#135

# Differential Gene Expression of Bovine Long Noncoding RNAs in Single Oocytes Aspirated from Small and Large Follicles

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## INTRODUCTION

Cattle have a single large follicle present on each ovary from days 5-10 of an estrous cycle (Matton *et al.* 1981). During these days, the concentration of estradiol is similar to concentrations during estrus (Lukaszewska and Hansel 1980). In a study conducted by Ireland and Roche in 1982, follicles with higher concentrations of estradiol than progesterone and androgens in follicular fluid were classified as estrogen active (EA). Based on their results, EA follicles demonstrated characterized developmental changes in ovulatory follicles as they increased in size from 6-20 mm in diameter after a single $PGF_{2\alpha}$ injection (Ireland and Roche 1982).

As folliculogenesis progresses, the follicle grows in size as the oocyte increases in diameter. Several studies over the past two decades, in different mammalian species, have proven that oocyte developmental competence is highly correlated with oocyte diameter and follicle size (Gad *et al.* 2019). To achieve reproductive success, the oocyte must be able to display developmental competence; meaning, it must be capable to resume meiosis, cleave upon fertilization, sustain early development (namely to activate its genome), establish pregnancy, and sustain fetal growth and development until birth (Gilbert *et al.* 2015). During early embryonic activation, maternal transcripts that accumulated in the oocyte during oogenesis and folliculargenesis, play important roles in embryonic genome activation (Hamatani *et al.* 2004). Some maternal transcripts are oocyte-specific and known as maternal effect genes which are required for the early developmental events post fertilization (Dean 2002; Zheng and Dean 2007).

Long Non-Coding RNAs (lncRNAs) are maternal transcripts believed to regulate a wide range of biological processes including gene expression and epigenetic status (Wilusz *et al.* 2009; Pauli *et al.* 2011). Important mammalian lncRNAs have been identified in humans and mouse embryonic development including *Malat1*, *Xist,* and *Hotair.* Very little information is available on bovine oocyte-specific lncRNAs.

## MATERIALS AND METHODS

Single oocytes were aspirated from small (<4 mm) (SF) and presumably estrogen active (6–18 mm) (EA) follicles and were denuded at the germinal vesicle (GV) or MII stage.

MII stage was determined by cumulus expansion and the extrusion of the first polar body.

Real-time quantitative PCR analysis was performed, using RPL-19 as an endogenous control for data normalization analyzed using the standard curve method.

Average expression of each lncRNA from SF was used as a calibrator in determination of fold change.

Effect of follicle size (SF, EA) and maturity stage (GV and MII) and their interaction on the lncRNA expression were examined using a two-way factorial ANOVA followed by Tukey's HSD *post hoc* test.

