can both impair bacterial growth and prevent formation of sticky bacterial aggregates, called biofilms, that are inherently more resistant to antibiotics. To date, no studies have systematically evaluated mucin-type proteins in synovial fluid.

Research Plan

Our studies will provide both epidemiological and mechanistic insight into equine synovial fluid sepsis, with the potential to inform clinical antibiotic selection, minimize antibiotic resistance, and develop techniques that could enhance our ability to isolate bacteria from infected synovial fluid structures. The specific goals of this proposal are two-fold: (1) to identify changes in clinical bacterial isolates, antimicrobial sensitivity patterns and positive culture rates in equine synovial fluid sepsis over the past two decades and [(2) to investigate the ability of mucins, including the mucin-like glycoprotein lubricin, to inhibit bacterial growth and biofilm formation.] Because there are no recent studies from the United States reporting on clinical bacterial isolates and antimicrobial susceptibility profiles from equine synovial fluid sepsis and because antimicrobial resistance is known to vary across geographic regions with distinct antimicrobial stewardship policies, the first aim of this study is to gather relevant epidemiological data on equine synovial fluid samples with suspected synovial sepsis submitted to the Animal Health Diagnostic Center (AHDC) Bacteriology Laboratory, one of the largest of its kind in the world. Goals include comparing changes in bacterial and antibiotic susceptibility profiles across decades (2000-2009 to 2010-2020) and evaluating associations between the likelihood of positive synovial fluid culture and relevant variables such as horse age, duration of clinical signs, culture techniques and synovial fluid clinical pathology data. [Bacterial isolates and antibiograms will be compared both within and between AHDC synovial samples obtained from: (1) Cornell-affiliated hospitals, including the Cornell University Equine Hospital (Ithaca, NY), the Cornell Ruffian Equine Specialists Hospital (CRES, Elmont, NY) and the Cornell University Ambulatory service (Ithaca, NY and surrounding upstate NY region) and (2) equine private practice submissions obtained from a wider geographic region (predominantly northeastern US).] Recent data suggests that mucins may be able to disrupt bacterial biofilms and reduce bacterial virulence10–12. Our lab has preliminary data to suggest that the mucin-like glycoprotein lubricin can both inhibit bacterial growth and disrupt biofilm formation in *P. aeruginosa*. Glycomics data from our lab also suggests that lubricin is the predominant *O*-glycosylated protein in equine synovial fluid13, and we hypothesize that synovial fluid mucins and lubricin inhibit bacterial growth and biofilm formation in synovial fluid. [In order to answer these questions, we will deplete lubricin and mucins from equine synovial fluid *in vitro* to determine whether we can enhance our ability to culture common model bacteria from synovial fluid, including *Pseudomonas aeruginosa* (*P.aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli (E. coli)*. We will also assess whether mucins, including lubricin and porcine gastric mucin, inhibit bacterial growth and biofilm formation.

Principal Investigator:	Dr. Bettina Wagner
Title:	Inflammatory biomarkers for prediction of breakdown injuries in horses
Project Period:	1/1/22-12/31/23

LAY SUMMARY

The goal of this project is to identify serum biomarkers for breakdown injuries in Thoroughbred (TB) racehorses. These biomarkers can become predictive indicators for recognizing horses that are at risk for breakdown injuries before catastrophic events happen. Predictive biomarkers will benefit the racing industry and prevent future catastrophic breakdown injuries at racetracks in NYS and beyond.

Fatal musculoskeletal injuries experienced by TB racehorses involve distal limb fractures, including those of the proximal sesamoid bone fetlock joint. About 85% of these fractures are associated with previous bone changes. Early in the process, local chronic inflammation drives changes in bone remodeling and supports bone erosion. Abnormal remodeling of the subchondral bone leads to changes in bone strength and increased risk of fracture. Normal bone remodeling in TBs is essential to prepare the bones of racehorses to withstand the rigors of training.

Reliable biomarkers for catastrophic breakdown injuries can help veterinarians and trainers to identify horses at increased risk for breakdown injuries and can be used as an indicator for modification of the horse's training program to mitigate the risk for injury. Previous and ongoing studies on biomarkers for catastrophic breakdown injuries have focused on molecules that are associated with joint and bone repair as indicators of normal or abnormal bone remodeling. In one longitudinal study by Frisbie and co-workers (2010), some of the musculoskeletal biomarkers tested correlated with the injury in up to 74% of the horses. However, none of these musculoskeletal biomarkers made it into prognosticators of catastrophic breakdown in TB horses.

In humans, chronic inflammation is one of the most evident pathological causes leading to deregulated bone remodeling. **Why have we not yet looked at inflammatory biomarkers as indicators for predicting breakdown injuries in TBs?** The answer is quite simple: the tools and reagents for detecting these inflammatory molecules in horses have not been available for a long time. We have recently overcome this gap by developing a novel diagnostic assay tool for inflammatory markers in horses.

During the past couple of years, Dr. Wagner's equine immunology research group has worked on reagent development for equine cytokines and chemokines (= inflammatory biomarkers). This work was mainly funded by USDA/NIFA since 2005 and also in shorter projects supported by the Morris Animal Foundation and the Zweig Memorial Fund. The equine reagent development project has resulted in a variety of monoclonal antibodies (mAbs = reagents) to advance immunological research and to enable testing for immune markers in horses (https://courses2.cit.cornell.edu/wagnerlab/research/reagents.htm). These reagents have been used by many equine researchers around the world. Most recently, our group developed a panel of reagents for measurement and quantification of inflammatory biomarkers which are easily accessible in equine blood.

In addition, Dr. Wagner's diagnostic Serology Laboratory at the Animal Health Diagnostic Center (AHDC) has used the reagents to develop and validate Luminex based multiplex assay for equine cytokines (Wagner and Freer 2009, Perkins et al 2014), chemokine (not yet published), and sCD14 (Wagner et al. 2013). This 'inflammatory marker assay' is a novel tool to identify biomarkers with clinical relevance to specific equine diseases and inflammatory conditions. The assay is a sensitive test and fast to perform. It simultaneously detects multiple inflammatory markers in the same sample and results in quantification of all markers. It provides a sensitive testing platform that allows for rapid screening and high-throughput of serum samples. The assay was first used by research Dr. Wagner's group to

evaluate innate immunity after equine herpesvirus type 1 (EHV-1) infection. This illuminated on the potential of the assay to evaluate inflammation in horses in general. During the past two years, the assay was subsequently used in many collaborations with other equine researchers to evaluate inflammatory markers for a broad array of equine diseases (Table 1).

The collaborative work has widely expanded with the validation of the most recent assay for chemokines (IL-1β, TNF-α, CCL2, CCL3, CCL5, CCL11) which identifies key markers of acute and chronic equine inflammation. The accumulated preliminary data by using the novel inflammatory marker assay during the past two years confirmed the value of this sensitive diagnostic test for identifying a wide array of inflammatory processes in horses, including those related to orthopedic issues like osteoarthritis (Watkins et al. 2021, Fasanello et al. submitted).

APPENDIX B
Final & Progress Reports from 2022

PI	Project Title	Term Date	Report Type
Cheetham, Jonathan	Accelerating Recovery after Laryngeal Nerve Graft in Horses	12/31/22	Final
Delco, Michelle	Investigation of Mitovesicles as a new Equine Orthobiologic	06/30/23	Progress
Delco, Michelle	Synovial fluid extracellular vesicles in equine joint disease and therapy (Year 2)	12/31/22	Final
Diel de Amorim, Mariana	Inflammatory markers from endometrial swab/cytobrush as a screening test for equine endometritis and endometrial fibrosis	12/31/23	Progress
Felippe, Julia	Diagnostic markers in mares with placentitis	12/31/23	Progress
Perkins, Gillian	Equine gamma herpesviruses and equine gastric ulcer syndrome (EGUS) – is there a link?	6/30/22	Final
Pigott, John	Multi-modal screening to identify Thoroughbred racehorses at increased risk for catastrophic injury of the metacarpophalangeal joint	12/31/23	Progress
Reesink, Heidi	Unraveling lubricin signaling in equine joint injury	12/31/22	Final
Todhunter, Rory	Genomics of Autopsy–Negative Sudden Cardiac Death in Racing Thoroughbreds	12/31/23	Progress
Wagner, Bettina	Intranasal biomarkers of EHV-1 susceptibility and protection	12/31/23	Progress

Principal Investigator:	Dr. Jonathan Cheetham
Title:	Accelerating Recovery after Laryngeal Nerve Graft in Horses
Project Period:	1/1/19 – 12/31/22
Reporting Period:	1/1/19 – 12/31/22

Project Title: Accelerating recovery after Laryngeal Nerve Graft in Horses

Principal Investigators: Jonathan Cheetham

A. Specific Aims of the Study and Modifications

This study aimed to evaluate the efficacy of a graft of the Recurrent Laryngeal nerve (RLn) with a donor nerve in reinnervating the dorsal cricoarytenoid muscle (CAD). A second aim was to assess the safety of IL-10 as nerve repair modulator when injected at the site of nerve anastomosis. The left RLn was transected, grafted with a C1 branch, and injected sub-epineurally with an agarose hydrogel loaded with recombinant equine IL-10. This protocol was applied in 5 horses that after surgery were monitored via endoscopy at rest, endoscopy during exercise, laryngeal muscles ultrasonography and transcutaneous electrical stimulation of the C1. As expected, transection of the RLn initially induced complete loss of function of the left CAD muscle and consequently left sided arytenoid paralysis. One horse, already affected by recurrent laryngeal neuropathy at advanced stage at the time of surgery showed severely impaired laryngeal function before surgery and never recovered function after performing the nerve graft. The other four horses instead showed signs of progressive reinnervation through C1 fibers. The first signs of CAD reinnervation were detected starting from 10-16 weeks post-op, in the form of arytenoid abduction induced by electrical stimulation of C1. Gradually, the horses also showed spontaneous twitching of the left arytenoid and improved left laryngeal function during incremental exercise. Already at 10 weeks post-op, the arytenoid function improved, the horses were able to reach a higher speed compared to the early post-operative period, and despite a reduction of the arytenoid angle at end exercise, the arytenoid did not further collapsed indicating an improvement in the CAD strength and ability to tolerate higher inspiratory load. Left arytenoid angle continued to improve at 18weeks post-op suggesting a higher degree of reinnervation. Despite the promising outset, the arytenoid function during exercise declined after 22 weeks, indicating an insufficient recruitment of C1 during exercise.

The second phase of the study was conducted after a long pause due to COVID-19 restrictions, and several delays due to restrictive surgery and anesthesia schedule on the large animal hospital. Considering the performance of the RLn-C1 graft in the first 5 horses, we evaluated an alternative nerve donor, the ventral branch of the accessory nerve (XI cranial nerve), and an alternative graft technique, the end-to-side (ETS) anastomosis. The accessory nerve is larger in size compared to C1, potentially providing a higher number of axons for reinnervation. We opted for an end-to-side anastomosis approach to prevent a complete resection of the RLn and the complete loss of laryngeal function for several weeks after surgery. Preventing a complete loss of function would be a more palatable option for application of this reinnervation in client-owned horses.

