Title: Development of a Diagnostic Test for Ovarian Follicular Dysplasia (OFD)
Principal Investigator: Tim D. Braden, Auburn University
Date: Sept 1, 2017

This is the final research report from the above work. Although funding and duration of work was planned for 12 months, due to delays from the funding source, this time-frame was compressed to 4 months.

Summary of Findings

The overall goal of this research is to characterize and develop a diagnostic test for OFD, as well as disseminate our findings. To this end, a variety of samples were collected this year in order to identify differences between affected and unaffected cows. Collection of ovarian tissue (post-mortem) confirmed differences found previously in the population of ovarian follicles between affected and unaffected cows. The sizes of these follicles are too small to be detected with in vivo ultrasound procedures, but mineralization was found to be prominent in affected cows. Ultrasound testing from this year suggests that OFD at levels 2, 3 and 4 may be identifiable (see below).

Analysis of metals from liver samples, collected post-mortem, revealed some dramatic differences between affected and unaffected cows. Levels of sodium, cadmium, copper, and barium appear to have potential for diagnostic value in identifying affected and unaffected cows. While there is a correlation between these liver metal concentrations and OFD, it is unknown if this is a cause or an effect of OFD.

DNA has been collected from several tissues (blood, ovary, uterus, cheek, vagina) as well as body fluids (blood, saliva) of affected and unaffected cows. Preparation of those samples for genomic and endocrine analyses has not been completed, but materials have been secured for this purpose. Due to the delay in securing funding, these samples have not yet undergone genomic analyses, and funds for this portion of the project were not encumbered.

Dissemination of our results have been provided to owners of the animals used in these studies. Moreover, six presentations of our results have been made at local, national, and international meetings attended of professionals in the animal industries affected by this disorder. A draft manuscript, to be submitted to an international pathology journal, has been prepared.

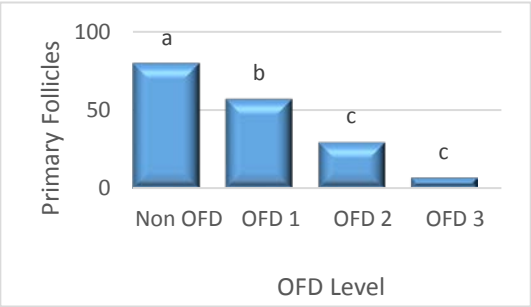
An estimate of categorized expenditures (direct costs only) is attached to the end of this document.

Detailed Results

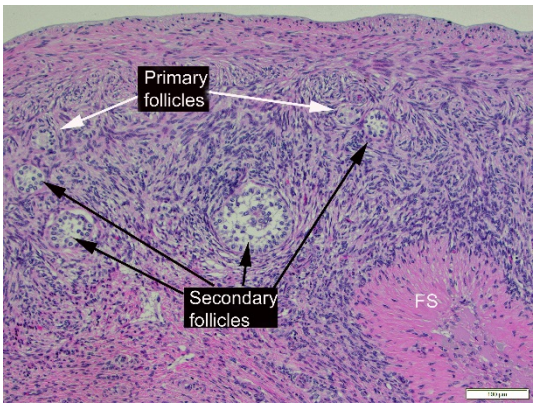
We sampled cattle from 2 ranches in Florida. A third was scheduled, but due to the delay in funding, we were unable to reschedule sampling from this ranch. For these ranches, we obtained samples from cows and heifer including: blood samples, saliva samples, cheek swabs, vaginal swabs, and ultrasound of the reproductive tract. Thirty cows and heifers were followed to slaughter, and tissues were collected (ovaries, uterine biopsy, liver biopsy, etc). Histological analysis of the ovarian tissues indicated that OFD was diagnosed in 10/15 (66.7%) and 14/15 (93%) in the first and second ranches respectively.

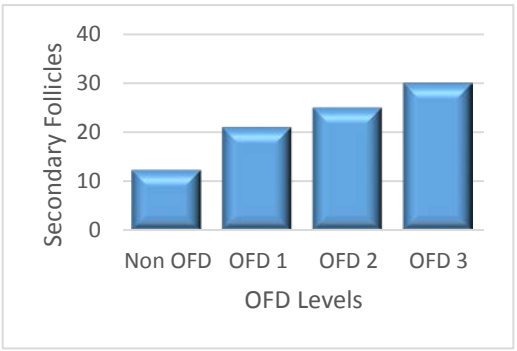
Ovarian Characteristics

Data from analysis of ovaries identified the following characteristics based on the severity of the OFD diagnosis:

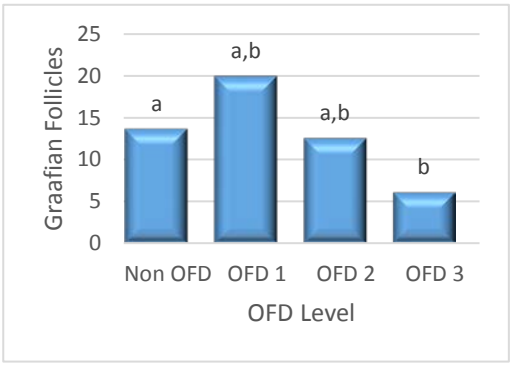


Primary follicles are ovarian follicles that have just begun to grow. They can only be identified by microscopic evaluation of the ovaries. These data indicate that as the severity of OFD increases, the number of these small ovarian follicles is decreased. For this and all following graphs, bars with different letters are statistically significantly different.



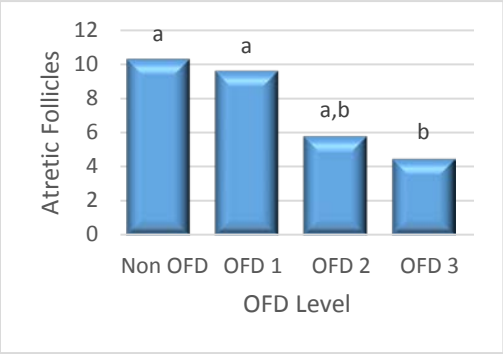


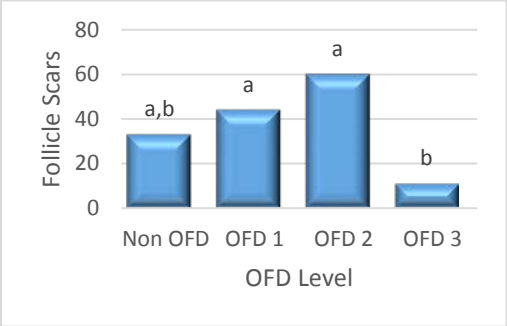
Secondary follicles are ovarian follicles that are in the early phases of growth. They can only be identified by microscopic evaluation of the ovaries. There data, while not showing statistically significant differences, indicate a trend accumulation in OFD affected cows. An accumulation of this size of ovarian follicles, and them not progressing, would lead to decreased and/or lack of fertility.



