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Epidermal growth factor (EGF) promotes ovarian steroidogenesis and epidermal growth factor receptor (EGFR) signaling is required for gonadotropin-induced steroid production in common carp *Cyprinus carpio*

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BACKGROUD

- The vertebrate ovary is an extremely dynamic organ, development and function of which are primarily regulated by pituitary gonadotropins-FSH and LH

- However, various locally produced growth factors, particularly, IGFs, insulin and EGF are reported to play important role either independently or synergistically with gonadotropins in modulating gonadal steroidogenesis

- Understanding the independent role of EGF in modulating gonadal steroidogenesis and the signaling pathways induced by gonadotropins is essential to know how EGF takes part in these processes

- Studies on role of EGF in mammalian ovary is extensive

- Mature EGF (55 amino acids) is released from large precursor anchored to the plasma membrane and EGF act by binding to a specific EGFR on the cell surface with high affinity
- EGFR is a glycosylated transmembrane protein (170 kDa), which represents the prototypes of RTKs and binding of EGFR by EGF activates its intrinsic receptor tyrosine kinase and rapidly elicits the downstream signaling
- EGF production has been demonstrated in the ovarian follicles of mammal and birds
- It modulates ovarian steroidogenesis and granulosa cell differentiation by increasing progesterone production and inhibit FSH-induced 17β-estradiol production
- EGF triggers steroidogenesis in gonadal cells, including Leydig cells and granulosa cells
- Whereas, EGF promotes steroidogenesis in these models, an essential role of EGF and EGFR in regulating the gonadotropin-induced steroidogenesis in mammals have recently been described

- In non-mammalian vertebrates studies on EGF and its roles in the ovary are rather limited

- This was because EGF has not been cloned until 2004 in any non-mammalian species. It was Wang and Ge (2004) first cloned EGF and EGFR from zebrafish ovary

- Reports are available that in fish ovary, EGF plays role in controlling follicle survival and steroidogenesis as well as DNA synthesis in the vitellogenic follicles

- Taking fish as a model we, tried to explore the regulatory role of EGF on ovarian follicular steroidogenesis and how EGFR signaling is required for gonadotropin-induced steroid production

- For our study, we considered isolated theca-granulosa cells of late vitellogenic ovarian follicles

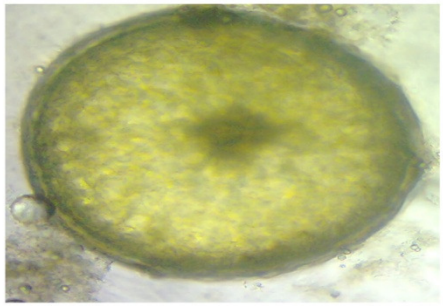
- Theca–granulosa cells were isolated by enzymatic (collagenase) digestion

- After isolation, theca and granulosa cells were mixed and pre-incubated for 6 h in 24 well culture plate in DMEM supplemented with 2% BCS-DMEM, streptomycin (100 µg/ml) and penicillin (100 IU/ml)

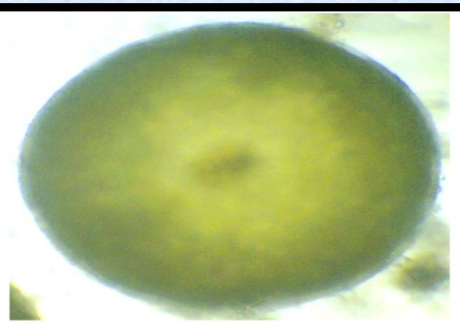
- Initial density of theca-granulosa cell in the incubation was 0.9 x 10^5 and 2.1 x 10^5 cell per well respectively

- After 6 h, BCS-DMEM was replaced by serum-free DMEM in each well containing effectors and inhibitors and incubated for different time in a metabolic shaker bath at 23 ±1^0C

