EXPRESSION PROFILES OF GTHβ SUBUNIT mRNAs DURING SEASONAL AND SPAWNING CYCLES IN FEMALE CHUB MACKEREL *SCOMBER JAPONICUS* AND *IN VITRO* STEROID POTENCY OF PURIFIED GTHS IN VITELLOGENIC OOCYTES

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Introduction:

The physiological functions of teleost gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are well established in salmonids, which are single spawning fish. In salmonids, FSH regulates vitellogenesis via estradiol-17β (E2) production, whereas LH has a specific role in stimulating final oocyte maturation (FOM) through 17α,20β-dihydroxy-4-pregenen-3-one (17,20β-P) production [1]. In contrast, their roles in multiple spawning fish are still poorly understood. To understand the role of GtHs during ovarian development of female chub mackerel, *Scomber japonicus*, which are multiple spawners, changes in mRNA expression of FSHβ and LHβ were investigated during seasonal and spawning cycles. In addition, the effects of purified chub mackerel GtHs (cmFSH and cmLH) on the steroid production were examined in the vitellogenic oocytes of chub mackerel *in vitro*.

Methods:

Gene expression analysis- Fish were reared in sea pens and sampled during November 2008, March, April and August 2009 to investigate seasonal changes in *fshβ* and *lhβ*. During the spawning season, fish showing multiple spawning in 3-ton concrete tanks were sampled at 1300, 1600, 2000, and 0600 to investigate those changes during the spawning cycle. For analysis of each sample, mRNA was isolated from the pituitary and template cDNA was synthesized. A real-time PCR reaction method was used to assay *fshβ* and *lhβ*. *In vitro experiments*- Fully grown post-vitellogenic and mid-vitellogenic oocytes were separated from the ovary and incubated in the absence or presence of purified cmFSH or cmLH (6, 25, 100, and 200 ng/ml), respectively. After incubation for 18 hr at 20 °C, culture media were frozen and later, E2 and 17,20β-P were measured using ELISA.

Fig. 1. Changes in *fshβ* and *lhβ* in the female chub mackerel pituitary during seasonal and spawning cycles. IM, immature; EV, early vitellogenesis; LV, late vitellegenesis; PS, post-spawning; GVM, germinal vesicle migration; HY, hydration; OV, ovulation; POV, post-ovulation.

