2024 Florida Veterinary Scholars Program
Faculty Application

Name	Amara Estrada
Email address	estradaa@ufl.edu
Proposed project title	In vitro modeling of cardiac diseases
Will you provide matching student stipend funding ($2500)?	Yes.
Source of project/research funding	Faculty IDC funds

Prior student research mentees (last 5 years, if applicable):

CLASS	STUDENT	PROJECT TITLE	STATUS
2026	Sasha Spada-O'Neill	CRISPR-Mediated Gene Editing in Canine Cells for Pyruvate Dehydrogenase Kinase 4 to Treat Dilated Cardiomyopathy	Project ongoing, Manuscript in progress
2026	Arielle Admonius	CRISPR Gene Editing in Feline Cells for Alstrom Syndrome Protein 1 Associated Hypertrophic Cardiomyopathy	Manuscript in progress
2025	Nirali Pathak	Optimizing Gene Editing in Canine Cells Using CRISPR/Cas9 for Pyruvate Dehydrogrenase Kinase	Poster presentation at NIH/Manuscript pending
2022	Barbara Berrios	Fibroblast cell isolation and expansion and iPSC derivation in purebred Maine Coon and Ragdoll cats	Poster presentations at Phi Zeta and ACVIM
2022	Deborah Grimaldi	Utility of routine pre-operative electrocardiographic recordings in canine patients using a computer-aided electrocardiographic algorithm	Poster presentations at Phi Zeta and ACVIM. Manuscript published in JVC
2022	Erica Castaneda	Utility of routine pre-operative electrocardiographic recordings in feline patients using a computer-aided electrocardiographic algorithm	Poster presentations at Phi Zeta and ACVIM. Manuscript published in JVC
2019	Katy Taggart	Pyruvate Dehydrogenase Kinase 4 (PDK4) and mitochondrial metabolism in Doberman Pinschers	Poster presentations at Phi Zeta and ACVIM. Manuscript published in Science

I agree to obtaining all necessary approvals (e.g. IACUC/IRB/EH&S/VHRRC – see below for specifics)

to conduct the project with the student PRIOR to the commencement of the summer program, as well as submitting documentation of these approvals to the FVSP board by 5/20/24

YES

I agree to assisting my student prepare for the summer program during the Spring semester, which will include preparation of a study outline, and training in relevant laboratory techniques

YES

I agree to plan for commencing the experiment/data collection by the beginning of the summer program (5/20/24)

YES

	Needed (Yes/No)	Approval by 5/20/24 (Yes/No)?
IACUC Approval and Training	Yes	Yes
IRB Registration and Training	No	
Biological Agent Registration	No	
Biopath Registration	No	
Veterinary Hospital Research	No	
FERPA Training	No	
Biohazardous Waste Training	No	
Laboratory Safety Training	Yes	Yes

Abstract of proposed student project.

Cardiac diseases in veterinary medicine are often quite similar to cardiac diseases in human medicine. Yet the ability to directly transfer diagnostic or therapeutic advances from human medicine to veterinary

medicine, or vice versa, is often a time intensive and expensive pathway. Our veterinary clinical faculty encounter these diseases on a daily basis and may have questions as to the efficacy or value in using new discoveries or ideas in an efficient manner. Our group of veterinary specialists and basic scientists have been working together to create an in vitro model of cardiac disease by utilizing either skin biopsies or peripheral blood samples to isolate and expand early progenitor cells and then reprogram these cells to crease induced pluripotent stem cells (iPSCs) that can then be differentiated into cardiomyocytes. These cardiomyocytes can then be used in vitro to test various diagnostic or therapeutic compounds for efficacy bench-side before proceeding to clinical trials. This is important as different species and different breeds have different clinical presentations, as well as different genetic mutations with variable penetrance. Thus, what might be an effective strategy for one genetic disease, may not be as effective in another type of genetic or non-genetic/spontaneous disease. Because of this, treatment or therapeutic strategies which are proven to be clinically effective for humans, or for one canine or feline breed, may not work as well in another breed even if the genetic mutation is similar. An in vitro cellular model allowing for more in-depth bench top assessment of novel therapeutic strategies could prove beneficial in these types of assessments.