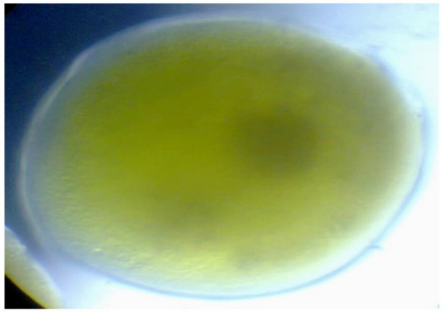
Cyprinus carpio



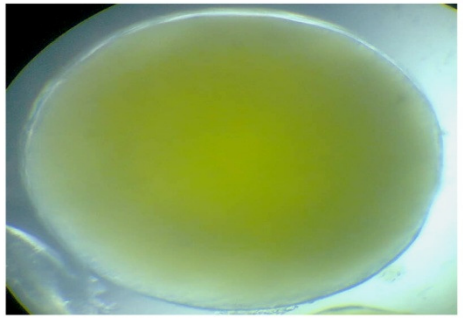
Pre-vitellogenic



Vitellogenic



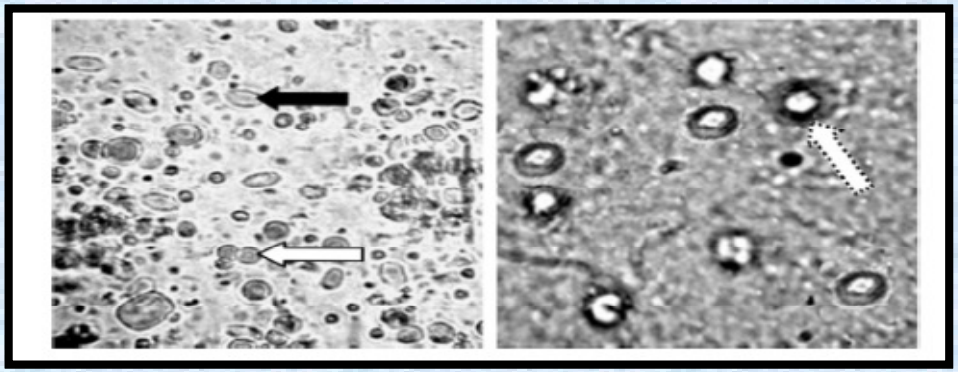
Post-vitellogenic

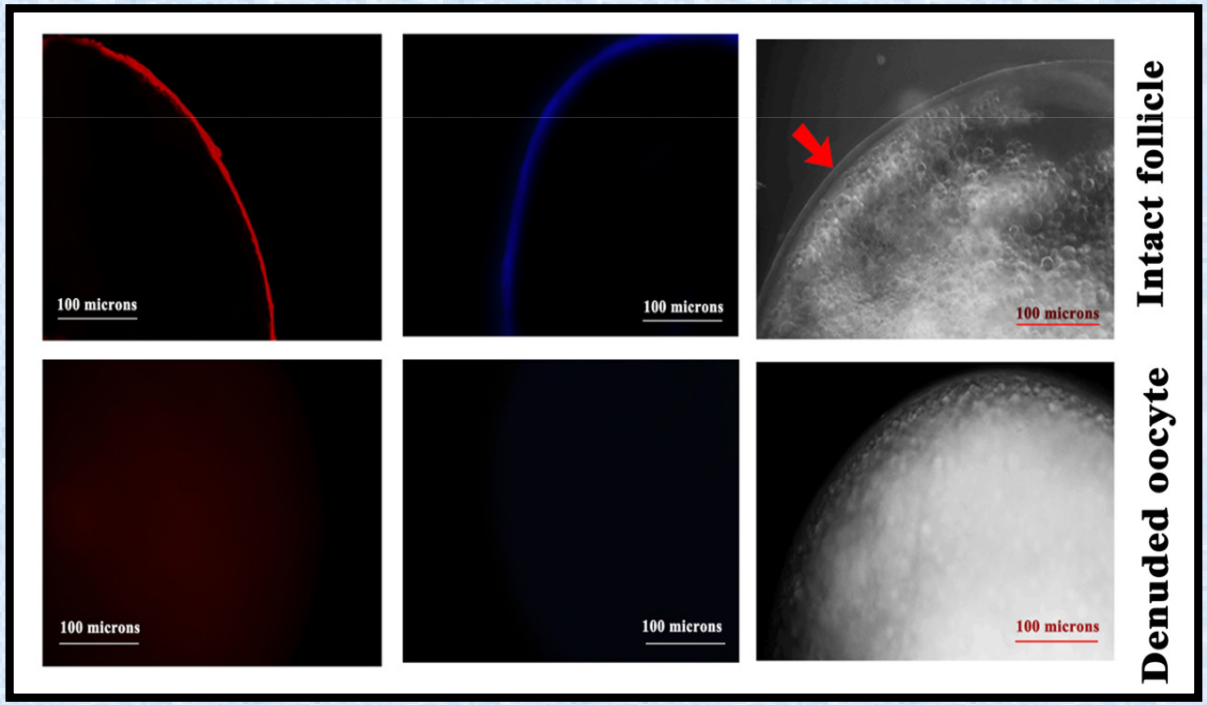


Spawning

Theca- granulosa cells

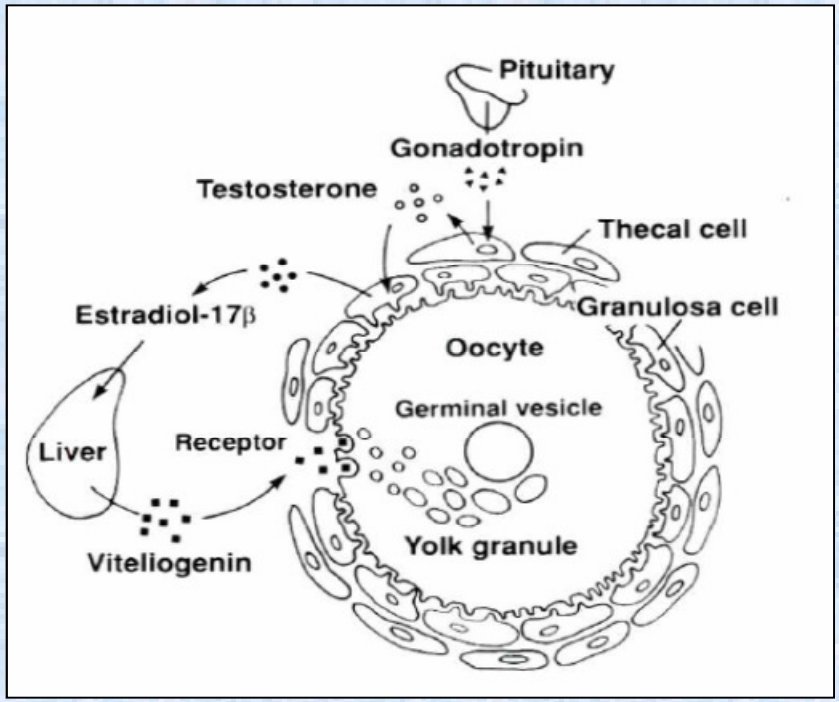
Granulosa cells stained with
P-nitroblue- tetrazolium