In the last year, we performed surgery on two horses and followed them for more than 7 months. One horse (left laryngeal function III.1-A) received a C1-RLn end-to-side anastomosis, while the second horse (left laryngeal function III.3-C) received an accessory-RLn end anastomosis. Both horses showed loss of left laryngeal function after surgery, but the first horse started already at 3 weeks to regain spontaneous movement of the left arytenoid and by 4 months he showed full recovery. The second horse had minimal laryngeal function before surgery and did not show any improvement of his condition after surgery.

Eight months after surgery, the horses were transferred to another study protocol with the aim of transferring back to this protocol for a longer-term evaluation of the laryngeal function. They were

scheduled for a repeat endoscopy exam at the end of 2022, but at the time of this report, they were not yet cleared to be re-enrolled in this study protocol.

Nerves and muscles collected post-mortem from the first five horses have been processed and sectioned by the AHDC, but the histopathologic evaluation is still ongoing, and no preliminary findings have been shared by the pathologist who has been recently collaborating in the study.

B. Summary of Scientific Findings

Even if the final data analysis is not completed, the preliminary clinical results of this study show that:

- agarose loaded with recombinant Equine IL-10 is well tolerated and does not impede the progression of reinnervation.
- reinnervation of the dorsal crico-arytenoid muscle via C1 graft to the RLn can be obtained via both an end-to-end and an end-to-side anastomosis.
- nerve graft of the RLn with branches of C1 and the accessory nerve is feasible with the horse standing under sedation via constant rate infusion of sedative and does not require the horse to undergo general anesthesia.
- advanced stage of recurrent laryngeal neuropathy seems to prevent an effective reinnervation. The pending histopathologic assessment of the graft should clear if the reinnervation is prevented by the deteriorated structure of the recurrent laryngeal nerve, or by the marked atrophy of the CAD muscle that represent a hostile environment for the reinnervating fibers.
- return to function after an end-to-side anastomosis is much faster than end-to-end anastomosis possibly thanks to the epineurial or perneurial window that allows to maintain intact most of the recipient nerve structure.

C. Significance

This study shows that RLn and CAD muscle can be reinnervated by C1 axons and potentially the accessory nerve via an end-to side anastomosis. Reinnervation timeline and efficacy is variable among horses based on the condition of the recurrent laryngeal nerve and muscle. This surgical approach can be safely performed in standing sedated horses.

D. Publications and Other Grant Submissions

Even if the clinical data collection is completed, the sample analysis is still ongoing. The funded study period is terminated, but the sample analysis will continue and be completed. A manuscript will be drafted as soon as the histopathologic evaluation is finalized.

Principal Investigator:	Dr. Michelle Delco
Title:	Investigation of Mitovesicles as a new Equine Orthobiologic
Project Period:	1/1/22 – 6/30/23
Reporting Period:	1/1/22 – 12/31/22

Osteoarthritis (OA) affects the quality of life and athleticism of horses worldwide. Currently, there are no therapies to prevent or treat the progression of OA. Joint cartilage heals poorly following injury and has no intrinsic capacity to regenerate following degenerative loss from OA. This cartilage loss results in pain and lameness in horses. Existing management strategies include intra-articular injections, where medications or biologic products are deposited directly into the joint to promote healing and to diminish painful inflammation. Common techniques include the injection of mesenchymal stromal cells (MSCs or stem cells) or platelet rich plasma; however, the mechanisms whereby these therapies reduce pain and promote healing capacity of cartilage remains unclear.

We are investigating an alternative, cell-free orthobiologic to prevent and treat OA in the horse. Recent evidence by our lab and others suggests that mitochondrial dysfunction is one of the earliest events in OA development and follows shortly after initial cartilage trauma. We have shown that healthy mitochondria can be transferred from MSCs to chondrocytes, the cells within cartilage. Further, MSCs package mitochondria for donation into nano-size membrane-bound particles called mitovesicles (mitoEVs). We have found that when MSCs are grown together with chrondrocytes, MSCs donate healthy mitochondria to chondrocytes through direct contact and through mitoEVs. However, cell-free mitochondrial transfer, whereby isolated mitoEVs are taken up by chondrocytes in the absence of parent MSCs, has not yet been investigated. Therefore, our objective during the funding period was 1) to assess the ability of chondrocytes to take up mitochondria from isolated mitoEVs and 2) to assess the ability of these mitoEVs to improve chondrocyte function.

We cultured chondrocytes and treated them with isolated mitoEVs from MSCs. We then assessed mitochondrial respiration outcomes which indicate mitochondrial and cellular function. During the assay, the cells are stressed, which allows assessment of their ability to respond to environmental changes (i.e. as they would in joint disease). When compared to untreated chondrocytes, chondrocytes treated with mitoEVs showed an increase in baseline respiratory capacity. This suggests mitoEVs are having a positive effect on the chondrocytes, likely by increasing mitochondria content per cell. Furthermore, the mitoEVs-treated chondrocytes showed an increase in mitochondrial ATP (energy) production, which is a measure of cellular health. Furthermore, we cultured one group of chondrocytes with mitoEVs from the same horse (matched pairs) and one group of chondrocytes with mitoEVs from a different horse (unmatched pairs). We found that there was no difference in baseline respiratory capacity between groups, suggesting no difference in mitochondrial transfer between matched and unmatched pairs. Similarly, we found no difference in energy production between the matched and unmatched pairs. Finally, we found a difference between the spare respiratory capacity of matched and unmatched chondrocyte-mitoEV pairs; spare respiratory capacity represents the amount of extra energy (ATP) that can be produced by a cell in response to a sudden stress increasing energy demand. Taken together, our results show that mitochondria can be transferred from mitoEVs to chondrocytes in culture, and that this transfer augments the function of chondrocytes, independent of the mitoEV source.

Principal Investigator:	Dr. Michelle Delco
Title:	Synovial fluid extracellular vesicles in equine joint disease and therapy (Year 2)
Project Period:	1/1/21 – 12/31/22
Reporting Period:	1/1/21 – 12/31/22

Project Title: Synovial fluid extracellular vesicles in equine joint disease and therapy

Principal Investigators: Michelle L. Delco

A. Specific Aims of the Study and Modifications

The goals of this work were to investigate mitochondria-containing extracellular vesicles (mitovesicles, mitoEVs) as potential regenerative therapeutics, as well as biomarkers of joint injury in the horse.

B. Summary of Scientific Findings

Cartilage heals poorly, and no treatments are currently available to prevent ongoing cartilage loss or improve repair after joint injury. Mesenchymal Stem/stromal cells (MSCs) are commonly used in equine sports medicine; however, outcomes are variable. To improve MSC-based regenerative therapies, we need to understand how they work. Our Zweig-funded research investigated one of the secreted products of MSC, extracellular vesicles (EVs). We were most interested in a subset of MSC-EVs that contain whole mitochondria, called mitovesicles (mitoEVs). Mitochondria produce energy that is required for healing. In other cell types, transfer of healthy mitochondria from MSCs to injured cells via mitoEVs can improve recipient cell viability and tissue healing. We are the first group to document MSC-chondrocyte mitochondrial transfer and investigate mitoEVs as a potential new joint therapy. Two manuscripts that include data generated by this funding were recently published (*see below, Thomas+ 2022, Fahey+ 2022*).

In summary, our most exciting findings include:

1) MSCs package functional mitochondria into extracellular vesicles and export them as mitoEVs.
2) MSC MitoEVs are taken up by injured chondrocytes and incorporated into the recipient cell's mitochondrial network.
3) MitoEV uptake improves chondrocyte health. Specifically, chondrocytes that were treated with mitoEVs had higher baseline respiratory function and increased ATP (energy) production, likely due to increased mitochondrial content.
4) In addition to our original Aims, we have begun to investigate the cellular mechanisms that govern the release and uptake of mitoEVs. Specifically, we are studying the role of Connexin 43 (Cx43, a.k.a. GJA1), a protein that is critical for cell-cell communication. We found that chemical inhibitors of Cx43 prevent the uptake of MSC mitochondria by chondrocytes. Further, chondrocytes that lack Cx43 (knockouts) take up fewer mitochondria. Further, we found that mitoEVs contain Cx43 protein, and Cx43 is concentrated at sites of MSC-chondrocyte MT transfer. Taken together, these findings suggest Cx43 plays an important role in the uptake of MSC mitoEVs by chondrocytes. We have bred a strain of mice that has an inducible, cartilage- specific Cx43 gene (GJA1) knockout, which will let us further investigate the role of this protein in cartilage degeneration and healing. Those studies are ongoing.

C. Significance

These findings strongly support our continued investigation of mitoEVs as a promising next-generation regenerative therapy to prevent degeneration and improve healing after joint injury in horses and humans.

D. Publications and Other Grant Submissions

Grants – *Funded*

Delco (PI) 06/01/2022 - 05/31/2023
Sponsor/Mechanism: ACVS Foundation Surgeon-in-Training Research Grant Title: Investigating Mitovesicles in a Model of Equine Osteoarthritis
Role: Role: Principal Investigator/Mentor (Dr. Brenna Pugliese)

Grant Applications - *Submitted*

Delco (PI) 05/01/2023 - 04/30/2028
Sponsor/Mechanism: NIH NRSA Dual-Degree (DVM/PhD) Predoctoral Fellowship (F30)
Title: MSC secretome fractions targeting mitochondrial function to treat degenerative disc disease
Role: Principal Investigator/Sponsor (Megan Fahey; *Resubmitted 2023*)

Cohen (PI), Delco (PI) 07/01/2023 - 06/30/2025
Sponsor/Mechanism: NIH-NIAMS R21
Title: Using STRAINS, a big data method that analyzes the spatiotemporal distribution of cell phenotypes, to investigate mechanotransduction pathways in injured cartilage (*Scored; 13th percentile*)
Role: Co-Principal Investigator

Delco (PI) 01/2023 – 12/2025
Sponsor/Mechanism: Morris Animal Foundation/Mitochondrial Function & Chronic Disease
Title: Targeting mitochondrial dysfunction in canine degenerative disc disease to develop novel regenerative therapeutics
Role: Principal Investigator

Delco (PI) 09/01/2023 - 08/31/2028
Sponsor/Mechanism: NIH Directors New Innovator Award (DP2)
Title: Next-Generation Mitochondria-targeted Regenerative Therapies to Tackle the Most Common Cause of Skeletal Pain and Disability
Role: Principal Investigator

Delco (PI) 12/01/2022 - 11/30/2027
Sponsor/Mechanism: NIH Mentored Research Scientist Development Award (K01)
Title: The Role of GJA1 in Cartilage Mitochondrial Function and Mechanotransduction
Role: Principal Investigator/Sponsor (Dr. Rebecca Irwin)

Grant Applications - *In Preparation*

Delco (PI) Due: June 5, 2023
Sponsor/Mechanism: NIH-NIAMS ESI R01
Title: Intercellular mitochondrial transfer: The next frontier in orthopedic regenerative medicine
Role: Principal Investigator

Manuscripts

Thomas MA, Fahey MJ, Pugliese BP, Irwin RM, Antonyak MA, **Delco ML**. Human Mesenchymal Stromal Cells Release Functional Mitochondria in Extracellular Vesicles. *Emerging Technologies for Musculoskeletal Disease Modeling and Regenerative Medicine, Front. Bioeng. Biotechnol.* 2022 Aug 19; 10:870193: 1-12. doi: 10.3389/fbioe.2022.870193

Fahey MJ, Bennett M, Thomas M, Montney K, Vivancos-Koopman I, Pugliese b, Browning L, Bonassar LJ, **Delco ML**. Mesenchymal Stromal Cells Donate Mitochondria to Articular Chondrocytes Exposed to Mitochondrial, Environmental, and Mechanical Stress. *Sci Rep*. 2022 Dec 13;12(1):21525. doi:10.1038/s41598-022-25844-5. PMID: 36513773

Seewald LA, Sabino IG, Montney KL, **Delco ML**. Synovial fluid mitochondrial DNA concentration reflects the degree of cartilage damage after naturally occurring articular injury. *Osteoarthritis Cartilage*. 2023 Apr 6:S1063-4584(23)00735-5. doi: 10.1016/j.joca.2023.03.013. Online ahead of print.

