The role of endothelins in regulating bovine granulosa cell function

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INTRODUCTION

- Ovarian steroidogenesis and steroid-mediated signaling are critical for normal ovarian processes such as follicle growth and ovulation (1).
- Endothelin 1 (ET-1) and endothelin 2 (ET-2) are isoforms of vasoactive 21-amino acid peptides known to affect steroidogenesis within the ovary in several species (2,3,4,5).
- The role of endothelins in bovine ovarian steroidogenesis is inconclusive, but based on studies in other species we hypothesize that ET-1 and ET-2 inhibit bovine granulosa cell steroid production.
- This study is designed to investigate the effects of endothelins on bovine granulosa cells.

MATERIAL & METHODS

- **Cell Culture:** Bovine ovaries were obtained from a local slaughterhouse. Follicular fluid was aspirated from small (1-5mm) follicles, and granulosa cells were isolated and exposed to various treatments (ET-1, ET-2, or ET-1+ET-2 with or without FSH or IGF1) in multiple experiments. FSH and IGF1 were treated at 30 ng/mL.
- **RIA and Cell Counting:** In replicated experiments, culture medium was removed and analyzed for steroid production via radioimmunoassays. Granulosa cells were harvested with trypsin and counted using a Coulter Particle Counter.
- **qPCR:** Cellular RNA was extracted & quantified using real-time PCR (18 S rRNA as housekeeping gene).
- **Statistical Analysis:** Statistical analyses were performed using ANOVA and the general linear models (GLM) procedure of SAS for Windows (version 9.3, SAS Institute Inc., Cary, NY). If a significant main effect was identified in the ANOVA, then mean differences were determined by Fisher's protected least significant differences test (6). The values were reported as the least squares means ± SEM.

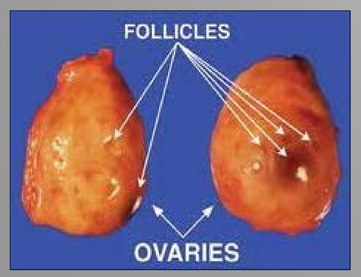

Figure 1: Bovine ovaries showing antral follicles.

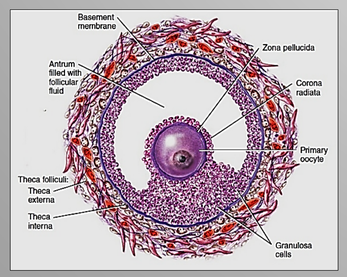

Figure 2: Cross-section of mature Graffian follicle showing oocyte, theca and granulosa cells.

RESULTS

Steroidogenesis

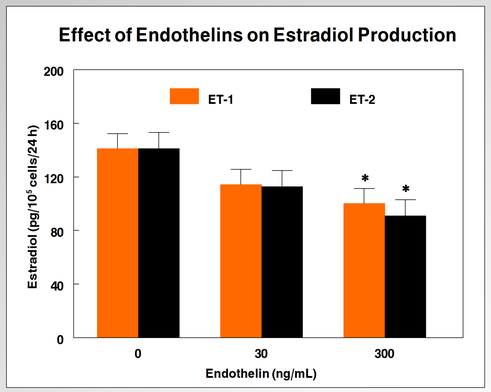

Figure 3: Dose response to endothelins in FSH plus IGF1-treated granulosa cells. * Means P<0.05 compared to control.

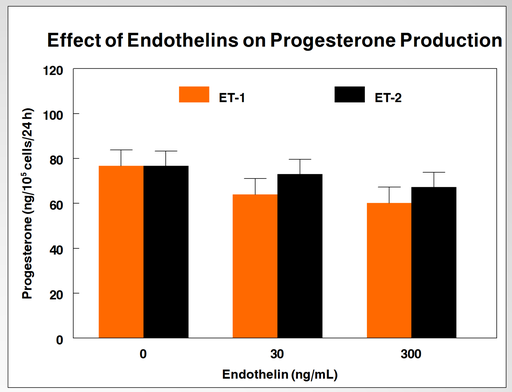

Figure 4: Dose response to endothelins in FSH plus IGF1-treated granulosa cells. Means did not differ P>0.05.