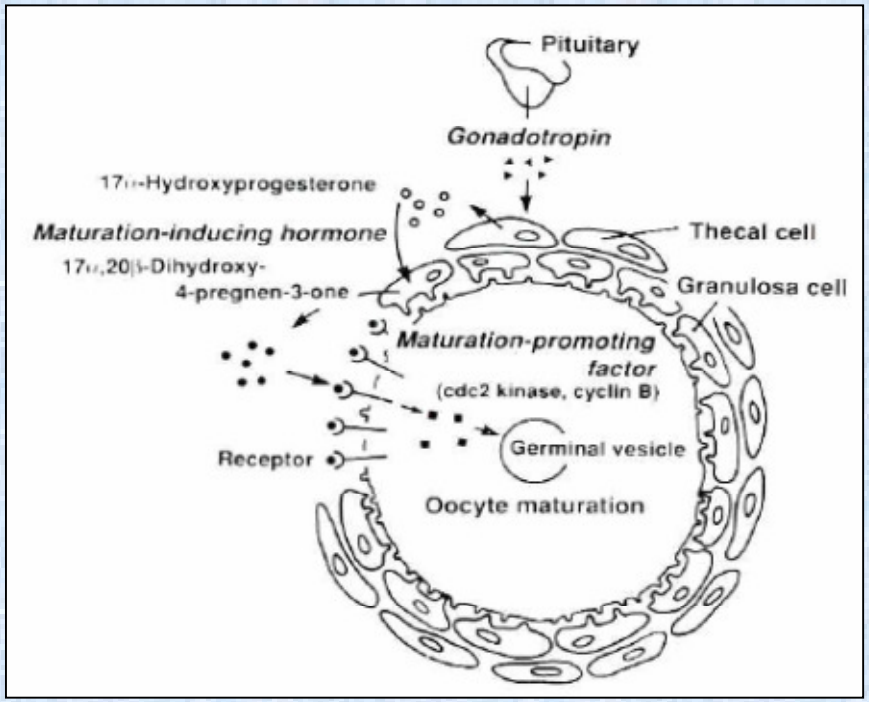


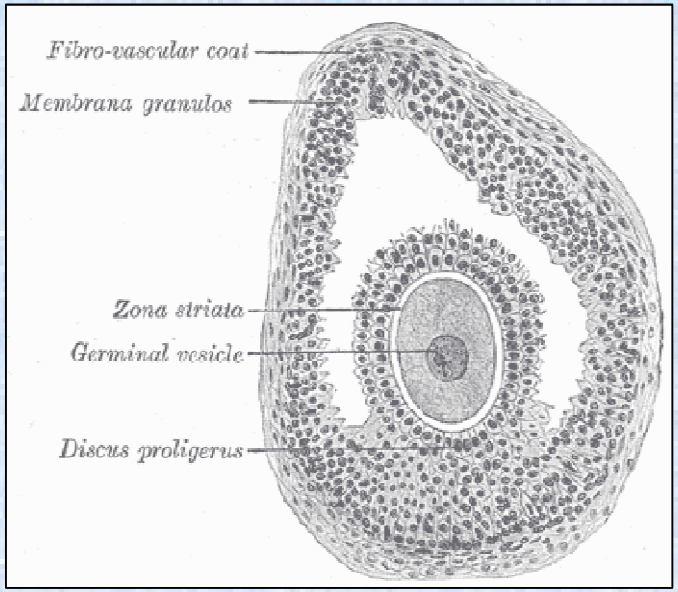
PI DAPI

Late- Vitellogenic



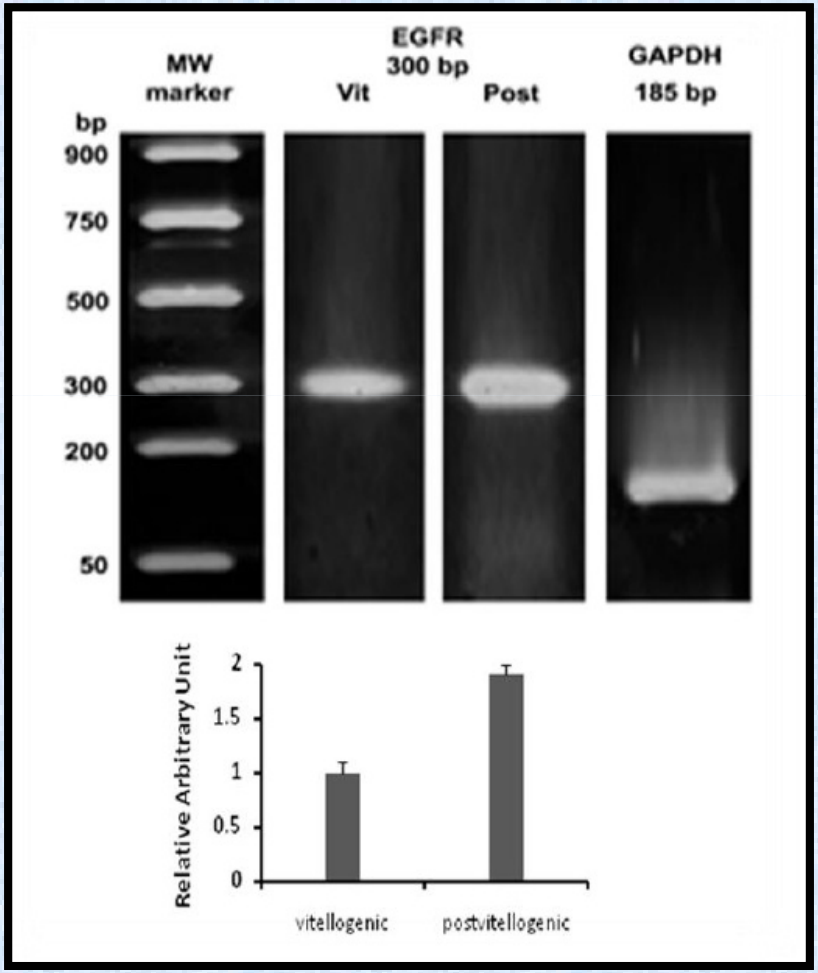
Post- Vitellogenic



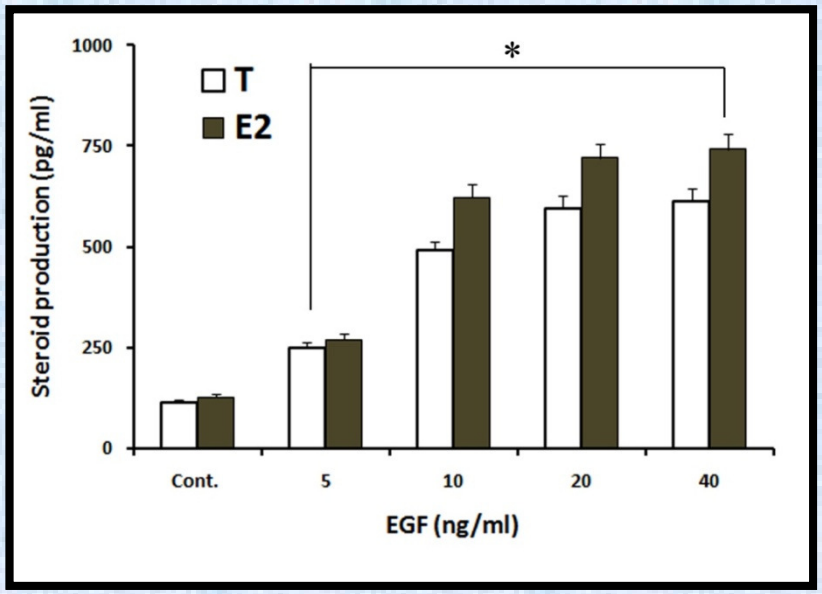


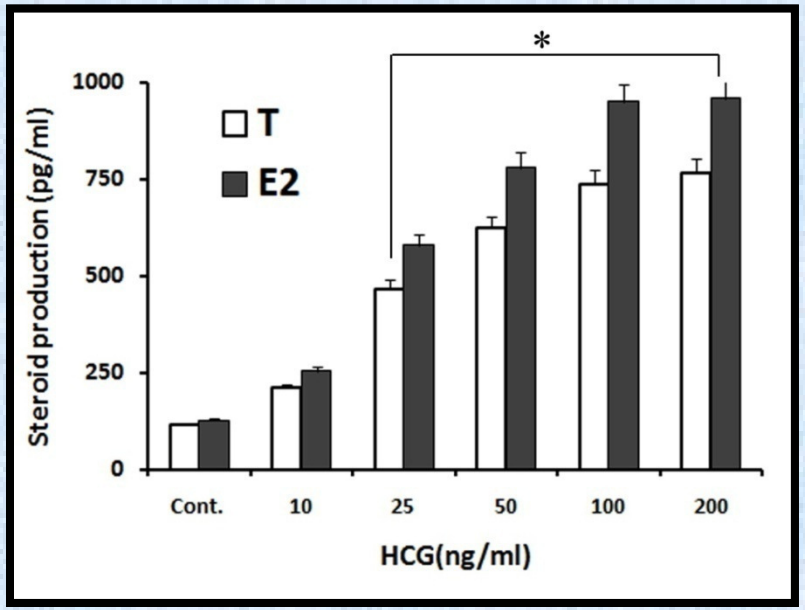
Gravid follicle of human

RT-PCR expression of EGFR in carp follicle

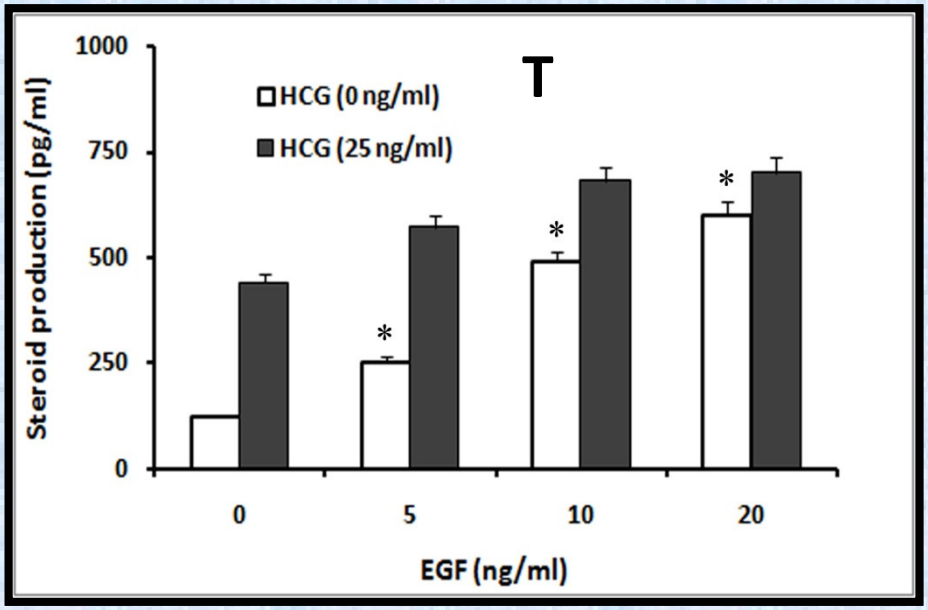


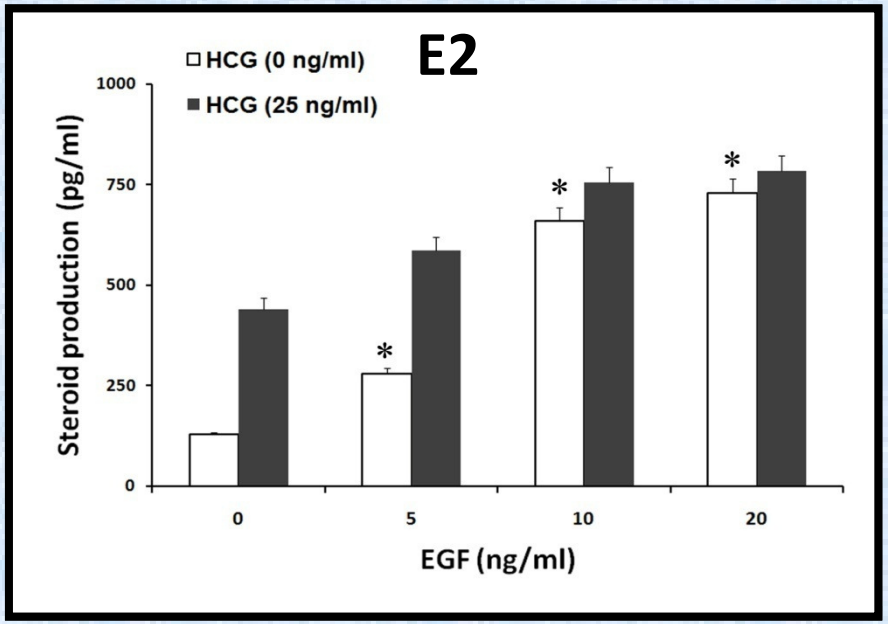
Effect of EGF and HCG on Testosterone (T) and 17β-estradiol (E2) production in carp ovarian follicles



