Ovarian Follicular Dysplasia (OFD): Early Detection and Origin

Final Report – Florida Cattlemen's Association (#22958-Auburn University) September 15, 2016

We sampled cattle from 5 separate ranches, two in North Central Florida, and three in South Central Florida. Across those ranches, approximately 470 cattle, selected for infertility, were subjected to ultrasound examination of the reproductive tract and blood samples taken for hormone analyses. From those 470 cattle, approximately 66 were taken to slaughter and ovaries collected for further analyses. From those 66 cattle, 10 ovarian samples were taken for analysis of gene expression.

OFD by Ranch

The proportion of cows/heifers affected by OFD differed across the ranches samples (Table 1).

Table 1: Proportion of Ovarian Follicular Dysplasia in 5 Florida Ranches as Determined by Ovarian Appearance Post-Mortem

Ranch	Area	OFD Mild (%)	OFD Severe (%)
A	South	20	70
B	Central	33	40
C	South	38	31
D	South	16	25
E	Central	25	6

Hormonal Detection of OFD

Circulating levels of progesterone (P4) and Anti-Mullerian Hormone (AMH) in serum were quantified in blood samples from approximately 200 cows. This was part of our initial hypothesis that AMH would differ in OFD due to the expected changes in ovarian follicle populations. Circulating P4 levels were quantified as a measure of general reproductive status.

Figure 1

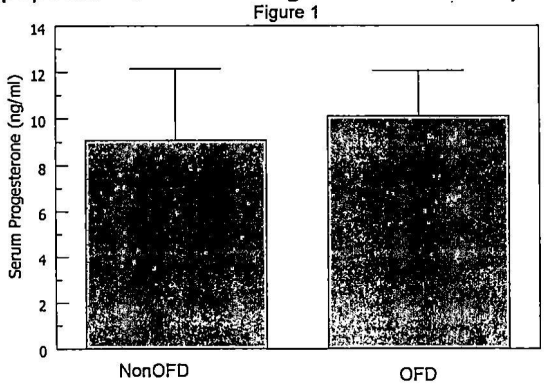
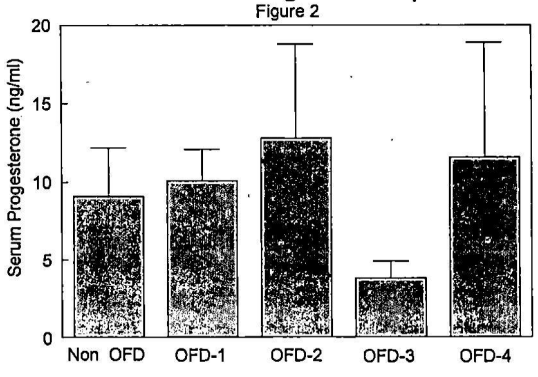


Figure 2



There were no differences in circulating P4 between unaffected (NonOFD) and affected (OFD) cows as evidenced in Figure 1. Moreover, after ovarian histological diagnosis of OFD, there was no relationship identified between circulating levels of P4 and degree of the OFD (Figure 2).

Additionally, there were no differences detected in the circulating levels of AMH when unaffected and affected animals were compared (Figure 3). Moreover, no relationship was identified between circulating levels of AMH and degree of the OFD effect (Figure 4).

Figure 3

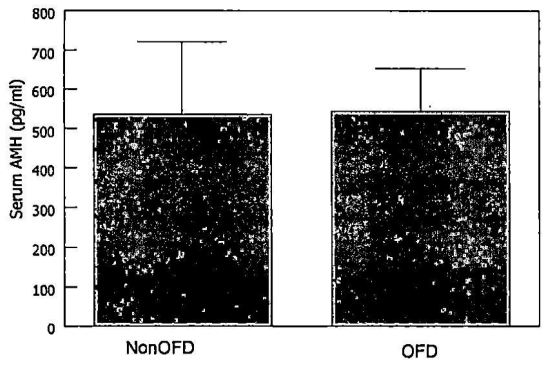
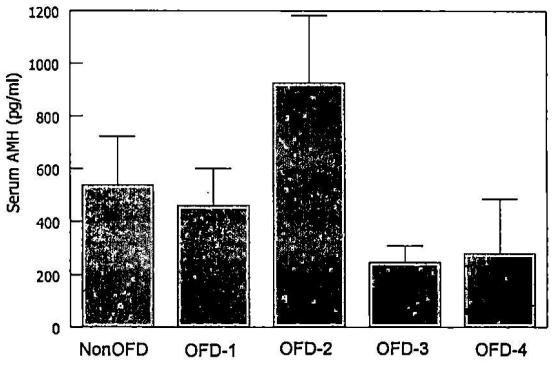
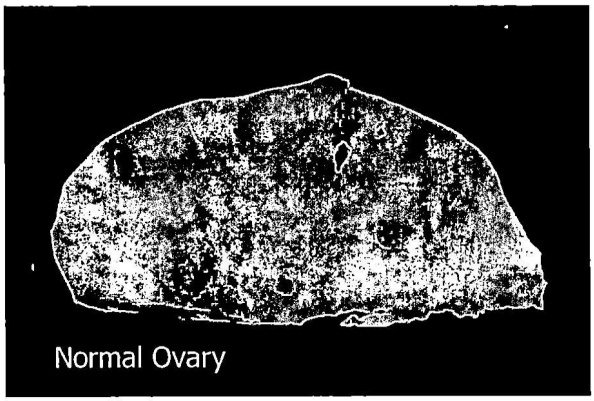


