Summer 8-2022

Effects of Hormones on Follicular Gata4 and Gata6 Expression

Danielle D. Oberpriller
Summer Undergraduate Research Program

Shyamal K. Roy Dr.
University of Nebraska Medical Center

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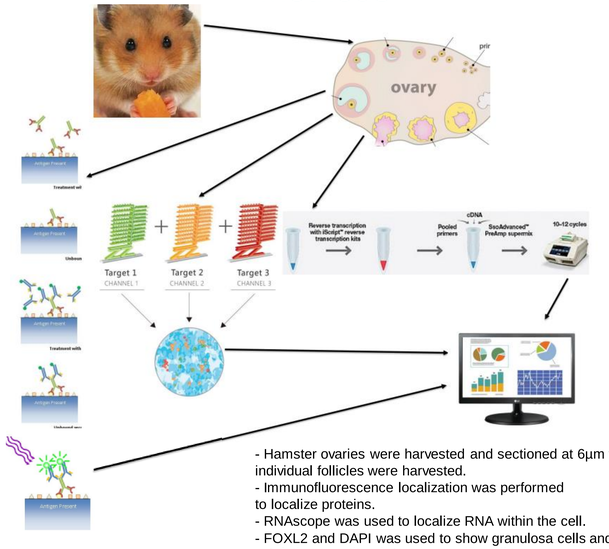
Danielle D. Oberpriller[1] and Shyamal K. Roy[2,3]
[1]UNMC SURP Program; [2]Department of Obstetrics and Gynecology, and [3]Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE

Abstract

The GATA gene family consists two sub-groups of six genes namely, gata1-3, and gata4-6 transcription factors. GATA4 increases in developing gonads in the context of sex determination. Both gata4 and gata6 are present primarily in granulosa cells, but also in the theca, germinal epithelium, and corpus lutem. However, little is known about hormones, which may affect GATA4 expression in specific cell types within the ovary. The regulation of both gata4 and gata6 expression seems to be controlled by several factors including hormones, calcium signaling, oxygen levels and transcriptional/translational mechanisms. Deletion of gata4 in mice lead to a 75% decrease in litter size while deletion of both gata4 and gata6 results in complete infertility due to inefficient primordial follicle formation, oocyte survival and follicular somatic cell development leading to defective folliculogenesis.

The objectives of this study were to determine (1) if gata4 and gata6 mRNA and protein expression in granulosa cells would change during preantral to antral follicle transition, and (2) if specific gonadotropins or ovarian steroid hormones influenced the expression of gata4 and gata6 mRNA and proteins in granulosa cells during antral follicle formation.

Methods



- Hamster ovaries were harvested and sectioned at 6µm thick or individual follicles were harvested.
- Immunofluorescence localization was performed to localize proteins.
- RNAscope was used to localize RNA within the cell.
- FOXL2 and DAPI was used to show granulosa cells and nuclei.
- Relative quantity of RNA in specific cell types were determined by RT-qPCR.
- Statistical analysis done using unpaired t-test.

Results

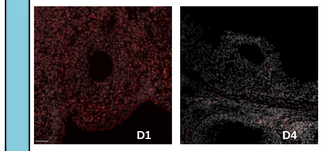


Fig. 1: GATA4 protein expression during the estrous cycle
GATA4, DAPI

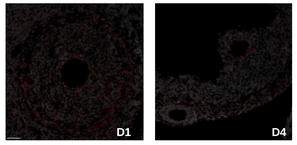


Fig. 2: gata4 mRNA expression during the estrous cycle
Gata4, DAPI

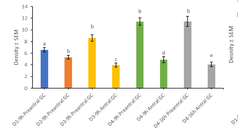


Fig. 5: GATA4 protein expression in granulosa cells across the ovarian cyclic cycle. Preantral granulosa cells compared to D1-9h preantral granulosa cells. Antral GC compared to preantral counterparts. Same letter p>0.05 Different letter p<0.05

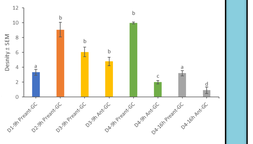


Fig. 6: gata4 RNA expression in granulosa cells across the ovarian cyclic cycle. Preantral granulosa cells compared to D1-9h preantral granulosa cells. Antral granulosa cells compared to preantral counterparts.
Same letter p>0.05 Different letter p<0.05

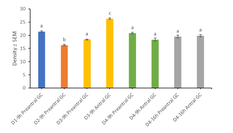


Fig. 7: GATA6 protein expression in granulosa cells across the ovarian estrous cycle. Preantral granulosa cells compared to D1-9h preantral granulosa cells. Antral GC compared to preantral counterparts.
Same letter p>0.05 Different letter p<0.05

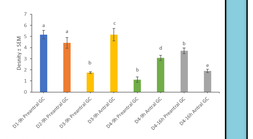


Fig. 8: GATA6 RNA expression in granulosa cells across the ovarian cyclic cycle. Preantral granulosa cells compared to D1-9h preantral granulosa cells. Antral GC compared to preantral counterparts.
Same letter p>0.05 different letter p<0.05

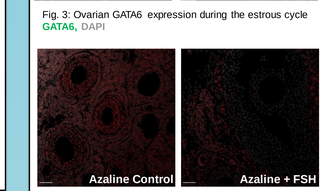


Fig. 3: Ovarian GATA6 expression during the estrous cycle
GATA6, DAPI

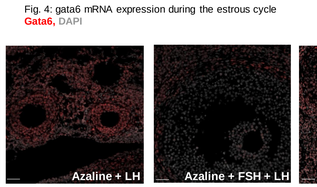
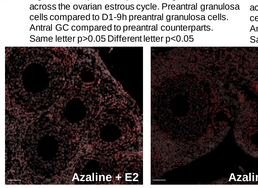


