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Characterization of Agouti-Signaling Protein and its Receptors in the Bovine Oocyte and Early Embryo

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Introduction

Bovine *In Vitro* Fertilization (IVF)

- For the first time, it was reported a greater number of bovine embryos were generated via IVF than *in vivo* in 2016 [1].
- Despite advancements in oocyte and embryo culture systems, only 20-40% of presumptive zygotes will reach the blastocyst stage *in vitro* [2].
- A limiting factor to IVF is lack of knowledge of the mRNAs and proteins necessary for oocyte maturation and early embryonic development [3,4].

Agouti signaling protein (*ASIP*):

- 132 amino acid secreted protein
- Highly abundant in the bovine oocyte (RNA-Sequencing data, Yao lab; **Fig. 1**).
- The function of ASIP in the ovary is unknown.
- A role in lipid metabolism has been established in other bovine tissues including adipose [5] and mammary epithelial cells [6].
- ASIP binds to melanocortin receptors (MC1R-5R) and the attractin (ATRN) receptor [6].

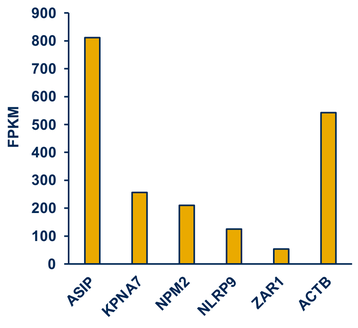

Figure 1. *ASIP* expression in the bovine oocyte in relation to other highly expressed oocyte genes.

Objectives:

1. Characterize *ASIP* expression throughout early embryonic development and within the ovarian follicle.
2. Determine the expression of putative receptors for *ASIP* in follicular cells and the oocyte.

Materials and Methods

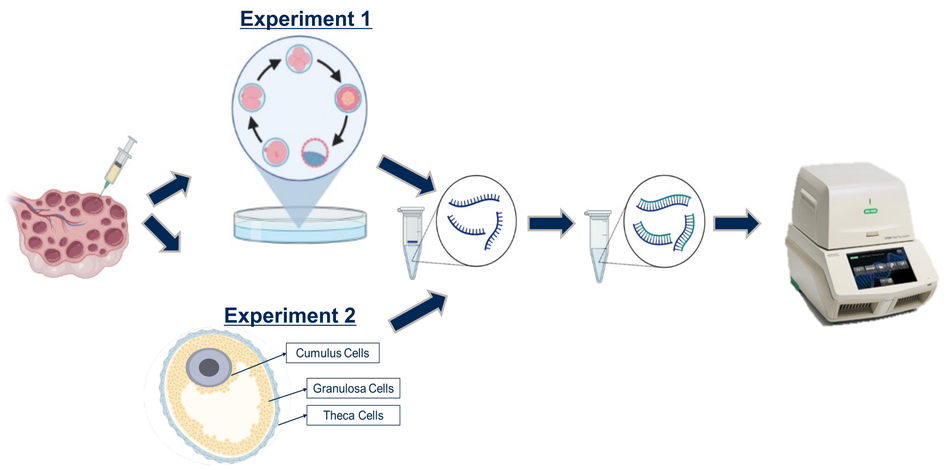

Figure 2. Workflow for experiments 1 and 2. Oocytes were obtained from slaughterhouse bovine ovaries and aspirated to obtain cumulus-oocyte complexes (COCs) or antral follicles 3-10 mm were dissected for cell isolation.

In vitro embryo production

- Cumulus-oocyte complexes (COCs) were cultured in IVM (IVF Bioscience) for 22-24 h and fertilized.
- Presumptive zygotes were placed in IVC (IVF Bioscience), and 2-, 4-, 8-, 16, morula, and blastocyst stage embryos were collected then stored at -80˚C until RNA isolation.

