rhAMH inhibits CYP19 and P450scc mRNA expression in granulosa-lutein cells treated with gonadotropin

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Introduction

- Anti-Mullerian hormone (AMH) is a member of transforming growth factor β (TGF-β)
- Produced by human granulosa cells
- AMH inhibits initiation of primordial follicle growth
- AMH inhibits FSH-stimulated follicle growth
- Negative correlation between AMH retrieved in fluid from small antral follicles and *Cyp19A1* mRNA
- AMH reduces the expression of aromatase *CYP19A1* induced by FSH
- Gonadotropins treatment (using LH or FSH) induce strong expression of both aromatases *Cyp19A1* and *P450scc*

Material and Methods

hGLCs were purified from ovarian follicles of women undergoing in vitro fertilization protocol through a Percoll density gradient then maintained in culture for 6 days to allow the recovery of response to gonadotropins.

The primary hGLCs colture were then incubated for further 24 hours with increasing dosage of rhAMH (range 2-200 ng/ml) to asses the basal transcriptional response of both enzymes. Alternatively, hGLCs were treated for 24 hours with 5 ng/ml of rLH or FSH alone or in combination, and then AMH at a concentration of 10 ng/ml was added to colture.

Samples collected from each treatment were processed for RNA extraction followed by retrotranscription to cDNA then evaluated by RT-qPCR using specific pairs of primers. The expression level of both *Cyp19A1* and *P450scc* genes expressed as number of fold changes was normalized by housekeeping gene RPS7. Negative controls were included.

Results

As shown in Figures 1 - 2 rhAMH was unable to modulate the basal expression of both *P450scc* and *Cyp19A1* in any concentration tested. *P450scc* (Fig. 3) and *Cyp19A1* (Fig. 4) genes were strongly up regulated by rhLH (blue), rhFSH alone (yellow) and by the two goandotropins when combined (green bar). The effect of 20 ng/ml rhAMH (gray) added to the colture medium in presence of gonadotropins is also showed in Figs 3 and 4. AMH completely inhibited the postive effect of gonadotropins on *P450scc* and *Cyp19A1* expression.

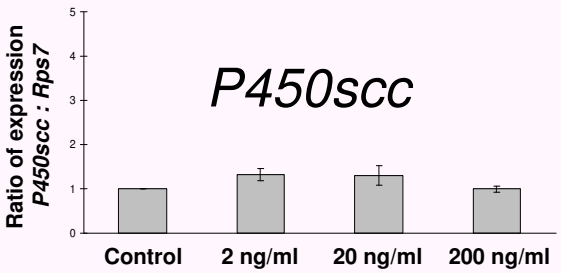

Fig. 1 Effect of increasing concentrations (range 2 – 200 ng/ml) of rhAMH on *P450scc* expression in hGLCs after 24 hours incubation

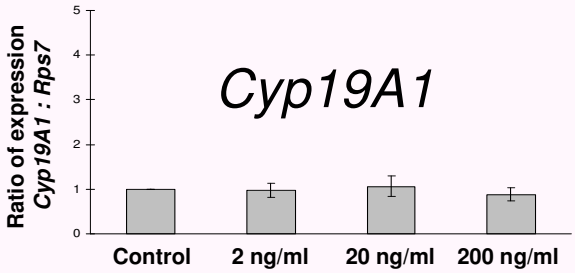

Fig. 2 Effect of increasing concentrations (range 2 – 200 ng/ml) of rhAMH on *Cyp19A1* expression in hGLCs after 24 hours incubation

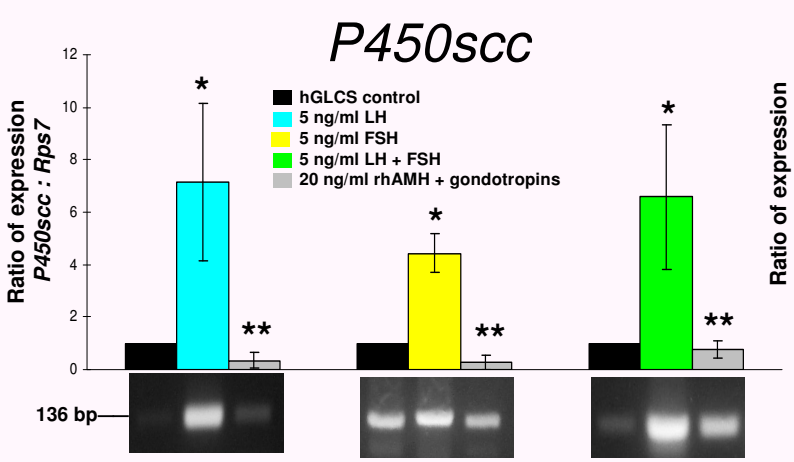

Fig. 3 Effect of gonadotropins and rhAMH alone or combined on *P450scc* expression in hGLCs after 24 hours incubation.

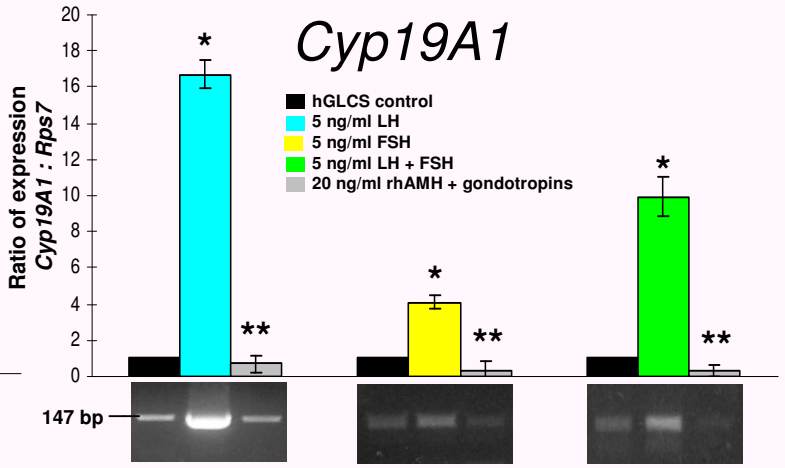

Fig. 4 Effect of gonadotropins and rhAMH alone or combined on *Cyp19A1* expression in hGLCs after 24 hours incubation.

Conclusion

rhAMH reduced the strong transcriptional up regulation of *P450scc* and *Cyp19A1* genes generated by gonadotropins treatment (alone and combined) impairing the enzymes response although rhAMH alone did not affect thier basal expression in any of the concentrations tested.