W167

Expression of WNT Signaling Transcripts at Specific Stages of Follicle Development in Bovine Granulosa Cells

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Introduction

- Wingless-type mammary tumor virus integration-site (WNT) signaling molecules are involved in development and growth of ovarian follicles in mammals (Hsieh et al., 2002).
- The WNT signaling pathway controls numerous developmental processes including fate of the cell, cell growth and differentiation and has been extensively studied in flies, rodents, and humans (Boyer et al., 2009).
- Pituitary gonadotropins are important in regulation of follicle maturation and ovulation.
- Several WNT family members are hormonally regulated in adult rodent ovaries.
- Recently, our laboratory demonstrated that the canonical WNT pathway is present in the bovine ovary and is stimulated by follicle stimulating hormone (FSH) (Castañon et al., 2012).
- To date, limited information is available on the role of WNT signaling in livestock reproduction.

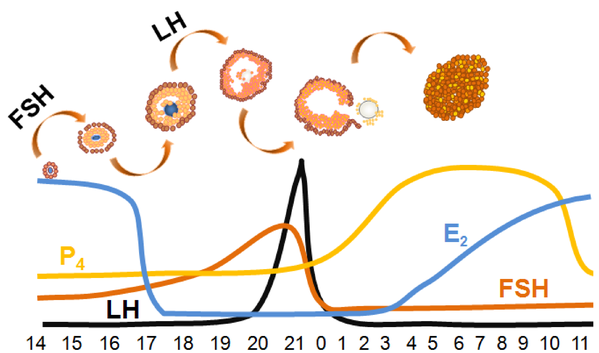
Objective

- To characterize expression of WNT signaling pathway molecules at specific stages of follicular development in bovine granulosa cells (GC).

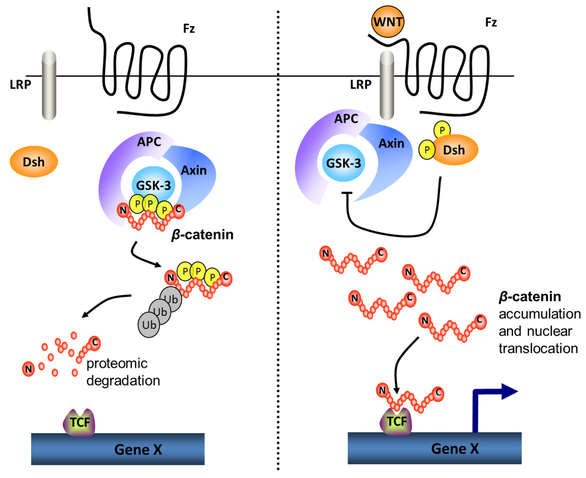
Hypothesis

- Expression of WNT signaling molecules will be differentially expressed throughout follicle development.

Ovarian Follicle Development Requires Coordinated Hormonal Input



Follicle Maturation Involves Regulation of Local Ovarian Factors Such as WNT/CNNTB1



Materials and Methods

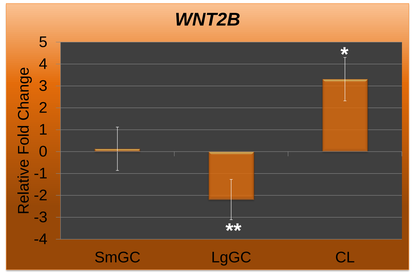
Experiment

- Mid-luteal ovaries pairs were collected from a local abattoir.
- GC and follicular fluid were aspirated from ovary pairs containing a stage III CL (d 11 to 17 of an estrous cycle).
- GC were isolated from small (1 to 5 mm) and large (8 to 22 mm) follicles, and the corresponding CL.
- Total ribonucleic acid was collected from TRIzol reagent and real time polymerase chain reaction was used to measure gene expression.
- Intra-follicular estradiol and progesterone concentrations were quantified by radioimmunoassay and used to identify large dominant follicles (estradiol:progesterone > 1).

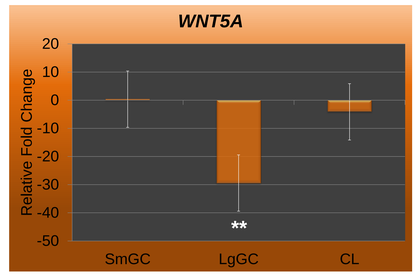
Statistics

- Changes in gene expression were measured via real-time polymerase chain reaction using cyclophilin A (*PPIA)* and ribosomal protein L19 (*MRPL19)* as housekeeping genes.
- All samples were analyzed using the ΔΔCt method to calculate fold change among tissues relative to small follicles.
- Gene expression was analyzed for significance using PROC GLM procedure of SAS with a significance value of $P \leq 0.10$ and data is presented by least squared mean ± mean standard error.

WNT Ligands Display Stage Specific Expression in Bovine Granulosa Cells

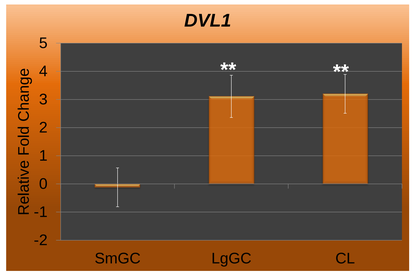


- *WNT2B* expression is lower in large dominant follicles ($P = 0.03$) but increased in CL ($P = 0.10$) compared to small follicles.

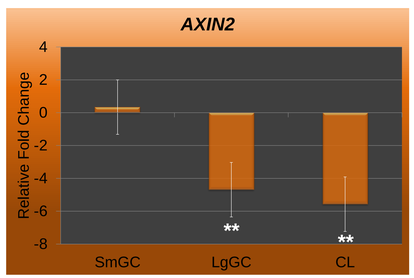


- A comparable pattern of expression was demonstrated for *WNT5A* as small follicles had greater expression compared to large follicles ($P < 0.01$) and was similar to CL ($P = 0.56$).

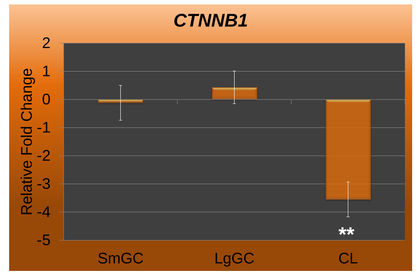
Evaluation of Canonical WNT Signaling Pathway Components



- Disheleved 1 (*DVL1*) is significantly increased in large follicles ($P < 0.01$) and CL ($P < 0.01$) compared to GC of small follicles in mid-luteal ovaries.



- Preliminary data for axin 2 (*AXIN2)* suggest a regulation of this signaling molecule in large follicles ($P = 0.05$) and CL ($P = 0.02$).



- Compared to small follicle GC, expression of the WNT transcriptional co-factor, β-catenin (*CTNNB1*) was similar in large dominant follicles ($P = 0.53$) but decreased in the CL ($P < 0.01$).

Conclusion

- Specific WNT and WNT pathway components are expressed in the adult bovine ovary.
- During folliculogenesis and luteinization WNT signaling is hormonally regulated and distinct WNT expression is dependent on stage of follicular maturation.
- Data suggest a specific function for these signaling molecules in the bovine ovary.

References

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- Hsieh, M. et. al. Endocrinology 2002 143(3):898-908.

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