Zheng J, Wyse Jackson T, Fortier LA, Bonassar LJ, **Delco ML**, Cohen I. STRAINS: A big data method for classifying cellular response to stimuli at the tissue scale. May 2022; BioRxiv doi:10.1101/2022.06.12.495830. *PLoS One*. 2022 Dec 8;17(12):e0278626. doi:10.1371/journal.pone.0278626. PMID: 36480531

Research Abstracts *(for podium presentations, presenter is underlined)*
Irwin RM, Thomas MA, Fahey MJ, Kondayapalepu PS, Mayan M, **Delco ML** (2023). Loss of Connexin 43 Impairs Chondrocyte Mitochondrial Bioenergetics and Decreases Mitochondrial Transfer from Mesenchymal Stromal Cells to Chondrocytes. Gordon Research Conference, Cartilage Biology and Pathology, Lucca, Italy
**GRC Poster Award*

Irwin RM, Thomas MA, Fahey MJ, Mayan M, Delco ML (2023). Connexin 43 Mediates Mitochondrial Transfer from Mesenchymal Stromal Cells to Chondrocytes. Orthopedic Research Society (ORS) Annual Meeting, Dallas, TX

Fahey MJ, Yerden R, Bonassar LJ, Delco ML (2022). Mitochondrial Transfer from Mesenchymal Stromal Cells to Intervertebral Disc Cells. Orthopedic Research Society PSRS 6th International Spine Research Symposium, Skytop, PA

Pugliese BR, Thomas MA, Fahey MJ, **Delco ML** (2022). Mitovesicles: A Potential New Orthobiologic. European College of Veterinary Annual Scientific Meeting, Porto, Portugal

Irwin RM, Thomas MA, Mayan M, **Delco ML** (2022). Connexin 43 Inhibition Decreases Mitochondrial Transfer Between Human Mesenchymal Stromal Cells and Articular Chondrocytes. International Gap Junction Conference, A Coruña, Spain

Thomas MA, Irwin RM, Fahey MJ, **Delco ML** (2022). MitoEVs Containing CX43 Transfer Mitochondria to Chondrocytes. International Gap Junction Conference, A Coruña, Spain

Pugliese BR, Thomas MA, Fahey MJ, Antonyak MA, **Delco ML** (2022). Mesenchymal Stromal Cell- and Plasma-derived Extracellular Vesicles Contain Mitochondria. ORS Annual Meeting, Tampa FL

Fahey MJ, Bennett MP, Browning L, Thomas MA, Bonassar LJ, **Delco ML** (2022). MSCs Transfer Mitochondria To Rescue Chondrocytes From Chemical, Environmental and Mechanical Stress. ORS Annual Meeting, Tampa, FL

Sabino IG, Seewald LA, Jacobs CA, Chen J, Leifer CA, Lattermann C, **Delco ML** (2022). Elevated Synovial Fluid Mitochondrial DNA is Associated with Inflammation After ACL Injury and Reconstruction. ORS Annual Meeting, Tampa, FL

Atkins PV, Bohner AM, Das M, **Delco ML** (2022). Stress Alters Chondrocyte Mitochondrial Dynamics In Situ. ORS Annual Meeting, Tampa, FL

*Our entire research group sincerely thanks the **Harry M. Zweig Memorial Fund for Equine Research** for supporting this work!*

Principal Investigator:	Dr. Mariana Diel de Amorim
Title:	Inflammatory markers from endometrial swab/cytobrush as a screening test for equine endometritis and endometrial fibrosis
Project Period:	1/1/22 – 12/31/23
Reporting Period:	1/1/22 – 12/31/22

Equine endometritis is a prevalent and challenging disease for practitioners to diagnose. Our objective was to investigate the utility of the endometrial swab/cytobrush to screen mares for acute or chronic endometritis using validated equine inflammatory markers and to compare the usefulness against cytology and culture.

This summer, we collected endometrial samples from 86 horses, in which, some of the same mares were sampled more than once in different cycles. A total of 197 samples were collected, in which 95 samples were cytobrush and the remaining 102 were endometrial swabs. Twenty-three samples were collected in diestrus and 171 were collected in estrus. Similarly to our previous investigation of inflammatory markers in LVL study, we found no differences in the inflammatory markers' concentrations between estrus and diestrus.

Inflammatory markers' concentrations did not differ between sample collection device (swab versus cytobrush), besides for IFN-γ. Therefore, all the data were analyzed stratified by sample collection devices to remove confounding factors.

A total of 96 endometrial cytologies were performed, which 27 of them were positive for inflammation ($\geq$ 1 neutrophil/hpf). IL1-β were significantly increased from endometrial swabs of mares with inflammation compared to mares that didn't have any neutrophils on cytology ($p<0.05$).

Regarding the endometrial culture, there were 139 samples that had cultures performed, which 62 had bacterial growth and 77 had no growth. Out of those positive cultures, 31 were identified to be gram-positive and 27 were gram-negative. sCD14 and TNF-α had a statistical trend to be increased in cytobrush samples from mares that had a positive culture, and that bacterial growth was a gram-positive compared to gram-negative, respectively ($p=0.06$). A larger scale data set is required to remove confounding factors and validate if sCD14 and/or TNF-α may have applications as a screening tool for bacterial endometritis, which we applied for the Grayson-Jockey Club for making a larger scale project.

We further analyzed endometrial biopsy gradings. Forty-six endometrial biopsies were collected, which 29 of them were graded as either grade I or grade IIA by the Kenney and Doig system, and the remaining 17 were graded as IIB or III. Comparably to the LVL study, IFN-γ was significantly increased in mares with poor endometrial biopsy grading (IIB or III) compared to good biopsy scores (I or IIA) in both swab and cytobrush samples ($p<0.02$).

Principal Investigator:	Dr. Julia Felippe
Title:	Diagnostic markers in mares with placentitis
Project Period:	1/1/20 – 12/31/23
Reporting Period:	1/1/22 – 12/31/22

Pregnancy loss and neonatal mortality have significant economic impact on the equine industry, with emotional implications to horse owners. The main goal of this investigation is to identify blood parameters that indicate imminent placentitis, before inflammation establishes and challenges the pregnancy and the health of the fetus. Early diagnosis of placentitis allows prompt treatment intervention and increases the chances of carrying on the pregnancy with normal fetal development.

Our investigations have tested two specific hypotheses focused on missing knowledge in the literature. By studying naturally occurring placentitis, we now better understand how clinical, immunological, inflammatory, and hormonal parameters change physiologically during normal pregnancies; we also learned the differences in these parameters between normal pregnancy and pregnancy with imminent placentitis.

In the past year, the studies moved at a fast pace. We are happy to inform that we are finalizing Aim 1 and completed Aims 2a and 2b, summarized below:

First Hypothesis (H1): *Physiological parameters change during the different phases of a healthy gestation.* **Aim 1:** to establish physiological parameters in mares with healthy pregnancies by longitudinally measuring clinical, hormonal, inflammatory, and immunological values (Table 1) before, during and after gestation (total of 10 time points per mare, throughout the peri-parturient and 330-day gestation). We have collected and processed samples for all time points from 6 mares (60 samples), and this aim will be completed in the Spring 2023 when the remaining samples from 3 mares (ongoing) are collected.

Second Hypothesis (H2): *Differences from physiological parameters of a healthy pregnancy indicate imminent placentitis.*

Aim 2a: to identify clinical, hormonal, inflammatory, and immunological parameters (Table 1) that indicate early stages of placentitis when compared to mares with healthy pregnancies of comparable stages. This aim remained as originally proposed and it is completed. We performed 219 prospective blood sample collections and reproductive exams, monthly, between 180 to 300 days of gestation from a total of 53 pregnant mares. In this cohort, we identified samples from 12 mares before the detection of clinical signs of placentitis (samples named *pre-onset*), and from the same mares when they developed signs of placentitis at the following examination (samples named *early onset*); in addition, samples from 12 mares with healthy pregnancies (gestationally matched controls) were collected at the same time points.

Aim 2b: to measure clinical, hormonal, inflammatory, and immunological parameters that are associated with severity of placentitis. Using a scoring system, the severity of placentitis from pregnant mares were classified, and the same parameters measured (Table 1). This is an added, bonus aim that became possible due to the large number of samples and clinical data set collected from the mares above, and it is completed. Statistical analyses were performed for each parameter comparing values between and within groups at pre-onset and early onset time points (Aim 2a), and between groups in each severity score (Aim 2b). Also, we calculated the accuracy of each individual parameter as a diagnostic test for placentitis.

Table 1: Clinical parameters for the scoring system of the ultrasonographic findings, and blood analyses performed in Aim 1, Aim 2a and Aim 2b.

clinical parameters (ultrasonographic scoring)	*normal:* no ultrasonographic abnormalities; *mild*: opening at the cervix; *moderate-severe*: increased combined thickness of the uterus and placenta, fluid collection, placental edema, and/or placental separation.
10 immunological parameters	lymphocyte subpopulation distribution (CD4 T cell, CD8 T cell, B cell, and ratios); cytokine profile (IL-4, IL-6, IL-10, IL-17, IFN-γ, IFN-α, TNF-α, and ratios)
4 inflammatory parameters	complete blood count (CBC); globulin, serum amyloid A (SAA); alpha-fetoprotein(AFP)
13 hormonal parameters	progesterone; pregnenolone; DHP; allopregnanolone; 17α-OHP; 20α-OHP; 3β,20α-DHP; DHEA; androstenedione; testosterone; cortisol; estradiol-17β; prostaglandin F(2alpha)

Principal Investigator:	Dr. Gillian Perkins
Title:	Equine gamma herpesviruses and equine gastric ulcer syndrome (EGUS) – is there a link?
Project Period:	1/1/20 – 6/30/22
Reporting Period:	1/1/20 – 6/30/22

Project Title: Equine gamma herpesviruses and equine gastric ulcer syndrome (EGUS) -- is there a link?