Analysis of gene expression via RT-qPCR (Fig. 2)

- RNA was isolated from all samples using the RNAqueous Total RNA Isolation Kit (Invitrogen).
- RNA was reverse transcribed using the High-capacity cDNA Reverse Transcription Kit (Applied Biosystems).
- RT-qPCR was conducted using PowerUp SYBR Green Master Mix (Applied Biosystems) according to published methods [7].
- Gene normalization:
  - Embryo panel samples were spiked with *GFP* RNA during RNA isolation, which was used for normalization.
  - *RPL19* expression was utilized for normalization of all other data.
- 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.
- Statistical analysis included either a Student's t-test (Fig. 4A) or one-way ANOVA followed by Tukey's HSD.
- $P \leq 0.05$ considered significant.

Results

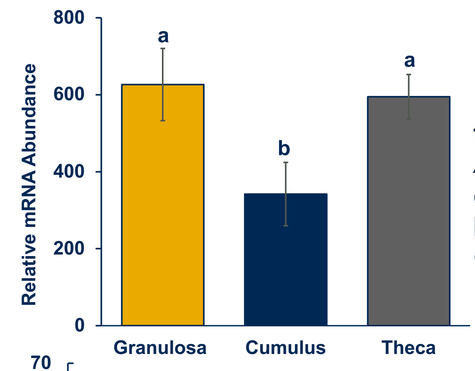

Figure 2. Expression of *ASIP* in ovarian follicular cells collected from antral follicles. *ASIP* expression was detected in granulosa, cumulus, and theca cells (n = 12-16) with higher levels in granulosa and theca cells ($P < 0.0001$). Gene expression is relative to *RPL19.*

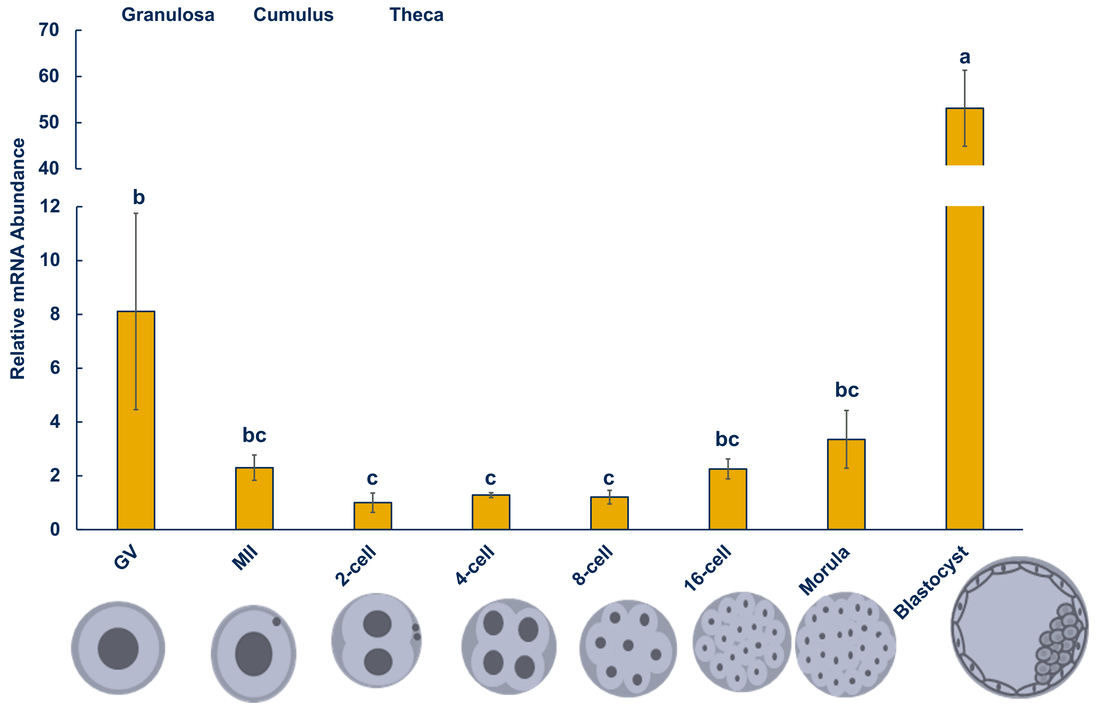

Figure 3. Expression of *ASIP* during oocyte maturation and early embryogenesis. Data are relative to *GFP* RNA levels. Expression was detected in all stages (n = 4 pools of 20 oocytes/embryos) with the highest levels present at the GV and blastocyst stages ($P < 0.0001$).

