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The Effects of Androgens on Bovine and Human Granulosa Cells

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Abstract

A subpopulation of cows in the Physiology herd has been identified as subfertile due to sporadic or chronic anovulation. This decrease in fertility could be lost profits for farmers and raises questions about ovulation disorders in women. Granulosa cells, a type of cell that is essential to ovarian follicle development, was investigated to determine if high concentrations of a hormone, androstenedione, could impact the follicular environment enough to cause anovulation disorders. Previous studies suggested that excess androgen may decrease the number of functioning granulosa cells by preventing them from proliferating within the follicle. Fewer granulosa cells mean fewer cells that are available to convert androstenedione to estrogen; and estrogen is required for the development of the follicle and ovulation. This research experience determined that when primary granulosa cells are subjected to high concentrations of androstenedione the rate of proliferation decreases based on the reduced proliferation promoting genes within the cells. These tests were done on primary granulosa cells from bovine as well as an immortalized granulosa cell line from humans.

Background of the Project:

Figure 1. In the ovary each oocyte has a follicle made up of granulosa cells and theca cells which make the steroid hormones. Theca cells make androgens while the Granulosa cells convert androgens to estrogen.

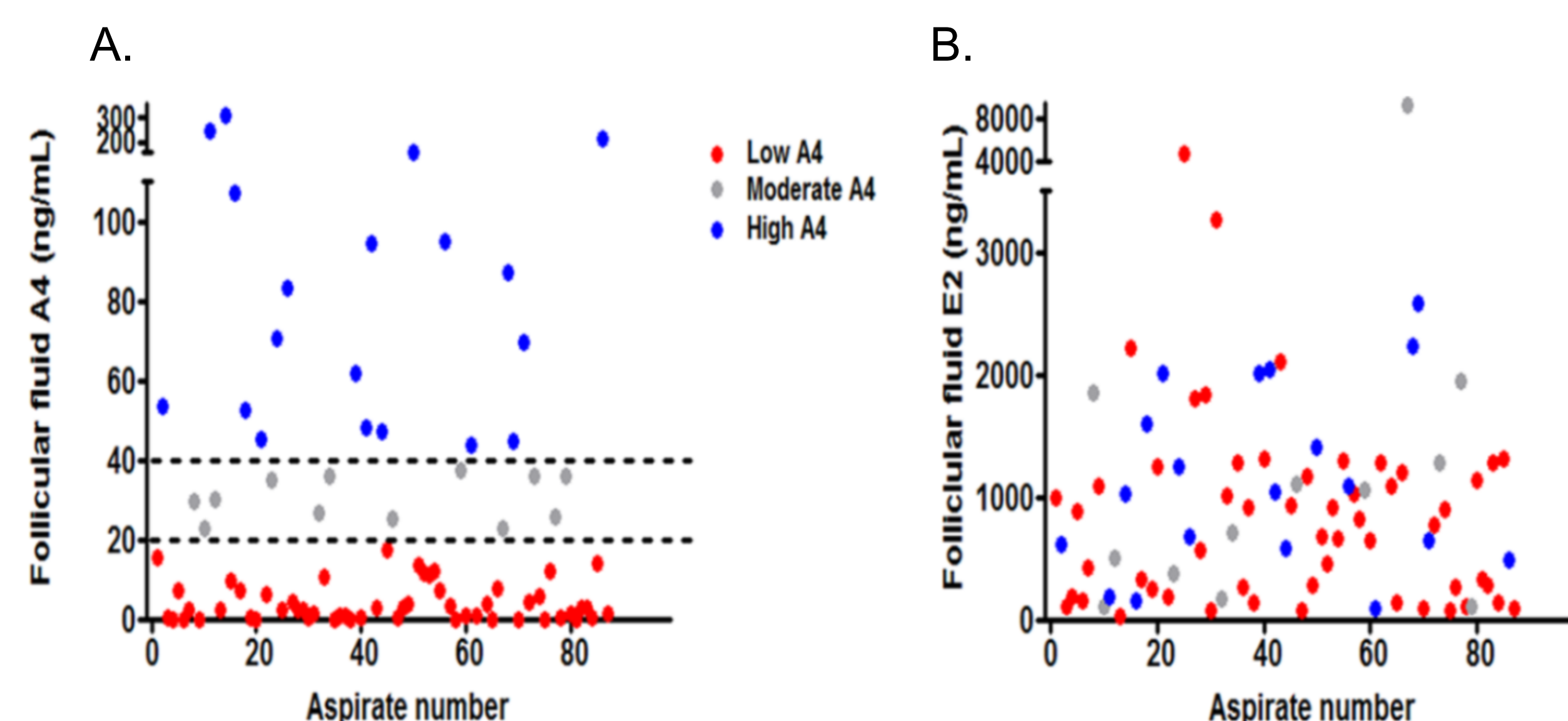
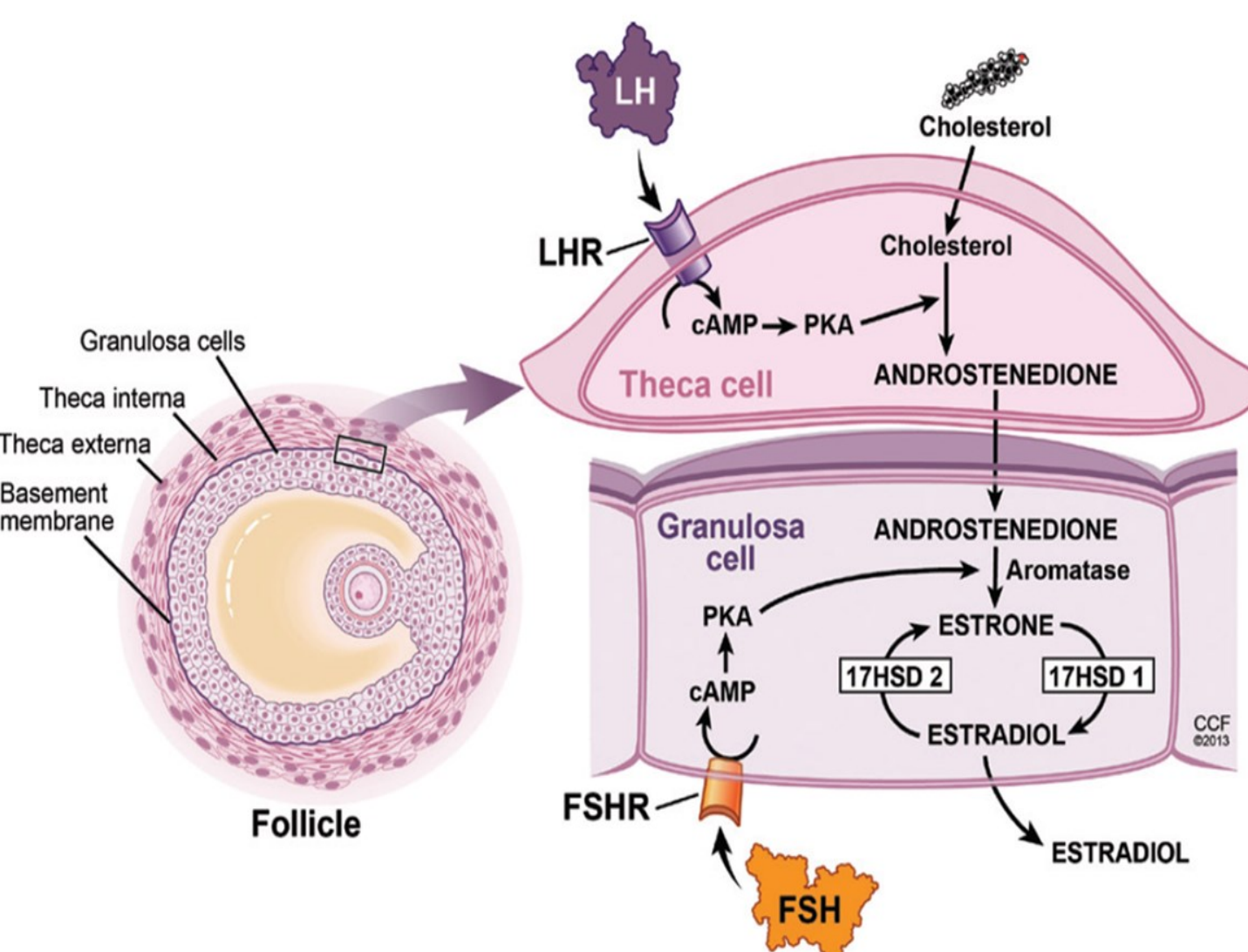