Principal Investigators: Gillian Perkins, DVM, DACVIM; with Co-Mentors Gerlinde Van de Walle DVM, PhD, Joy Tomlinson, DVM, DACVIM, PhD. Large Animal Internal Medicine Resident – Rachelle Thompson, DVM

A. Specific Aims of the Study and Modifications

Overarching hypothesis-- EHV-2 and -5 are more prevalent in horses with equine gastric ulcers compared to horses without ulcers or a normal stomach. And that within an individual horse's stomach, the gamma herpesviruses are more likely to be localized at the site of ulcerated tissue. We chose to focus on equine glandular gastric ulcer syndrome (EGGUD) due to the current lack of knowledge and suspected different pathophysiology for EGGUD than equine squamous gastric ulcer disease (ESGUS).

Modified Aim 1: To evaluate the viral load of EHV-2 and/or EHV-5 in the stomach of horses with ulcers versus horses without ulcers.

This aim compared horses that had EGGUD (n=17; 11 horses with EGGUD only and 6 horses with EGGUD plus ESGUS) to horses with normal stomachs, free of ulcers (n=11). Stomach mucosa samples collected either during endoscopic or post-mortem examinations at the Cornell University, Equine and Nemo Farm Animal Hospital (ENFAH) and Louisiana State University. The gastric tissue samples were evaluated using an RNAscope in situ hybridization assay (ISH) with probes targeting the glycoprotein B gene of either EHV-2 or EHV-5, previously validated in our lab (Pennington et al. 2017). ISH allowed us to detect the presence of virus, its localization within the stomach mucosa, and the viral load. Based on early observations that either or both viruses were detected at least in low levels in nearly all the tissues, the approach was altered to assess viral load rather than presence or absence of infection. To do this, positive cells per unit area was calculated for each sample.

Modified Aim 2: To evaluate the viral load of EHV-2 and/or EHV-5 in the normal glandular mucosa versus the glandular ulcer site in the same horse.

There are 11 horses with EGGUS and 6 horses with both EGGUS and ESGUS where we used ISH to compare the positive cells/mm2 from normal glandular mucosa versus ulcerated glandular mucosa within each horse. This could provide evidence that high viral load is related to ulcer pathology.

Additional Aim 3: To describe histopathologic changes associated with glandular gastric disease.

Much of the pathology observed in the glandular stomach of horses includes hyperemia, inflammation, and a raised appearance, rather than true ulceration or loss of the mucosa. Histopathology of this condition is poorly described in the literature. In addition to describing the gross appearance of the ulcers, histopathologic changes were assessed by Dr. Sean McDonough and descriptive analysis will be provided in the final report. In summary, we prioritized investigating the viral load of gamma herpesviruses in horses with glandular ulcers compared to those horses without ulcers. Our study focuses on the quantitation of EHV-2 and EHV-5 positive cells in the mucosa of horses using ISH. ISH is superior to qPCR as it also provides quantitative and qualitative information regarding the localization of the gamma herpesviruses within the mucosa and submucosal regions. To date, we have compiled the signalment and historical information on each horse along with the appearance of the stomach on endoscopy or post-mortem examination, histopathological findings of the stomach with Dr. Sean McDonough (Anatomic Pathologist, Cornell University). We must complete the counting of positive cells

by ISH in a handful of horses and then the statistical analysis will be done (estimated by the end of 2022).

B. Summary of Scientific Findings

Based on early observations during data analysis, we observed that either or both viruses were detected at least in low levels in nearly all the tissues. Therefore, the approach was altered to assess viral load rather than presence or absence of infection. To do this using ISH, the positive cells per unit area was calculated for each sample. Data appears to be right skewed. Samples for Aim 1 will be analyzed by Wilcoxon rank sum test, Aim 2 will be analyzed by mixed effects model with horse as random effect. A few samples are left to be counted. Statistical analysis at this point shows no statistical difference in viral load between horses with or without ulcers, or between healthy and ulcerated sites in horses with glandular ulcers.

Our grant had described identifying gamma herpesviruses in the stomach of horses by qPCR. Despite many attempts in the laboratory, we were unable to develop a qPCR for EHV-2 and EHV-5. Because of our findings with high proportion of infection based on ISH, we opted not to further pursue qPCR, but instead to perform ISH on all samples.

In addition, we had originally aimed to determine if the gamma herpesviruses found in these areas were in an active or latent state. However, despite multiple attempts and optimization, the ISH with DNase pre-treatment could not be assessed due to poor RNA quality in many of the tissue samples, based on results of our positive RNA controls.

C. Significance

This work explored the role of Equid gamma herpesviruses in equine glandular ulcer disease. Although gamma herpesviruses play a role in human gastric ulcers, and there is a high viral burden in equine stomachs, there is no clear evidence of a causal relationship between EHV-2 or EHV-5 and equine glandular gastric ulcers based on this work. The unexpectedly high rate of infection, with virus present in all horses, limits the ability to draw conclusions as to whether the viruses could play some part of a multifactorial process in ulcer development. Virus was observed frequently in the mucosal glands, indicating a plausible biological role in disrupting normal gastric defense mechanisms.

D. Publications and Other Grant Submissions

The goal is to complete the final data collection and statistical analysis by the end of November 2022. Upon completion, the author's plan to submit a manuscript to the Journal of Veterinary Internal Medicine in January of 2023.

Principal Investigator:	Dr. John Pigott
Title:	Multi-modal screening to identify Thoroughbred racehorses at increased risk for catastrophic injury of the metacarpophalangeal joint
Project Period:	1/1/21 – 12/31/23
Reporting Period:	1/1/22 – 12/31/22

This study has continued to progress well over the last year with enrollment of clinical and control Thoroughbred horses for the gait tracking, imaging, and biomarker aims of the project. Trainer and owner interest continues to grow as more clinical cases are enrolled. To date, we have completed 23 MRI imaging clinical cases and 2 MRI control cases. We have digital tomosynthesis and blood biomarker data on these 23 clinical horses, along with 15 tomosynthesis controls. As expected, the control MRI cases are more difficult to acquire but we are pursuing different strategies to encourage enrollment. We have completed the 3-year old clinical section and are half way completed with 2- and 4-year old clinical cases. There have been a number of landmark cases over the last year that highlighted the overall importance of this research, by identifying pathology prior to catastrophic breakdown in the metacarpophalangeal joint in those cases. Once we reach our target number of clinical (40) and control cases (20), we will proceed with data analysis of both the MRI and tomosynthesis images, and serum biomarkers. The StrideSAFE gait tracking aspect of the project has made progress but needs to be further validated through ongoing data acquisition. Progressive follow-up of horses with StrideSAFE green, yellow or red flag classifications during the 2021 Saratoga race meet revealed that horses with red alert classifications took longer to resume high speed exercise and were less likely to resume racing within 4 months of the recorded race than did horses with green classifications. An example of a red flag classification due to a fracture in the knee, which was then surgically repaired, was provided in the 2021 Zweig Continuation proposal. Based upon the analysis of the Saratoga data, the StrideSAFE algorithm was modified using guided artificial intelligence to better identify horses at increased risk for career ending or catastrophic injury. This new algorithm was used to classify horses that ran during the Saratoga 2022 race meet. Follow-up data from that meet are currently being gathered for analysis. Additional serial data are being pursued on horses at Belmont.

Principal Investigator:	Dr. Heidi Reesink
Title:	Unraveling lubricin signaling in equine joint injury
Project Period:	1/1/20 – 12/31/22
Reporting Period:	1/1/20 – 12/31/22

Project Title: Unraveling lubricin signaling in equine joint injury

Principal Investigators: Heidi Reesink, Matthew Paszek

A. Specific Aims of the Study and Modifications

The specific aims have not been modified from the prior reporting period updates in October 2021 and 2022. As reported previously, there were challenges associated with establishing *Prg4* knockout and overexpressing primary equine cell lines in which clones were generated and selected for but were not able to be culture expanded to perform the studies proposed in Aim 1. Therefore, efforts over the past year have been predominantly dedicated to finalizing and optimizing the recombinant equine lubricin (rEqLub) formulation for *in vivo* injection and evaluation of the safety of this formulation in horses in preparation for a future efficacy study as proposed in Aim 3.

B. Summary of Scientific Findings

Prior to this reporting period, recombinant equine lubricin (rEqLub) had been produced at laboratory scales of <1 L per batch, with yields of approximately 0.25 g/L. Over the past year, productivity and efficiency in the bioprocess pipeline were boosted to 10 L production batches with match scaled purification. Culture densities were improved by approximately 30-fold by moving to a perfusion feeding wave-mixed bioreactor, achieving yields of over 1 g/L. Higher product concentration and lower impurity levels enabled higher loading volume on a cation exchange column employed as a capture operation, which in turn yielded a higher concentration crude product suitable for polishing by size exclusion and hydrophobic interaction chromatography. Finally, 0.45 µm filtration with aliquotation into singleuse crimp-top serum vials was integrated to maintain product sterility. The frozen stores (-80°C) were tested in triplicate for sterility in fluid thioglycollate media (30 °C, anaerobes) and tryptic soy broth (22°C, aerobes) as per industry standards.

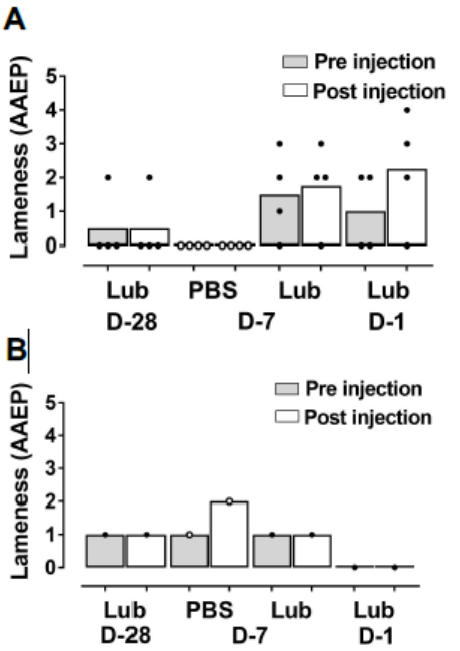


Figure 1. Subjective gait analysis based on the American Association of Equine Practitioners (AAEP) lameness scale. A) Subjective lameness following PBS (n=4 joints) or rEqLub (n=12 joints) injection in four horses from Phase 1. **B)** Subjective lameness following PBS (n=1 joint) or rEqLub (n=3 joints) injection in one horse from Phase 2.

An equine pilot study was initiated to evaluate the safety of rEqLub injections into healthy equine fetlock joints. This study was designed with the primary intent of identifying any adverse clinical (i.e., physical exam and lameness) and structural (i.e., synovial membrane and osteochondral histologic) responses to rEqLub injection, with generation of preliminary synovial fluid cytologic and biochemical data to motivate a larger efficacy study. To date, five horses have been enrolled in the pilot safety study (Phase 1: 4 horses, Phase 2: 1 horse), with the goal of enrolling an additional 5 horses to complete Phase 2. A blocked randomized study design was employed to assign each of 4 fetlock joints per horse to a treatment group following baseline physical and lameness examinations. For Phase 1 of the study, four adult lightbreed horses that were donated to the Cornell Equine Park were enrolled, including 3 geldings and 2 mares.