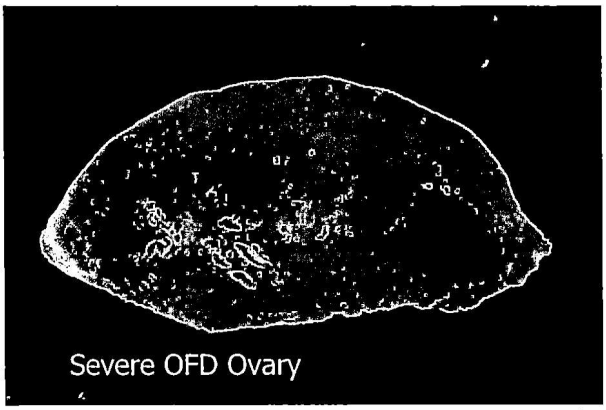
Figure 4



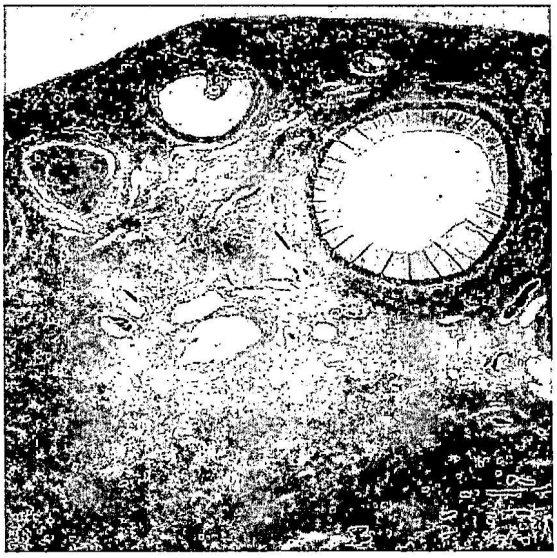
Histological Findings

Necropsy, histology findings and selection of tissue for genetic testing





Ovaries with OFD were graded I to IV. The distribution of OFD for 30 affected females was Gr I 16/30, Gr II 9/30, Gr III 4/30 and Gr IV 1/30. For genetic analysis 5 NonOFD ovaries were compared to 5 ovaries with Grade II and III OFD. In one herd where five heifers were selected for slaughter early OFD was identified in a heifer. OFD was identified in one cow that had undergone early embryonic death and was in process of abortion. When more detailed analysis of small follicles was conducted the ratio of primary/primordial follicles to secondary follicles was found to be inverse in OFD verses NonOFD ovaries.

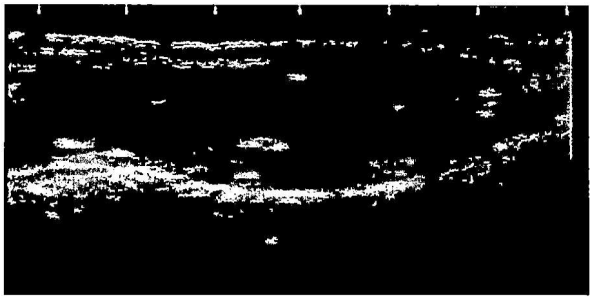


Normal ovary with follicle containing viable ova

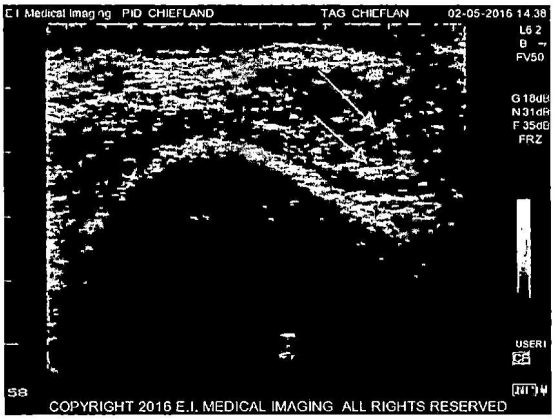
OFD ovary with clusters of dysplastic follicle containing non-viable follicles