Figure 2. A subpopulation of cows in the Physiology herd have excess androgen in their follicles (High A4 blue; Summers 2014). (A) Androstenedione (otherwise called A4) was found at different concentrations. (B) Estrogen did not display similar trends.

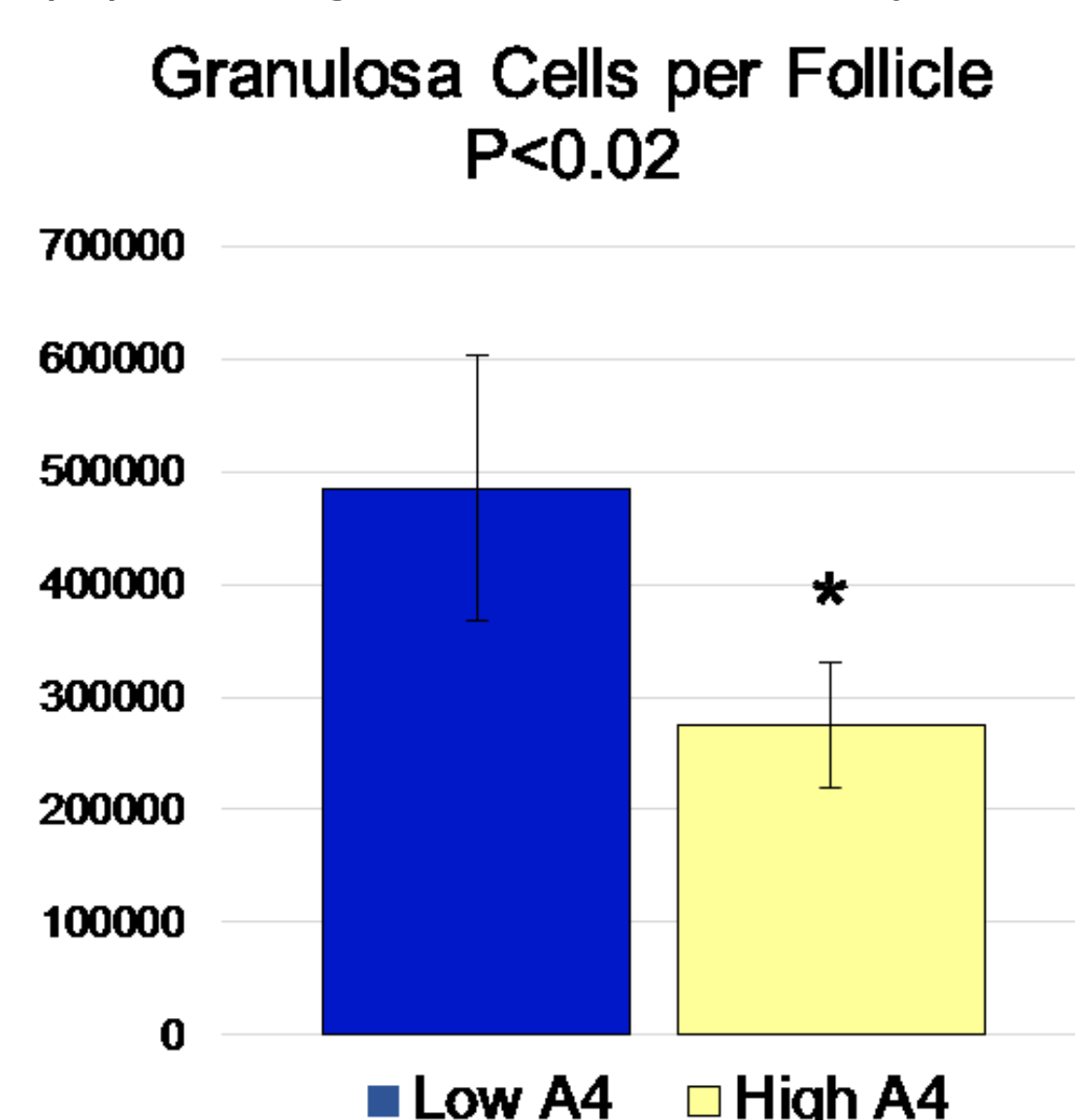


Figure 3. The granulosa cell population of those cows was half that of the control low androstenedione cows.

Hypothesis

Excess androstenedione causes granulosa cells to decrease their rate of proliferation.

Methods

Quantitative Real Time PCR (qPCR):

Quantify the transcription of regulatory genes involved in proliferation:

- AURKB
- CCNA2
- CDC20
- CYP19
- ECT2
- HDAC5

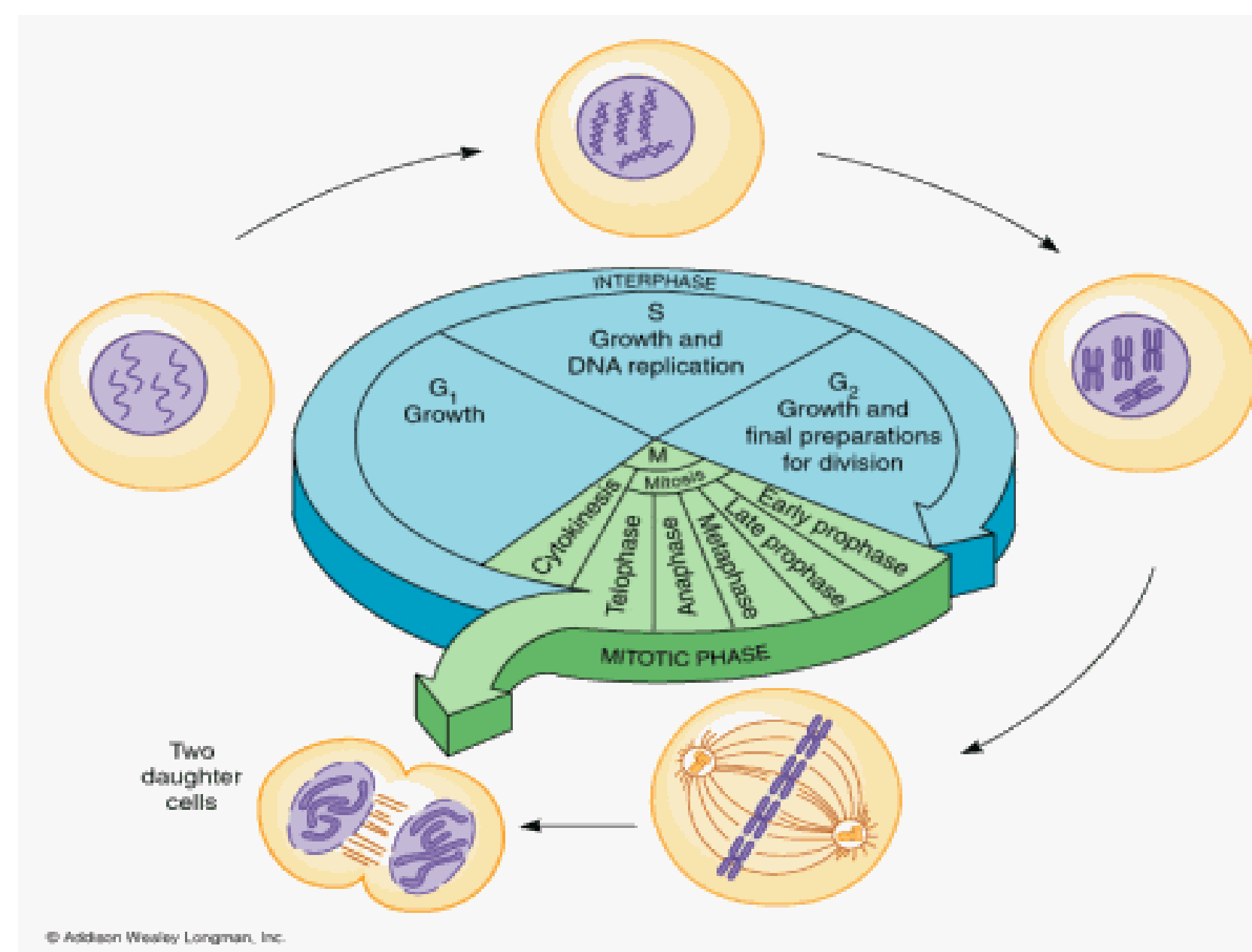


Figure 4. The cell cycle where the genes are selected to perform qPCR from the phase they play a role in.

Cell Culture:

- Commercially available cell line similar to the granulosa cell (KGN)