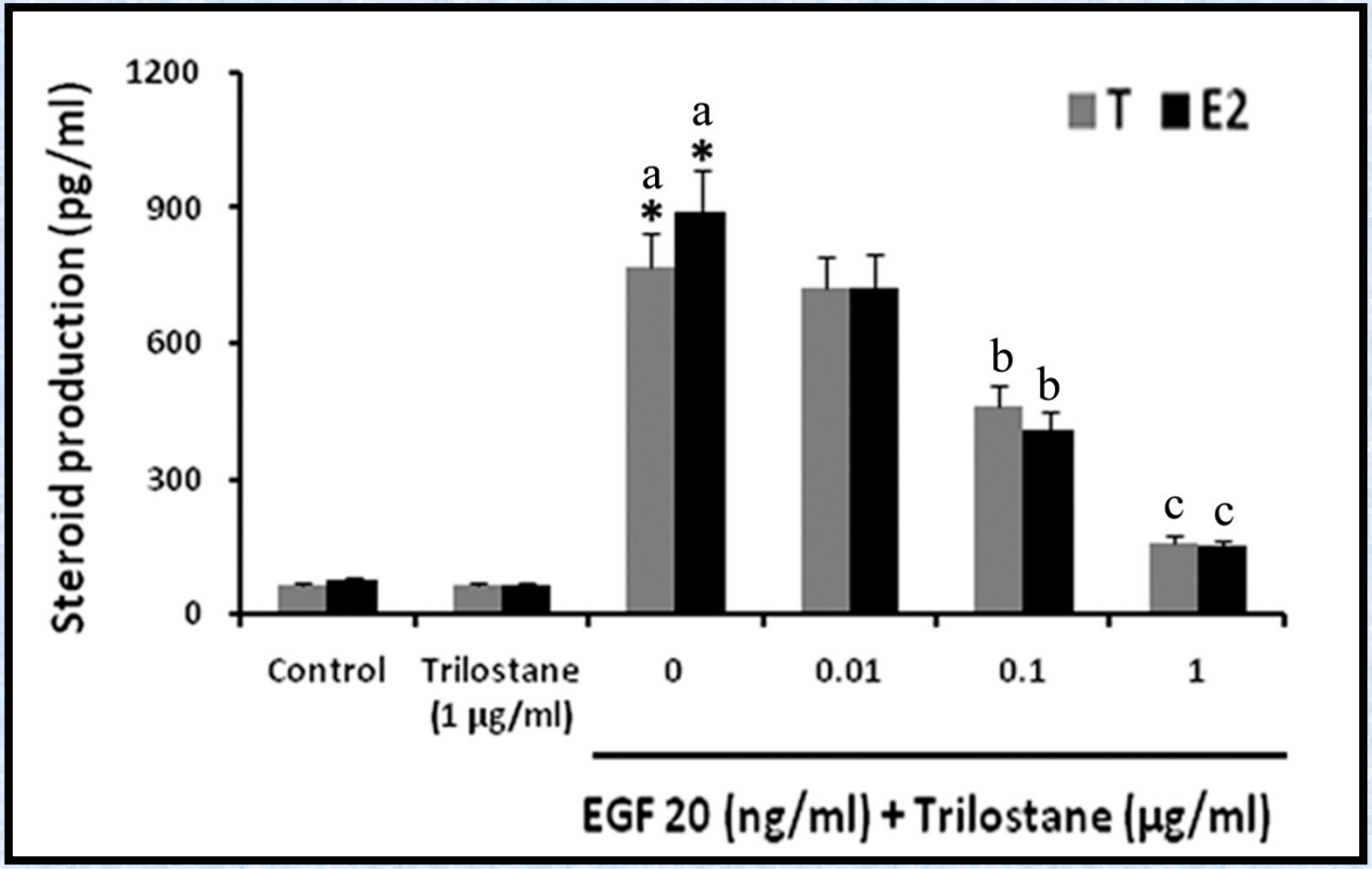


Dose response effect of EGF on basal (hollow bar) and HCG-stimulated (solid bar) in vitro release of steroids

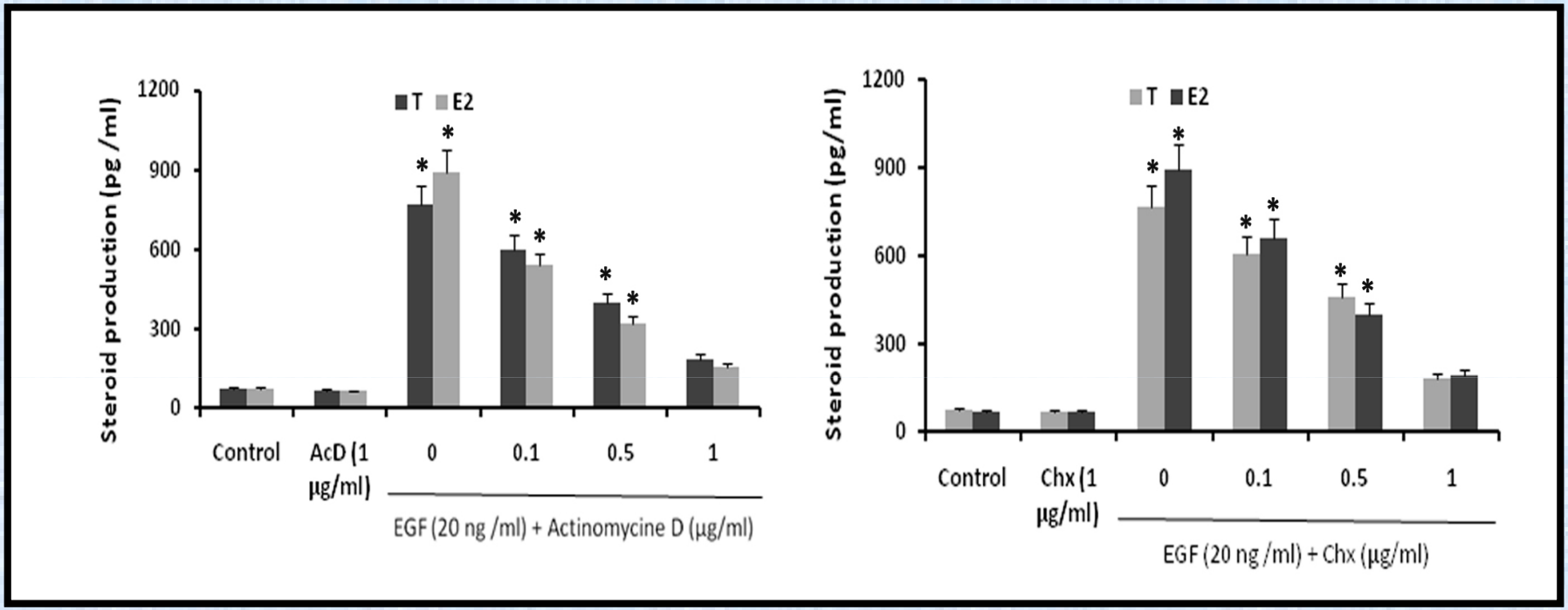




Effect of 3β-HSD inhibitor (Trilostane) on EGF- induced steroid production



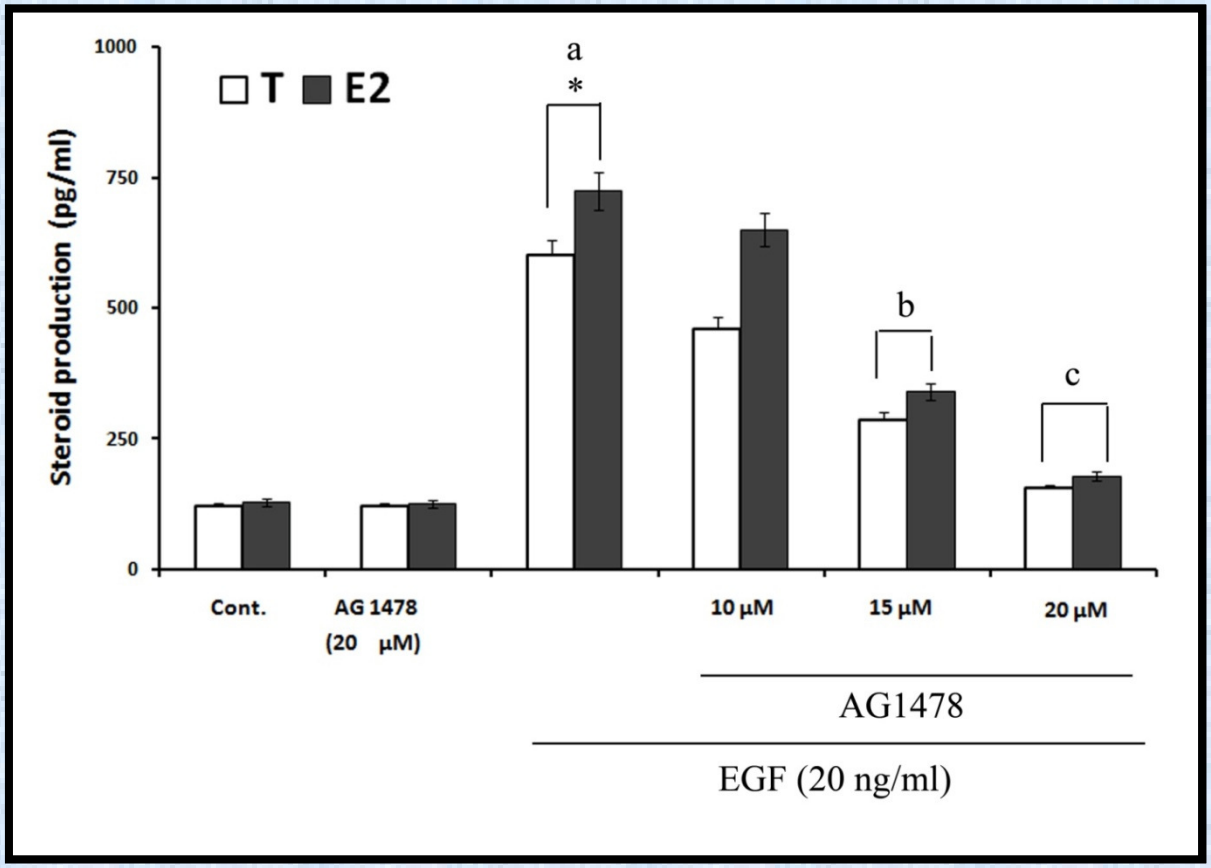
Effect of protein synthesis inhibitor on EGF-induced steroid production



