FUNCTION OF GONADOTROPINS IN ASYNCHRONOUS DEVELOPMENT OF OVARIAN FOLLICLES IN THE WRASSE *PSEUDOLABRUS SIEBOLDI*

Kitano H.[1], **Takeshita M.**[1], **Lee J.M.**[2], **Kusakabe T.**[2], **Yamaguchi A.**[1], and **Matsuyama M.**[1]

[1]Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan
Tel/Fax: +81 92 642 2888; E-mail: h-kitano@agr.kyushu-u.ac.jp
[2]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

Introduction:

Asynchronous development of ovarian follicles, the simultaneous progress of vitellogenesis and oocyte maturation in the ovary, is a rational reproductive strategy for achieving multiple spawning episodes in one reproductive season. The bambooleaf wrasse *Pseudolabrus sieboldi* is a typical daily spawner in which different developmental stages of ovarian follicles are found in the ovary for daily egg production. We previously reported that the expressions of both FSHβ and LHβ mRNA show diurnal fluctuation in the female pituitary, suggesting that ovarian follicles are likely to be exposed to both FSH and LH regardless of their developmental status [1]. Furthermore, we found that the mRNA of the receptors for FSH (FSHR) and LH (LHR) are highly expressed in the vitellogenic and postvitellogenic follicles, respectively [2]. In the present study, the recombinant gonadotropins of bambooleaf wasse were produced by baculovirus in silkworm *Bombyx mori* larvae. To reveal the mode of action of FSH and LH in asynchronous development of ovarian follicles, the steroidogenic potencies of the recombinant gonadotropins in ovarian follicles at different developmental stages were analyzed by in vitro bioassay. Moreover, the potency to induce final oocyte maturation was also evaluated.

Methods:

The single chain recombinant bambooleaf wrasse FSH (r-bwFSH) and LH (r-bwLH) were constructed by fusion of the nucleotide sequences encoding their β subunits and the GtHα subunit using spacer sequence in which two N-glycosylation sites contained, respectively. They were expressed in the silkworm system [3] and purified using affinity column. The vitellogenic or postvitellogenic ovarian follicles were isolated from mature females (n = 5) using stainless mesh filters, and 80 follicles of them were incubated in 1 ml **Leibovitz's** L-15 medium with (1-1000 ng/ml) or without r-bwFSH or r-bwLH, respectively. The concentration of Estradiol-17β (E2), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) in the culture media were determined by ELISA. These steroids of interest were selected on the basis of the steroidogenic pathways in the ovarian follicles during vitellogenesis and final oocyte maturation [4]. The percentage of germinal vesicle breakdown (GVBD) was

Fig. 1. In vitro effects of r-bwFSH and r-bwLH on E2 synthesis in the vitellogenic and postvitellogenic follicles. Asterisks represent significant differences from the control.

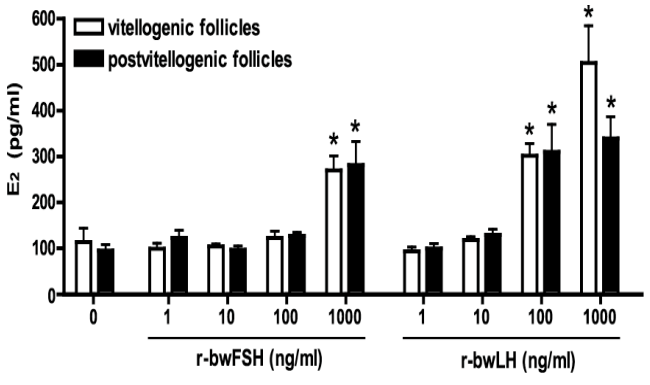


Fig. 2. In vitro effects of r-bwFSH and r-bwLH on 20β-S synthesis in the vitellogenic and postvitellogenic follicles. Asterisks represent significant differences from the control.