Graafian follicles are ovarian follicles which have accumulated a fluid filled compartment, and are in the final stages of development. These follicles can be seen with the naked eye, and many can be detected by ultrasound evaluation of the ovary in vivo. In severe cases of OFD, the number of the maturing follicles is decreased which would impair fertility.

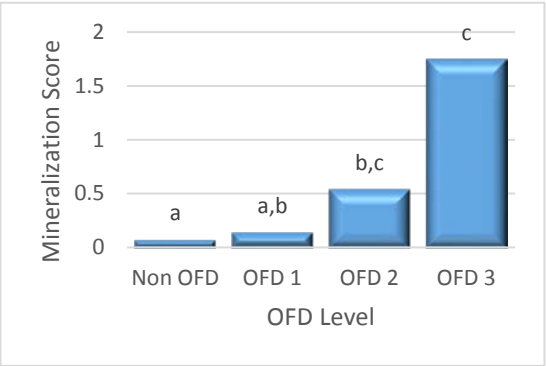
Atretic follicles are ovarian follicles which are not viable, and have begun to deteriorate. With severe cases of OFD, the number of atretic follicles is decreased. This decrease is likely due to the lack of follicles maturing appropriately, therefore, the pool of deteriorating follicles is also decreased.





Follicle scars are the remnants of ovarian follicles that have undergone atresia (deterioration). In this study, the number of follicle scars was reduced in the most severe cases of OFD. This is likely due to the lack of follicles undergoing the maturation process, therefore, there is a reduced pool of follicles that will undergo atresia.

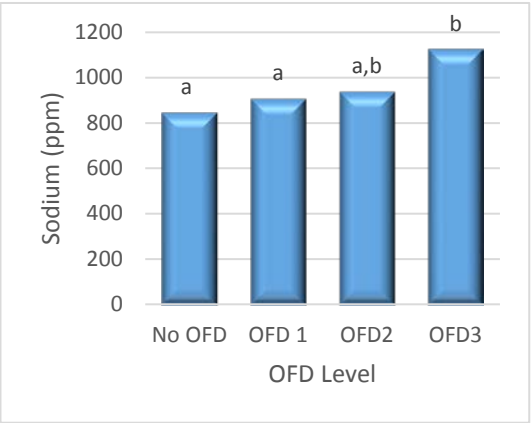
The ovaries were scored on their level of mineralization after removal from the animal. As we have identified previously, the mineralization of ovaries is increase with increasing severity of OFD.



Additional ovarian characteristics are presented in the Appendix. These were characteristics not found to be affected by OFD and/or are similar to those identified above

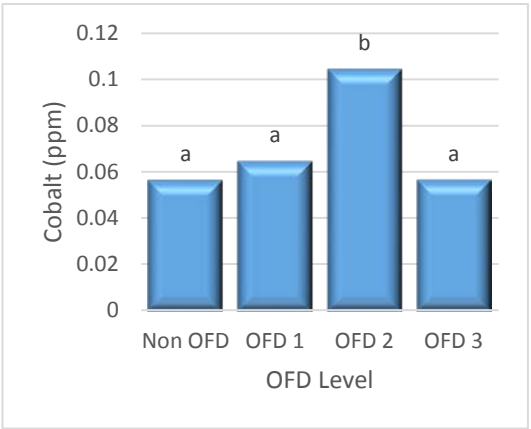
<u>Metal Analyses</u>

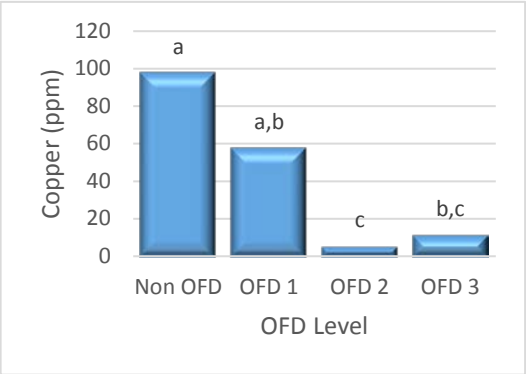
Liver samples from cows and heifers were analyzed for a number of metals. Significant findings are identified below. Bars with different letters are statistically different in the following graphs.



Levels of sodium were related to the severity of OFD with more severe cases exhibiting higher levels of sodium in the liver.

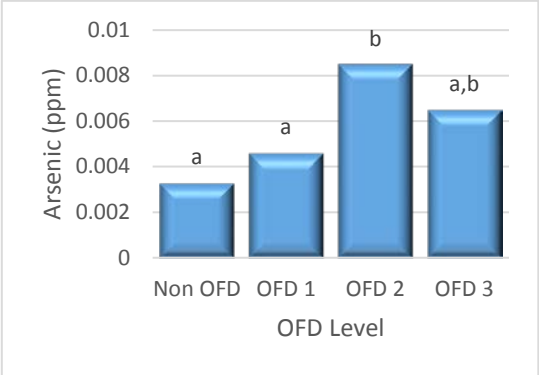
Levels of cobalt were dependent upon the severity of the OFD diagnosis with OFD level 2 exhibiting the highest cobalt in the liver.



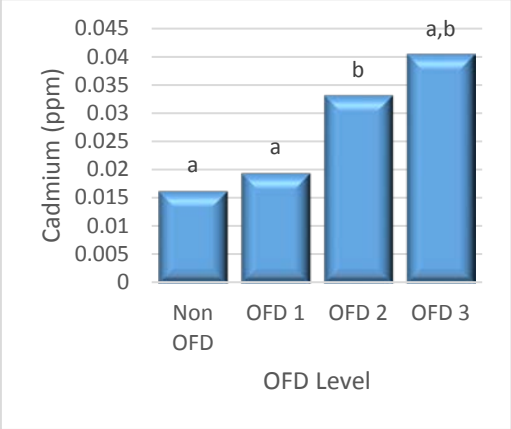


The levels of copper in the liver were related to the severity of the OFD with OFD levels of 2 and 3 having the lowest quantities compared to no OFD and slight severity.

