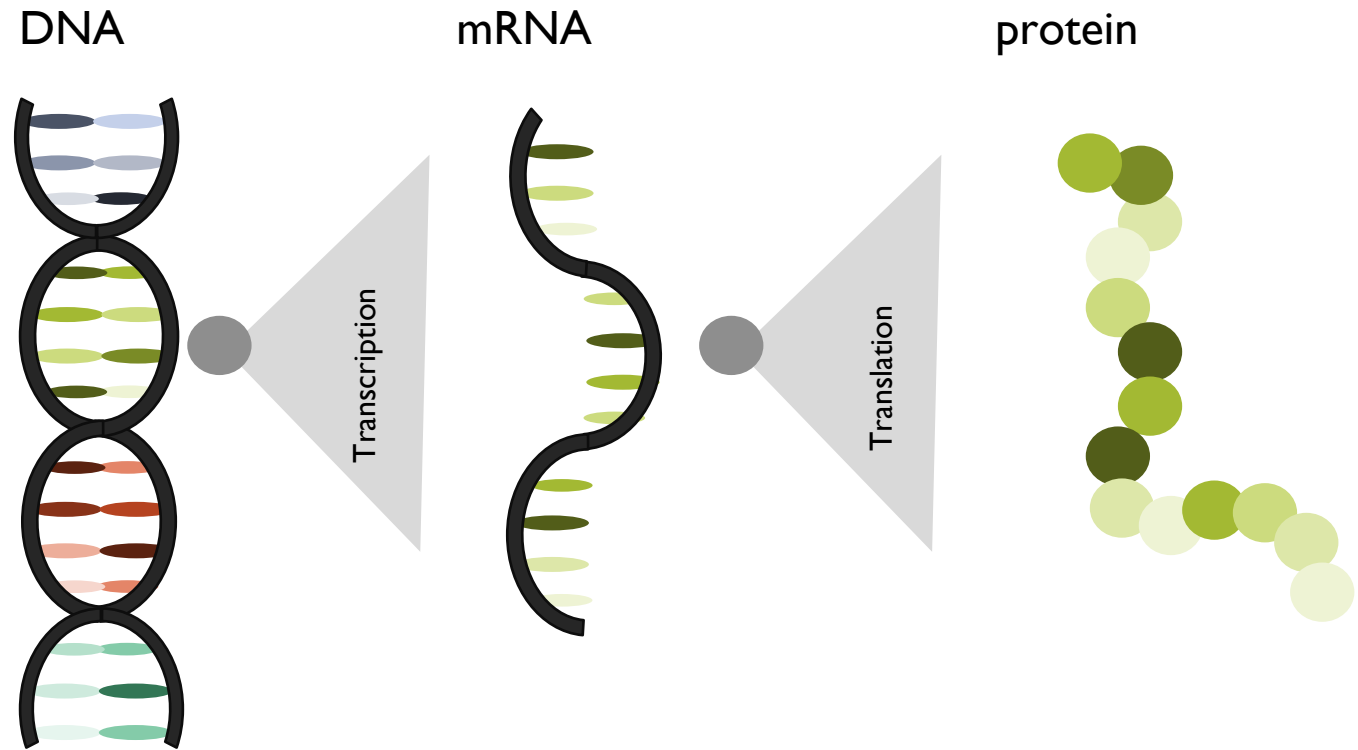




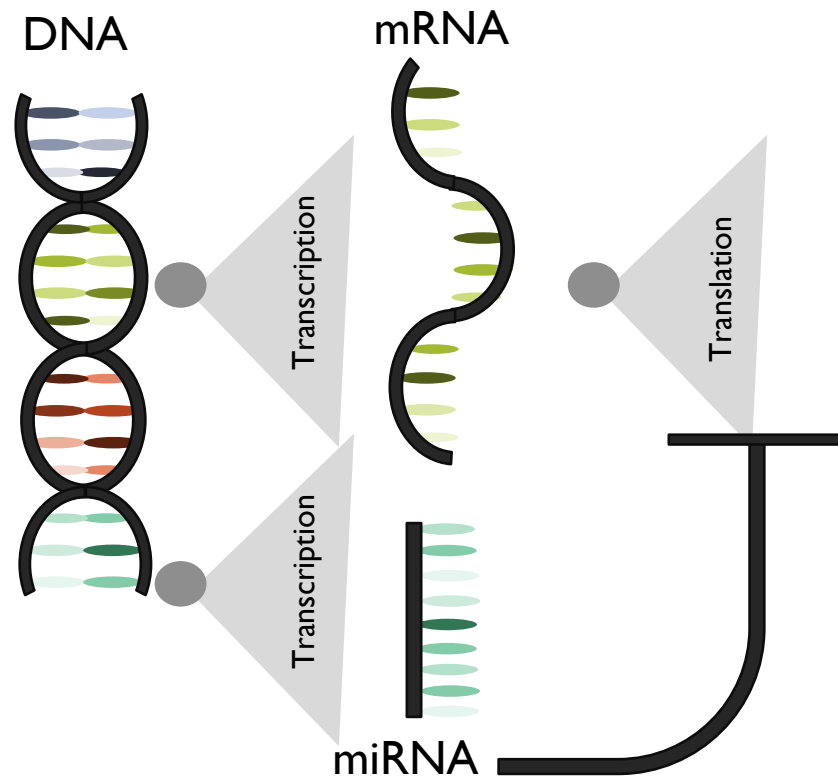
**Somatic cell nuclear transfer in early bovine embryo development is associated with changes in small non-coding RNA species**

# EPIGENETICS

- Genes can be turned **on** or **off**
  - Transcription
  - Translation
- Different cells have different epigenetic marks
  - Disease
  - Development



