



## POSSIBLE MEDIATORY ROLE OF TUMOR NECROSIS FACTOR ALPHA (TNF ALPHA) IN TROUT PREOVOVULATORY FOLLICLES: EFFECTS ON PREPARATORY EVENTS LEADING TO OVULATION

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### Introduction:

Ovulation, which is triggered by a surge of luteinizing hormone (LH), is a complex process leading to the release of the mature oocyte from the ovarian follicle. Several preparatory events must occur prior to the expulsion of the oocyte including follicle contraction, follicle separation and follicle rupture. In line with the notion that ovulation is an inflammatory-like process in mammals, we studied the mechanisms involved in the control of ovulation during the preovulatory period in a teleost fish, the brown trout (*Salmo trutta*). Specifically, we have tested the hypothesis that the pro-inflammatory cytokine tumor necrosis factor alpha (TNF alpha) could affect ovarian function and act as mediator of the effects of LH in preovulatory follicles.

### Methods:

For the follicle contraction experiments, preovulatory follicles punctured using a 25-gauge hypodermic needle were incubated in Hank's balanced salt solution (HBSS) containing 0.2% BSA (HBSS-BSA) in 6 cm culture dishes (10 follicles/4 ml) in the presence of the test compounds for 16 h at 15°C under shaking conditions (100 rpm). Follicle contraction was determined by measuring the weight of the 10 follicles in each replicate after incubation, as previously described and validated [1, 2]. Since contraction results in the expulsion of yolk through the puncture site, a decrease in follicle weight indicates an increase in follicle contraction. We also removed and stored at -20°C the culture medium at the termination of the incubation period to determine by enzyme immunoassay the *in vitro* prostaglandin (prostaglandin F2 alpha, PGF2 alpha) production. To collect ovarian tissue for RNA extraction and gelatinase/collagenase activity determination, preovulatory follicles from each of a total of three-five females were incubated (20 follicles/5 ml in triplicate) in HBSS-BSA in the absence or presence of test compounds, at 15°C for 16 h with gentle shaking (100 rpm). At the end of the incubation follicles were removed, flash frozen in liquid nitrogen and stored at -80°C until assayed.

### Results and Discussion:

We examined the *in vitro* effects of coho salmon LH (sLH) on trout preovulatory ovarian follicles and showed that sLH significantly increased follicle contraction and

that this effect was blocked by indomethacin (a prostaglandin synthesis inhibitor) and TAPI-1 (an inhibitor of TNF alpha-converting enzyme or TACE/ADAM17, that blocks TNF alpha secretion). Furthermore, sLH treatment increased the expression of *Tnf alpha*, *Tace/Adam17* and prostaglandin synthase 1 and 2 (*Cox-1* and *Cox-2*) in the trout follicle, as well as the production of PGF2 alpha into the culture medium. Interestingly, sLH increased gelatinase/collagenase activity in the trout follicle. We also used a salmonid-specific microarray platform (GEO GPL10706) and observed that sLH induced the expression of genes known to be involved in inflammation, proteolysis and tissue remodeling. In order to further study the possible involvement of TNF alpha as a mediator of sLH in the trout ovary, we incubated trout preovulatory follicles in the presence of recombinant trout TNF alpha (rtTNF alpha). First, rtTNF alpha caused a significant increase in follicle contraction and indomethacin blocked this effect, suggesting a possible involvement of prostaglandins in rtTNF alpha action. Second, rtTNF alpha stimulated the expression of *Cox-1* and *Cox-2*. Third, rtTNF alpha stimulated the *in vitro* production of PGF2 alpha. Fourth, rtTNF alpha stimulated gelatinase/collagenase activity. Interestingly, PGF2 alpha directly stimulated follicle contraction, confirming the results from other studies, as well as gelatinase/collagenase activity.

### Conclusion:

In view of these results we propose that TNF alpha may be a potential mediator of the effects of LH in the ovulatory process in trout probably through its stimulation of the production of PGF2 alpha. Specifically, TNF alpha could have an important role in the biomechanics of follicle weakening, ovarian rupture and oocyte expulsion during ovulation in teleosts.

### References:

- [1] GOETZ, F.W., BRADLEY, J.A. 1994. Stimulation of *in vitro* ovulation and contraction of brook trout (*Salvelinus fontinalis*) follicles by adrenaline through alpha-adrenoreceptors. *J. Reprod. Fertil.*, 100(2): 381-385.
- [2] HSU, S.Y., GOETZ, F.W. 1992. The effects of E and F prostaglandins on ovarian cAMP production and follicular contraction in the brook trout (*Salvelinus fontinalis*). *Gen. Comp. Endocrinol.*, 88(3): 434-443.