The liver levels of arsenic were also associated with severity of OFD.

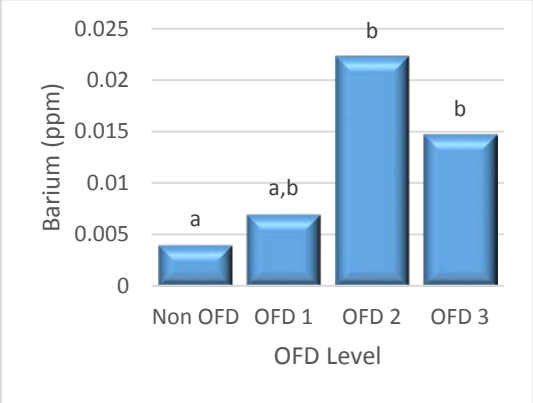


Generally, higher levels of arsenic were associated with diagnosis of more severe OFD.

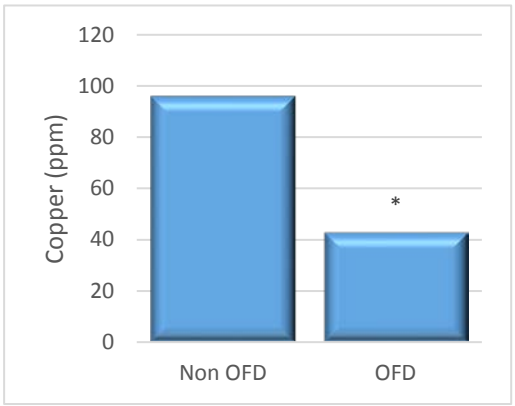


In addition, cadmium levels in the liver were increased in more severe cases of OFD.

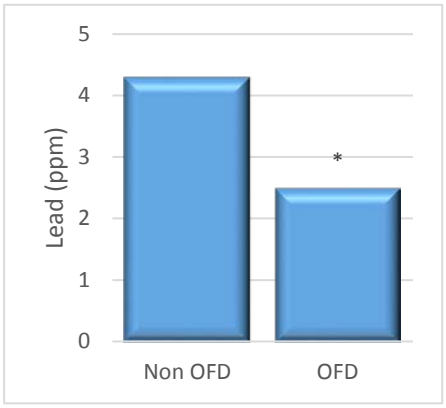
Barium levels in the liver were also associated with more severe cases of OFD.



When considering development of a diagnostic test for OFD, comparing liver levels of metals between animals with and without OFD was also considered. When this type of comparison was made, liver levels of copper and lead were identified as being different between unaffected and affected animals. An asterisk indicates significant differences between Non OFD (unaffected) and OFD (affected) animals.



Remaining data related to analyses of liver metals is included in the Appendix.



Ultrasound Results

All cows utilized in this project were scanned ante-mortem and graded for OFD via ultrasound. Ovaries collected at the slaughter house were ultrasounded post-mortem. Type 3 and Type 4 ovarian follicular dysplasia (OFD) can reliably identified and graded with ultrasound ante-mortem and post-mortem. Ovaries with Type 3 and 4 OFD have decreased numbers of Graffian follicles which can be noted on ultrasound. Additionally, significant mineralization within the ovary can also be visualized via ultrasound. Abnormal folliculogenesis occurs with OFD. The abnormal follicular development can result in calcium influx within the follicle resulting in mineral deposits within the ovary. Then areas of mineralization can be seen throughout the ovary on histologic analysis and ante-mortem via ultrasongraphy. The areas of mineralization appear hyperechoic, similar to bone density on the ultrasound. Ovarian cysts, small chronic cystic follicles (approximately 0.5 to 1.0 cm) and mucometra can all be associated with OFD and visualized via ultrasound. Ultrasound is a valuable tool in detect sentinels within a herd ante-mortem. There are a number of good quality ultrasounds available. The Ibex EVO ultrasound with the 8.5 MHZ 66mm linear array transducer was utilized in this research project. This ultrasound provides greater detail and visualization than many of the older ultrasounds. It appears that ultrasound can detect grade 2 OFD cows as well if an 8 to 10 MHZ ultrasound is utilized.

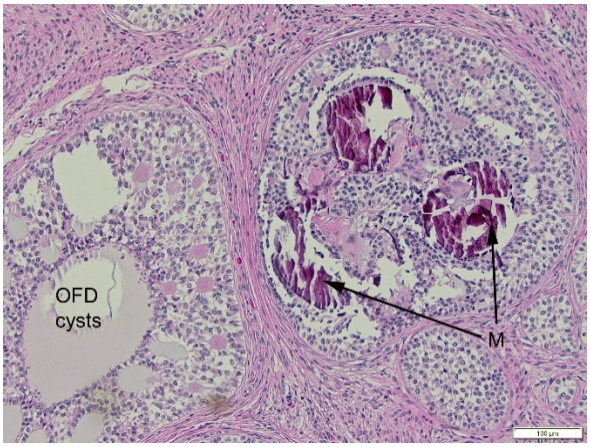


Image 1. The histologic image (left) and two ultrasound images below (image 2&3) are of 11S. The cow, 11S, was diagnosed via ultrasound ante-mortem and histologically independently as having a grade 4 OFD.

Image 2. The trans-rectal ultrasound image is the right ovary from 11S. The multiple areas of mineralization are present in the ovary along with decreased numbers of Graffian follicles indicative of a grade 4 OFD cow.

Image 3. The trans-rectal ultrasound image is the left ovary . There are multiple areas of mineralization, and small cystic areas seen in the ovary indicative of a grade 4 OFD cow.