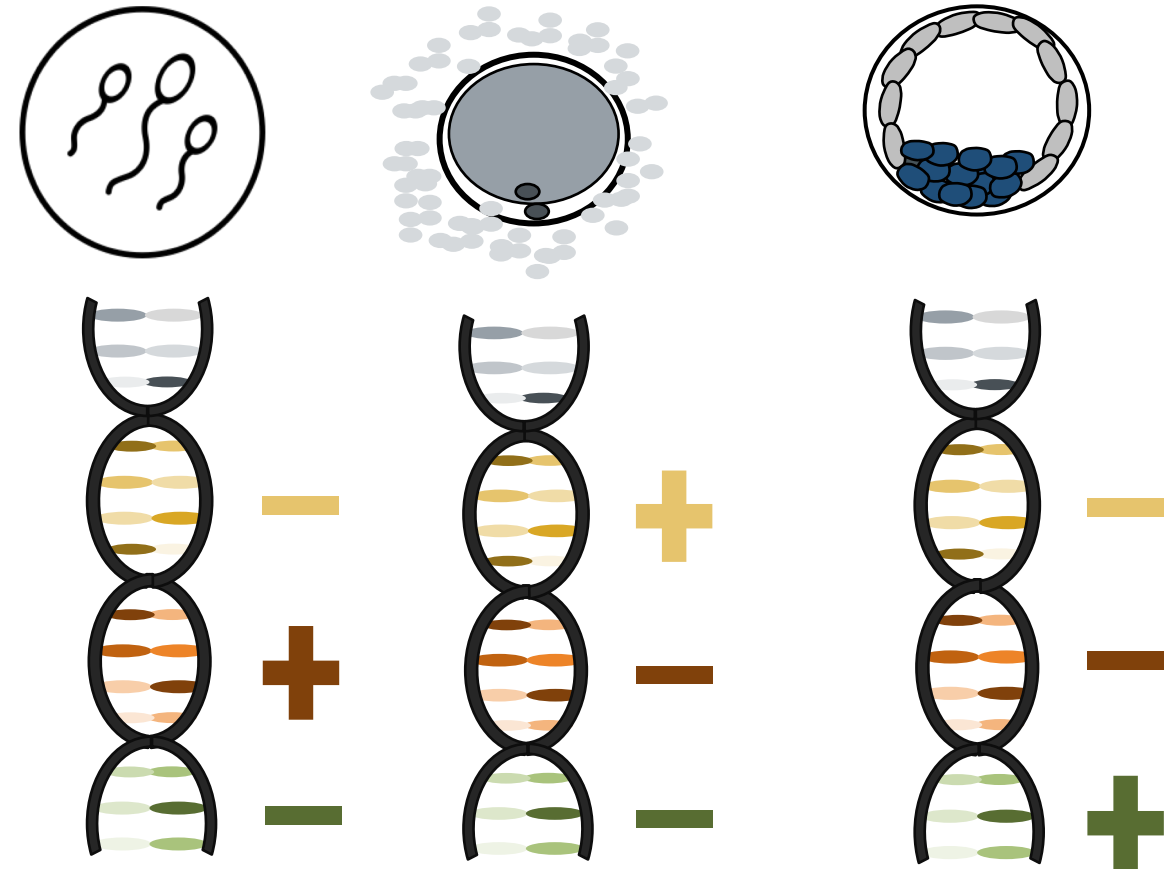
# MICRO-RNA



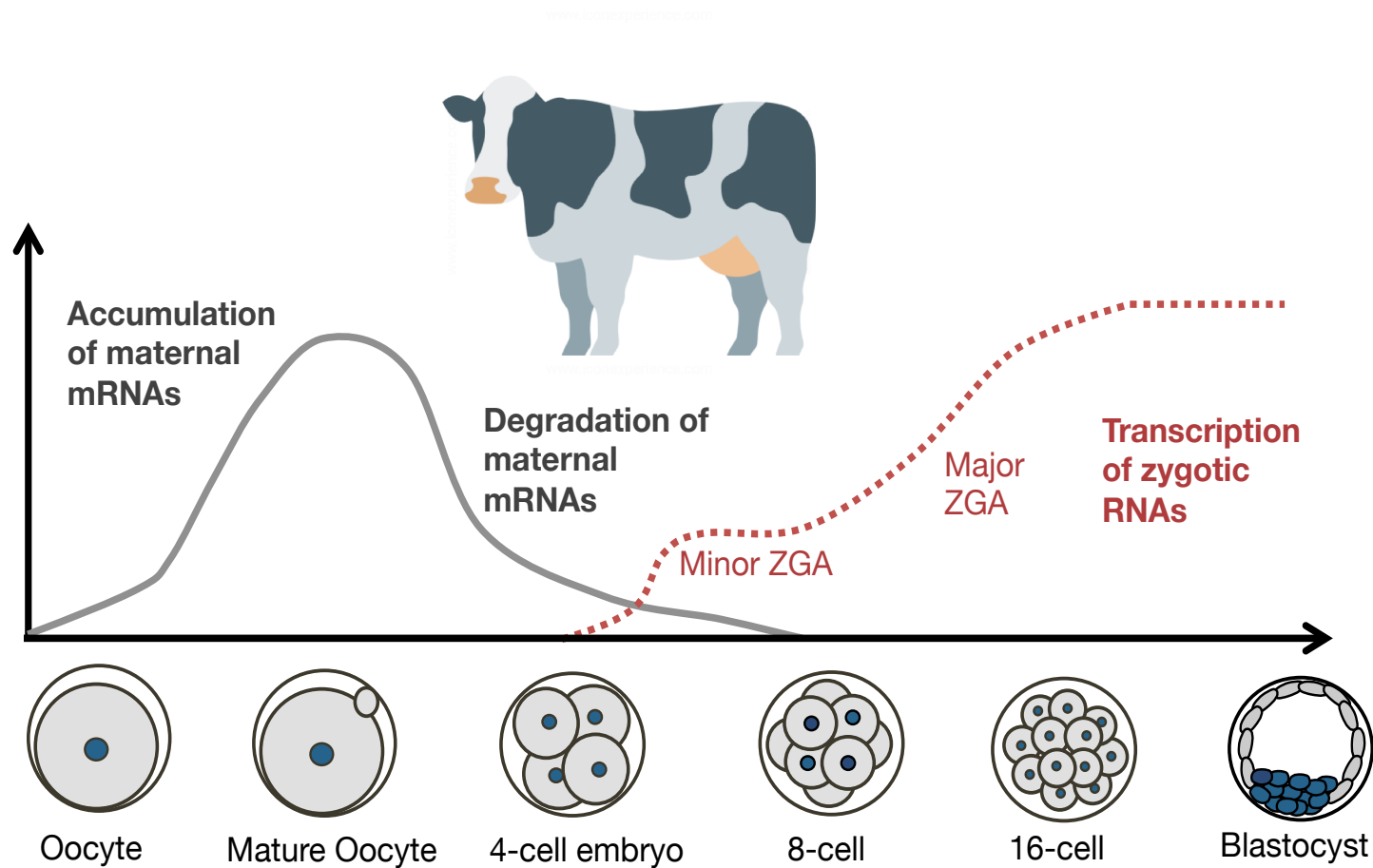
- Small non-coding RNA
- Bind complementarily with mRNA
- Highly conserved
- Turn genes **off** by blocking translation
- Non-coding RNA expanding field

