The Expression of Agouti-Signaling Protein During Folliculogenesis and Oocyte Maturation in Cattle

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Heather L. Chaney, Brady M. Nicewarner, Jaelyn Z. Current, Mingxiang Zhang, and Jianbo Yao

Davis College of Agriculture, Natural Resources and Design, Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV, USA

Introduction

Oocyte competence:

- Regulates the oocyte's ability to resume meiosis, cleave when fertilized, and promote embryonic development [1].
- Is determined by the mRNA and protein present in the oocyte [1].
- Acquired by oocytes at the follicular size of 3 mm, however, the proportion of competent oocytes greatly increases once reaching the follicular size of 8 mm [2].

Agouti signaling protein (*ASIP*):

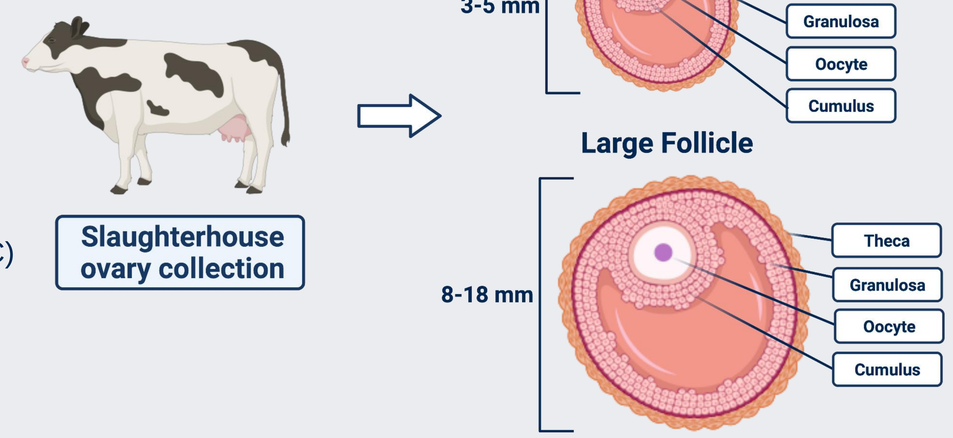
- Adipose cell expression of *ASIP* is associated with lipid metabolism and insulin resistance [3].
- Highly abundant in the germinal vesicle (GV) bovine oocyte [*Unpublished RNA-Seq data, Yao lab].*
- The function of ASIP in the bovine ovary is currently unknown.

Objectives:

1. Examine the effect of follicle size on *ASIP* expression in the bovine oocyte (GV and MII stage), and theca, granulosa, and cumulus cells.
2. Determine the oocyte and cumulus cell *ASIP* expression pattern following *in vitro* maturation (IVM).

Materials and Methods

Figure 1. Luteal phase ovary collection. Small (3-5 mm; SF) and Large (8-18 mm; LF) follicles were aspirated for cumulus-oocyte complex (COC) isolation. Additional follicles were dissected for granulosa cell (GC) and theca cell (TC) collection.



Analysis of *ASIP* expression via RT-qPCR

- RT-qPCR was conducted using PowerUp SYBR Green Master Mix (Applied Biosystems) according to published methods [4].
- *RPL19* expression was utilized for normalization and relative expression values were calculated using the standard curve method.
- Data are presented as mean ± SEM.
- Log-transformed RT-qPCR data were used for statistical analysis.

Experiment 1: *Effect of follicle size on granulosa and theca cell ASIP levels*

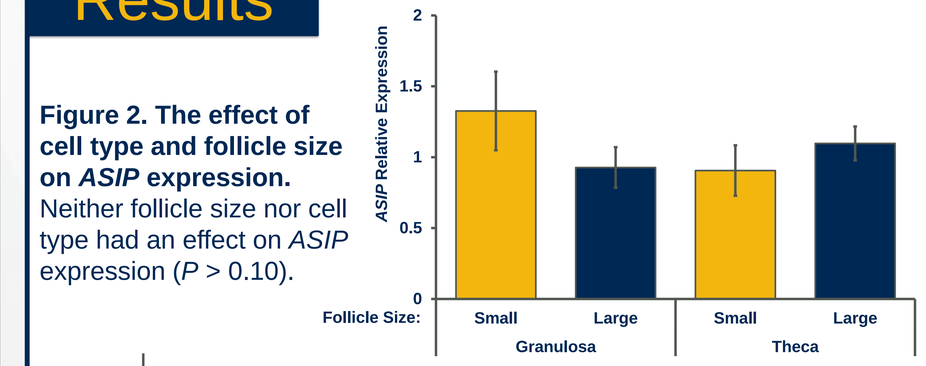
- GC and TC from individual SF (n=6) and LF (n=6) were analyzed for *ASIP* expression.
- Statistics: Split-plot analysis with the main plot of follicle size and subplot of cell type.