Sequential physical and orthopedic examinations in combination with fetlock arthrocentesis have been performed prior to (pre-) and postinjection of rEqLub or phosphate buffered saline (PBS) over a study period of 28 days. Three fetlocks per horse were injected with rEqLub at one of three time points [1 month (D-28), 1 week (D-7) or 1 day (D-1)] prior to study completion. The remaining fetlock joint was assigned as a placebo control and injected with PBS on D-7. Synovial fluid was collected prior to injection (D-28) or at study completion (D-28, D-7, and D-1), and synovial membrane and osteochondral samples were harvested for biochemical and (immuno-) histological assessments, respectively, upon study completion.

During Phase 1 of the study, no horses demonstrated any adverse effects following the first or second rEqLub injections. However, two out of the first four enrolled horses demonstrated evidence of joint effusion and mild edema/lameness following the third and final rEqLub injection, suggesting that there may be a mild immune response to repeated exposure to the formulation. Clinical gait analysis (AAEP) revealed mild increased lameness 24 hours after the third and final rEqLub injection but not after the first two injections (Fig 1A), with an increase in synovial fluid total protein concentrations (Fig 2A).

In response to the Phase 1 data, the lubricin bioprocess chain was modified by adding an intermediate purification operation before the final polishing step. This new, 3-step process removed residual impurities that we suspect were causing the observed sensitization response to the third injection, resulting in a >99% purity formulation (Fig 3). With the retooled biomanufacturing and purification pipeline in place, we produced a new batch of injection-quality rEqLub. Following sterility testing, we injected three fetlocks in one horse in Phase 2 with the reformulated compound, with no adverse responses detected after any of the injections. Clinical gait analysis and synovial fluid total protein concentrations were similar pre- and post-injection (Fig. 1B, 2B). Note that the increased synovial fluid total protein concentration on D-1 in Phase 2 was caused by iatrogenic blood contamination during the arthrocentesis process, with similar concentrations measured before and after rEqLub injection. Synovial fluid and joint tissues have been collected for batch biochemical and histochemical analyses once study enrollment is complete. Based on these findings, we are optimistic that this final formulation will be appropriate for future efficacy testing, and enrollment is underway to include an additional 5 horses to complete the final phase (Phase 2) of the ongoing safety study.

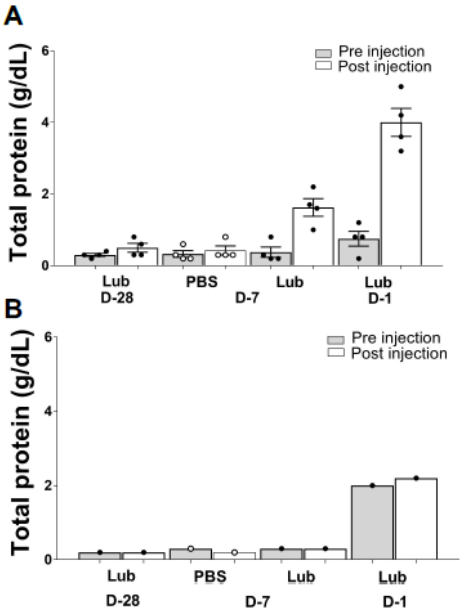


Figure 2. Synovial fluid total protein (TP) analysis following rEqLub and PBS intra-articular injections. A) Total protein (g/dL) in n=4 horses in Phase 1, and **B)** total protein (g/dL) in n=1 horse in Phase 2, revealing a protein response to injection in Phase 1 but not Phase 2.

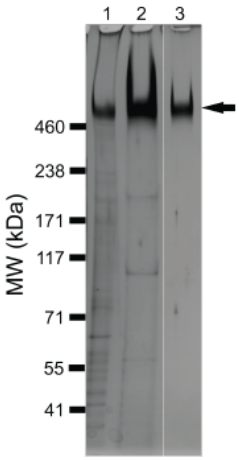


Figure 3. Product purity assessment by SDS-PAGE and silver staining. Lanes: **1)** Phase 1 formulation, **2)** Phase 2 compound after intermediate purification, **3)** Phase 2 final formulation. The arrow denotes rEqLub. The light bands below 460 kDa correspond to the trace impurities suspected to have caused sensitization in Phase 1.

C. Significance

We have successfully produced recombinant equine lubricin (rEqLub) at scales sufficient for *in vivo* equine studies and clinical trials, developing a biomanufacturing process that is appropriate for scale up and production via a contract manufacturer. Preliminary safety data have enabled us to improve upon the purification and bioprocessing strategy and suggest that intra-articular injections of rEqLub are well tolerated in horses, even after repeated administration (Phase 2). Safety data obtained in an additional 5 horses will bolster the sample size, and histopathological and biochemical analyses will be performed subsequently in batch-wise fashion.

These pilot safety and feasibility data are critical to support and prepare for future efficacy studies in the horse, which bring us one step closer to elucidating the mechanisms of action by which lubricin functions as a chondroprotective agent in joints.

Goals for the remainder of the study include enrolling 5 additional horses in Phase 2 to achieve a sample size of 6 horses for safety evaluation of the final rEqLub formulation. Sample processing (osteochondral decalcification, synovial membrane and osteochondral paraffin embedding and sectioning), hematoxylin & eosin staining (synovial membrane and osteochondral sections), safranin O-fast green staining (articular cartilage), lubricin immunohistochemistry, and histological scoring will be performed for all samples. ELISAs (prostaglandin E2 (PGE2) and lubricin) will also be performed on all synovial fluid samples. Once data collection is complete, we plan to submit an abstract to a national orthopedic meeting and prepare a manuscript for publication.

D. Publications and Other Grant Submissions

Publications

Watkins A, Fasanello D, Stefanovski D, Schurer S, Caracappa K, D'Agostino A, Costello E, Freer H, Rollins A, Read C, Su J, Colville M, Paszek M, Wagner B, **Reesink HL**. Investigation of synovial fluid lubricants and inflammatory cytokines in the horse: a comparison of recombinant equine interleukin 1b-induced synovitis and joint lavage models. *BMC Vet. Res.* 2021 May 12;17(1)189. doi: 10.1186/s12917-021-02873-2. PMID: 33980227.

Vishwanath K, Secor EJ, Watkins AR, **Reesink HL**, Bonassar LJ. Loss of effective lubricating viscosity is the primary mechanical marker of joint inflammation in equine synovitis. In preparation for submission.

Ysebaert MP, Secor EJ, Colville MJ, Gier CJ, Cervero IJ, Paszek MJ, **Reesink HL**. Evaluation of the safety of repeated intra-articular injections of recombinant equine lubricin in healthy horses. In preparation.

Grant Proposals Submitted and Funded

Secor (Trainee) / Reesink (Mentor) 1/1/2022 - 12/31/2023 0.1 CM
Hong Kong Jockey Club Equine Welfare Research Foundation
Research Training Scholarship Application
Investigation of equine fetlock joint immunopathology and the immunomodulatory effects of intra-articular therapeutics
The goal is to illustrate the immune cell distribution in equine osteoarthritis, correlate lubricin glycosylation with immune cell populations, and evaluate the immunomodulatory effects of common intra-articular therapies.
Role: PI (Supervisor)

1R03AR078961-01A1 (Reesink) 4/1/2022 - 3/31/2024 0.6 CM
National Institutes of Health
Engineering recombinant lubricin to combat orthopedic infection
The goal of this project is to investigate how lubricin attenuates orthopedic biofilms and to determine whether distinct lubricin *O*-glycans mediate these anti-biofilm properties.
Role: Principal Investigator

Reesink (PI) 5/1/2022-4/30/2024 0.01 CM
Veterinary Orthopedic Society (VOS)
Investigation of equine osteoarthritis immunophenotypes and immune responses to intra-articular therapeutics
The goal of this project is to compare immune cell distributions in early and late equine osteoarthritis and evaluate the effects of intra-articular therapeutics on macrophage polarization.
Role: Principal Investigator

Reesink (PI) 4/1/2023-3/31/2025 0.1 CM
Grayson-Jockey Club Research Foundation
Efficacy of recombinant equine lubricin for OA
The goal of this project is to evaluate the efficacy of recombinant equine lubricin injections in the equine carpal osteochondral fragment model and to identify transcriptomic and proteomic changes in response to recombinant equine lubricin.
Role: Principal Investigator

Principal Investigator:	Dr. Rory Todhunter
Title:	Genomics of Autopsy-Negative Sudden Cardiac Death in Racing Thoroughbreds
Project Period:	1/1/20 – 12/31/23
Reporting Period:	1/1/22 – 12/31/22

The goal of this ongoing research project is to use genomic advances to characterize the genetic causes of autopsy-negative sudden death in racing Thoroughbreds to help guide the identification of associated genes that will inform test development and/or educate breeding decisions to ultimately reduce sudden death prevalence.

Sample collection was slower than expected, but has now been completed. We accumulated heart and/or spleen tissue samples from 64 affected and 91 unaffected racing Thoroughbred horses. These tissue samples were collected and shipped from collaborators in Kentucky, California, Sydney (Australia), and New York. DNA from these samples has been extracted and quantified. Pathology reports from each sample have been checked to ensure they meet the criteria for exercise-associated autopsy-negative sudden death to be included as either affected or unaffected.

With all samples now in hand, the labwork for the remaining two aims can proceed. The targeted exome-sequencing library preparation is nearing completion, with 26 of the 40 samples processed so far. For genotyping on the Illumina Equine Plus 80k array, 120 DNA samples (52 affecteds, 68 controls) have been selected and these data will be merged with genotype data from our preliminary study and from collaborators.

Principal Investigator:	Dr. Bettina Wagner
Title:	Intranasal biomarkers of EHV-1 susceptibility and protection
Project Period:	1/1/21 – 12/31/23
Reporting Period:	1/1/22 – 12/31/22

The goal of this project is to identify novel biomarkers for host immunity and protection against EHV-1. To discover these biomarkers, we looked at differences in gene expression between sick and protected horses. We used banked samples from previously infected horses, isolated RNA and submitted the samples to RNAseq analysis. We analyzed several samples from both groups of horses prior to infection and at different time points post EHV-1 infection. The data analysis identified multiple genes with high differences between the two groups. In this project, we then focused on differences in immune system genes. In particular, we identified two secreted proteins called granulysin (GLYN) and secretory leukocyte proteinase inhibitor (SLPI), as well as seven markers that are located on the surface of T-cells. The analysis of these markers during early, mid and late EHV-1 infection identified a novel biomarker candidate, SLPI, that can distinguish early and late infection stages between the two groups, infection stages within the sick group, and that can serve as indicator of protection at different time points post EHV-1 exposure. In summary, we identified a novel biomarker that is characteristic for EHV-1 protected horses and distinguishes the EHV-1 immune status of a horse during EHV-1 outbreaks.

APPENDIX C
Summary of 2022 Expenditures

2022 Research Awards	$681,407
FY23 Public Relations and Administrative Budget	$41,200
2022 Incentive Awards	$1,792
TOTAL EXPENDITURES:	**$724,399**

APPENDIX D
2022 Research Presentations

The Harry M. Zweig Memorial Fund for Equine Research

Zweig 2022 Faculty Research Presentations
Wednesday, November 9, 2022, 3:00-4:30 PM

Everyone is welcome to attend. Reception and poster session to follow in Takoda's Run 1st floor Atrium.

