The expressions of ovarian steroidogenic enzymes do not increase proportionally after FSH, creating a shunting that promotes progesterone output in the granulosa cells without luteinization

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Study Question: If premature rise in serum P before ovulation may occur in stimulated IVF cycles can gonadotropin stimulation possibly play a role in this scenario?

Summary answer: Yes. FSH alters the expression patterns of the enzymes involved in ovarian sterodiogenesis that facilitates premature rise in serum P in a dose-dependent manner.

What is known already: Serum progesterone may prematurely rise before ovulation trigger during multi-follicular development and reduce pregnancy rate in stimulated IVF cycles. The underlying molecular pathogenic mechanism is unclear. We aimed in this study to explore is gonadotropin stimulation alters the expression of the ovarian steroidogenic enzymes (stAR, SCC, 3B-HSD, 17a-OH, 17B-HSD and aromatase) in a way that creates a relative shunting in steroidogenetic pathways leading to premature rise in serum P before ovulation.

Design: A translational research study combining in vivo and in vitro models of human ovarian cortical samples and granulosa cells.

MATERIALS & METHODS

Ovarian cortical samples (n=10) and non-luteinizing mitotic granulosa cells (HGrC1) were stimulated with rec-FSH at 12,5 – 25 – 50 mIU/mL concentrations for 24 and 48 hours. The expressions of steroidogenic enzymes were compared at mRNA level by real-time quantitative PCR and protein level by western blot and ELISA.

Key words: Premature progesterone elevation; gonadotropin stimulation; steroidogenic enzymes, ovary enzymes, granulosa cells

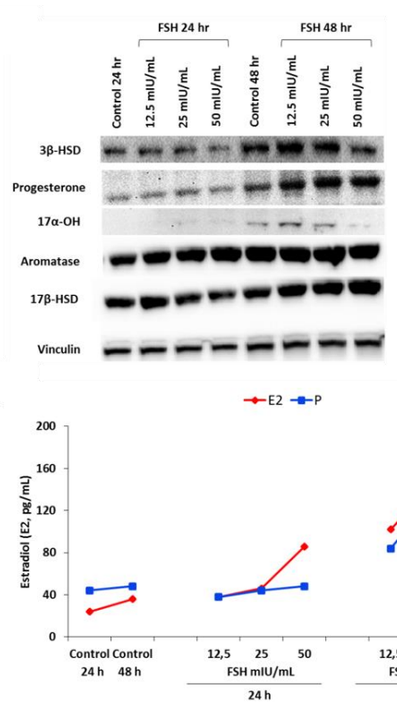
RESULTS

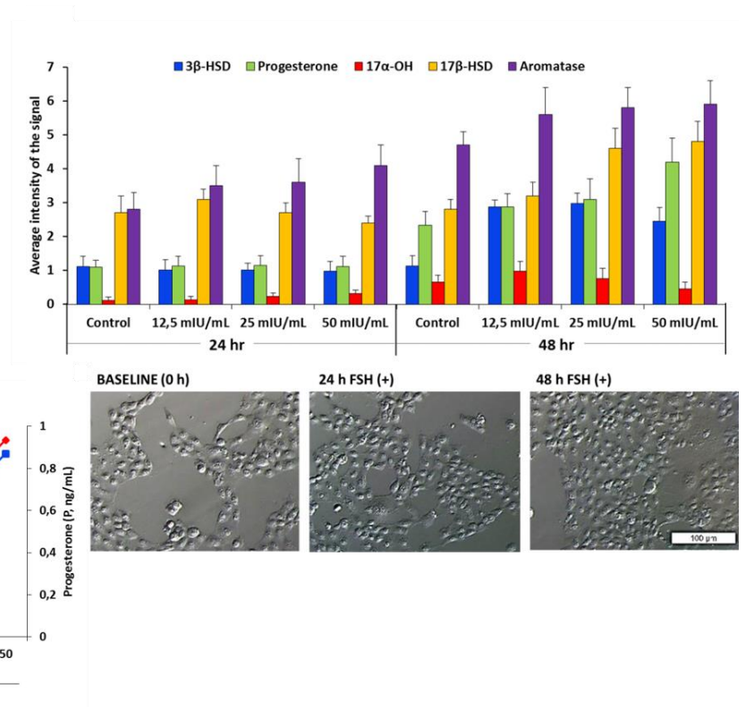
Stimulation of the granulosa cells with FSH resulted in a dose-dependent increase in the mRNA and protein level of 3β-HSD. Overall, when all time points and FSH doses were analyzed collectively, FSH significantly up-regulated the mRNA expression of its own receptor (3.73 fold, p<0.001), stAR (1.7 fold, p<0.01), SCC (1.75 fold, p<0.01), aromatase (4.49 fold, p<0.001), 3β-HSD (1.68 fold, p<0.01) 17β-HSD (2.16 fold, p<0.01) in the granulosa cells with a notable exception of 17α-OH (1.03 fold p>0.05) on quantitative real-time RT-PCR analysis.