# EARLY DEVELOPMENT

- Parental epigenome must be erased
- Embryonic epigenome must be correctly established
  - Requires period of transcriptional silence
  - Maternal transcripts drive early development during this period

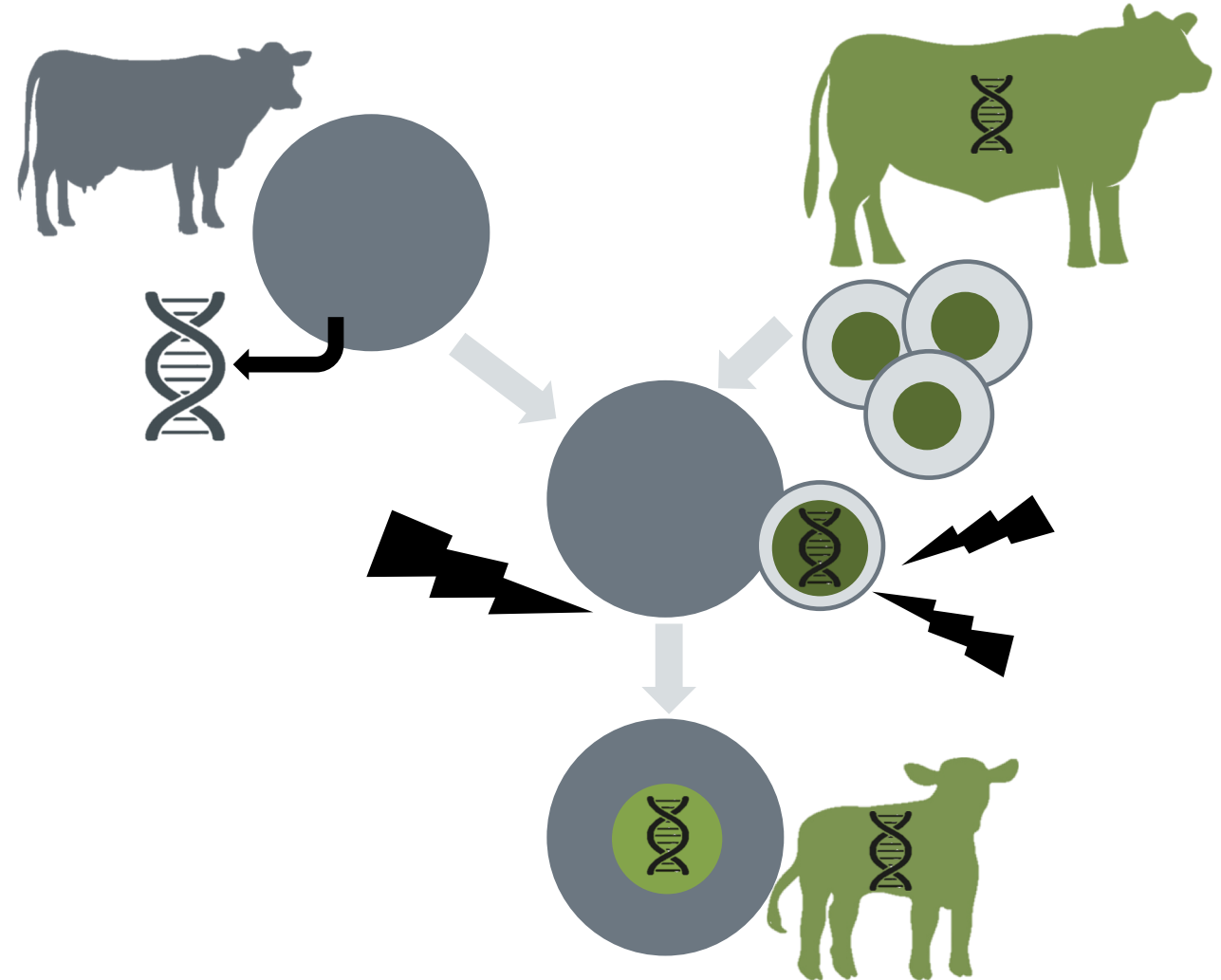


# POTENTIAL ROLE FOR MIRNA



# 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

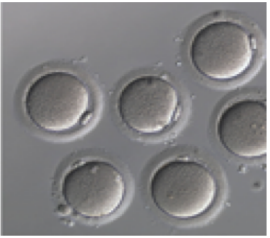


# STUDY DESIGN

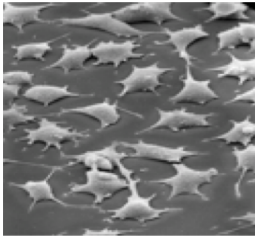
## Sample types

Embryo stages (IVF & scNT)

Oocytes



Fibroblasts



2-cell



8-cell



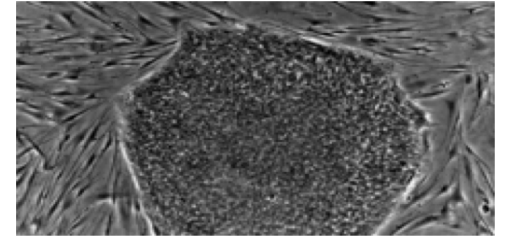
Morula



Blast.