- The results were collected as the expression of genes involved in cell proliferation



Figure 5. Primary granulosa cells.

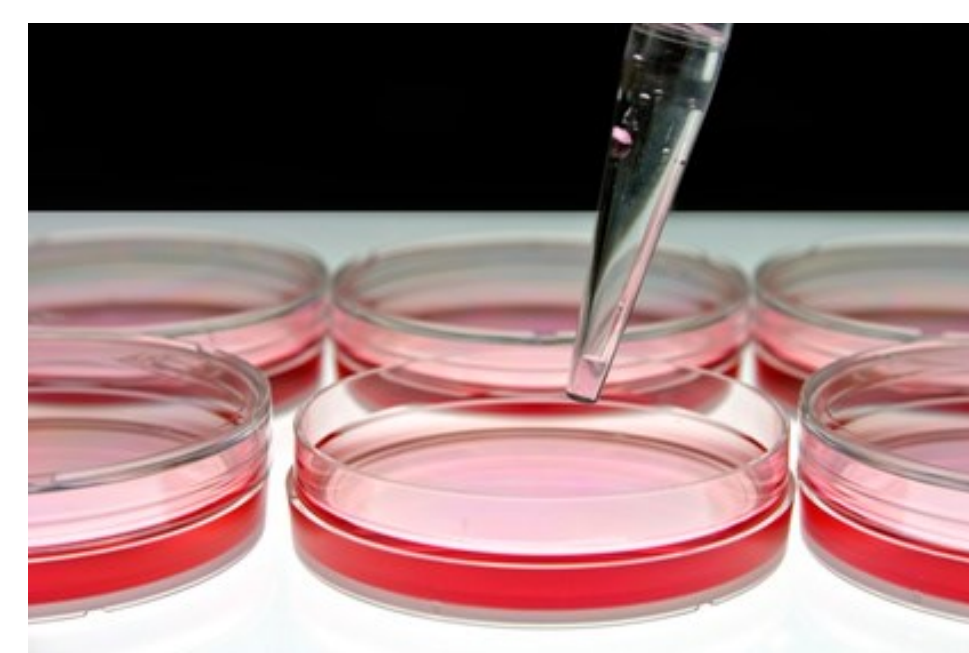


Figure 6. Both Primary granulosa cells and human immortalized cells were treated with high androstenedione.

Western blotting:

Quantify the protein production of genes involved in proliferation.