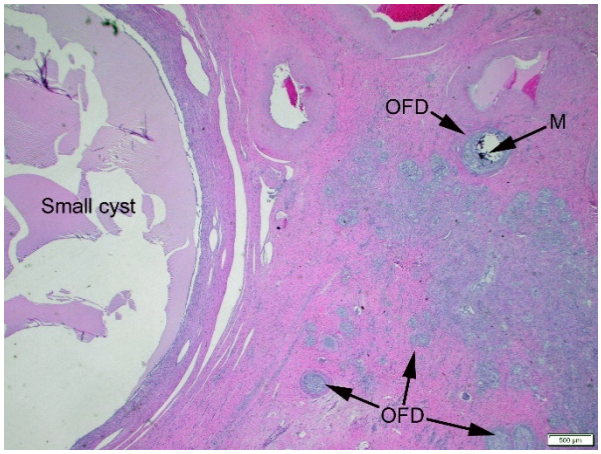
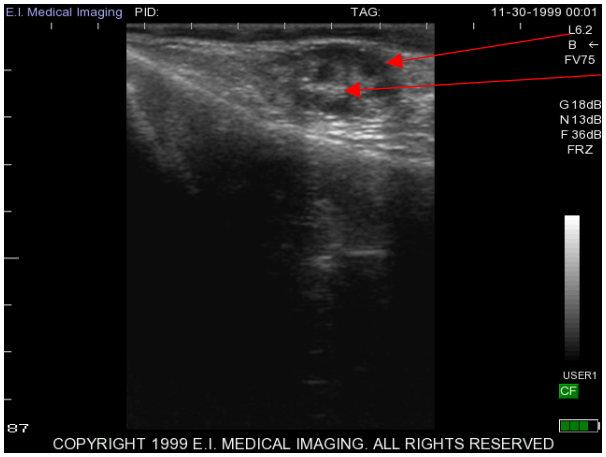
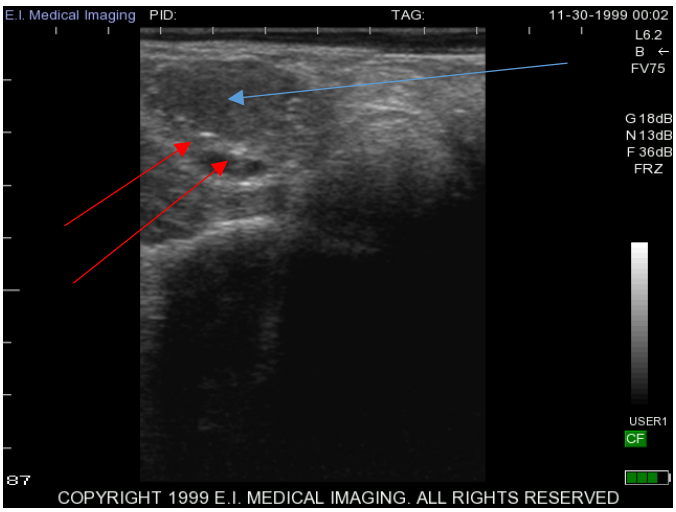


Image 4. The histologic image (left) and two ultrasound images below (image 5&6) are of 8S. The cow, 8S, was diagnosed via ultrasound ante-mortem as having a grade 2 OFD. The histologic analysis was confirmed that the cow had grade 2 OFD.

Image 5. The image shows a cow with grade 2 OFD. There are early cystic follicles (red arrows) with small areas of mineralization in the echoic rim. The cow was diagnosed with grade 2 OFD via ultrasound and was confirmed via histological analysis (Image 4).

Image 6. The image shows a cow with grade 2 OFD. There is a corpus luteum (blue arrow) with small areas of mineralization. The cow was diagnosed with grade 2 OFD via ultrasound and was confirmed via histological analysis (Image 4).





Pathogen Analyses

Analyses of several pathogens for each cow, including bovine viral diarrhea virus, have been completed and communicated to the appropriate ranches. No specific relationship between OFD and any tested pathogens have been noted.

Survey Results

Surveys have been completed for 3 ranches. The data are being compiled. Concern has been voiced regarding the use of low quality plastic feed tubs. Of note, several ovarian genes associated with OFD are also associated with endocrine disruptors derived from the environment.

Hormone Analyses

Blood and saliva samples have been collected from cattle sampled this year. Additionally, blood is being sampled from some normal cows in Alabama for comparison, and we have blood samples archived from previous studies. While the hormonal analyses are not completed at this time, the materials to quantify inhibin and activing as potential diagnostic aids for OFD have been purchased. Analysis is awaiting the final blood collection from normal cows for comparison, and will be completed with no additional costs required.

DNA Analyses

Whole blood, buccal (cheek) swabs, vaginal swabs, and uterine tissues have been collected from experimental animals. Similar tissues are being collected from normal animals for comparison. The materials for purifying the DNA have been purchased. Some DNA samples have been purified to date, but as not all tissues have been collected, analysis of the DNA has not been performed from these experimental or control animals. While purification of DNA for genomic analyses will be completed with no additional costs required, the actual genomic analyses cannot be completed without additional support. Funds allocated for these analyses have not been encumbered.

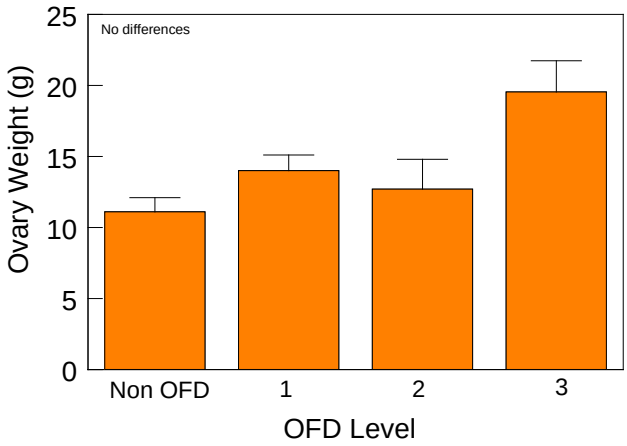
Dissemination of Information from these studies.