Ultrasound Findings

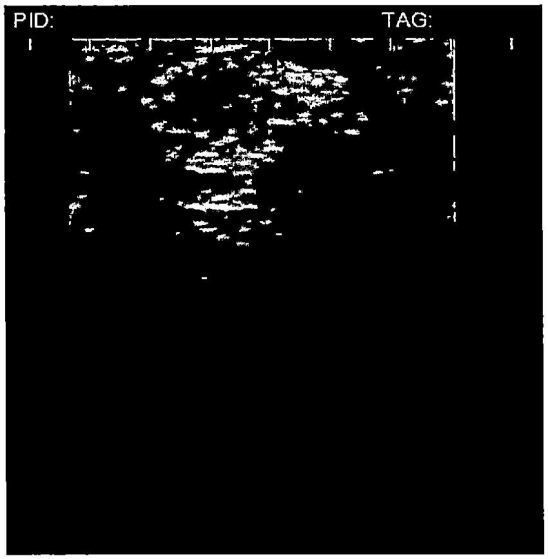
The presence of mineral densities (dystrophic mineralization) and decrease in Graffian follicles can be used to provide a means of identification of Grade III and IV OFD with routine trans-rectal ultrasound. Additionally, ovary cysts might also signify OFD in an animal. However, low grades of OFD I and II cannot be reliably detected on routine ultrasound.



This ultrasound image shows a few small follicles and hyperechoic areas indicating mineralization more consistent with a Grade II or Grade III OFD.

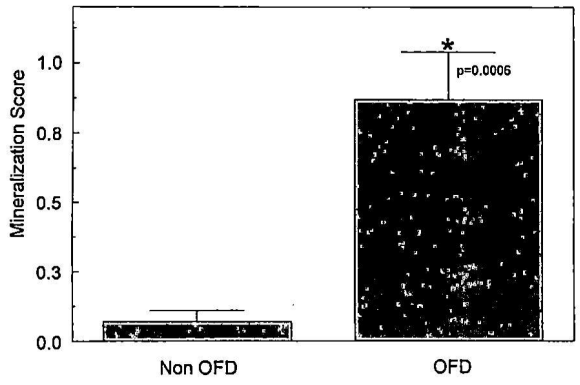


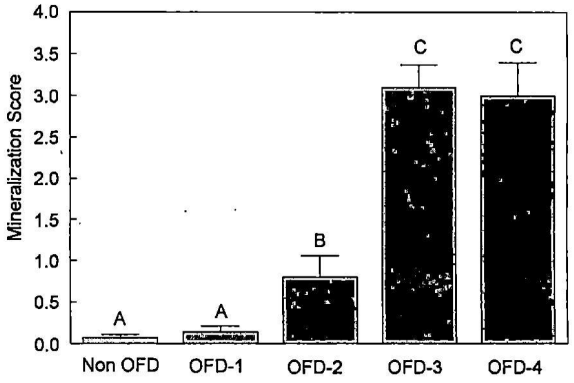
Ultrasound of an ovary with numerous mineral densities (blue arrows) and lack of follicular activity, especialy Graffian follicles seen with grade III and IV OFD.



An additional ultrasound image of an ovary with multiple mineral densities and one large follicle (potentially cystic follicle), indicative of Grade IV OFD.