- Promoting cell cycle progression and the regulation of mitosis
 - cyclin D2
 - cyclin A2
 - CDC 25A
- DNA synthesis: PCNA

Other skills/lab techniques:

- Blood sample collecting
- Assisting with ovariectomy procedures
- Ultrasound follicle detection

Results

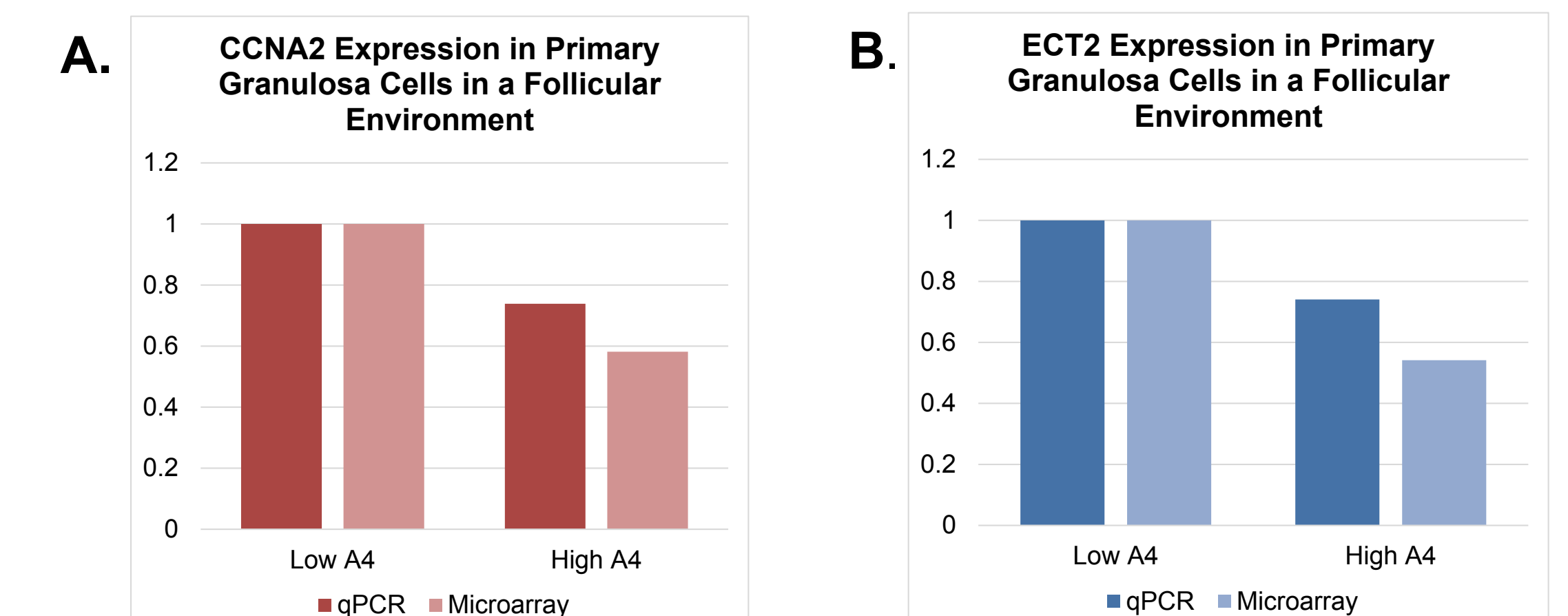


Figure 7. High A4 follicles have granulosa cells with less expression of proliferation promoting genes. (A) CCNA2 gene. (B) ECT2 gene.