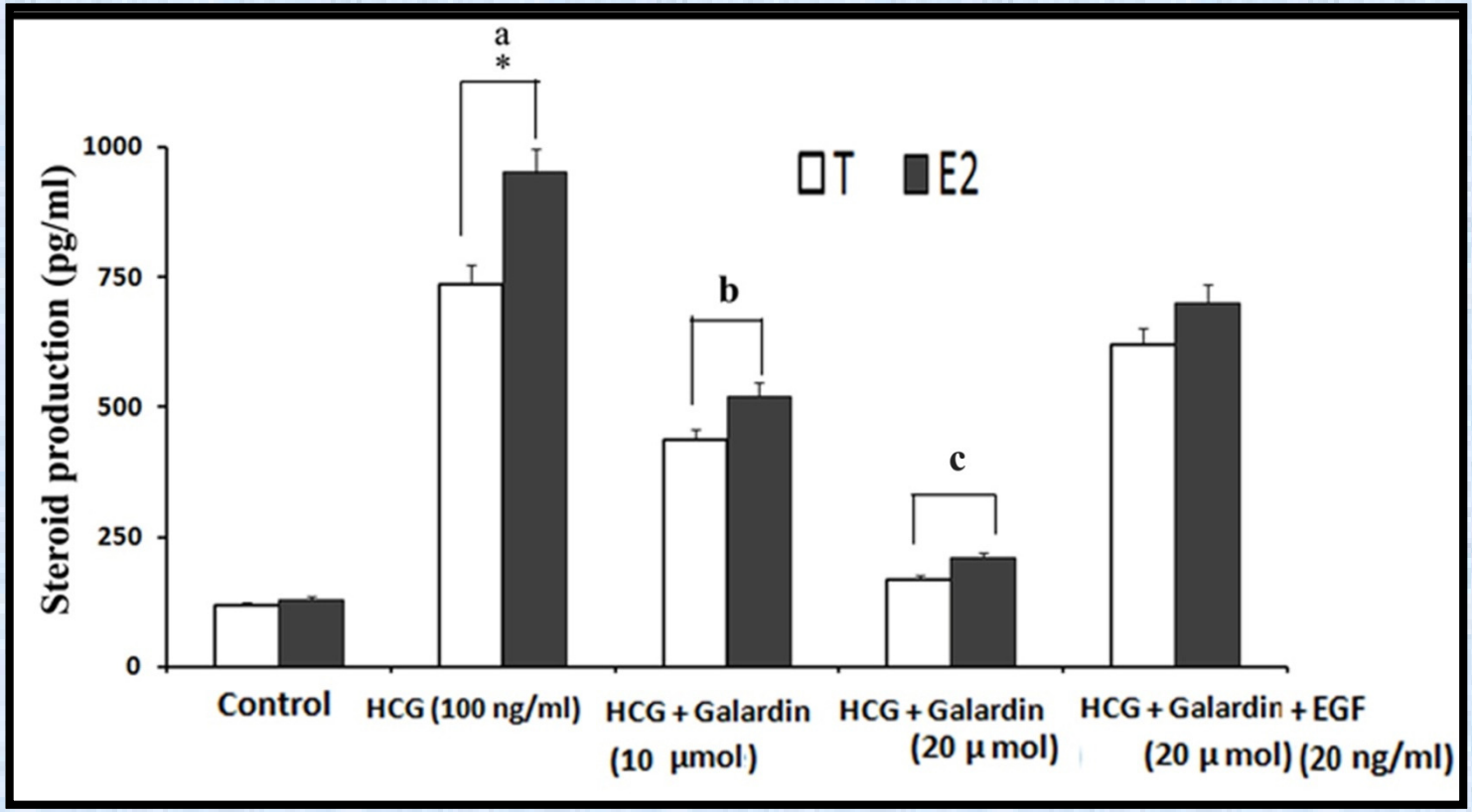
Inference

- EGFR mRNA was expressed in vitelligenic and post-vitellogenic carp ovarian follicle cells
- EGF independently can stimulate T and E2 production by the follicle cells and the extent of production was little less than that of HCG
- EGF could stimulate both basal and HCG stimulated T and E2 release.
- 3b-HSD inhibitor could effectively attenuate steroidogenesis
- Both transcription and translation inhibitor could block the steroidogenesis

Effects of EGFRI inhibitor-AG1478 on EGF-induced steroid production



Effect of MMP inhibitor, Galardin on HCG-stimulated steroid production and rescue by EGF



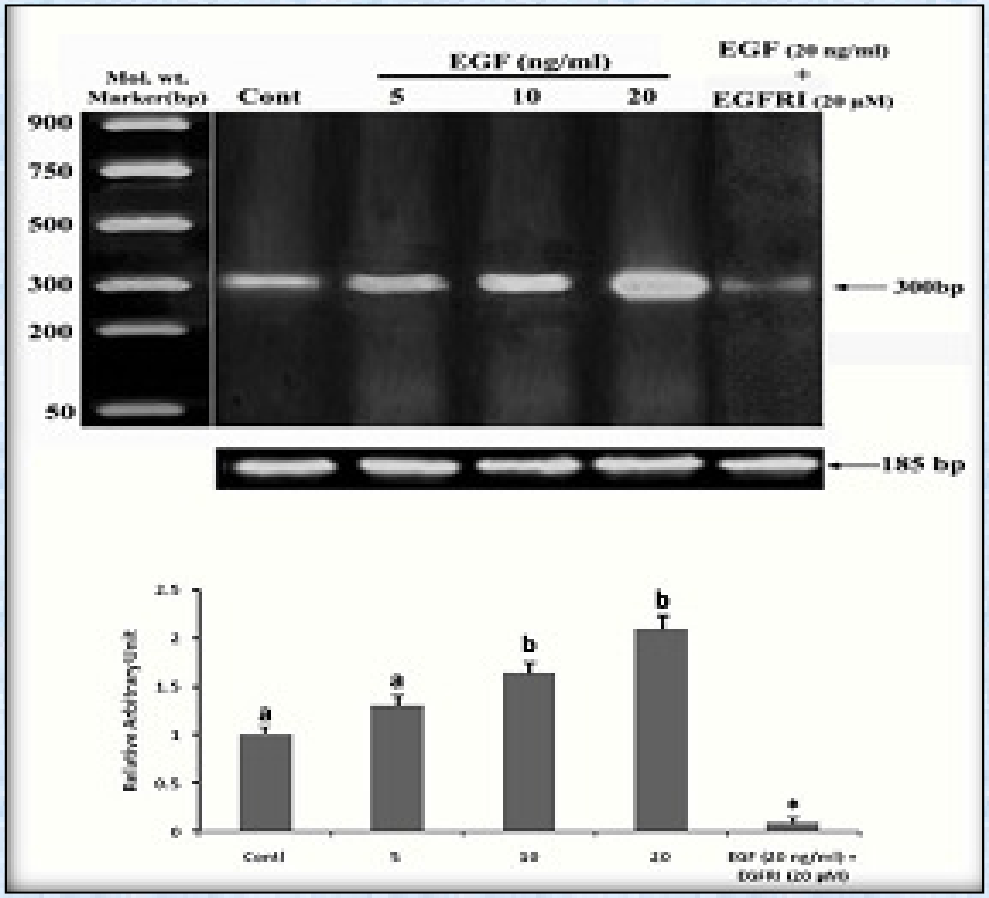
Inference

- EGFR signaling is necessary for EGF-stimulated steroidogenesis
- EGFR signaling is also necessary for HCG-induced follicular steroidogenesis
- HCG-mediated steroidogenesis in ovarian follicles requires cleavage of membrane-bound EGF family members and activation of the EGFR