Fig. 4: gata6 mRNA expression during the estrous cycle
Gata6, DAPI



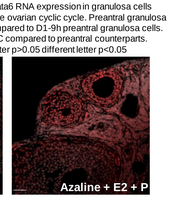


Fig. 9: GATA4 protein expression in ovaries through the Azaline treated cycle **GATA4,** DAPI — 50µm

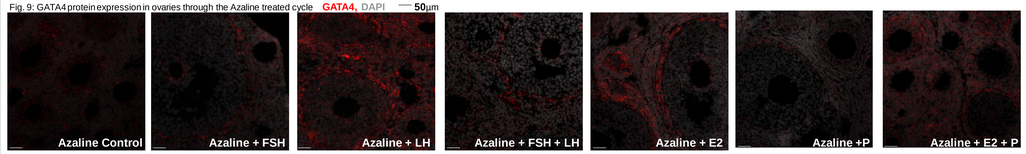


Fig. 10: gata4 mRNA expression in ovaries through the Azaline treated cycle **gata4,** DAPI

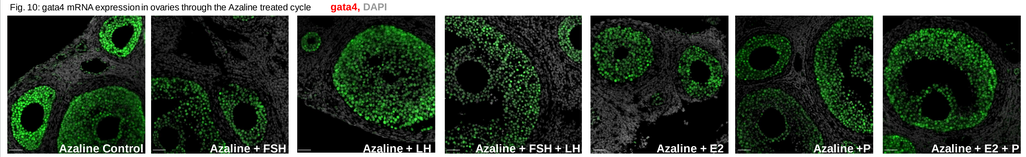


Fig. 11: GATA6 protein expression in ovaries through the Azaline treated cycle **GATA6,** DAPI

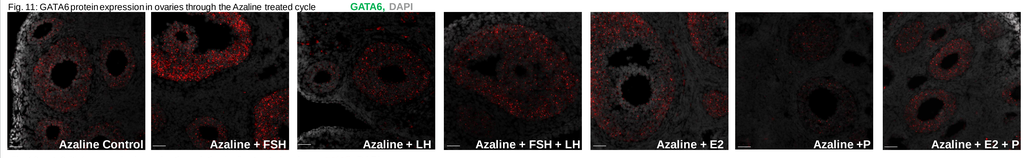


Fig. 12: gata6 RNA expression in ovaries through the Azaline treated cycle **gata6,** DAPI

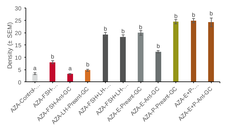


Fig. 13: Effect of hormones on GATA4 protein in granulosa cells in Azaline treated hamsters. Comparison was done with Azaline control.
Same letter =p<0.05 Different letter =p>0.05

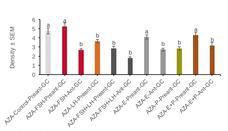


Fig. 14: Effect of hormones on gata4 mRNA in granulosa cells in Azaline treated hamsters. Comparison was done with Azaline control.
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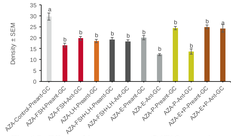


Fig. 15: Effect of hormones on GATA6 protein in granulosa cells in Azaline treated hamsters. Comparison was done with Azaline control.
Same letter =p<0.05 Different letter =p>0.05

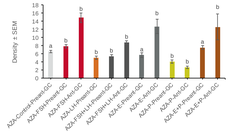
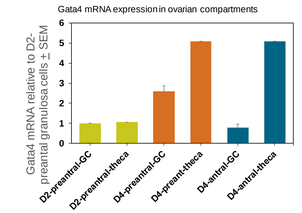
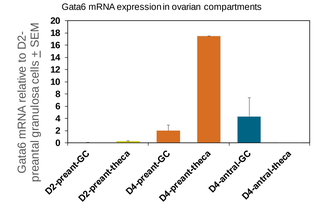
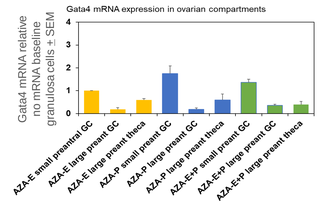


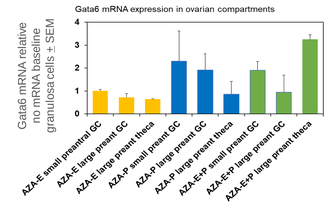
Fig. 16: Effect of hormones on gata6 mRNA in granulosa cells in Azaline treated hamsters. Comparison was done with Azaline control.
Same letter =p<0.05 Different letter =p>0.05

Results









Summary

- GATA4 mRNA and protein were expressed in all follicular cells and in the interstitium; however, GATA4 expression declined remarkably with follicular transition to antral stage.
- GATA6 mRNA and protein were expressed almost exclusively in the granulosa cells.
- Gonadotropins and ovarian steroids, especially estrogen upregulated Gata4 and Gata6 mRNA and protein in granulosa cells in varied degrees.

Conclusions

- GATA4 and GATA6 are differentially expressed in follicular granulosa cells.
- Whereas GATA4 expression markedly declines in antral follicular granulosa cells, GATA6 expression increased significantly suggesting different control mechanisms exist for these transcription factors.
- The differential expression of GATA4 and GATA6 also suggest that they regulate different function of granulosa cells as follicles mature from preantral to antral stage.

References

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