## PRELIMINARY RESULTS

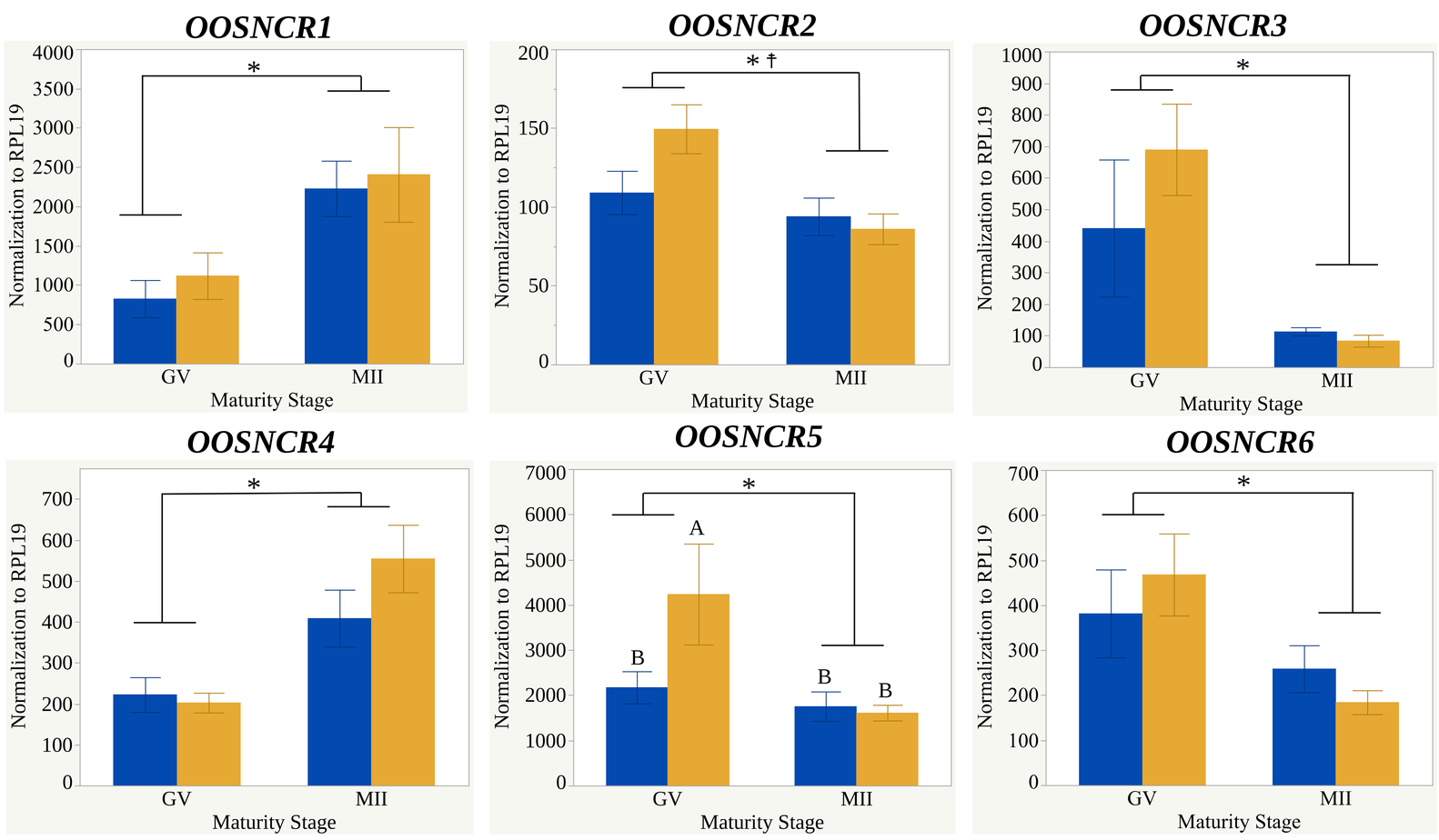
**Table 1. Expression of lncRNAs in bovine oocytes relative to 9 other tissues (FPKM)**

| LncRNA | Oocyte | Testes | Spleen | Skeletal Muscle | Lungs | Liver | Kidney | Heart | Colon | Brain |
|---|---|---|---|---|---|---|---|---|---|---|
| **OOSNCR1** | 252.236 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **OOSNCR2** | 114.618 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **OOSNCR3** | 85.1764 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **OOSNCR4** | 190.441 | 0.06401 | 0.05130 | 0.06824 | 0.18655 | 0 | 0.07378 | 0.94259 | 0.07336 | 3.58942 |
| **OOSNCR5** | 267.845 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **OOSNCR6** | 146.852 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Our lab previously identified 1535 lncRNAs in bovine oocytes. **Table 1** presents the top six highly abundant lncRNAs in bovine oocytes relative to 9 other tissues using their calculated FPKM scores.

***The objective of this study was to verify our RNA-seq data and if present, characterize the expression of six highly abundant lncRNAs in single oocytes aspirated from varying size follicles at different developmental stages.***

## RESULTS




*** Denotes significance of maturity stage (P < 0.05)**
**A,B denote significant interaction of maturity stage and follicle size (P < 0.05). Groups with diverse letters differ from each other.**
**† denotes tendency for interaction of maturity stage and follicle size (P < 0.1)**

Maturity stage was significant (P < 0.05) in all 6 lncRNAs. ***OOSNCR1*** and ***OOSNCR4*** displayed opposite expression patterns compared to the other four lncRNAs.

There was a significant interaction of stage and size in expression of ***OOSNCR5***. (P < 0.05)

- SF oocytes at the GV stage had the highest relative expression.
- Significant difference was detected between SF MII and SF GV oocytes, indicating a higher expression in the earlier developmental stage (P < 0.05).
- SF oocytes at MII had a 0.63-fold decrease in its expression relative to the SF oocytes at GV.

There was a tendency for significant interaction between stage and size in expression of ***OOSNCR2***. (P < 0.1)

- ***OOSNCR2*** exhibited a similar expression patterns as ***OOSNCR5***.
- SF oocytes at MII had a 0.43 -fold decrease in its expression relative to the SF oocytes at GV.

**Follicle Size**

**Large – Estrogen Active (EA) (6-18mm)**
**Small – Estrogen Inactive (SF) ( < 4mm)**

## CONCLUSION

The literature indicates that follicle size can be an indicator of oocyte quality and that RNAs accumulated or degraded during maturation are crucial to achieve developmental competence. Therefore, lncRNAs *OOSNCR2* and *OOSNCR5* show promise to be linked with oocyte quality.

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