ESCs from IVF & scNT

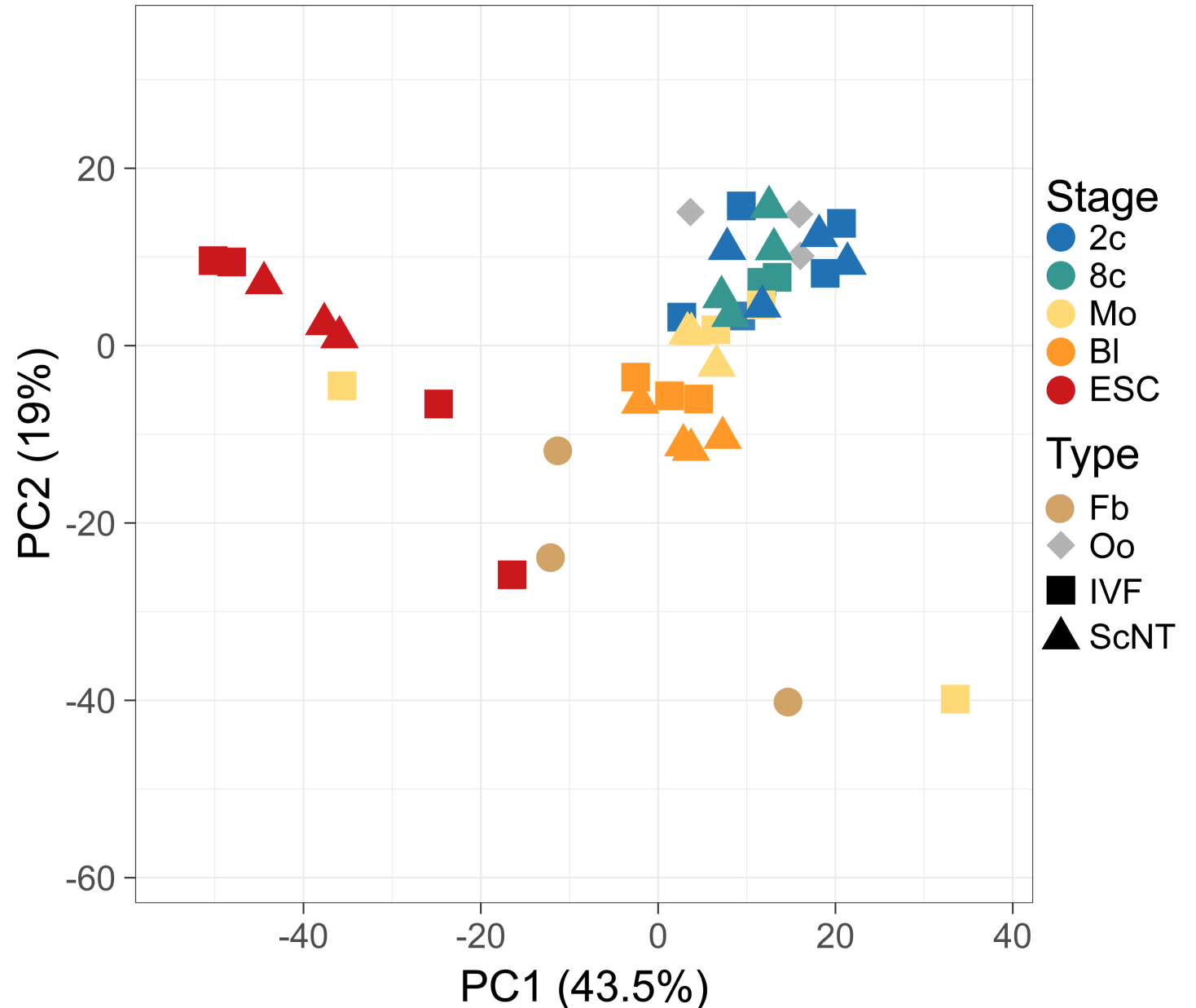


## Sample processing

- 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