Experiment 2: *Effect of follicle size and IVM on oocyte and cumulus cell ASIP expression*

- COCs containing GV oocytes from SF and LF were denuded to collect GV oocytes (n=8 pools of 10) and cumulus cells (n=8 pools of cells from 10 COCs; CC-GV).
- Additional SF and LF COCs underwent IVM for 22-24 h to collect MII oocytes (n=8 pools of 10) and cumulus cells (n=8 pools of cells from 10 COCs; MII-CC)
- Statistics:
  - 2x2x2 factorial design to analyze the effects of cell type, follicle size, and maturity level.
  - Post hoc: Tukey's HSD

Results

Figure 2. The effect of cell type and follicle size on *ASIP* expression. Neither follicle size nor cell type had an effect on *ASIP* expression ($P > 0.10$).



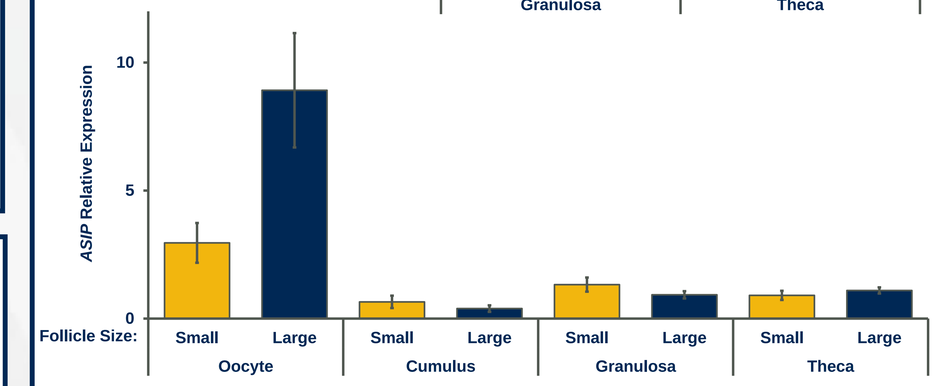


Figure 3. Follicular *ASIP* expression. Comparison of expression levels for all cell types analyzed in this study. Oocytes from large follicles highly express *ASIP*.

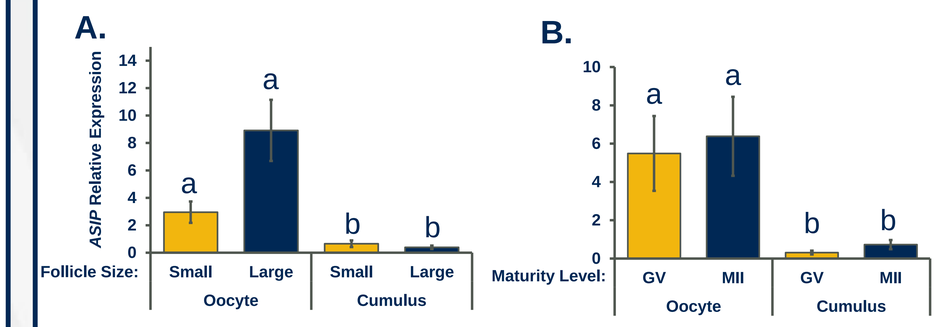


Figure 4. The effect of COC cell type, follicle size, and maturity level on *ASIP* expression. (A) There was an effect of cell type ($P < 0.0001$) and an interaction between cell type and follicle size ($P = 0.03$). (B) Maturity level did not affect *ASIP* expression.

Conclusions

- Bovine oocytes highly express *ASIP*.
- While *ASIP* expression is increased in oocytes from large vs. small follicles, cumulus cell expression of *ASIP* decreased in large follicles.
- Future work will aim to elucidate the function of ASIP in the bovine oocyte, particularly during oocyte maturation and early embryonic development.

Acknowledgements

Funding for this research was provided by:

- National Institute of Food and Agriculture, U.S. Department of Agriculture, award number 2020-38640-31520 through the Northeast SARE program under subaward number GNE21-252-35383.
- USDA National Institute of Food and Agriculture, Multistate Research Project 1014002.

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*Email: hebaldwin@mix.wvu.edu