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 culture were then incubated for further 24 hours with increasing dosage of rhAMH (range 2-200 ng/ml) to assess 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 culture.

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 gonadotropins when combined (green bar). The effect of 20 ng/ml rhAMH (gray) added to the culture medium in presence of gonadotropins is also showed in Figs 3 and 4. AMH completely inhibited the positive effect of gonadotropins on P450scC and Cyp19A1 expression.

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 their basal expression in any of the concentrations tested.