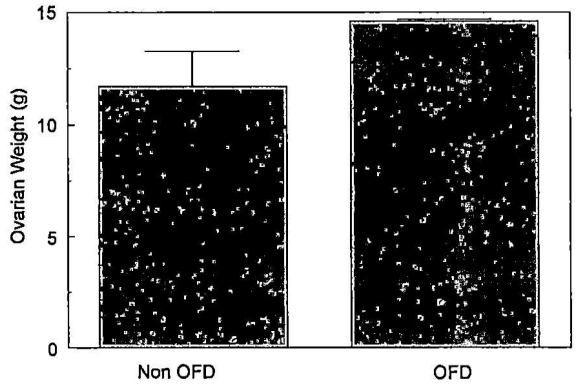
When a mineralization score was assigned to each ultrasound exam of the ovaries of each animal, significant differences between NonOFD and OFD cows were identified. There was significantly greater mineralization in OFD cattle versus NonOFD cattle. Additionally, there was increasing mineralization scores with increasing severity of OFD. *Bars with different superscripts are significantly different.*

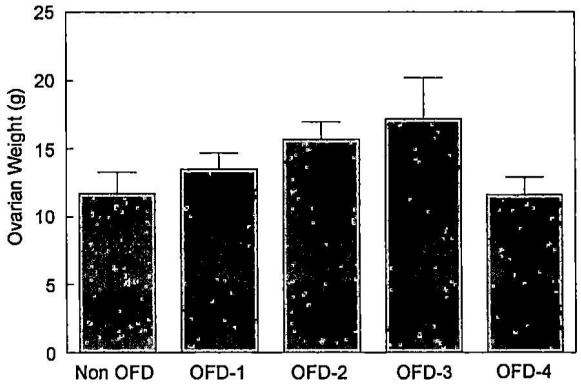




Ovarian Follicular Characteristics

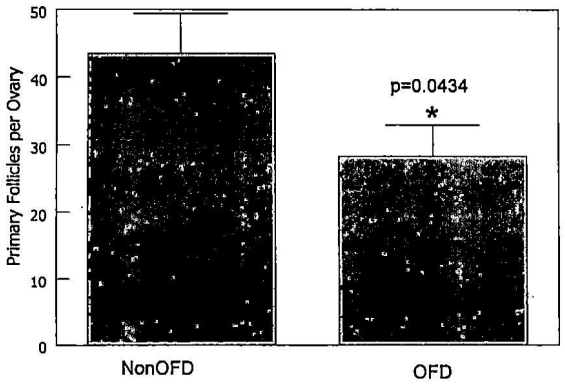
There were no significant differences identified between NonOFD and OFD ovaries with regard to weight. Additionally, there was no relationship between severity of OFD and ovarian weight.

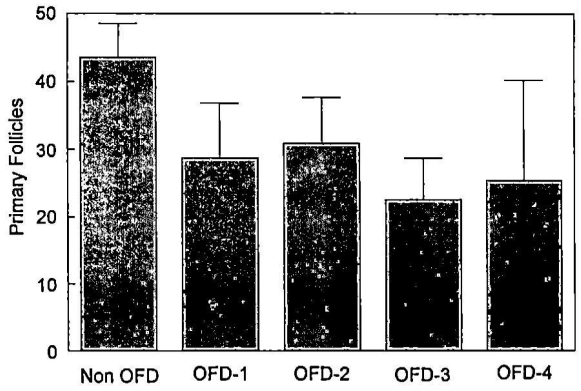


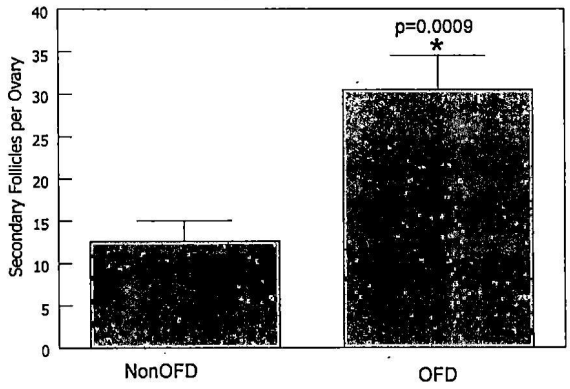


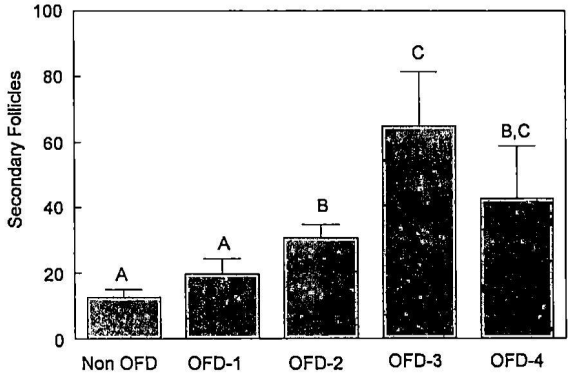
Ovaries were evaluated for the populations of various follicles present on the ovaries. As can be seen in the figures below *(bars with different superscripts are significantly different)*:

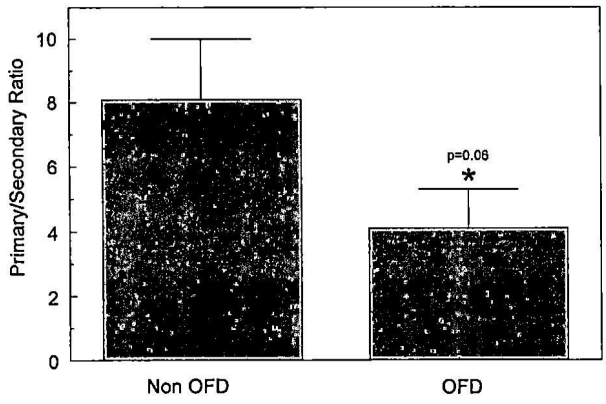
1. Ovaries from NonOFD cows had more primary follicles than ovaries from OFD cows, however, this difference was not related to the severity of the OFD.
2. There were more secondary follicles in ovaries from cows with OFD than NonOFD cows. Moreover, this difference was related to the severity of the OFD.
3. The difference in the ratio of primary/secondary follicles was very pronounced and ovaries from NonOFD cattle had a much higher ratio than ovaries from OFD cattle. This ratio was related to the severity of the OFD.
4. The number of Graffian follicles (those follicles with a fluid compartment) was not different between ovaries from NonOFD and OFD cattle. However, when the severity of OFD was considered, ovaries from the most severe cases of OFD had significantly fewer Graffian follicles than ovaries from NonOFD cattle or less severe OFD.