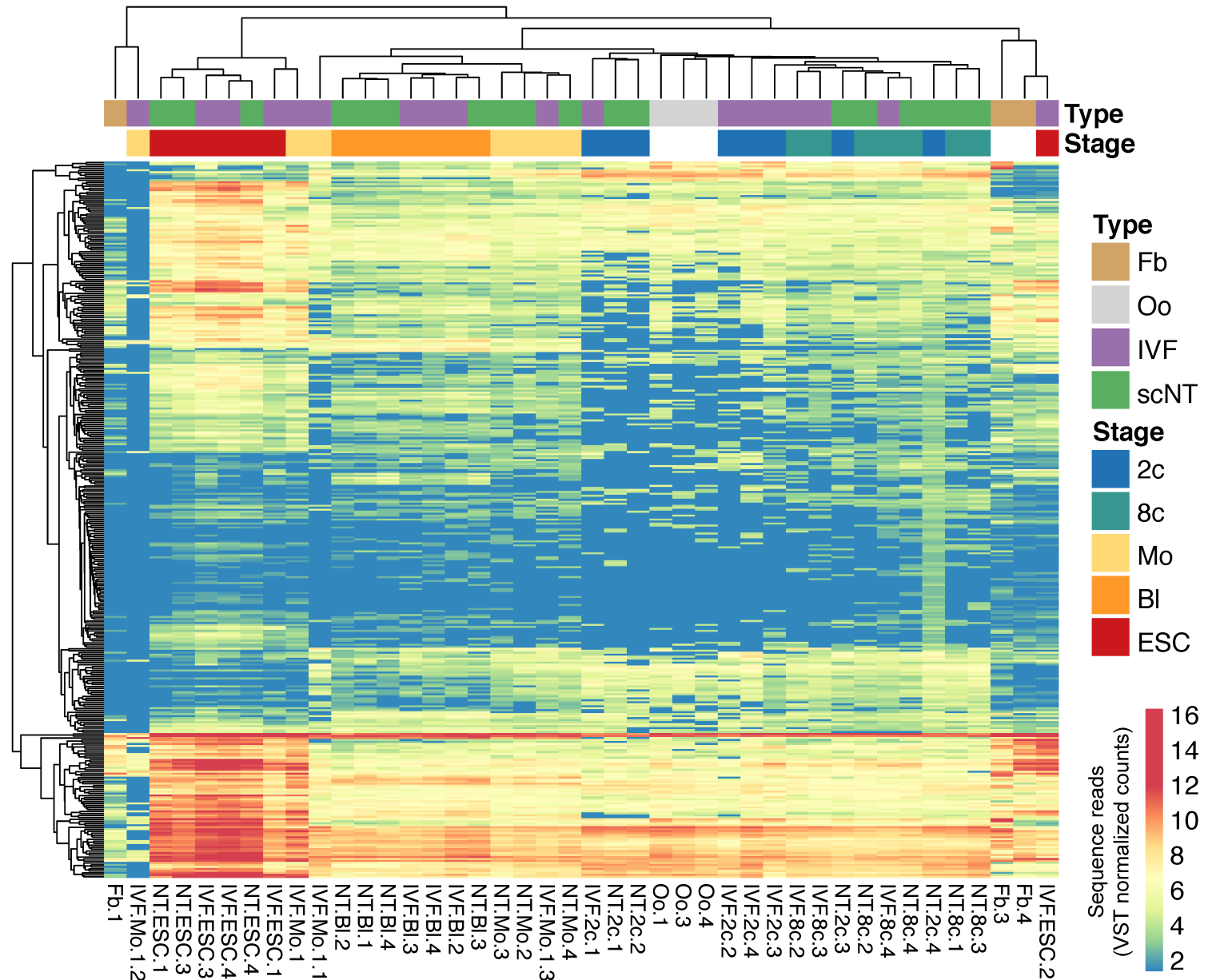
- Samples tend to cluster by stage
- No obvious differences between IVF and ScNT embryos
- Several samples do not cluster well (morula)





