REGULATION OF DEVELOPMENT OF LATE PRIMARY AND EARLY SECONDARY OVARIAN FOLLICLES OF COHO SALMON BY SEX STEROIDS

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Introduction:

Relatively few studies have examined the endocrine control of pre-vitellogenic ovarian follicle development in teleosts. Follicles of hypophysectomized teleosts can develop to the late perinucleolar/early cortical alveolus stage in the absence of the pituitary, suggesting that pituitary gonadotropins are not mandatory for completion of primary development of the follicle [1]. However, several lines of evidence suggest that sex steroids regulate growth of pre-vitellogenic follicles. Estradiol-17β (E2) stimulated development of cortical alveolus stage ovarian follicles in hypophysectomized goldfish, and several in vivo and in vitro studies on anguillid eels provide strong evidence for a role of 11-ketotesterone (11-KT) in the regulation of primary ovarian follicle development [2]. Since female anguillid eels display uniquely high blood levels of 11-KT at the start of the migration to oceanic spawning sites [2],it is not known if androgenic control of primary ovarian follicle development is a general phenomenon. In this study, we addressed the hypotheses that: 1) Androgens regulate primary ovarian follicle development; 2) E2 stimulates development of cortical alveolus stage follicles; and 3) plasma steroid levels reflect the roles of androgens and estrogen during primary and early secondary ovarian follicle development.

Methods:

In vitro experiments: Ovaries containing follicles at the late (Balbiani bodies absent) perinucleolar and early alveolus stages were removed from one year-old coho salmon. Ovarian fragments were cultured for 7, 14 and 21 days without or with 0.03-30 ng/ml 11-KT, E2 or testosterone (T), fixed, and processed for histology. The circumference of oocytes sectioned through the center of the nucleus was measured, and oocyte volume was calculated. Abundance of cortical alveoli was quantified. Further experiments examined whether androgen (flutamide) or estrogen (tamoxifen) receptor antagonists inhibited the growth response to steroids, and a specific aromatase inhibitor (exemestane) was used to determine if the effects of T were partially due to its aromatization to E2. *In vivo experiments:* Females with ovaries containing late perinucleolar or early cortical alveolus stage oocytes were injected with sustained release implants containing either 11-KT or E2, and sampled after 10 and 20 days. Effects on follicle size were determined as above, and plasma 11-KT and E2 levels were measured by immunoassay.

Plasma sex steroid levels during ovarian follicle development: Females were sampled at six stages: chromatin nucleolar, early and late perinucleolar, early and late cortical alveolus, and lipid droplet. Plasma 11-KT, T and E2 levels were measured by immunoassay.

Results and Discussion:

In vitro experiments: Low concentrations of 11-KT had significant growth-promoting effects on late perinucleolar follicles in vitro, with maximum size reached by 7 days of culture. Flutamide inhibited this growth promoting effect. T, although causing a significant increase in volume of late perinucleolar follicles, was less effective that 11-KT. Blocking aromatization to E2 enhanced the growth promoting effect of T. E2 had no effect on growth of perinucleolar follicles but by 21 days, E2-treated follicles displayed a few cortical alveoli. At the early cortical alveolus stage, 11-KT increased follicle volume but there was no effect on the abundance of cortical alveoli. E2 strongly promoted growth and stimulated synthesis of cortical alveoli, actions that were inhibited by tamoxifen. T had modest growth-promoting effects and stimulated a moderate increase in cortical alveoli abundance. These results indicate that completion of primary follicle growth is under androgenic control, while early secondary growth (cortical alveolus stage) is predominantly under the control of E2.

In vivo experiments: Modest increases in plasma 11-KT resulted in a significant increase in volume of late perinucleolar follicles by 10 days and in contrast to in vitro treatment, induced the appearance of a few cortical alveoli. E2 had no observable effect by day 10, but by day 20 induced increases in volume similar to those seem with 11-KT, and was also more effective than 11-KT in stimulating cortical alveoli formation. By contrast, in females with ovaries that contained early cortical alveolus stage follicles at the time of implantation, E2 was much more effective than 11-KT in stimulating follicle growth, and strikingly, the ooplasm of follicles from E2-treated fish was filled with cortical alveoli. These results are generally consistent with those from in vivo experiments; but with more overlap between the

stage-specific effects of steroids, likely due to steroids actions on the brain and/or pituitary.*Plasma sex steroid levels during ovarian follicle development:* Plasma levels of T were constant during follicle development, aside from a decrease at the early cortical alveolus stage. E2 levels, consistent with previous studies [4] became elevated at the early cortical alveolus stage. Although we used 11-KT in experimental studies as a non-aromatizable androgen without assumptions about levels in vivo and its potential role in normal development, 11-KT levels increased significantly at the late perinucleolar stage, suggesting that this steroid participates in the regulation of primary growth of follicles.

Conclusion:

These studies demonstrate the stage-specific effects of androgens and E2 on development of late primary and early secondary ovarian follicles of coho salmon. The former appears to be under predominantly androgenic control while the latter appears to be largely due to the effects of E2. (This study was supported by grant IOS-0949765 from NSF).

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