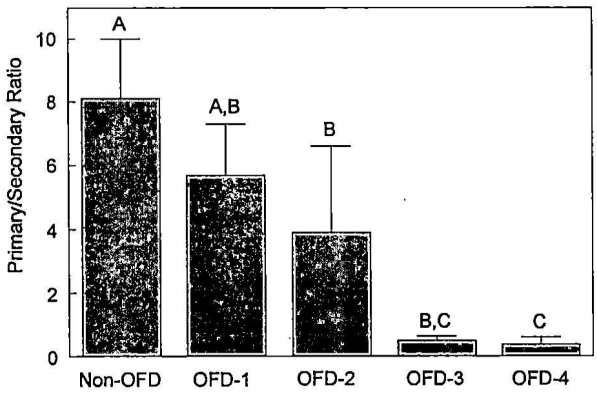

Primary Follicles per Ovary
50
40
30
20
10
0
p=0.0434
*
NonOFD
OFD

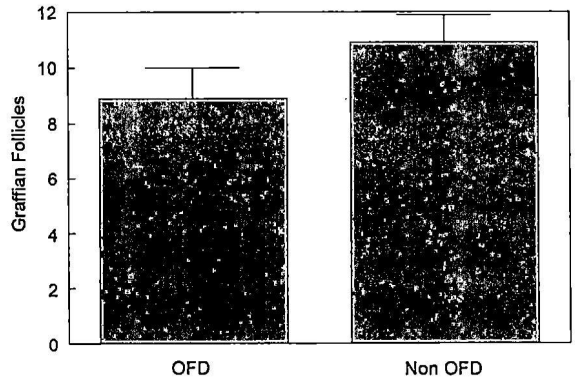

Primary Follicles
50
40
30
20
10
0
Non OFD
OFD-1
OFD-2
OFD-3
OFD-4

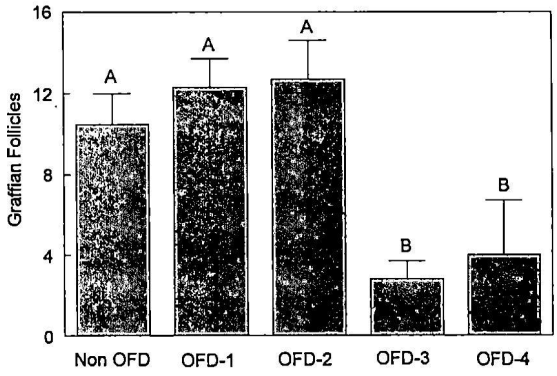

Secondary Follicles per Ovary
40
35
30
25
20
15
10
5
0
p=0.0009
*
NonOFD
OFD

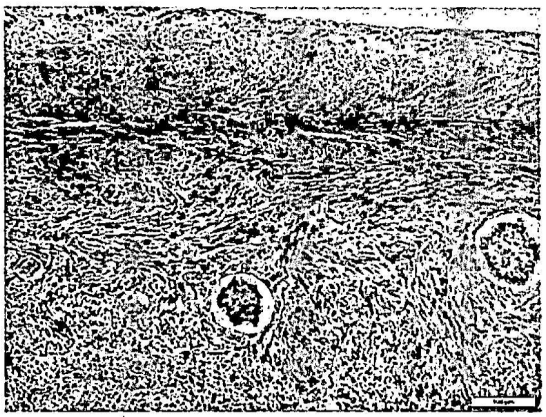

Secondary Follicles
100
80
60
40
20
0
A
A
B
C
B,C
Non OFD
OFD-1
OFD-2
OFD-3
OFD-4


Primary/Secondary Ratio
10
8
6
4
2
0
p=0.06
*
Non OFD
OFD

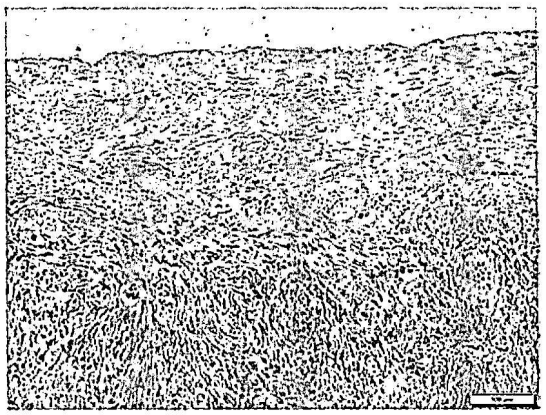

Primary/Secondary Ratio
10
8
6
4
2
0
A
A,B
B
B,C
C
Non-OFD
OFD-1
OFD-2
OFD-3
OFD-4


Graffian Follicles
12
10
8
6
4
2
0
OFD
Non OFD


Graffian Follicles
16
12
8
4
0
A
A
A
B
B
Non OFD
OFD-1
OFD-2
OFD-3
OFD-4



OFD ovary with increased number of secondary follicles



NonOFD ovary with many viable small follicles

Gene Expression Identification

RNA Sequencing

Ten ovarian samples (5-NonOFD, 5-OFD) were submitted to HudsonAlpha for sequencing in May, 2016. The first set of results were received September 9, 2016. Thus, the analysis of these results is just beginning. However, HudsonAlpha has reported that 1085 genes were differentially expressed between ovarian samples of NonOFD and OFD cattle. Of those 1085, 628 genes were up-regulated in OFD and 457 were down-regulated.

MicroRNA Relationships Identified

Approximately 706 microRNA sequences of ovarian tissue were evaluated for differences between NonOFD and OFD. microRNA are small non-coding molecules that function in suppressing the production of specific proteins. These data are just received, but 28 microRNAs were found to differ between NonOFD and OFD ovarian samples. Of those 28, 23 were increased in expression in OFD and 5 were decreased in expression. Subsequent analyses of these data will be used to identify specific genes which are targeted by these microRNAs. While these data are still being analyzed, at least four of these microRNAs are associated with carcinomas.

Conclusions

OFD was the leading cause of infertility identified at slaughter in Florida beef cows. Single analysis of progesterone and Anti-Mullerian Hormone in serum did not serve for ante mortem diagnosis of OFD.

However, cows with severe OFD may be identified with ultrasound evaluation and may serve as sentinels to identify OFD in affected herds. Additionally, we have identified distinct differences in the populations of follicles in the ovary which are related to OFD and OFD-severity. Finally, we have identified over 1000 genes and 28 microRNAs which differ between NonOFD and OFD cattle ovaries. These differential characteristics may provide for a useful tool in identification of OFD.

Presentation of Results

Presentations of the data from this project. Items 2, 3, and 4 are accompanied by Abstracts to be contained in Proceedings from the specific meetings.

1) Infertility caused by Ovarian Follicular Dysplasia (OFD) in Beef Cattle. Florida Cattlemen's Association, June 14, 2016
2) Morphological characteristics of Ovarian Follicular Dysplasia (OFD) observed by ultrasound in five Florida beef herds. Society for Theriogenology, North Carolina, July 28, 2016
3) Histological characteristics of Ovarian Follicular Dysplasia (OFD) observed through ultrasound in Florida beef herds. America Association of Beef Practitioners, North Carolina, September 15, 2016.
4) Assessment of Ovarian Follicular Dysplasia utilizing ultrasound and histologic examination. International Embryo Transfer Society, scheduled for presentation in 2017.