RESULTS

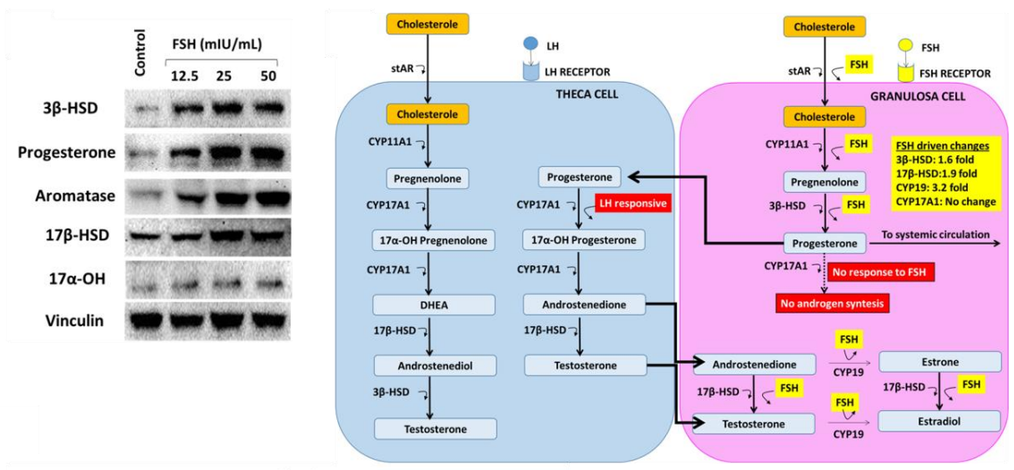
Similar changes were observed in the protein expression analysis of these enzymes on western blotting after FSH stimulation. Protein expression of P was increased along with 3β-HSD after FSH stimulation. In line with these findings P output (1.05±0.3 vs. 0.2±0.1 ng/mL, respectively, p<0.001) from the samples stimulated with FSH were significantly increased along with E_2 (1918±203 vs. 932±102 pg/mL, respectively, p<0.001) compared to unstimulated controls (Figure).

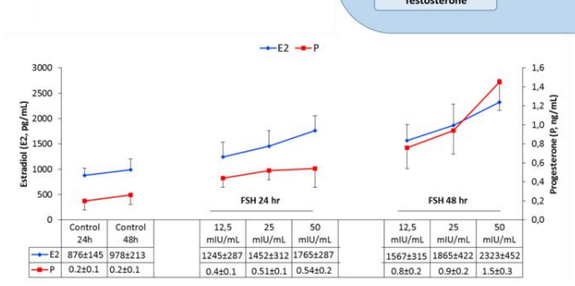
Granulosa cells





Ovarian tissue samples





CONCLUSION

When direct stimulatory effect of FSH on 3β-HSD and P biosynthesis is combined with unresponsiveness of 17α-OH to FSH, high input precursor steroids generated during multi-follicular development under the influence of tonically elevated FSH level in stimulated IVF cycles may exceed the ability of the ovary to effectively convert them into estrogen pathway, creating a relative shunting at 17 hydroxylation step that diverts these precursors into progesterone pathway for conversion to androgenic substrates for final estrogen synthesis.