Abstract of proposed student project (1 page limit. This should mirror the aims page of a grant and CLEARLY indicate the student's role.)

willHistologic quantification and gene expression for ovarian follicular dysplasia (OFD). Short term aim for student: Quantitative analysis of normal vs bovine disease ovaries. Long term aim of project: To develop a rapid diagnostic biomarker test that can be used on peripheral blood or cervical-vaginal mucus to allow detection of early (sub-clinical) ovarian follicular dysplasia (OFD). OFD was determined to be the leading cause of female infertility on twelve Florida beef ranches (Braden 2016. Braden 2017, Nobre 2019). Preliminary transcriptome analysis show correlation between genes involved in vascular formation. Previous and current work is supported by the Florida Cattlemen's Association (FCA) and funded by Florida Cattle Enhancement Fund (FCEF). All tissues needed to complete our year one study were collected under Institutional Animal Care and Use Committee-Auburn University guidelines during the 2015-2017 FCEF Project. A full set of tissues were collected at three locations from 36 females (two private Florida ranches and control cows an Auburn University farm) and were preserved and transported to University of Florida College of Veterinary Medicine (UFCVM). From this group, we selected a characteristic subset of tissue samples from four cows with late stage OFD, four heifers with early stage OFD, one cow with a Sertoli-type sex cord tumor, and four control heifers and four control cows with normal ovaries. These tissue sets including sagittal sections of both ovaries, blood samples including serum, plasma, and buffy coat (cells), as well as cervical mucus samples, and all were preserved for subsequent molecular and microscopic studies.

The student will train algorithm in HALO by Indica labs with artificial intelligence (AI) to annotate up to 10 structures from both ovaries of 36 cattle (72 ovaries total). Structures will include area of follicular dysplasia, areas of neoplasia, follicular cyst, Graafian follicles, atretic follicles, secondary and primary follicles, mesenchymal composition and vascular structures. Selected ovaries will be stained with Vascular endothelial Growth Factor and area of immuno-expression for VEGF will also be quantitated using HALO. At the laboratory of Chris Martyniuk transcriptome analysis will be conducted on ovary tissue and blood from 17 cattle (8 controls and 9 effected). To identify circulating RNA biomarkers global analysis of RNA levels (RNAseq) will be conducted on blood (buffy coat), the cell free serum, and the cervical mucus (which can contain epithelial cells). Ovarian structures will be compared to locally expressed genes in ovary tissue and circulating long RNA. We will then thus carry out an integrated computational analysis of the multiple data sets to provide potential insight into the pathogenesis of OFD using an analysis program (e.g., Pathway Studio). The molecular results will be compared to quantified structural data from of ovary tissue. Initial algorithm training by the student is anticipated to take 4 weeks. Analysis of data is expected to take two weeks and final preparation of abstract expected to take two week. With the algorithm created by the student ovaries from and additional 120 cows will be analyzed. The results of this study will contribute to the foundation paper for ovarian follicular dysplasia in Florida beef cattle.