Validation of Sex-Biased Transcriptomic Differences in *in vitro* Bovine Embryos

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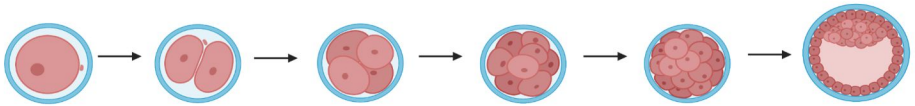
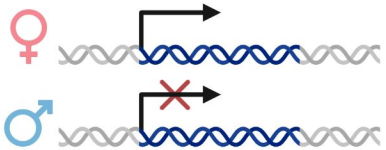
Background

- Early embryonic development is a process of single-celled zygote develop into a blastocyst via cleavage and blastulation.
- Male and female *in vitro* bovine embryos exhibit sexually dimorphism in their growth/cleavage rate, with males developing faster than females.
- This phenomenon has been observed in human, mice, cattle, and sheep embryos
- This study aims to investigate underlying mechanism driving this sex-specific difference in early embryo development by identifying differentially expressed genes and differential alternative splicing events.

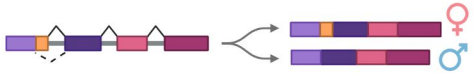
Early Embryonic Development



Differentially Expressed



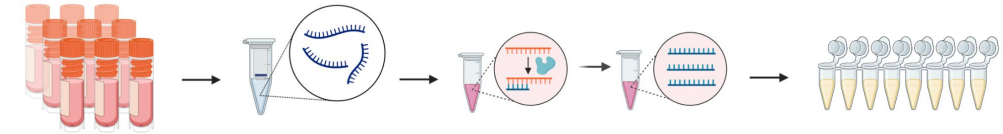
Sex-Biased Alternative Splicing



<u>IISAGE REU project objective</u>: To validate the RNA-seq identified differential gene expression (DEG) and differential alternative splicing (DAS) event in males vs. females during early embryonic development.

Methods

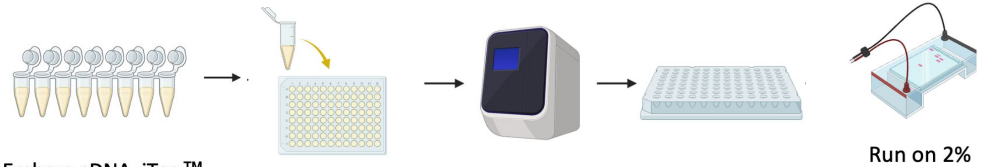
RNA Extraction & Reverse Transcription



Frozen IVF embryos → RNA extraction → Reverse transcription with iScript reaction mix → Embryo cDNA

35 cycles **PCR** with DAS primers

qPCR / PCR & Gel electrophoresis



Embryo cDNA, iTaq™ Universal SYBR® Green mix, H2O, DEG primer → Pipette into qPCR plate → Run qPCR → qPCR product → **DEG Validation:** Collect CT values, compare concentrations by sex

Run on 2% agarose gel → **DAS Validation:** Compare band intensities by sex

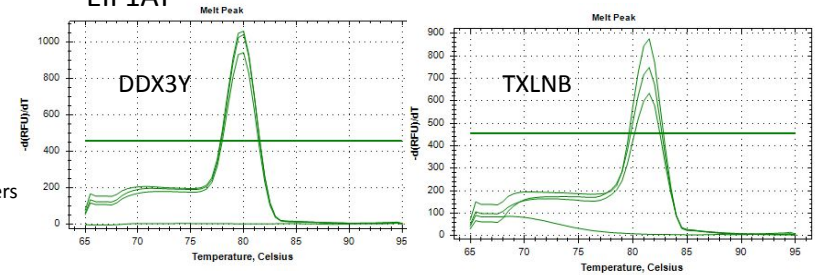
(Validation experiments in progress)

Testing which primers to use for validation:

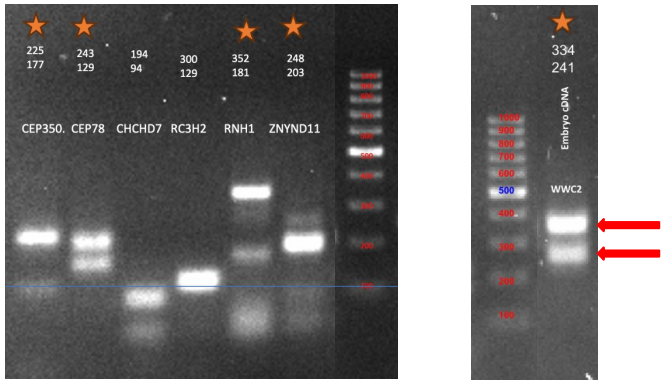
- **"Good" DEG primers = 1 peak on qPCR melting curve**
- **"Good" DAS primers = 2 bands on gel (with difference of brightness)**

Results

Good DEG primers to use for validation: XIST, MAGEB1, MAGEB16, TXLNB, SOX21, DDX3Y, ZRSR2Y, EIF1AY



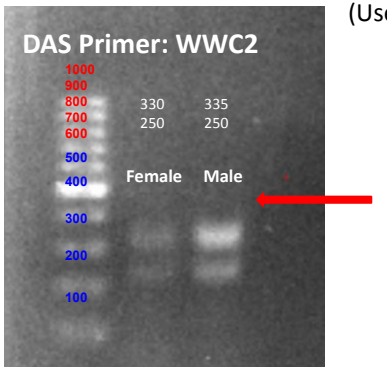
Good DAS primers to use for validation: CEP350, CEP78, RNH1, ZMYND11, WWC2



★ = good primers to use (Used mixed sexed embryos)

Results Cont.

WWC2: Male-biased DAS gene. AS event: skipped exon



(Used sexed embryos)

Upper band in male sample is brighter, showing more expression of the transcript without exon skipped

Experiments in progress:

1. DAS Validation
2. DEG Validation

Conclusions & Future Work

In conclusion, these primers are good to use for the validation

DEG Name	XIST	MAGEB1	MAGEB16	TXLNB	SOX21	DDX3Y	ZRSR2Y	EIF1AY
Sex w/ higher expression	F	F	F	F	M	M	M	M

DAS Gene Name	CEP350	CEP78	RNH1	ZMYND11	WWC2
Sex w/ higher expression	M	F	M	M	M

WWC2 is validated as male-biased differential alternative spliced gene in *in vitro* bovine embryos.

The DEG/DAS validation experiments are still in progress.

Next steps:

- Use imaging software to quantify intensity of the bands.
- Apply this validated protocol to the sexed-embryos samples used in generating RNA-seq library.