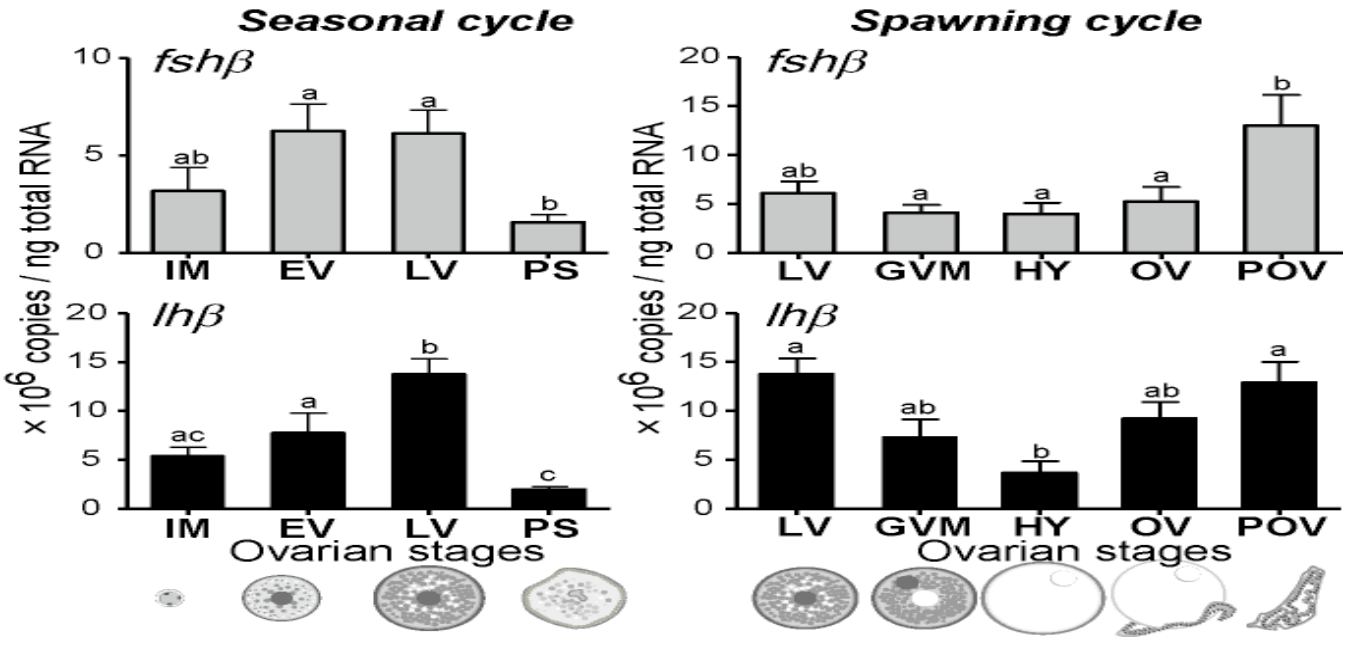
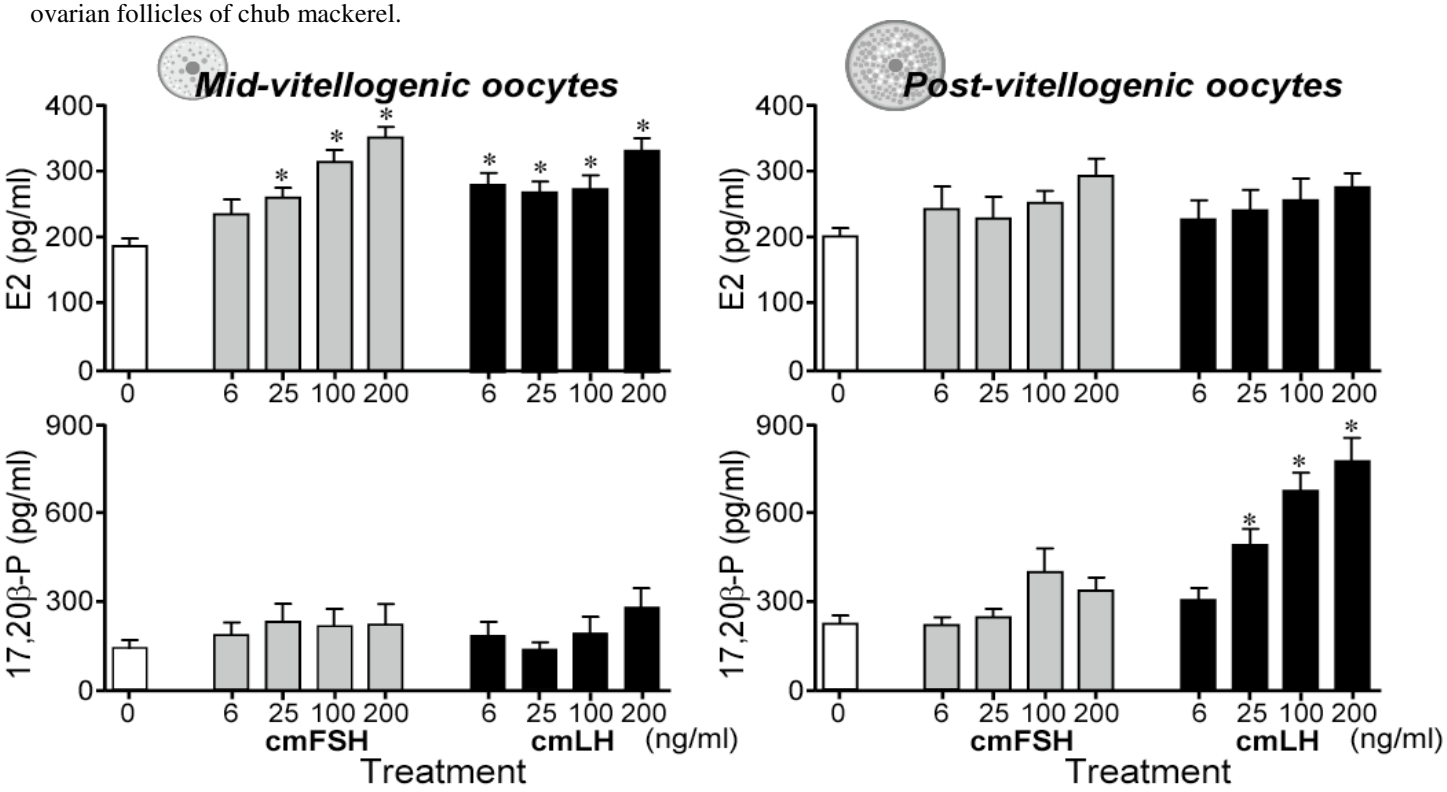


Fig. 2. Effects of purified chub mackerel GtHs (cmFSH and cmLH) on E2 and 17,20β-P production in the different stages of ovarian follicles of chub mackerel.



Results and Discussion:

The levels of *fshβ* remained high during vitellogenesis while *lhβ* increased in association with ovarian development and reached a peak at late vitellogenesis (Fig. 1). Both *fshβ* and *lhβ* levels decreased during the post-spawning period. The *in vitro* cultivation of oocytes demonstrated that cmFSH stimulated E2, but not 17,20β-P production, whereas cmLH stimulated both E2 and 17,20β-P synthesis (Fig. 2). It has been revealed that in ovarian follicles of chub mackerel, when hormonal stimulation was investigated by radiolabeled precursors *in vitro*, E2 was synthesized during vitellogenesis while 17,20β-P was produced during FOM [2]. Therefore, in chub mackerel, both FSH and LH may be involved in the vitellogenic process through the stimulation of E2 production, whereas FOM may be regulated by the LH, regulating the synthesis of 17,20β-P production. Interestingly, during the spawning cycle, high expression of *fshβ* further increased after ovulation (POV, in Fig. 1), suggesting that the FSHβ transcription may be promoted to accelerate the vitellogenesis. Conversely, high expression of *lhβ* declined from germinal vesicle migration (GVM) to hydration (HY) and increased after ovulation (Fig. 1).

Conclusion:

Seasonal expression profiles of GtHβ subunit mRNAs and *in vitro* steroid productions by cmFSH and cmLH suggests that chub mackerel FSH may act on vitellogenesis and LH on both vitellogenesis and FOM. Moreover, changes in *fshβ* and *lhβ* during the spawing cycle indicate that the synthesis of FSH and LH may be differently regulated during FOM, ovulation and spawning in the multiple spawning fish.

References:

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