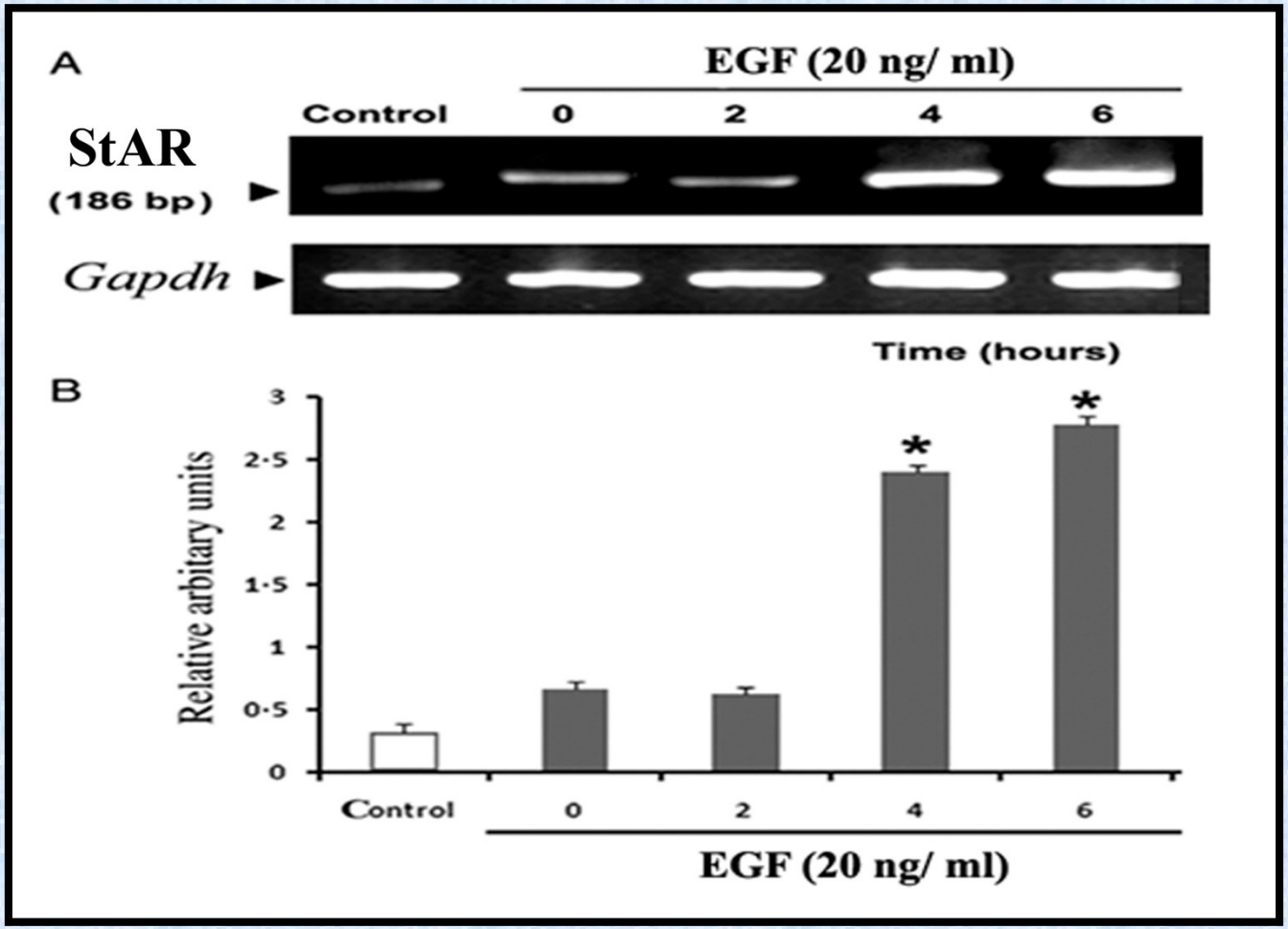
We proposed that,

- HCG activates its receptor located on the follicle cells resulting in to activation of MMPs and cleavage of the membrane-bound EGFs
- Soluble EGF molecule then bind to EGFR of the follicle cells to enhance steroid production

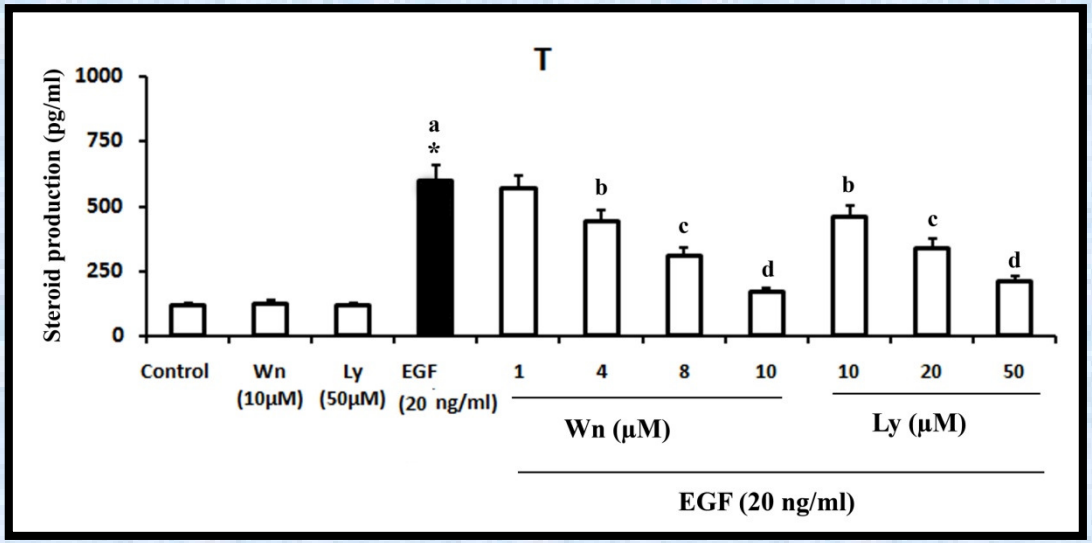
RT-PCR expression of EGFR mRNA and effects of EGF and EGFRI