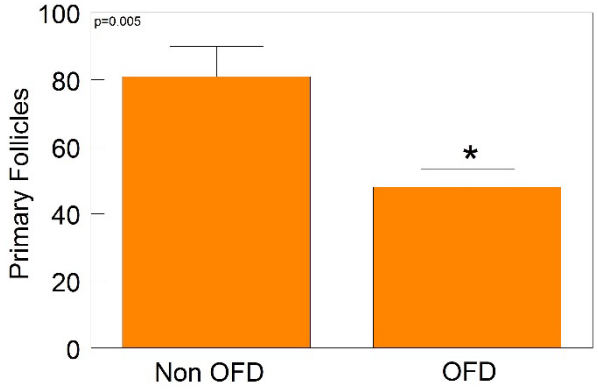
1. Ranches from which these samples were taken have been informed of the results of the study. Results of the presence or absence of numerous reproductive pathogens have also been reported to the ranches and their veterinarians.
2. “Assessment of Ovarian Follicular Dysplasia utilizing ultrasound and histologic examination.” Presented to the International Embryo Transfer Society.
3. “Characterization of Ovarian Follicular Dysplasia (OFD) in five Florida beef herds.” Presented to the College of Veterinary Medicine, Auburn University.
4. “Histological characteristics of Ovarian Follicular Dysplasia (OFD) observed through ultrasound in Florida beef herds.” Presented to the American Association of Bovine Practitioners. Charlotte, NC.
5. “Bovine ovarian follicular dysplasia (OFD) and beyond.” Presented to the College of Veterinary Medicine during the Annual Conference, Auburn University, AL.
6. “Evaluation of diagnostics for ante-mortem testing of ovarian follicular dysplasia (OFD) in cattle.” Presented to the Society for Theriogenology, Fort Collins, CO.
7. “Genetic comparison of Florida beef cows with and without ovarian follicular dysplasia (OFD).” Presented to the American Association of Beef Practitioners, Omaha, NE
8. A draft manuscript has been prepared for publication of OFD pathology.

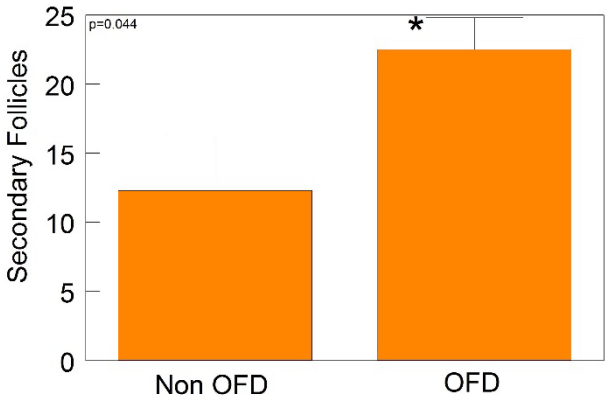
Appendix:

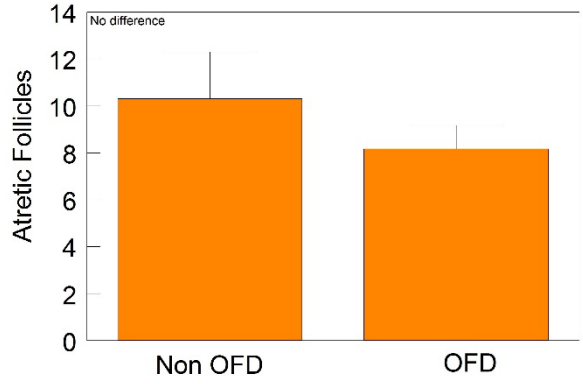
Ovary Characteristics

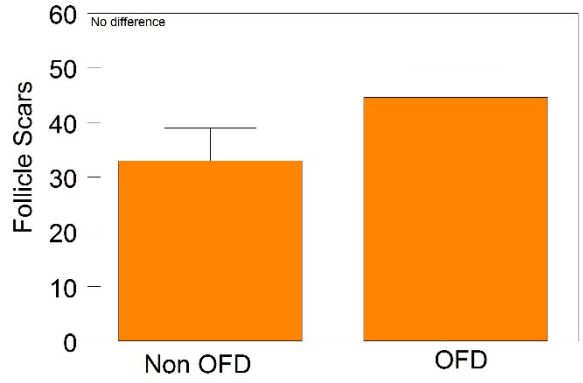
An asterisk indicates statistical difference between groups.