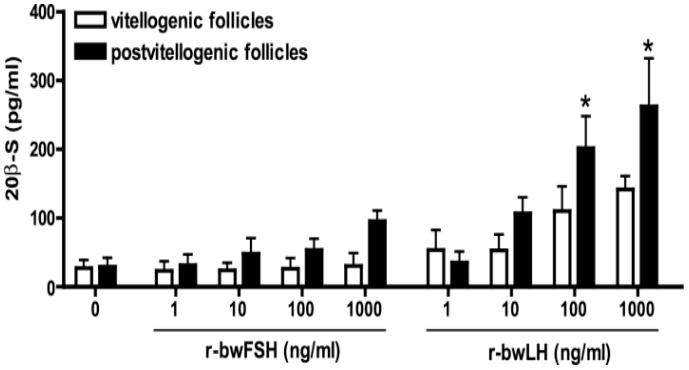
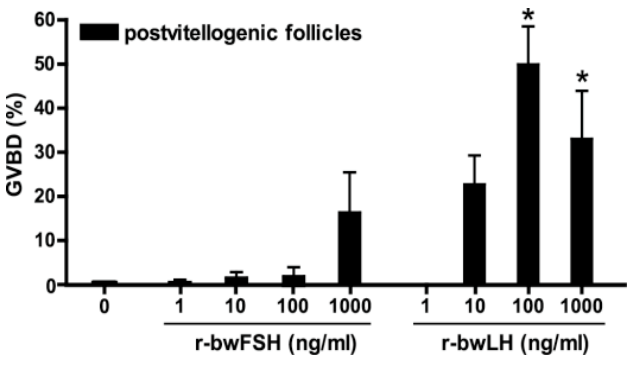


Fig. 3. In vitro effects of r-bwFSH and r-bwLH on GVBD in the postvitellogenic follicles. Asterisks represent significant differences from the control.



calculated to evaluate the potency of r-bwFSH or r-bwLH on oocyte maturation.

Results and Discussion:

E2 synthesis was induced in the vitellogenic and postvitellogenic follicles by both r-bwFSH and r-bwLH, respectively (Fig. 1). 20β-S synthesis and GVBD were specifically induced in postvitellogenic follicles by r-bwLH in a dose dependent manner (Fig. 2 and 3). Interestingly, 17,20β-P, which is a major maturation-inducing hormone (MIH) in many teleosts, was not induced by r-bwFSH and r-bwLH, indicating that principal MIH in the present species is 20β-S. These results suggest that the vitellogenesis is induced by both FSH and LH. On the other hand, oocyte maturation is evoked by LH only in the bambooleaf wrasse. Furthermore, the present results imply that the simultaneous advance of vitellogenesis and oocyte maturation in the ovary of bambooleaf wrasse is defined by the follicular stage-selective receptivity of two gonadotropins. Since the FSHR mRNA is highly expressed in vitellogenic follicles [2], E2 synthesis is suggested to be regulated by FSH and cross-reactive LH via FSHR. On the other hand, the LHR mRNA is elevated in postvitellogenic follicles [2], oocyte maturation followed by MIH synthesis is likely to be facilitated specifically by LH via abundant LHR in postvitellogenic follicles.

Conclusion:

In the bambooleaf wrasse, E2 synthesis was induced in vitellogenic and postvitellogenic ovarian follicles by both r-bwFSH and r-bwLH. On the other hand, 20β-S synthesis and GVBD were specifically induced in the postvitellogenic follicles by r-bwLH. Here we propose that the asynchronous development of ovarian follicles in the bambooleaf wrasse is regulated by 1) co-regulation of vitellogenesis by FSH and LH via FSHR, and 2) the postvitellogenic stage-selective induction of the oocyte maturation by LH via LHR.

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References:

[1]OHTA, K., MINE, T., YAMAGUCHI, A., MATSUYAMA, M. 2008. Sexually dimorphic expression of pituitary glycoprotein hormones in a sex-changing fish (*Pseudolabrus sieboldi*). J. Exp. Zool., 309A: 534-541.

[2]KITANO, H., IRIE, S., OHTA, K., YAMAGUCHI, A., MATSUYAMA, M. 2008. Regulation of asynchronous development of ovarian follicles by the expression levels of two gonadotropin receptors in a daily spawning teleost, a bambooleaf wrasse, *Pseudolabrus sieboldi*. Proc. 5th World Fisheries Congress: 3a-11.

[3] KAWAKAMI, M., LEE, J.M., MON, H., KUBO, Y., BANNO, Y., KAWAGUCHI, Y., MAENAKA, K., PARK E.Y., KOGA, K., KUSAKABE, T. 2008. Efficient protein expression in *Bombyx mori* larvae of the strain d17 highly sensitive to *B. mori* nucleopolyhedrovirus. Mol. Biotech., 40: 180-185.

[4]OHTA, K., MINE, T., YAMAGUCHI, A., MATSUYAMA, M. 2001. Steroidogenic pathway to estradiol-17β synthesis in the ovarian follicles of the protogynous wrasse, *Pseudolabrus sieboldi*. Zool. Sci., 18: 937-945