In vitro models of heart disease are not readily available and most pre-clinical work in this area have relied on mouse models as an alternative. For this study, we seek to create a benchtop platform with which different therapeutic modalities (nutritional, environmental, pharmacogenetic) can be tested for efficacy prior to initiating such therapy in a patient enrolled in a clinical trial. The ability to reprogram early progenitor cells derived from many different tissues has been previously described. The goal of the study presented in this report was to identify a somatic cell source that can be collected quickly and easily in canine and feline patients with naturally occurring heart disease. One such cell source is the fibroblast but this technique requires a skin biopsy which can be stressful or not possible in some patients without sedation. The dermal papillae of vibrissae (whiskers) and urine derived renal epithelial cells are potential sources of somatic cells that can be collected quickly and non-invasively with minimal restraint for the patient. This investigation aims to develop the first protocol for isolation and identification of somatic cells from dermal papillae and renal epithelial cells in canine and feline patients. Additionally, it investigates cell expandability to determine the efficiency and practicality of differentiating these cell cultures into beating cardiomyocytes for use of these cells in future studies.

The objectives of the study will include the following: (1) Collect vibrissae and urine derived renal epithelial cells from canine and feline patients with underlying heart disease, (2) Modify published human protocols to isolate and expand stem cell populations and identify populations with pluripotency and, (3) Modify published human protocols to allow for cardiac differentiation of these iPSCs resulting in autologous contracting cardiomyocytes

Approach: Our lab has been working with Doberman, Maine Coon, Ragdoll and Sphynx peripheral blood mononuclear cells and fibroblasts to create iPSCs and this project will continue this work and protocol modifications which have already proven effective in creating beating cardiomyocytes from these new cell sources.

The significance for Veterinary Medicine and the student.

The student participating in this project will work alongside other veterinary students, graduate students and post-doctoral students working on similar or inter-related projects in our laboratory centered several funded projects investigating CRISPR/Cas9 gene editing. They will learn cell culture and reprogramming techniques but most important will work in a Team Science atmosphere where all projects are somehow related to each other and are small off-shoots of larger projects happening in the lab. We have weekly lab meetings and work as a team on all projects. The student can expect to have their own

project/poster/manuscript but will likely also be learning about the other projects occurring around them as well. We encourage trainees to present their research at the UF Genetics Institute symposium in the Fall, participate in the Phi Zeta Day at our own college and travel to the research symposium to present their own projects at the end of the summer. Students will develop their own posters and present to the wider UF community, representing our College in research on campus which is very important.

References:

1. Xu J, Yu L, Guo J, et al. Generation of pig induced pluripotent stem cells using an extended pluripotent stem cell culture system. Stem Cell Res Ther. 2019 Jun 27; 10 (1).
2. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007 July 19; 448(7151).
3. Zhang H, Tian L, Shen M et al. Generation of Quiescent Cardiac Fibroblasts From Human Induced Pluripotent Stem Cells for In Vitro Modeling of Cardiac Fibrosis. Circ Res. 2019 Aug 16;125(5).
4. Yang J, Liu H, Sun H et al. Construction of induced pluripotent stem cell line (ZZUi0017-A) from the fibroblast cells of a female patient with CACNA1A mutation by unintegrated reprogramming approach. Stem Cell Res. 2020 Oct 48.
5. Zhou T, Benda C, Dunzinger S et al. Generation of human induced pluripotent stem cells from urine samples. Nat Protoc. 2012 Dec; 7(12).
6. Steinle H, Weber M, Behring A, et al. Reprogramming of Urine-Derived Renal Epithelial Cells into iPSCs Using srRNA and Consecutive Differentiation into Beating Cardiomyocytes. Mol Ther Nucleic Acids. 2019 Sep 6 (17).