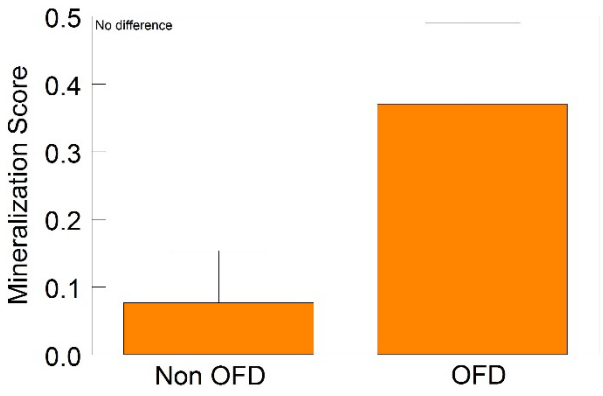




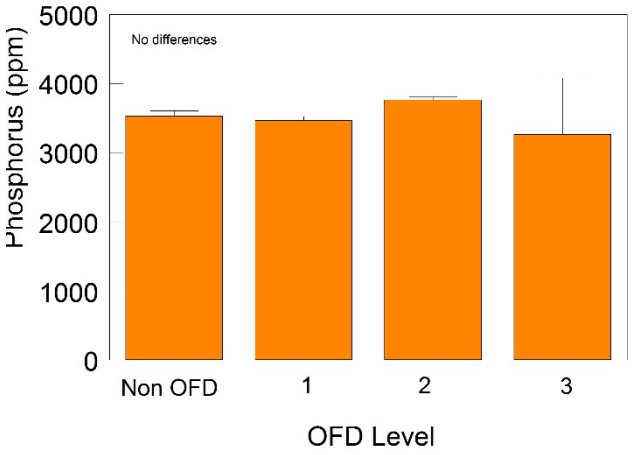


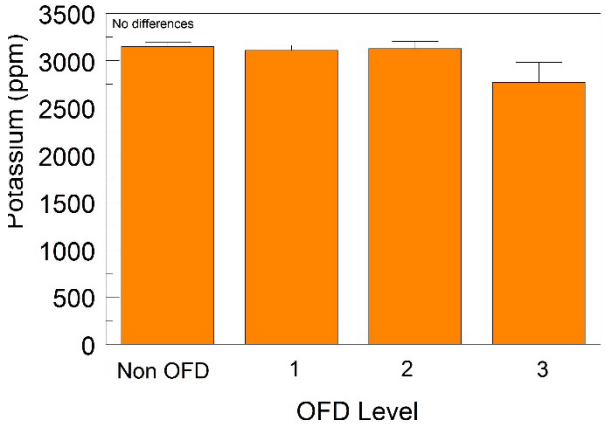


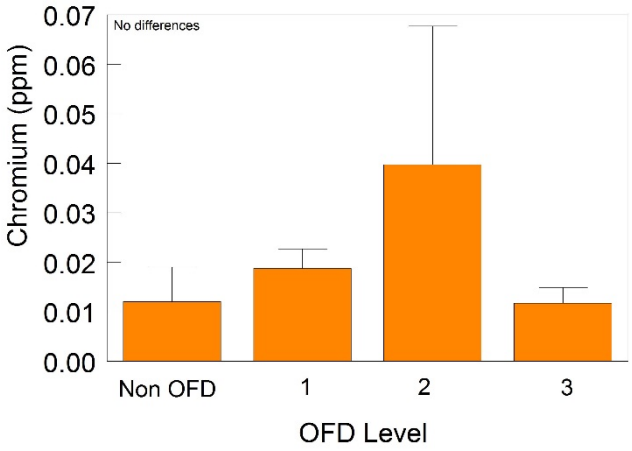


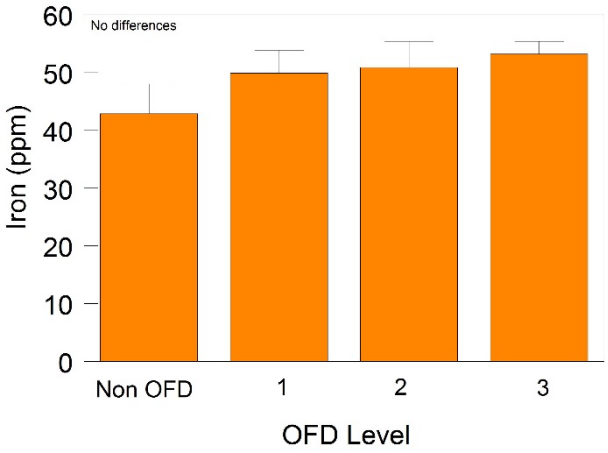


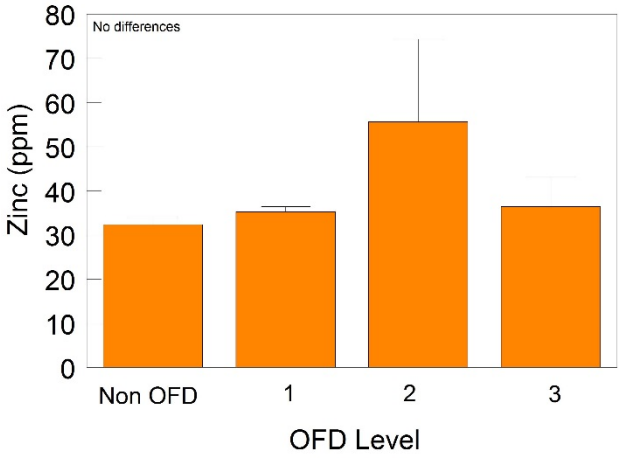
Metal Analyses

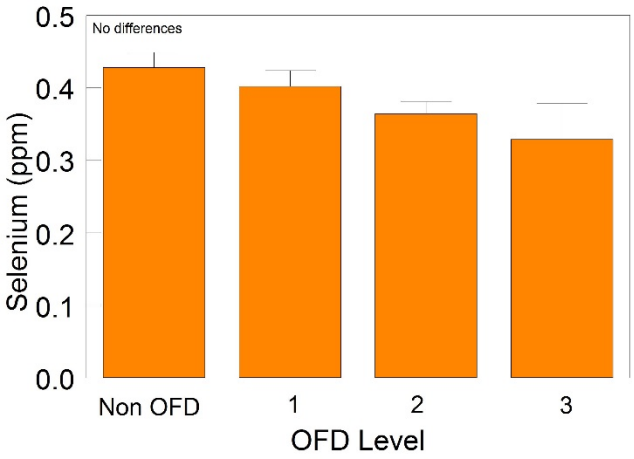


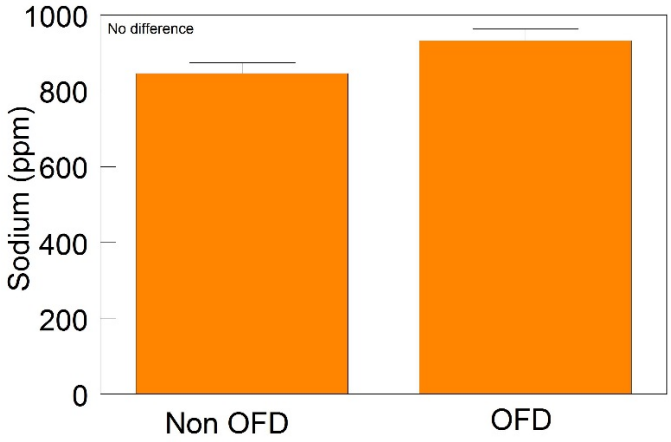


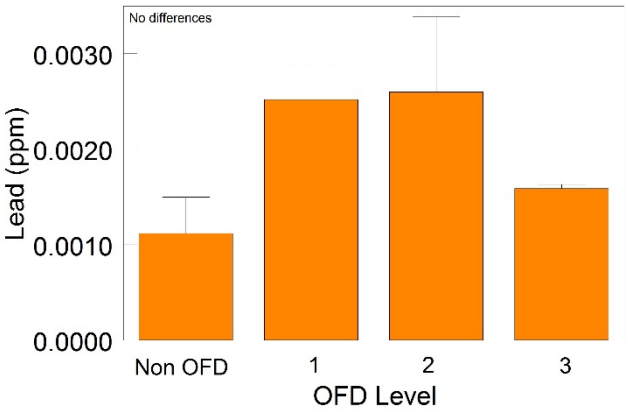


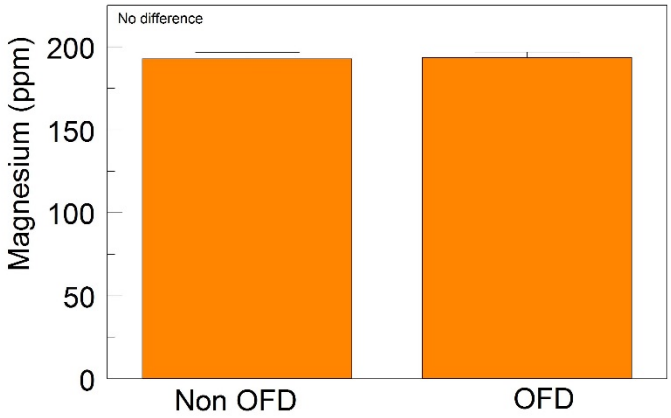


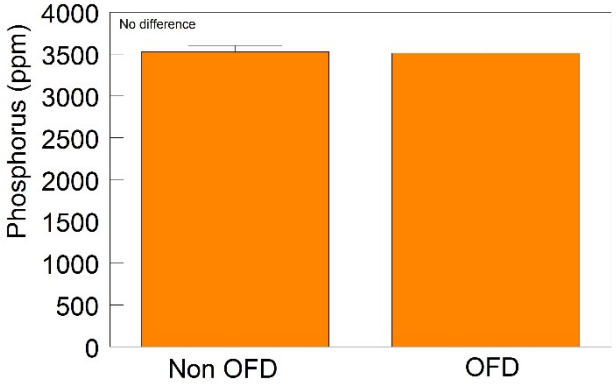


