



PREVITELLOGENIC OOCYTE GROWTH IN A TELEOST FISH, *ANGUILLA AUSTRALIS*: CROSSTALK BETWEEN INTRAOVARIAN FACTORS AND GROWTH AXIS

Shoae A., Setiawan A. N., Lokman P. M.

Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand. Fax +64-34797584, e-mail: ashoae@yahoo.com

Introduction:

It is well-established that oocyte growth and follicular development in vertebrates involves communication between the germ cell and its surrounding follicular layers. This communication is believed to be controlled by a combination of intraovarian factors, such as growth factors and sex steroids, and extraovarian factors, such as growth hormone (GH), insulin-like growth factor-1 (IGF-1) and follicle-stimulating hormone (FSH). While a substantial number of publications has focused on regulatory factors of advanced stages of oogenesis (i.e. vitellogenesis and final oocyte maturation), less attention has been paid to early stages of oocyte growth (previtellogenesis) in teleost fish. In this study, we addressed the relationship among intraovarian mRNA levels of *igf-1*, growth differentiation factor-9 and bone morphogenetic protein-15 (*gdf-9* and *bmp-15*), growth hormone receptor-1 (*ghr-1*), insulin-like growth factor type-1 receptor (*igf-1r*) and serum levels of sex steroids (estradiol-17 β , E₂ and 11-ketotestosterone, 11-KT). We further included a suite of extraovarian factors (pituitary *gh* and hepatic *igf-1* mRNA abundance) in our analyses in order to tease out the factors that are likely to play the most prominent role during previtellogenesis in the shortfinned eel, *Anguilla australis*.

Methods:

Immature wild shortfinned eels of different size classes were caught from Lake Ellesmere, South Island, New Zealand during spring and autumn of 2008/09. The fish were euthanized, weighed and a piece of ovary, liver and pituitary removed for histological and molecular

analyses. Blood samples were processed to enable measurement of E₂ and 11-KT by radioimmunoassay. The target genes were partially cloned and subjected to real-time qPCR assay. On the basis of histological observations of ovarian tissues, the eels were subdivided between chromatin nucleolus (CN), perinucleolus (PN) and oil droplet (OD) stages of previtellogenesis. Using SPSS-16, steroid and transcript abundance means for the three previtellogenic stages (two-way ANOVA with season and stage of oocyte development as fixed factors) were compared and correlation coefficients calculated.

Results and discussion:

Oocyte size significantly increased during previtellogenesis (Fig-1A). Furthermore, we observed significant increases in serum 11-KT levels (Fig-1B) and mRNA abundance of ovarian *gdf-9*, *bmp-15*, *ghr-1* and *igf-1r* with advancing previtellogenesis in wild eels (Fig-2A-D). These results point to the involvement of metabolic factors (GH-IGF-1 axis) during previtellogenic oocyte growth. Our previous *in vitro* experiments similarly identified a stimulatory role of 11-KT and IGF-1 on previtellogenic oocyte growth in eel [1]. Although an effect of 11-KT on *Gdf-9* expression was not previously apparent in eel [2], we detected a strong correlation between oocyte diameter and *gdf-9*, *bmp-15* and 11-KT ($r=0.66$, $r=0.49$ and $r=0.68$, $P<0.01$, respectively). No relationship was found between intraovarian and hepatic *igf-1* mRNA levels. Likewise, pituitary *gh* and *fsh* did not significantly correlate with oocyte diameter; however, oocyte diameter *did* show a correlation with body weight ($r=0.83$, $P<0.01$).

Fig. 1: Oocyte diameter (A) and serum level of 11-KT (B) of New Zealand shortfinned eel during different stages of previtellogenesis in autumn (closed bars) and spring (open bars) of the 2008/09 austral summer; values are mean $\pm$ SEM. See text for abbreviations.