Yarnell Lecture Hall 4 (S1-210)

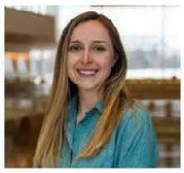
Zoom Link

Meeting ID: 970 8362 6698 - Passcode: 439076

3:00 pm *Opening Remarks by Robert Weiss, Associate Dean for Research & Graduate Education*

3:05 pm **PUTTING SOME EVIDENCE BEHIND SURGICAL TREATMENT OF PHARYNGEAL COLLAPSE**
Dr. Eileen Hackett
Department of Clinical Sciences
Professor of Surgery

3:25 pm **USING GENETICS TO IMPROVE EQUINE OCULAR HEALTH**
Dr. Kelly Knickelbein
Department of Clinical Sciences
Assistant Clinical Professor, Section of Ophthalmology



3:45 pm **UPDATE ON THE USE OF WEARABLE BIOMERIC SENSORS TO IDENTIFY HORSES AT RISK FOR CATASTROPHIC INJURY**
Dr. Scott Palmer
New York State Equine Medical Director
Department of Population Medicine & Diagnostic Sciences
Adjunct Professor

4:05 pm **EARLY DIAGNOSIS OF PLACENTITIS IN MARES**
Dr. Julia Felippe
Department of Clinical Sciences
Professor of Medicine
Provost's Fellow for Public Engagement

4:25 pm A brief overview of the Cornell Student Chapter of the American Association of Equine Practitioners (SC-AAEP) Program,
Dr. Gillian Perkins, Department of Clinical Sciences, Clinical Professor

APPENDIX E
2023 Research Awards

CONTINUATIONS

Principal Investigator	Project Title	2023 Award
Antczak, Douglas	Factors Affecting Durability in Standardbred Racehorses	$61,584
Reesink, Heidi	Equine synovial joint sepsis and fluid mucins	$55,030
Wagner, Bettina	Inflammatory biomarkers for prediction of breakdown injuries in horses	$57,914
	SUBTOTAL:	**$174,528**

NEW AWARDS

Principal Investigator	Project Title	2023 Award
Antczak, Douglas	A Rosetta Stone for Equine Immune System Genotyping	$28,203
Araos, Joaquin	Cardiopulmonary effects of a novel mechanical ventilation method in anesthetized horses	$94,092
Cercone, Marta	Evaluation of the celiac plexus block in treating paralytic ileus	$71,387
Diel de Amorim, Mariana	Investigation of interleukin 1 beta (IL1B) role in equine early pregnancy	$77,088
Felippe, Julia	Cytotoxic immune competence in the equine neonate and foal	$64,887
Van de Walle, Gerlinde	Equine gastric organoids to study the role of equine gammaherpesviruses in equine gastric ulcer syndrome (EGUS)	$74,674
Wagner, Bettina	Protecting horses better against equine herpesvirus type 1	$90,000
	SUBTOTAL:	**$500,331**
		$674,859

APPENDIX F
Zweig News Capsules

Issue #73 (June2022) and #74 (December 2022) are attached.

The full archive (1988–present) is available online at https://ecommons.cornell.edu/handle/1813/22528.

Zweig

From the Harry M. Zweig Memorial Fund for Equine Research at the Cornell University College of Veterinary Medicine

No. 73 June 2022

Delco named Harry M. Zweig Assistant Research Professor in Equine Health

By Melanie Greaver Cordova

In recognition of her dedication to extending the healthy lifespan of horses and improving their quality of life, Michelle Delco '98, D.V.M. '02, Ph.D. '16, has been named the next Harry M. Zweig Assistant Research Professor in Equine Health. This is a three-year, endowed position for a junior faculty member at the College of Veterinary Medicine who shows great promise for advancing equine research.

"Dr. Delco is an accomplished and highly valued member of our college community," said Dr. Susan Fubini, senior associate dean for academic affairs and professor in large animal surgery. "Her research program is on a tremendous upward trajectory, and its focus on equine joint disease and osteoarthritis aligns well with the objectives of the Zweig Memorial Fund."

Delco is a board-certified large animal surgeon and assistant research professor in the Department of Clinical Sciences whose time spent in clinical practice treating equine athletes for sports injuries has motivated her to discover new ways to treat and prevent osteoarthritis.

"We've domesticated this amazing species. Horses have worked alongside humans since the beginning of civilization," Delco said. "It's our job to keep them sound and healthy."

After receiving her bachelor's and D.V.M. degrees from Cornell, Delco completed internship training at Rood & Riddle Equine Hospital in Lexington, Kentucky, followed by residency training at the University of California at Davis. She then served in a faculty position at Kansas State University before working for a number of years in private practice in the Pacific Northwest.

"I did a lot of work on sport horses with complicated lameness issues," Delco said. "I got more and more frustrated diagnosing career-ending orthopedic injuries without effective treatment

Michelle Delco '98, D.V.M. '02, Ph.D. '16
Photo: Lauren Cahoon Roberts/CVM

options to offer clients and their horses."

This spurred her return to Cornell in 2012, where she studied post-traumatic arthritis in the lab of Lisa Fortier, Ph.D. '98, the James Law Professor of Surgery. Noted Dr. Robert Weiss, associate dean for research and graduate education, "With superb training as a veterinary clinician scientist, Dr. Delco is extremely well-positioned to conduct rigorous, cutting-edge biomedical research and then translate those findings for the benefit of animal health."

Dr. Michelle Delco with a patient. Photo: Carol Jennings/CVM

"I wanted to know what we were missing," Delco says. "Why weren't we making progress treating this disease?" She consequently did her Ph.D. research on the function of mitochondria, the energy generating powerhouses of cells, and their links to joint injury. Now a faculty member at Cornell with a lab of her own, Delco has developed an innovative niche in the area of mitochondrial biology within the fields of osteoarthritis and regenerative medicine. In particular, she is exploring new research questions that build on the concepts of mitochondrial dysfunction as a driver of osteoarthritis and enhancement of mitochondrial function as a new therapeutic strategy.

Delco's goal is to prevent chronic joint pain and dysfunction in both horses and humans. "For decades, lifespan has been steadily increasing — largely thanks to scientific discoveries in human and veterinary medicine," Delco said. She notes, however, that healthspan — the number of high-quality years lived — has not similarly increased. "We're developing new approaches to stop joint degeneration after injury. Whereas arthritis in human athletes can be career-ending and painful, for equine athletes, it can be life-threatening."

Delco is also keen to bring this scholarly expertise to her role as a surgeon at the Cornell Equine Hospital, where she works on the orthopedic surgery service. She's especially interested in minimally invasive surgery techniques like arthroscopy in standing horses. In both the hospital and her research lab, Delco collaborates with a team of skilled surgical residents, students, technicians and researchers.

"Beyond her many research accomplishments, Dr. Delco also is active in teaching and mentoring, and her passion for helping to develop young scientists is an asset to all of us," Fubini said.

"There's such a richness that comes from a diverse group of people working together," Delco said. "For example, my research group is a collection of talented and engaged people, all at different stages of their training, who are excited about science and progress. They inspire me. I feel lucky to work with such a great team."

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry Zweig, a distinguished veterinarian known for his contributions to New York's equine industry. In 1979, the New York State Legislature created the Zweig Fund to support and promote equine research at the Cornell University College of Veterinary Medicine. Read more about past Harry M. Zweig Assistant Professors online at bit.ly/ZweigFund ●

Harry M. Zweig Memorial Fund for Equine Research Awards

New

$91,661 to Bettina Wagner for "Inflammatory Biomarkers for Prediction of Breakdown Injuries in Horses"

$83,229 to Michelle Delco for "Synovial Fluid Extracellular Vesicles in Equine Joint Disease and Therapy"

$67,973 to Heidi Reesink for "Equine Joint Sepsis and Synovial Fluid Mucins"

$65,827 to Douglas Antczak for "Factors Affecting Durability in Standardbred Racehorses"

$47,948 to Mariana Diel de Amorim for "Inflammatory Markers from Endometrial Swab/Cytobrush as a Screening Test for Equine Endometritis and Endometrial Fibrosis"

Continued

$99,297 to John Pigott for "Multi-modal Screening to Identify Thoroughbred Racehorses at Increased Risk for Catastrophic Injury of the Metacarpophalangeal Joint"

$86,451 to Bettina Wagner for "Intranasal Biomarkers of EHV-1 Susceptibility and Protection"

$79,352 to Julia Felippe for "Diagnostic Markers in Mares with Placentitis"

$49,672 to Rory Todhunter for "Genomics of Autopsy–Negative Sudden Cardiac Death in Racing Thoroughbreds" ●

2022 Harry M. Zweig Memorial Trot

July 9 | Post time 12:10 p.m. | Vernon Downs Casino

In search of the 'Iron Horse'

By U.S. Trotting News

Research out of the Antczak lab at the Baker Institute for Animal Health will focus on understanding the factors that may contribute to the durability and longevity of career harness racehorses, by studying their genes. A recent award through the Harry M. Zweig Memorial Fund for Equine Research at Cornell University has brought the collaboration between the Baker Institute and the United States Trotters Association (USTA) to life.

The Baker Institute
Photo: Jason Koski/Cornell

The goal of the project is to advance the understanding of the genetic factors that could contribute to Standardbred harness horses' career success. Those horses who go on to have successful racing careers of five, six, seven-years in duration, with limited injuries, are the sought-after 'Ironman' of the horse racing industry.

Studies such as this, revealing if genetics play a role in a horse's predisposition to avoiding catastrophic injury suffered in racing or training, could help reduce wastage in the horse racing industry, and allow horses and their owners to have longer, more successful careers. This is good for the horse, good for the owners and good for the industry as a whole.

A study with this unique focus has not previously been done. Life-threatening injuries suffered by horses while racing or training are tragic for the horse and their rider, owner or trainer. Previous investigations have implicated many factors that may contribute to these types of injuries, including physical fitness, training regimens, track surfaces, use of illegal drugs and genetics. With so many variables, and relatively few affected horses, it has been difficult to separate the genetic from the environmental factors.

Recently published work does support the premise that genes do play a role in racing career success and durability. In this study, an interdisciplinary team of investigators that include members of the Horse Genome community of geneticists who have worked together for years, along with other veterinary scientists and clinicians will dig deeper.

In Dr. Doug Antczak's study, "Factors Affecting Durability in Standardbred Racehorses," the focus will remain on racehorses that have demonstrated durability and longevity in their careers, proposing that these horses are predisposed to their success due to their genetics coupled with excellent care and training. In this project, researchers will use genome-scale and epidemiological methods to identify genetic signatures correlated with longevity of racing career.

Using a Genome Wide Association Study (GWAS) and the 670k Equine Single Nucleotide Polymorphism (SNP) array, Antczak's lab will

compare three groups of horses, including: 100 aged Standardbreds with long racing careers and competitive race records; 100 aged Standardbreds with long racing careers but relatively weak earnings records; and 100 or more control Standardbreds selected from previous studies. They will test the hypothesis that the durable horses will show a GWAS peak on chromosome 7 that has been associated with racing starts in Thoroughbred horses. They may also be able to identify genetic regions that distinguish competitive from non-competitive horses with long racing careers. The lab will also obtain 20 x coverage whole genome sequence from 10 selected durable and 10 control Standardbreds for fine-scale comparison of regions identified in part one.