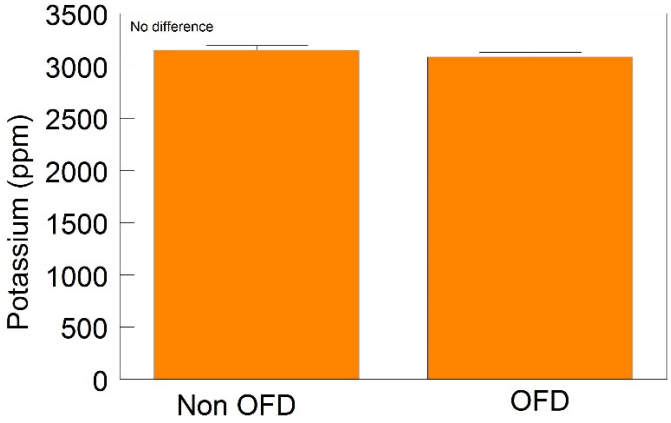


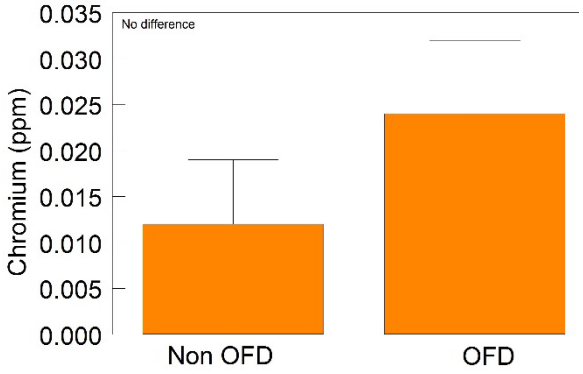

No difference
Sodium (ppm)
1000
800
600
400
200
0
Non OFD
OFD

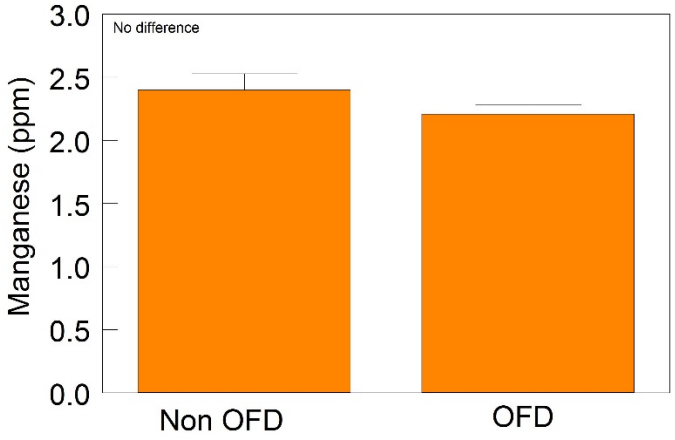

No differences
Lead (ppm)
0.0030
0.0020
0.0010
0.0000
Non OFD
1
2
3
OFD Level

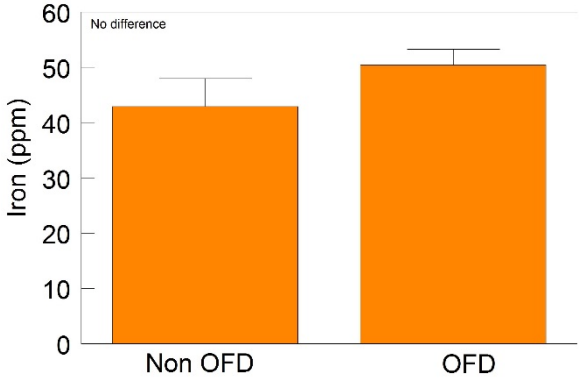

No difference
Magnesium (ppm)
200
150
100
50
0
Non OFD
OFD

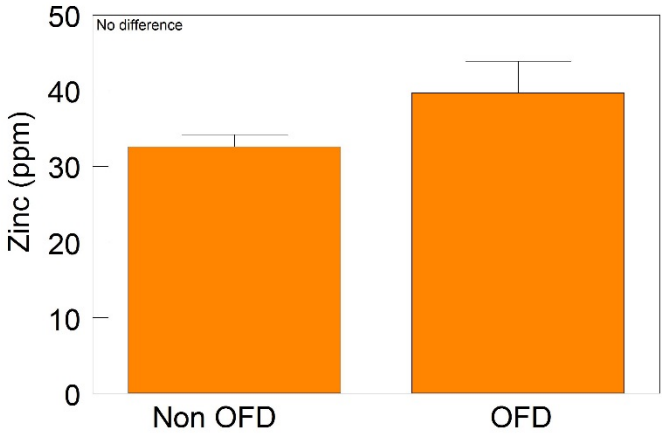

No difference
Phosphorus (ppm)
4000
3500
3000
2500
2000
1500
1000
500
0
Non OFD
OFD

