Pooling DNA to Investigate Cattle Infertility

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**Background**

- Reproductive efficiency
  - Lifetime production of the cow
  - Important element of cow-calf component of cattle industry
  - Female becomes a liability in the herd with no calf for producer to market
Objective

* Identify regions of the genome associated with reproductive efficiency in beef cattle
Challenges when evaluating reproductive traits

- Low heritability (0.10-0.40)
- Complex trait
  - Influenced by multiple genes
  - Creates difficulty when identifying genomic regions
Tools for overcoming challenges

Pooling

770 K SNP chip
Steps in creating DNA pools

- Extract DNA
  - Tissue
  - Blood
  - FTA cards

- Determine quality and quantity

- Pool equal amount of each individual

- Properly mix pool to ensure consistency of individuals in pool
100 animals for each pool

Each pool run in duplicate on 770 SNP chip
Cattle populations

- USMARC population
  - Genetically diverse populations
    - British and Continental breeds
  - 1000 females of low reproductive efficiency
  - 1000 females of high reproductive efficiency
  - 10 pools of 100 individuals for each phenotype
Cattle populations

- Central Florida population
  - Bos indicus influence
    - Brangus
    - Simbrah
    - Braford
  - Reproductive records from 2 breeding seasons
    - Open/open
    - Open/pregnant
    - Pregnant/open
    - Pregnant/pregnant
Additional populations

- Western Nebraska population
  - Records from 2007 born heifers
    - Records for three consecutive breeding seasons
    - Open females are culled after first failure

- Milt Thomas at NMSU
  - Records from 7 small populations
MARC population

- Identified multiple regions across genome associated with reproductive efficiency
  - BTA 1, 9, 17
  - Chromosome Y?
Central Florida population

* Identified multiple regions across genome associated with reproductive efficiency

* BTA 5, 8, 9, 14, 17, and 20

* Chromosome Y
Y SNP in males and females?

* Only see Y SNP in open and low reproductive pools

![Box plot of Y SNP in the Central Florida Populations](image)
Western Nebraska population

* Identified multiple regions across genome associated with reproductive efficiency
  * BTA 1, 17, Y
Additional populations

* Identified multiple regions across genome associated with reproductive efficiency
* BTA 5, 11, and Y
SNP across the Y chromosome
Why do we see Y SNP in the open females?

- Can we determine which females that possess the Y SNP?
- Evaluate individual females that make up open pool
  - Are all the open females contributing to the Y SNP?
Y chromosome in the pools

- Evaluate individuals of pools
- PCR test specific to Y chromosome
- PCR test is used to sex embryos
- Only positive for males

% pool positive for sexing test

<table>
<thead>
<tr>
<th>Breed</th>
<th>% Positive</th>
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<tbody>
<tr>
<td>Brangus</td>
<td>21%</td>
</tr>
<tr>
<td>Simbrah</td>
<td>27%</td>
</tr>
<tr>
<td>Braford</td>
<td>29%</td>
</tr>
<tr>
<td>USMARC</td>
<td>21%</td>
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Y SNP in pools

- Evaluate specific Y SNP on 770 K SNP chip
- Developed PCR tests for Y SNP that are significant
- We see different patterns in females that are positive for sexing PCR test (3-10% open females positive)
- Different fragments of Y chromosome?
What is causing the Y SNP in females?

- Undetected freemartins?
  - Females commonly calve on pasture
    - May miss calves that are born twins
  - Reproductive tracts are checked in Central Florida population at prebreeding check
  - Twins are recorded at USMARC
Freemartins in pools?

- Use PCR to test for genomic regions of Y in individuals that made up pool
- Sexing PCR test
Freemartins in pools?

- Use PCR to test for Y SNP that are significant
- All females that are positive for sexing primers are also positive for Y-SNP tests
- Are freemartins the only cause of the Y?

<table>
<thead>
<tr>
<th>% + Sexing primer test</th>
<th>% + Y-SNP primer test</th>
</tr>
</thead>
<tbody>
<tr>
<td>96%</td>
<td>96%</td>
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Freemartin females
Conclusions

- Identified regions of the genome associated with reproductive efficiency
- Able to replicate these results in additional populations
- Identified Y SNP in individuals of open pools that may contribute to low reproductive efficiency (approx. 3-29% of the open population)
Current status

- Evaluate Y-chromosome anomaly further
- Fine map regions on autosomes identified in current project
- Evaluate other chromosomal abnormalities
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