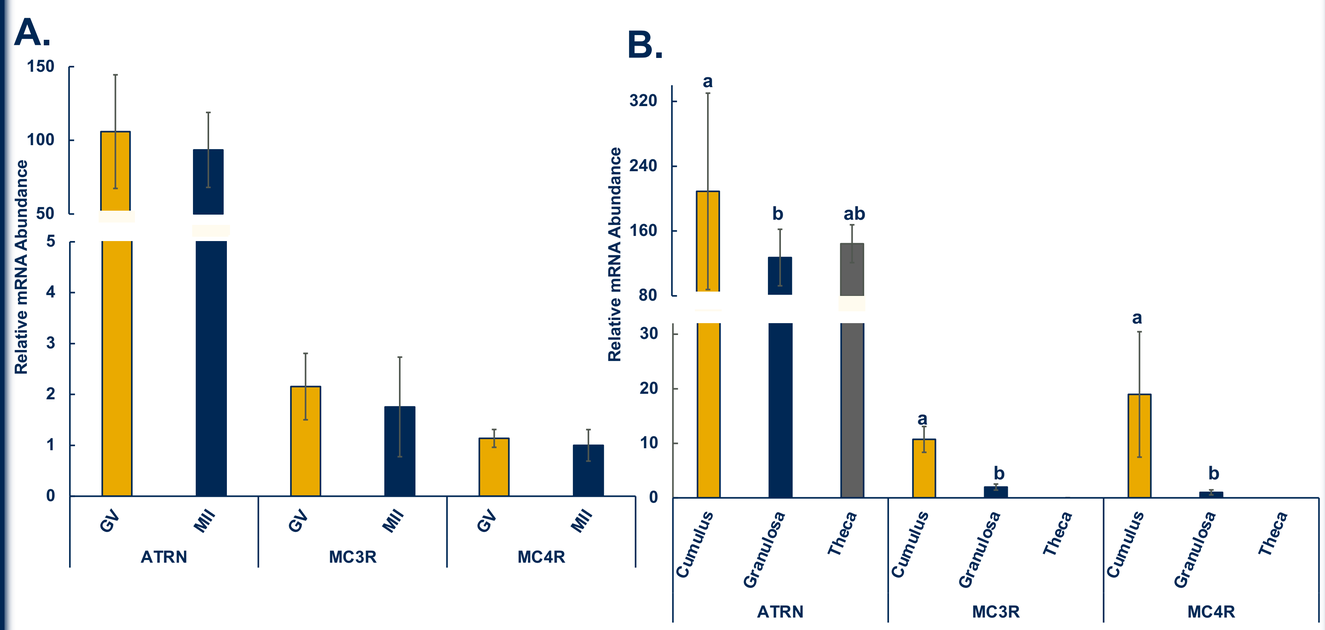

Figure 4. Putative ASIP receptors detected in oocytes (A) and follicular cells (B). (A) Expression of *ATRN*, *MC3R*, and *MC4R* was present in both GV and MII oocytes (n = 4 pools of 10). No statistical difference between stages (GV vs. MII; $P > 0.05$). (B) *ATRN*, *MC3R*, and *MC4R* were also detected in ovarian follicular cells including cumulus, granulosa, and theca cells isolated form antral follicles (n = 12-16) . Cumulus expression of receptors *ATRN*, *MC3R*, and *MC4R* was the highest ($P < 0.0001$).

Conclusions

- ***ASIP* is expressed in follicular cells including cumulus, granulosa, and theca cells.**
- ***ASIP* expression decreases following oocyte maturation and rises following activation of the embryonic genome at the 8-16 cell stage.**
- **ASIP putative receptors, *ATRN*, *MC3R*, and *MC4R* are expressed in both GV and MII oocytes. *ATRN* was detected at high levels in cumulus, granulosa, and theca cells while *MC3R* and *MC4R* were detected in cumulus and granulosa cells.**
- **Future work will characterize the function of ASIP during oocyte maturation and early embryonic development in cattle.**

Acknowledgements

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