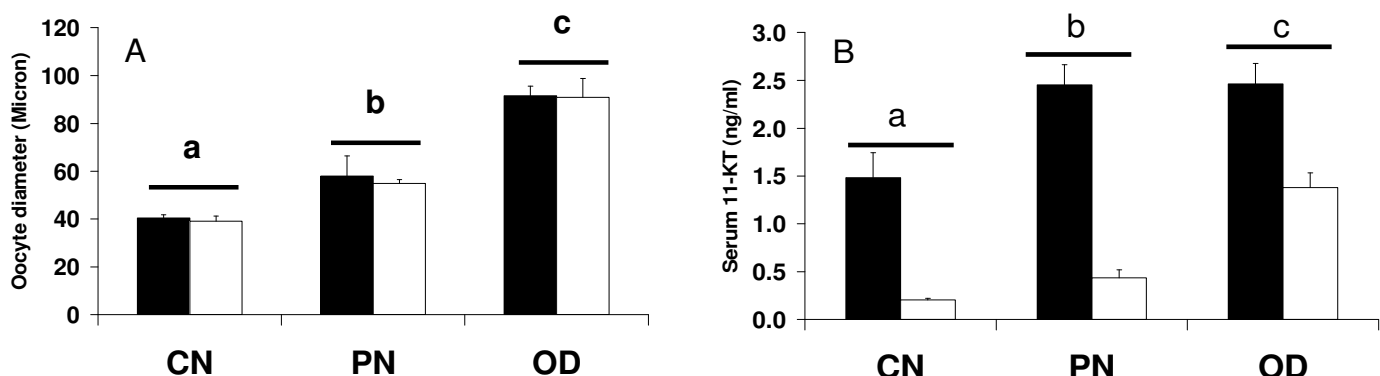
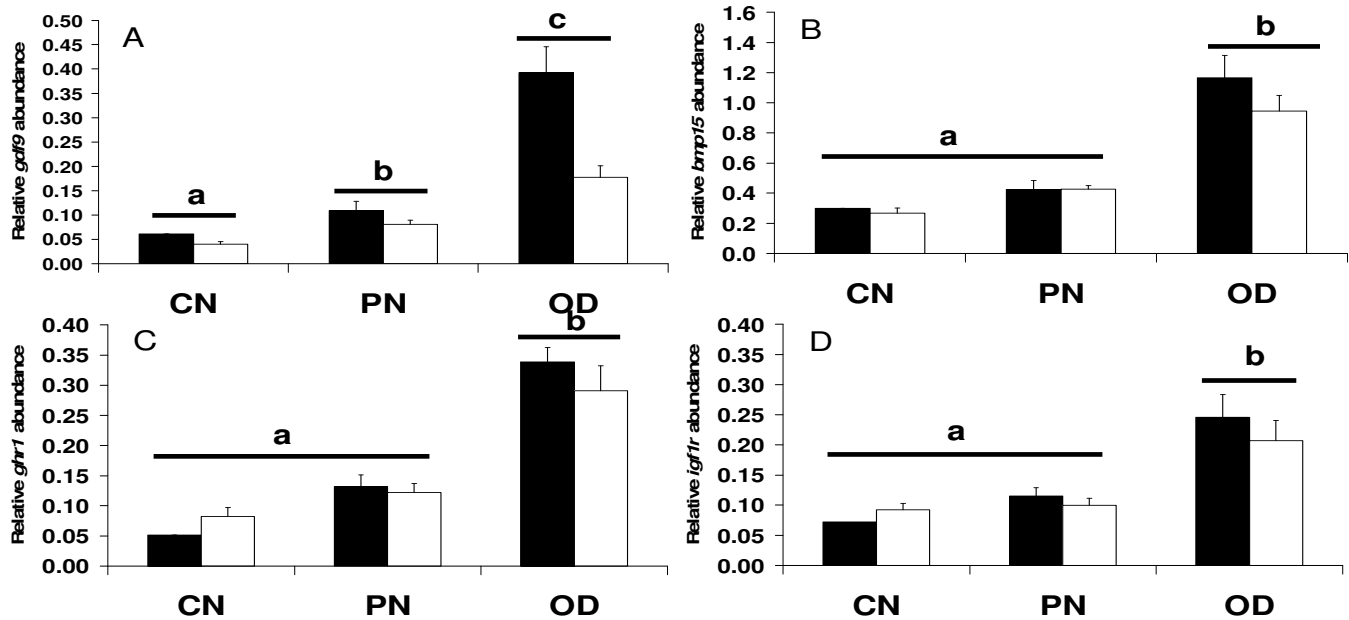


Fig.2: Relative mRNA abundance of *gdf-9* (A), *bmp-15* (B), *ghr-1* (C) and *igf-1r* (D) of New Zealand shortfinned eel during different stages of previtellogenesis in autumn (closed bars) and spring (open bars) of the 2008/09 austral summer, values are mean $\pm$ SEM. See text for abbreviations.



Conclusion:

The results of this study show a strong association between oocyte growth and mRNA levels of ovarian-derived factors, such as *gdf-9* and *bmp-15*, and 11-KT. In addition, the increasing abundances of transcripts from genes that are components of the growth axis (IGF-1R and GHR) may reflect increasing ovarian sensitivity to metabolic factors with advancing stages of early oocyte growth in teleost fish.