Gene Expression

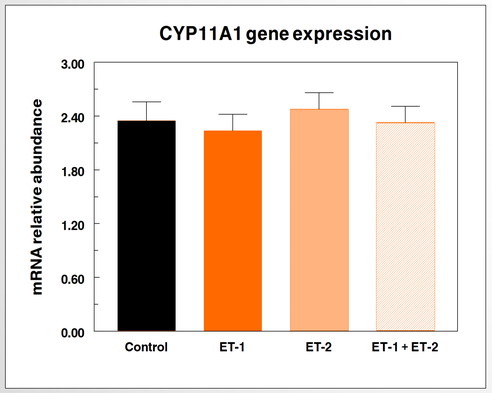

Figure 5: CYP11A1 response to various treatments in FSH plus IGF1-treated granulosa cells. Means did not differ P>0.05.

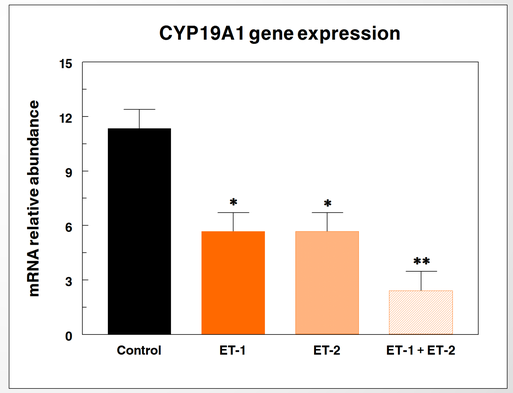

Figure 6: CYP19A1 response to various treatments in FSH plus IGF1-treated granulosa cells. *Means P<0.01 compared to control. **Mean P<0.01 compared to all other means.

SUMMARY & CONCLUSIONS

Steroidogenesis

- Both ET-1 and ET-2 inhibited estradiol production in a dose-dependent manner.
- Neither ET-1 nor ET-2 affected progesterone production.

Gene Expression

- CYP11A1 mRNA abundance was not affected by ET-1, ET-2 or ET-1+ET-2.
- CYP19A1 mRNA abundance was down-regulated by ET-1, ET-2 and ET-1+ET-2.

Conclusions

Endothelins suppress estradiol production by bovine granulosa cells, suggesting a role for endothelins in bovine ovarian function. Further investigation is necessary to fully understand the function of ET-1 and ET-2 in the bovine ovary and their role in regulating reproductive efficiency.

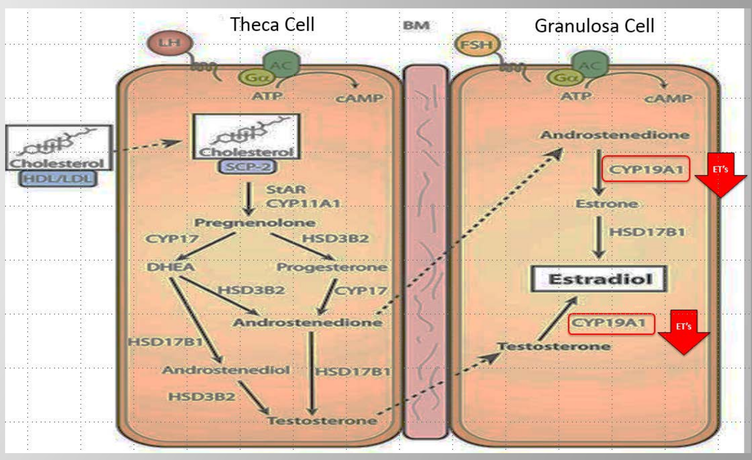

Figure 7: Enzymatic control of steroidogenesis in the growing follicle.

RESULTS

- Estradiol production was inhibited by 300 ng/mL of ET-1 (P<0.05) and ET-2 (P<0.001) (Fig. 3). While the 30 ng/mL treatments caused intermediate effects, they were not significantly different from controls (0 ng/mL) (P>0.05).
- ET-1 and ET-2 did not affect progesterone production at either dosage (P>0.05)(Fig. 4).
- Gene expression for side-chain cleavage enzyme (CYP11A1) was unaltered by ET-1, ET-2 or ET-1+ET-2 when compared to the control (Fig. 5). Gene expression for aromatase (CYP19A1) was inhibited by ET-1 (P≤0.01), ET-2 (P≤0.01) and ET-1+ET-2 (P<0.01)(Fig. 6).

REFERENCES

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