The USTA, an entity that governs Standardbred racing, will play an instrumental role in this study as well, providing access to data on samples from USTA studies on horses that are still racing at the age of seven years old. The organization is also raising awareness of this important work happening, revealing a better understanding of the durability of the breed, and by helping to deploy a 'citizen science' approach to promoting the collection of more samples from appropriate candidates across the Standardbred racing industry.

Researchers will seek out additional genome samples from racehorses, and will be seeking geldings with the appropriate balance of starts and wins throughout their career, while also being of the appropriate age. Those who feel their horses may fit the model and are interested in being involved can contact the Baker Institute.

The study will begin this summer with the examination of samples from previous USTA studies, followed by the study of the new collections gathered via the call for samples. ●

Dr. Doug Antczak
Photo: Rachel Philipson/CVM

Q&A series: Meet our new equine faculty

Dr. Eileen Hackett, professor in the Section of Large Animal Surgery

Q What has been your academic/career path leading to Cornell?

After completing veterinary training in my home state of Illinois and an internship at Rood and Riddle Equine Hospital in Lexington, Kentucky, I underwent surgical training at the University of Pennsylvania's New Bolton Center. I spent just over 19 years as a surgeon and professor at Colorado State University before joining the Cornell faculty in the fall of 2021.

Q What drew you to CVM?

Cornell has a tradition of excellence and innovation, especially in the field of large animal surgery.

Q What is your area of expertise?

I am very passionate about soft tissue surgical innovation, especially in the area of upper airway and minimally invasive surgery.

Q What drew you into this area? Any specific experiences, mentors or influences that helped guide you?

Great mentorship in my early career gave me a deep appreciation and love for this specialty. Drs. Norm Ducharme and Susie Fubini have been highly influential and exemplify ingenuity, dedication and skill.

Q What past professional work are you most proud of and why?

There are so many fond memories that I have of meeting horses and their owners, working through problems and improving outcomes. I am happy that I get to make a difference in the health of horses and their owners.

Q What about your clinical work, research or teaching innovations are you most excited for or proud of and why?

I focus on research that is highly applicable and results in publications that I would like to read. There are still so many unanswered questions in our field and it is important to be part of the conversation and contribute to the knowledge of our profession.

Q What impacts or applications do you hope to see your work have on the world, human/animal/planetary health?

A big of part my focus on soft tissue surgical innovation is making a difference in your very next case with quicker healing times, less pain associated with treatment and better outcomes.

Q What clinical/scientific questions are you looking to answer next or areas you plan to explore?

Our big push at the moment is to better understand equine pharyngeal function and surgical treatments, building on all of the tremendous work that has already been completed right here at Cornell.

Q What's something most people don't know about you?

I'm an 'A' Pony Clubber!

Q What's the best part of being a clinician/scientist?

Really all the parts!

Q What's the most challenging part?

We believe here at Cornell that the only limit is our imagination. It's important to keep pushing the envelope and to always question why we do things.

Q What are the benefits of working at CVM? At Cornell?

Our community is our most precious resource to do the greatest good! ●

Dr. Eileen Hackett
Photo provided

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The Harry M. Zweig Memorial Fund Committee extends its gratitude to exiting committee members Dr. Robert Tugel and Ms. M. Kelly Young.

We are pleased to welcome back Mr. Ronald Ochrym as treasurer.

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Visit us online
bit.ly/ZweigFund

Our site provides information on the projects and publications resulting from the Zweig Memorial Fund, and demonstrates the objectives of the Fund in promoting equine health in the racing industry. The Zweig News Capsule is published twice a year, and can be downloaded at bit.ly/ZweigNews. Please encourage other equine enthusiasts to visit the site.

Zweig

From the Harry M. Zweig Memorial Fund for Equine Research at the Cornell University College of Veterinary Medicine

No. 74 December 2022

Antczak attends Ascot horse races with the royals

By Olivia Hall

The recent death of Britain's Queen Elizabeth in early September had special poignancy for one Cornell alumnus. In April of this year, Dr. Doug Antczak '69, the Dorothy Havemeyer McConville Professor of Equine Medicine at the James A. Baker Institute for Animal Health, received an unexpected email. The message was from the Deputy Master of the Household and Equerry to Her Majesty the Queen of England, who inquired politely whether Antczak and his wife Wendy "might be persuaded to come over to the UK to join Her Majesty as her guests" for lunch at Windsor Castle and an afternoon of horseracing at the nearby Ascot Racecourse. "Her Majesty appreciates that it might be a tall order to come over from Cornell just for a day's racing but would be delighted if you could," the letter added.

Wondering if this might be a scam, Antczak telephoned Buckingham Palace the next day. It was confirmed: The invitation from the queen, who had a notable passion for horses, was genuine. "The queen was interested in learning about new discoveries in the fields of equine genetics and genomics," Antczak said. The invitation then made sense to Antczak, who is recognized internationally for his research achievements in equine reproduction, immunology and genetics.

For over two decades, Antczak has been part of the Horse Genome Project, an international collaboration between more than two dozen laboratories working on horse genetics. The Antczak Lab has made important contributions at many stages of the global effort to sequence the genome of the horse. Perhaps most notable is the Cornell horse-breeding program that produced the Thoroughbred mare, Twilight. Since the early 1980s, Antczak has bred horses selected for genes of the Major Histocompatibility Complex, the genomic region that controls many aspects of immune responses. These special Cornell-bred horses have helped advance knowledge of equine immunity, reproductive biology and regenerative medicine. Twilight was selected as the DNA donor for the horse genome sequence, known as the Equine Reference Sequence. This was the first full horse genome sequence ever completed. Today, equine geneticists from across the world use the Twilight Reference Sequence to help them interpret a wide array of equine genetic studies. "Twilight is the most famous research horse in the world," Antczak explained. "That puts Cornell on the map for our contribution to the Horse Genome Project."

"But why invite me and not one of the many other outstanding equine genomics specialists from

Dr. Doug Antczak '69 and Wendy Antczak arrive at the summer 2022 Royal Ascot Races in an open Ascot Landau carriage with Queen Elizabeth's granddaughter, Princess Beatrice, and her husband, Edoardo Mapelli Mozzi. Photo: Getty Images

around the world?" he said. They never learned the answer from the queen's staff at Windsor Castle, but it may have been in part because of Antczak's personal involvement with horse sports, and the Ph.D. that he earned at Cambridge University in the 1970s. "One of my favorite Winston Churchill quotes is this – A polo handicap is a passport to the world," said Antczak, who was captain of the polo team as a Cornell undergraduate. Antczak later met and played with then-Prince Charles a few times during his years at Cambridge.

On June 18, Antczak and his wife — in traditional top hat and tails and an elegant dress — drove into Windsor Castle for what would be a magical day. Prior to their arrival in England, little information was provided to Antczak and his wife, other than the dress code and very general expectations of the day's events. This resulted in several surprises. "I had anticipated a large luncheon group, and that perhaps I would meet the queen and have a short conversation, and that would be all," said Antczak. Upon arrival at Windsor Castle, Antczak learned that the luncheon party was very small, only three tables of eight, and that he would be seated at the right hand of the queen herself. Many of the guests had strong interest and deep involvement with horses. "It dawned on me that this event had been arranged by the queen so that she could be surrounded by people who shared her passion for the horse," Antczak reflected. "The realization that I was among fellow horsemen and horsewomen made conversation easy."

Dr. Doug Antczak '69 and Wendy Antczak at the summer 2022 Royal Ascot Races. Photo: Mr. Sandy Dudgeon/Provided

Antczak spent his time with the queen discussing the state of horse genetics, his research at Cornell, and many other topics. "She was very, very knowledgeable about horses, and through her passion she elevated the stature of horses in England and the world. The queen was very well informed and kept me on my toes with her questions. She was gracious, down to earth and refreshingly direct." Even the queen's beloved corgis made an appearance, coming in at the end of the hour to collect some table scraps.

While the queen — limited in her mobility — remained at the castle to watch the races on TV in her private rooms, the remaining guests were transported first by car, and then horse-drawn carriages for the 45-minute, seven-mile ride through Windsor Great Park to the Ascot Racecourse. For the journey, the Antczaks were seated in an open Ascot Landau carriage with the queen's granddaughter Princess Beatrice and her husband Edoardo Mapelli Mozzi. "We had no idea we'd be riding in carriages," Antczak said, "let alone with members of the royal family." Along the way, members of the public lined up to catch a glimpse of royalty. "So, we went along for the ride and waved to people as if we did this regularly," Antczak remembered.

The racecourse itself was packed with some 70,000 people, and as one of the most formal of the British horseracing calendar, "Royal Ascot was fancy beyond belief," Antczak said. Royal Ascot dress code calls for morning dress for men — top hat and tails — and elegant attire for women. Racing enthusiasts filled the grandstand and surrounding areas, and the Antczaks enjoyed a spectacular backdrop to a day of racing that featured many of the finest Thoroughbreds in the world.

Reflecting on the event, Antczak described it as, "a bittersweet memory — the queen was such a great advocate for the horse throughout her life, and she had sincere concern for horse welfare. The equine world is diminished by her passing. It was an honor to have been invited by the queen to represent the global community of equine geneticists who have worked together for over a quarter century to advance the state of horse genomics. I wish that my colleagues could have joined me on that day." ●

Twilight was the star attraction at the International Havemeyer Foundation Horse Genome Workshop that Cornell hosted in July of this year. Photo: John Enright/CVM

Recent publications from Zweig-funded projects

Jager MC, Tomlinson JE, Henry CE, Fahey MJ, Van de Walle GR. "Prevalence and Pathology of Equine Parvovirus-Hepatitis in Racehorses from New York Racetracks." Virology Journal, Nov. 2022.

Luedke LK, Ilevbare P, Noordwijk KJ, Palomino PM, McDonough SP, Palmer SE, Basran PS, Donnelly E, Reesink HL. "Proximal Sesamoid Bone Microdamage is Localized to Articular Subchondral Regions in Thoroughbred Racehorses, with similar Fracture Toughness Between Fracture and Controls." Veterinary Surgery, Aug. 2022.

Miller JL, Kanke M, Rauner G, Bakhle KM, Sethupathy P, Van de Walle GR. "Comparative Analysis of MicroRNAs that Stratify in Vitro Mammary Stem and Progenitor Activity Reveals Functionality of Human miR-92b-3p." Journal of Mammary Gland Biology and Neoplasia, Oct. 2022.

Rojas-Núñez I, Gomez AM, Selland EK, Oduol T, Wolf S, Palmer S, Mohammed HO. "Levels of Serum Phosphorylated Neurofilament Heavy Subunit in Clinically Healthy Standardbred Horses." Journal of Equine Veterinary Science, March 2022.

Thomas MA, Fahey MJ, Pugliese BJ, Irwin RM, Antonyak MA, Delco ML. "Human Mesenchymal Stromal Cells Release Functional Mitochondria in Extracellular Vesicles." Frontiers in Bioengineering and Biotechnology, Aug. 2022.