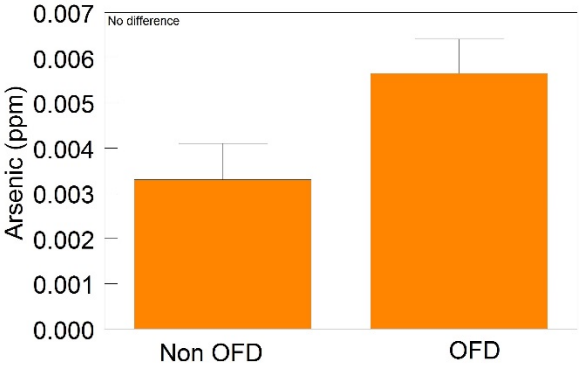

No difference
Potassium (ppm)
3500
3000
2500
2000
1500
1000
500
0
Non OFD
OFD

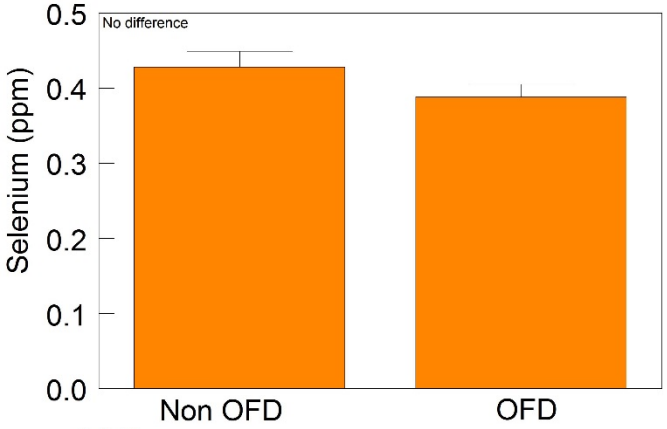

No difference
Chromium (ppm)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Non OFD
OFD

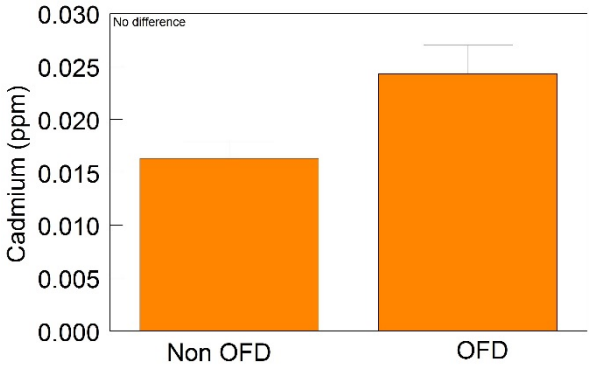

No difference
Manganese (ppm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Non OFD
OFD

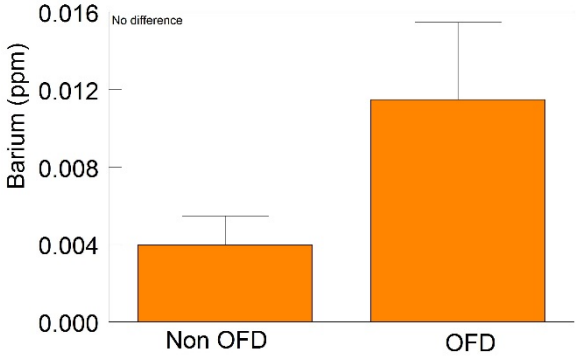

No difference
Iron (ppm)
60
50
40
30
20
10
0
Non OFD
OFD

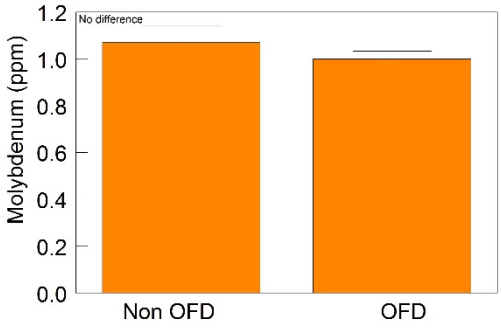

No difference
Zinc (ppm)
50
40
30
20
10
0
Non OFD
OFD

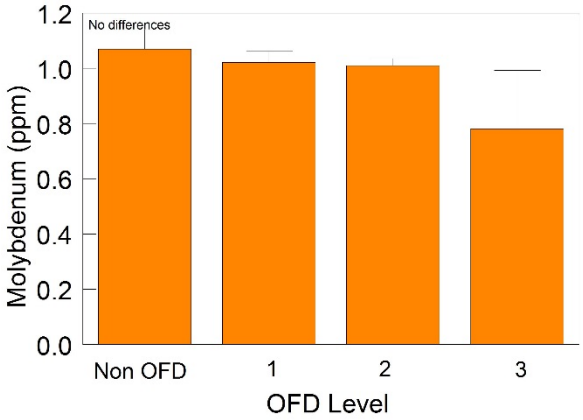

No difference
Arsenic (ppm)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Non OFD
OFD


No difference
Selenium (ppm)
0.5
0.4
0.3
0.2
0.1
0.0
Non OFD
OFD


No difference
Cadmium (ppm)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Non OFD
OFD


No difference
Barium (ppm)
0.016
0.012
0.008
0.004
0.000
Non OFD
OFD


No difference
Molybdenum (ppm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Non OFD
OFD


No differences
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Molybdenum (ppm)
Non OFD
1
2
3
OFD Level

Florida Cattle Ehancement Board					
Project Title:	Development of a Diagnostic Test for Ovarian Follicular Dysplasia (OFD)				
Detailed Line Item Description	QTY	Unit Price	Total	Explanation/Justification of Deliverable	Completion Date
General Laboratory Items					
Hormone Assays	125	$100.00	$12,500.00	Activin/Inhibin Assays	9/1/2017
Supplies	5	$999.99	$4,999.95	General Supplies	9/1/2017
Diagnostics					
Histology	30	$1,000.00	$30,000.00	Ovary and other tissue structure analyses	9/1/2017
Elemental	47	$96.84	$4,551.48	Tissue elemental analyses	9/1/2017
BVDV	30	$100.00	$3,000.00	Disease monitoring	9/1/2017
Ranch Services					
Veterinary Services	3	$2,999.99	$8,999.97	Veterinary assistance for sample collections	
Survey	3	$100.00	$300.00	Data on cattle management	9/1/2017
Normal Animals	7	$2,200.00	$15,400.00	Normal bovine tissues	9/1/2017
Ranch Services	3	$1,500.00	$4,500.00	Ranch assistance in tissue collections	
Equipment					
ultrasound	1	$18,295.00	$18,295.00	high resolution ultrasound for diagnostics	6/15/2017
Personnel	Costs incorporated into costs listed above				9/1/2017
Graduate Student					
2 Faculty					
Grand total			$102,546.40		