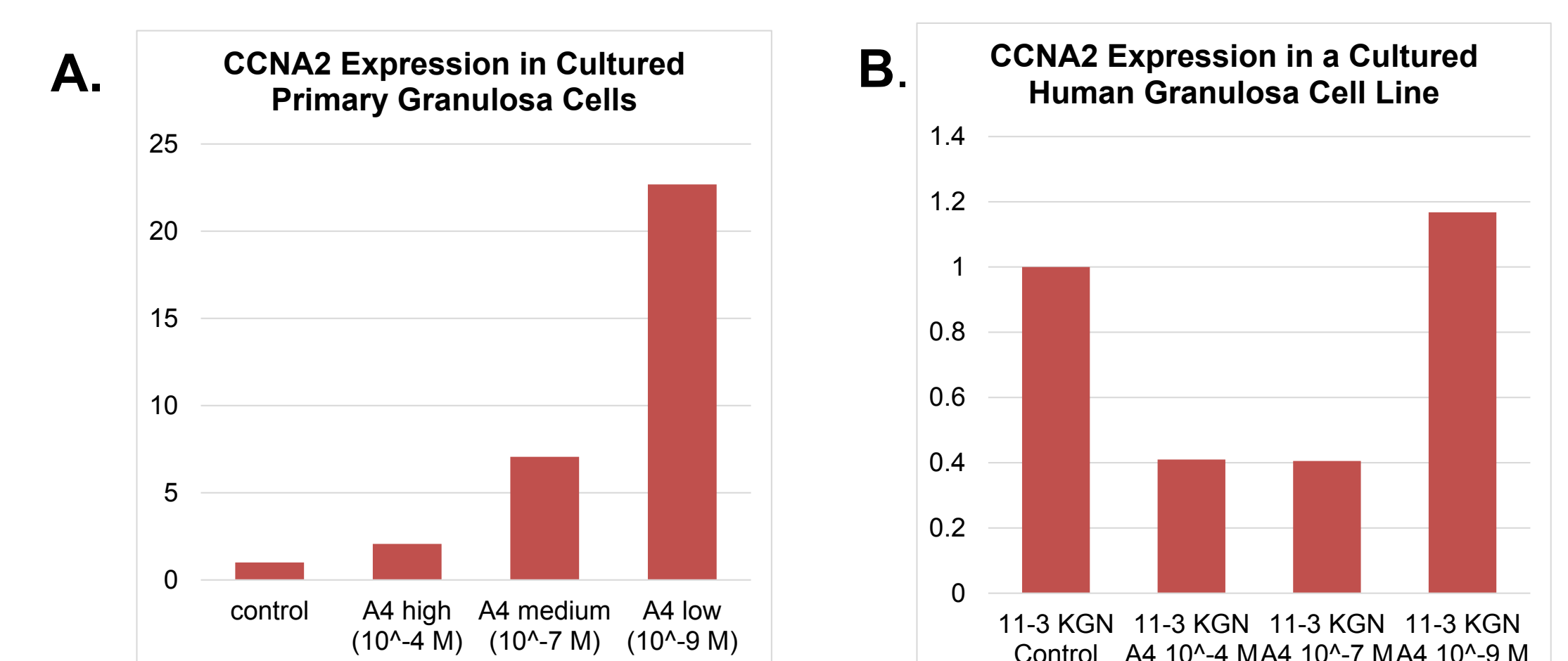


Figure 8. A) Primary Granulosa cells treated with lower dose A4 in culture show the most proliferation (similar to the physiological levels in the low A4 cows) and have less expression with increasing A4; B) and C) A human immortalized granulosa cell line responds with similar expression changes of CCNA2 and ECT2 which are cell proliferation genes.

Conclusion

These results support the hypothesis that granulosa cells respond to high androstenedione environments with decreased proliferation. Because granulosa cells are crucial for oocyte development and health this may explain our HighA4 ovulation disorders.

Impact

UCARE Experience Goals:

- To understand what it is to be a scientist/researcher.
- To help with as many projects to further my knowledge of the research methods.

References and Acknowledgements

Summers, A.F., et al. (2014). Altered Theca and Cumulus Oocyte Complex Gene Expression, Follicular Arrest and Reduced Fertility in Cows with Dominant Follicle Follicular Fluid Androgen Excess. PLoS One 9, e110683.

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