Van de Walle, GR. "The Potential of the Mesenchymal Stromal Cell Secretome in Equine Regenerative Medicine." Tissue Engineering, Part A, Vol. 28, April 2022.

Wang Z, Chivu AG, Choate LA, Rice EJ, Miller DC, Chu T, Chou SP, Kingsley NB, Petersen JL, Finno CJ, Bellone RR, Antczak DF, Lis JT, Danko CG. "Prediction of Histone Post-Translational Modification Patterns Based on Nascent Transcription Data." Nature Genetics, March 2022.

From roaring to racing, Zweig researchers showcase latest discoveries at annual meeting

By Lauren Cahoon Roberts

On Nov. 9, the Harry M. Zweig Memorial Fund for Equine Research held its annual meeting at the Cornell University College of Veterinary Medicine. College faculty, trainees, students and Zweig committee members gathered to present and discuss the latest research in horse health.

After welcoming remarks from Dr. Robert Weiss, associate dean for research and graduate education, attendees listened to a series of research presentations that covered a wide gamut of equine research.

Poster presentations at the annual Zweig event. Photo: Carol Jennings/CVM

Dr. Eileen Hackett, professor of surgery in the Department of Clinical Sciences, gave her talk, "Putting Some Evidence Behind Surgical Treatment of Pharyngeal Collapse." She noted that there are no studies on airway dynamics in horses with pharyngeal collapse. There are several procedures that practitioners employ to treat the issue, but "despite routine performance, no studies to date have evaluated surgical treatment of this condition," said Hackett. "We need evidence-based recommendations." For next steps, Hackett will be looking for ways to reproduce the condition and measure success of each surgical technique in a study setting.

Dr. Kelly Knickelbein, assistant clinical professor in the Department of Clinical Sciences, presented her talk, "Using Genetics to Improve Equine Ocular Health," in which she discussed how some of the most common eye diseases found in horses can have a genetic connection. For example, ocular surface squamous cell carcinoma is associated with a gene mutation that is prevalent in certain breeds, including Haflingers, Rocky Mountain Horses and Belgians. Knickelbein has also studied congenital cataracts, and through whole genome sequencing, is investigating variants in relevant genes and hopes to accumulate enough cases to conduct a genome-wide association study.

Next, Dr. Scott Palmer, New York state equine medical director and adjunct professor in the Department of Population Medicine and Diagnostic Sciences, presented "Update on the Use of Wearable Biometric Sensors to Identify Horses at Risk for Catastrophic Injury," in which he shared the latest findings from work done in collaboration with college colleagues, including Drs. John Piggott and Alan Nixon, in using Stride Safe GPS biometric sensors to measure racehorses' acceleration on the track. The sensor can create a 'fingerprint' of an elite racehorse's gait at high speed, showing what a sound horse should look like while racing. From this, Palmer created three levels — red, yellow and green — each associated with the level of an equine's deviation from the ideal G-forces while running. Sensors were placed on every horse that raced at Belmont and Saratoga during the summer of 2022, yielding key insight. "We learned that these sensors are reliable screening tools," said Palmer. "We can detect gait abnormalities before catastrophic breakdowns."

Moving forward, Palmer will investigate the correlation of data between different types of

exercise, and will continue to refine his algorithm for an 'animal welfare index' for each racehorse that will identify each animal's overall risk for breakdown.

Dr. Julia Felippe, professor in the Department of Clinical Sciences, then discussed her work in her presentation, "Early Diagnosis of Placentitis in Mares," describing her efforts to pin down biomarkers to identify the condition before it's too late. "Initial clinical signs of placentitis can be detected using transrectal ultrasonography, but this type of diagnosis relies on ongoing inflammation of placental tissues," Felippe said. Her research looked to identify blood parameters in mares that would sign for the early stages of ascending placentitis, before inflammation establishes. She found estradiol 17-beta concentration was a potential candidate for an early diagnosis, which would allow immediate treatment and improved pregnancy and foaling outcomes.

The final presentation was given by Dr. Gillian Perkins, clinical professor in the Department of Clinical Sciences, who gave a brief overview of the Cornell student chapter of the American Association of Equine Practitioners. Perkins has been the faculty advisor for the group for roughly two decades, and has overseen the group's many events, including a welcome-back barbecue and a 5K around the Cornell Equine Park track. Students have also had chances to participate in equine-specific labs on colic, reproduction, lameness, acupuncture and dentistry. "It's been 20 great years of working with wonderful students," Perkins said.

Dr. Robert Weiss, associate dean for research and graduate education, welcomes everyone to the annual Zweig event. Photo: Carol Jennings/CVM

The symposium wrapped up with a poster session and reception, during which attendees dined, mingled and discussed the broad array of research projects on display. The session featured a contest for best poster, with Ph.D. student Erica Secor '09, D.V.M. '13, winning the popular vote.

"Once again, the Zweig meeting inspired and energized everyone there," said Weiss. "It's gratifying to see the breadth and depth of research going on all in the name of equine health, and I look forward to seeing the next series of discoveries when we all gather again."

The Harry M. Zweig Memorial Fund for Equine Research Committee, excepting absent members Laura Javsicas, Patricia Wehle and William Wilmot. Photo: Tessa Brown/CVM

Cornell Ruffian veterinarians save older horse from severe colic

By Christina Frank

Colby Prokop and her horse, Astrid, essentially grew up together. The two are both 25 years old now, but they met when they were 13.

At the time, Prokop had a job exercising Astrid — a retired Thoroughbred racehorse — at a farm in Jamesport, New York, near her home on Long Island. Astrid's original owner lived in Manhattan and was finding it difficult to visit her on a regular basis. Eventually, Prokop became her owner. "She's my heart horse," says Prokop. "She's such a special nugget."

In December 2018, Prokop was studying for finals at the University of Richmond in Virginia when she got a call from her mother saying that Astrid was suffering from severe colic, a general term for experiencing abdominal pain. Their local veterinarian felt strongly that Astrid needed to be evaluated by doctors at Cornell Ruffian Equine Specialists (CRES) in Elmont, New York — a two-hour drive from where the family lives.

Astrid and Colby Prokop at Cornell Ruffian Equine Specialists. Photo provided.

Prokop flew home immediately. By the time she arrived, Astrid had been admitted and was undergoing surgery. An ultrasound had revealed thickening and distension of her small intestine due to a bowel obstruction.

According to John Pigott, D.V.M. '09, hospital director of CRES, many horses with colic are successfully treated with an anti-inflammatory medication and fluids on the farm, with about 30 to 40 percent needing more aggressive treatment in the hospital. Pigott cared for Astrid after the procedure, which was performed by Michelle Delco '98, D.V.M. '02, Ph.D. '16, the Harry M. Zweig Research Professor in the Department of Clinical Sciences at the Cornell University College of Veterinary Medicine.

"The surgery went great," says Pigott. "They found an adhesion in the front of her abdomen and the bowel got trapped against that scar tissue. They were able to remove the scar tissue and free up the bowel. Nothing needed to be cut out, which improved the prognosis."

While recovering, however, Astrid experienced some complications. She developed ileus, a transient decreased motility in the gut. This was followed by an aggressive case of pneumonia. "Pneumonia after severe colic can happen in some horses, particularly with cases of small intestinal obstruction," Pigott says.

With aggressive treatment, Astrid recovered completely. "She is a very tough horse. She had a severe colic event and pneumonia and was able to heal with intensive therapy," Pigott says.

In total, Astrid was at CRES for almost a month. She was admitted Dec. 8, 2021, and discharged Jan. 2, 2022.

"The worst day was Christmas Eve," says Prokop. "That was when Dr. Pigott told me that if she didn't turn a corner, it was not looking good. It was the pneumonia that almost got her."

When Astrid did turn a corner, she turned it quickly. Prokop, who drove two hours each way to see Astrid every day during her hospitalization, went to visit her horse on Christmas morning. "She was up at the gate and wanted to eat carrots and looked so great," Prokop says. "She was like our Christmas miracle."

On the day she was discharged, Prokop says, the staff had a little going away party for her. Two

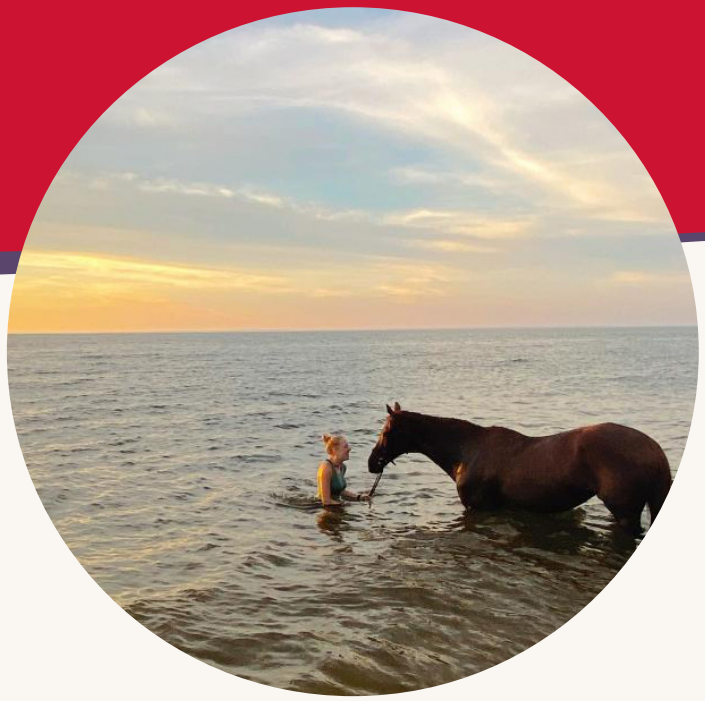
of her technicians even gave her handfuls of peppermints, which are her favorite treats.

It took another three months of stall rest, daily hand walking and overall monitoring of her health before she was fully healed.

"They definitely set us up for success at Cornell Ruffian," says Prokop. "The vets there were just completely out-of-this-world impressive. I never once doubted that she was in the best of hands when she was there."

Almost four years after her ordeal, Astrid is currently living the good life in California. Prokop was offered a dream job as an animal care crew manager at the Marine Mammal Center in Sausalito, and the two picked up and drove across the country.

"I never wanted to put her through something that would potentially be adverse to her health, but at 25 [approximately 75 in human years], she shipped across the country like a well-seasoned traveler," says Prokop. "She's still a handful for me under the saddle. She has so much energy that I can barely hold her back because she wants to gallop everywhere!"



Astrid with Colby Prokop. Photo provided.

Prokop continues, "Our family will be forever thankful to Dr. Pigott, Dr. Delco and the entire team. Without them, I would never have been able to bring Astrid with me." ●

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The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State Legislature created the fund to promote equine research at the College of Veterinary Medicine at Cornell University. The Harry M. Zweig Committee is 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.

Visit us online
bit.ly/ZweigFund

Our site provides information on the projects and publications resulting from the Zweig Memorial Fund, and demonstrates the objectives of the Fund in promoting equine health in the racing industry. The Zweig News Capsule is published twice a year, and can be downloaded at bit.ly/ZweigNews. Please encourage other equine enthusiasts to visit the site.