Somatic cell nuclear transfer in early bovine embryo development is associated with changes in small non-coding RNA species.
- Genes can be turned on or off
  - Transcription
  - Translation
- Different cells have different epigenetic marks
  - Disease
  - Development
MICRO-RNA

- Small non-coding RNA
- Bind complementarily with mRNA
- Highly conserved
- Turn genes off by blocking translation
- Non-coding RNA expanding field
Parental epigenome must be erased

Embryonic epigenome must be correctly established

- Requires period of transcriptional silence
- Maternal transcripts drive early development during this period
POTENTIAL ROLE FOR MIRNA

Accumulation of maternal mRNAs

Degradation of maternal mRNAs

Minor ZGA

Major ZGA

Transcription of zygotic RNAs

Oocyte

Mature Oocyte

4-cell embryo

8-cell

16-cell

Blastocyst
SOMATIC CELL NUCLEAR TRANSFER

- Allows one superior animal to have a much higher impact of the genetic population
- Valuable tool
- ScNT has very low efficiency rates
- Widespread, variable epigenetic errors
Sample types

- Oocytes
- Fibroblasts

Embryo stages (IVF & scNT)

- 2-cell
- 8-cell
- Morula
- Blast.

ESC from IVF & scNT

Sample processing

- Pool of 20 embryos combined from multiple cloning sessions
- Isolated Nucleic Acids
  - small RNA and large RNA
- miRNA to Ion Torrent for miRNAseq
MIRNA PROFILE IN SCNT AND IVF EMBRYOS

- Samples tend to cluster by stage
- No obvious differences between IVF and ScNT embryos
- Several samples do not cluster well (morula)
MIRNA PROFILE IN SCNT AND IVF EMBRYOS

- Can see overall expression patterns in several groups
- “Somatic cell” cluster includes some Morulas
- Blastocysts cluster well
- Rest of embryos tend to cluster by stage
- No clustering by IVF or ScNT
ScNT epigenetic errors are very variable

- May be preventing us from getting significant miRNA changes due to high variability
  - 8 significant miRNA by IVF vs. ScNT

Next steps:

- Map mRNA expression in these same embryos to sncRNA expression
- Micro-inject miRNAs into embryos that are lower in ScNT embryos than IVF embryos
- Expect to see increases in developmental rates
QUESTIONS?

Funded by:
- Utah State University Office of Research and Graduate Studies Research Catalyst Grant
- USDA NIFA AFRI pre-doctoral grant
- USU Presidential Doctoral Research Fellowship

Thanks to:
- Dr. Kenneth White's lab
  - Qinggang Meng
- Dr. Abby Benninghoffs lab
- Dr. Stewart Russell (CReATe IVF)
## SIGNIFICANT MiRNA

### IVF vs. ScNT

<table>
<thead>
<tr>
<th>Stage</th>
<th>#</th>
<th>Top miRNAs of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cell</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8-cell</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Morula</td>
<td>6</td>
<td>miR-2340-3p; miR-345-5p</td>
</tr>
<tr>
<td>Blast</td>
<td>1</td>
<td>miR-497-5p</td>
</tr>
</tbody>
</table>

### Between stages

<table>
<thead>
<tr>
<th>Stages</th>
<th>#</th>
<th>Top miRNAs of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte vs. 2-cell</td>
<td>3</td>
<td>miR-451; miR-133a; miR-29b</td>
</tr>
<tr>
<td>2-cell vs. 8-cell</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8-cell vs. Morula</td>
<td>22</td>
<td>miR-378; miR-19b-3p; miR-378-5p; miR-6119-5p; miR-378-3p</td>
</tr>
<tr>
<td>Morula vs. Blastocyst</td>
<td>56</td>
<td>miR-30e-5p; miR-497-5p; miR-125b-1-5p; miR-138-1-5p; miR-138-2-5p</td>
</tr>
<tr>
<td>Fibroblasts vs 2-cell</td>
<td>365</td>
<td>miR-205-5p</td>
</tr>
</tbody>
</table>
### SIGNIFICANCE IN OTHER SNCRNAs

#### Significant piRNA

<table>
<thead>
<tr>
<th>Stages</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte vs. 2-cell</td>
<td>1</td>
</tr>
<tr>
<td>2-cell vs. 8-cell</td>
<td>0</td>
</tr>
<tr>
<td>8-cell vs. Morula</td>
<td>24</td>
</tr>
<tr>
<td>Morula vs. Blastocyst</td>
<td>0</td>
</tr>
<tr>
<td>Fibroblasts vs 2-cell</td>
<td>4,671</td>
</tr>
</tbody>
</table>

#### Significant tRNA

<table>
<thead>
<tr>
<th>Stages</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte vs. 2-cell</td>
<td>2</td>
</tr>
<tr>
<td>2-cell vs. 8-cell</td>
<td>0</td>
</tr>
<tr>
<td>8-cell vs. Morula</td>
<td>21</td>
</tr>
<tr>
<td>Morula vs. Blastocyst</td>
<td>139</td>
</tr>
<tr>
<td>Fibroblasts vs 2-cell</td>
<td>393</td>
</tr>
</tbody>
</table>