# 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



# TAKE HOME AND FUTURE DIRECTIONS



## ScNT epigenetic errors are very variable

- May be preventing us from getting significant miRNA changes due to have 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



OFFICE OF  
**RESEARCH**  
**UtahStateUniversity®**

## Thanks to:

- Dr. Kenneth White's lab
  - Qinggang Meng
- Dr. Abby Benninghoffs lab
- Dr. Stewart Russell (CReATe IVF)



COLLEGE *of*  
**AGRICULTURE *and***  
**APPLIED SCIENCES**  
**UtahStateUniversity®**



# SIGNIFICANT MIRNA

## IVF vs. ScNT

| Stage  | # | Top miRNAs of interest  |
|--------|---|-------------------------|
| 2-cell | 1 |                         |
| 8-cell | 0 |                         |
| Morula | 6 | miR-2340-3p; miR-345-5p |
| Blast  | 1 | miR-497-5p              |

## Between stages

| Stages                | #   | Top miRNAs of interest  |
|-----------------------|-----|---|
| Oocyte vs. 2-cell     | 3   | miR-451; miR-133a, miR-29b  |
| 2-cell vs. 8-cell     | 0   |   |
| 8-cell vs. Morula     | 22  | miR-378; miR-19b-3p; miR-378-5p; miR-6119-5p; miR-378-3p          |
| Morula vs. Blastocyst | 56  | miR-30e-5p; miR-497-5p; miR-125b-1-5p; miR-138-1-5p; miR-138-2-5p |
| Fibroblasts vs 2-cell | 365 | miR-205-5p  |

# SIGNIFICANCE IN OTHER SNCRNA

## Significant piRNA

### Between stages

| Stages                | #     |
|-----------------------|-------|
| Oocyte vs. 2-cell     | 1     |
| 2-cell vs. 8-cell     | 0     |
| 8-cell vs. Morula     | 24    |
| Morula vs. Blastocyst | 0     |
| Fibroblasts vs 2-cell | 4,671 |

### IVF vs. ScNT

| Stage  | # |
|--------|---|
| 2-cell | 0 |
| 8-cell | 0 |
| Morula | 4 |
| Blast  | 1 |

## Significant tRNA

### Between stages

| Stages                | #   |
|-----------------------|-----|
| Oocyte vs. 2-cell     | 2   |
| 2-cell vs. 8-cell     | 0   |
| 8-cell vs. Morula     | 21  |
| Morula vs. Blastocyst | 139 |
| Fibroblasts vs 2-cell | 393 |

### IVF vs. ScNT

| Stage  | # |
|--------|---|
| 2-cell | 1 |
| 8-cell | 1 |
| Morula | 4 |
| Blast  | 0 |