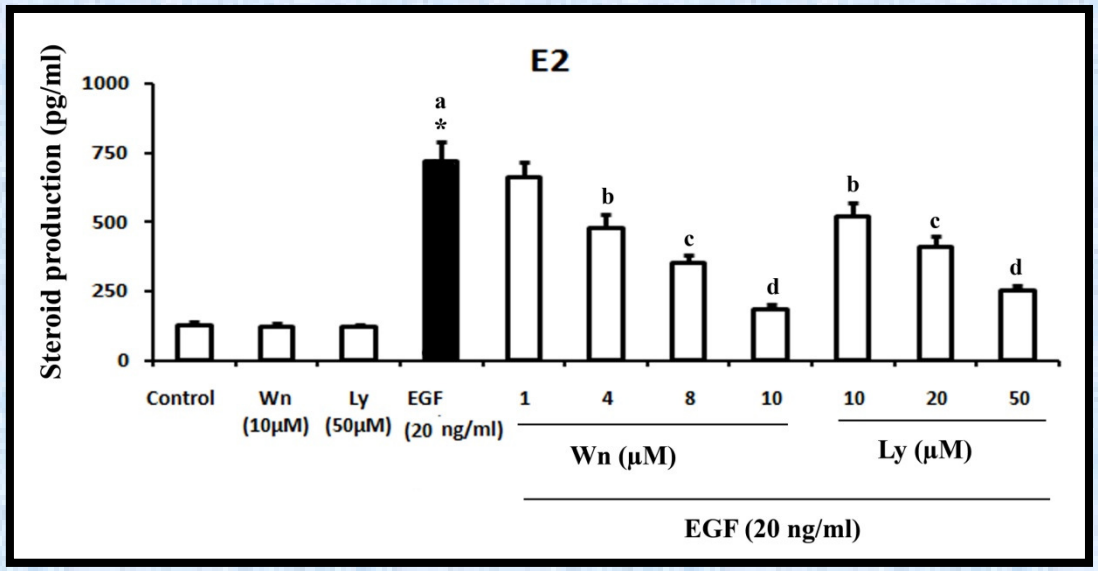


EGF-induced StAR mRNA expression

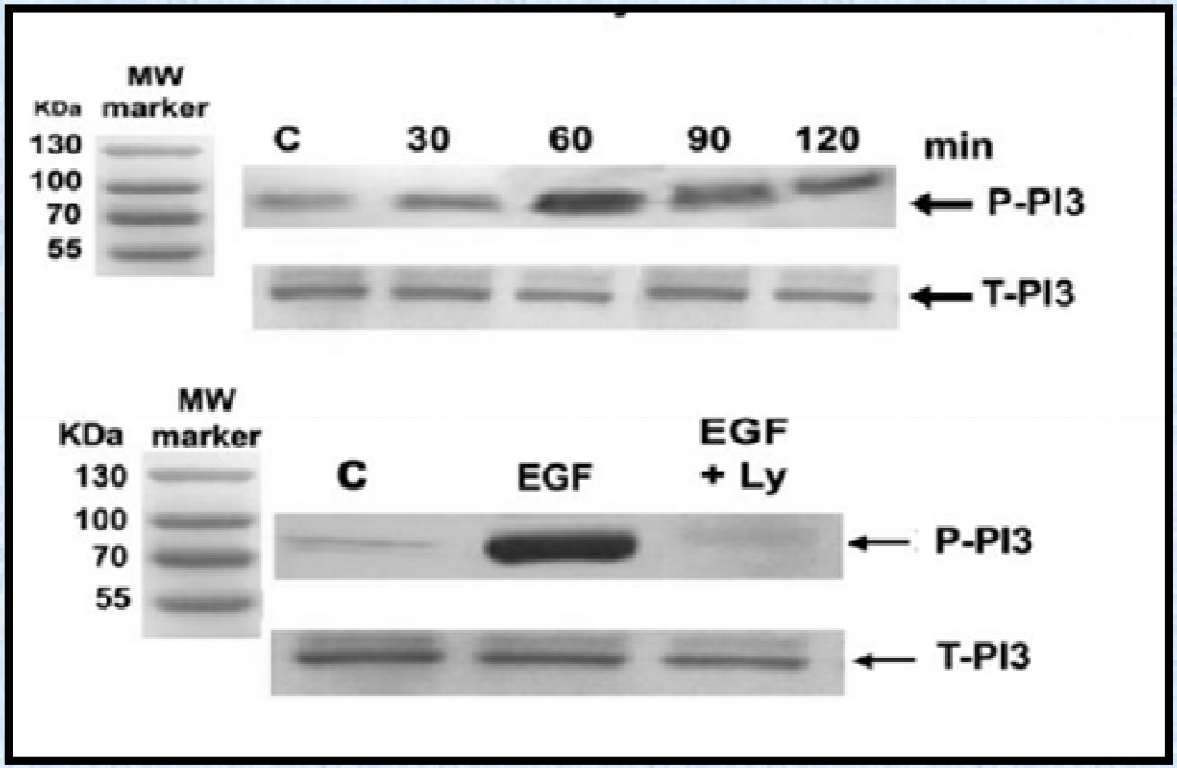


Effect of Wortmannin (Wn) and Ly294002 (Ly) on EGF-induced steroid production

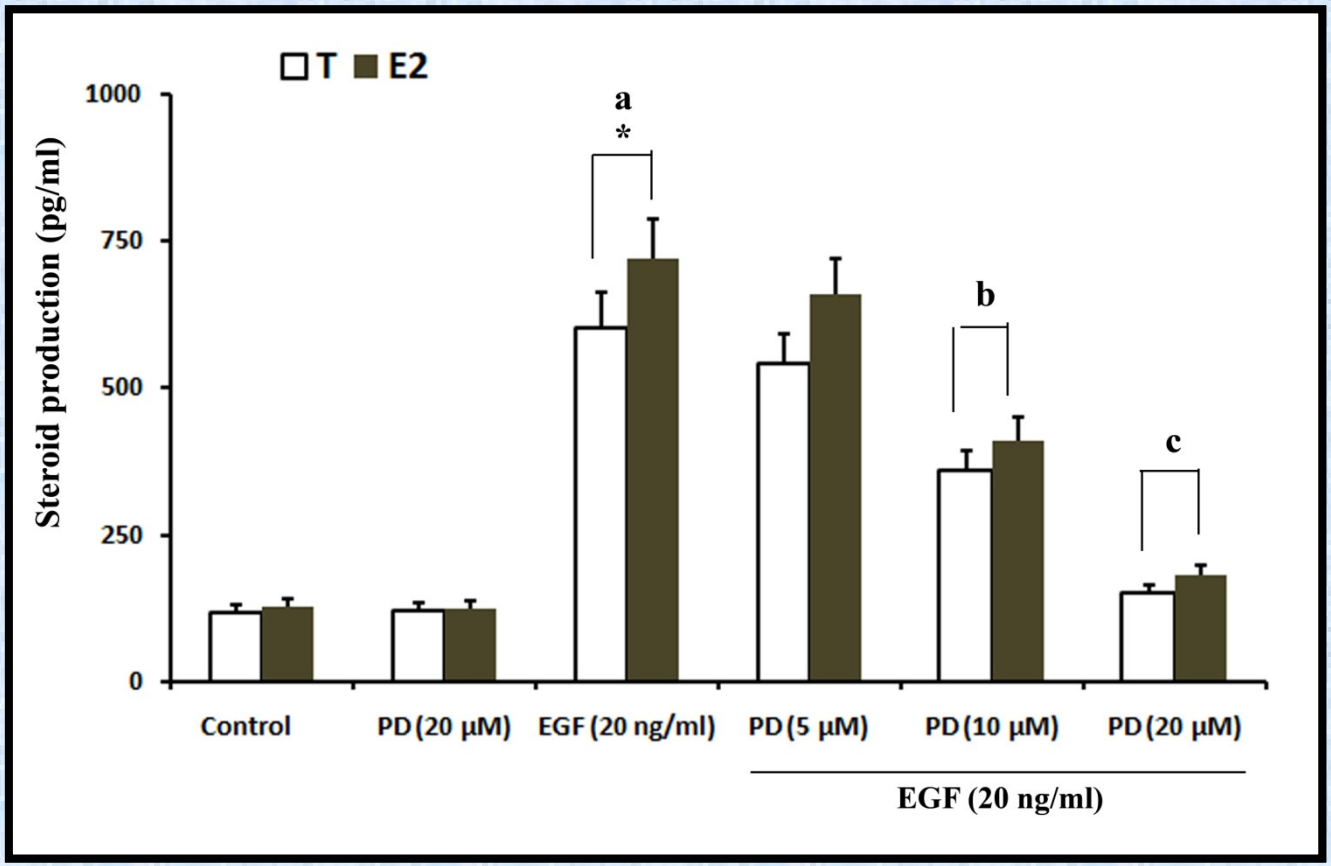




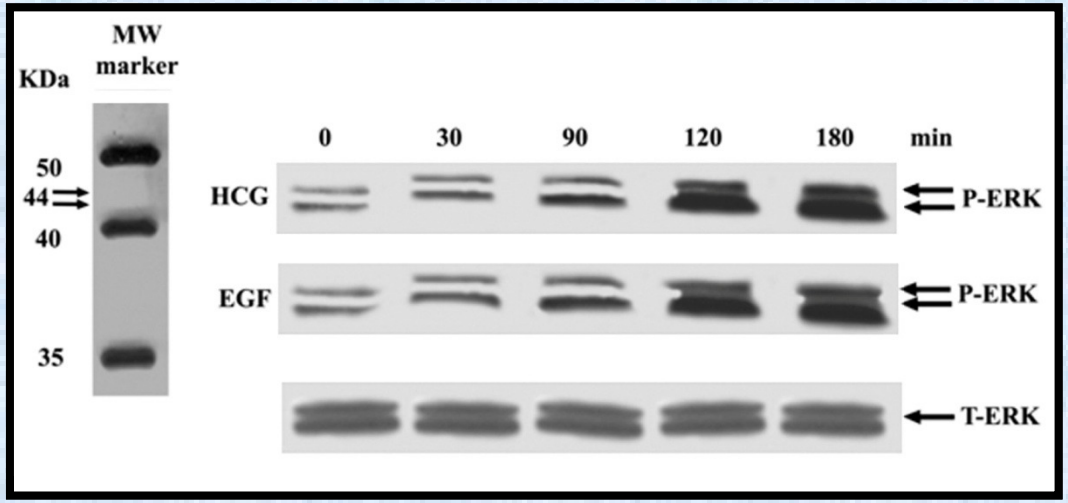
EGF-stimulated P^{85} associated PI3 kinase activation and effect of PI3 kinase inhibitor

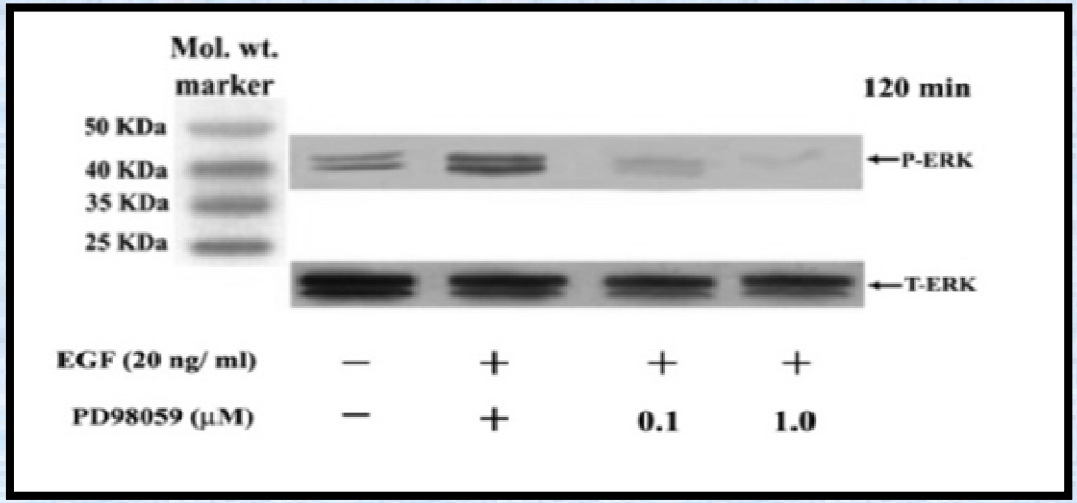


Effect of MEK inhibitor PD98049 on EGF-stimulated steroid production



Time- and MEK1/2-dependent activation of ERK1/2 by EGF

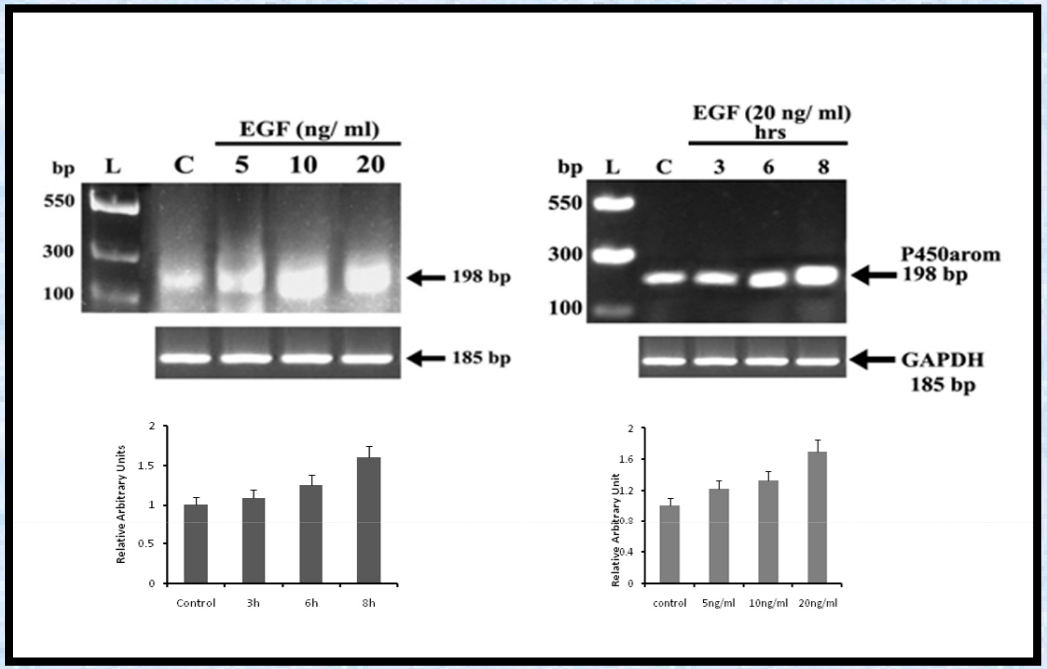


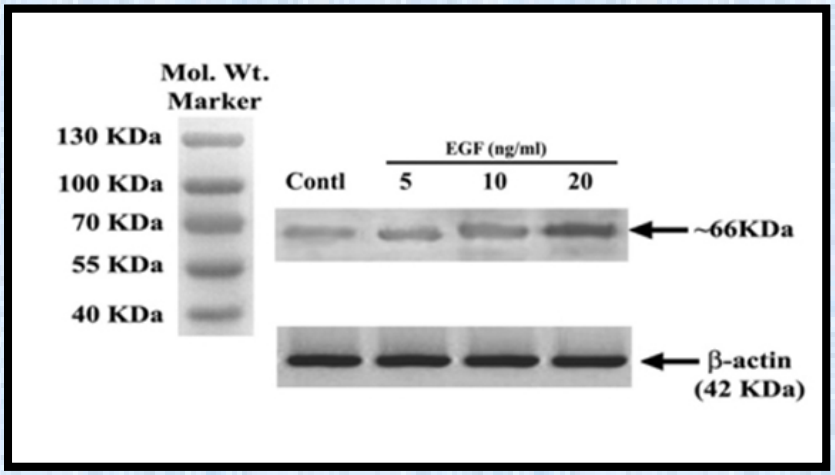


Inference

- EGF stimulates the EGFR expression in carp ovarian follicular cells
- EGF can induce StAR gene expression
- EGF effects on steroid production is mediated through activation of PI3 kinase and MAP kinase

P450 mRNA and its expression in follicular cells and effects of EGF





Summary

- Like mammal, EGF is a potent regulator of fish ovarian steroidogenesis
- EGF induces steroidogenesis both independently and synergistically with gonadotropins
- EGF-mediated steroid production requires an active EGFR and EGFR-mediated signaling is critical for normal gonadotropin-induced steroidogenesis in fish ovarian follicles
- EGF signaling is mediated through PI3 kinase and MAP kinase activation
- EGF-mediated steroid production in fish ovarian follicle is dependent on StAR gene expression

Co-workers

Sumana

Buddhadev

Sujata

Puja

Suravi

Shreyasi

Payel

Piyali

Arindam

Koushik

THANK YOU

Sisters and Brothers of America,

It fills my heart with joy unspeakable to rise in response to the warm and cordial welcome which you have given us. I thank you in the name of the most ancient order of monks in the world; I thank you in the name of the mother of religions; and I thank you in the name of millions and millions of Hindu people of all classes and sects. My thanks, also, to some of the speakers on this platform who, referring to the delegates from the Orient, have told you that these men from far-off nations may well claim the honour of bearing to different lands the idea of toleration. I am proud to belong to a religion which has taught the world both tolerance and universal acceptance……