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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation **Studies of Egg Formation and Egg-Sperm Interaction in Teleost Spawn Pelagic Eggs**
（浮遊性卵を産む硬骨魚類における卵形成および卵―精子相互作用に関する研究）

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Teleost is one of the most diverse vertebrate groups having a wide range of reproductive strategies. Japanese anchovy (*Engraulis japonicus*, EJ) is an oviparous species having oval-shaped pelagic eggs belonging to a lower taxonomic group of the teleost. However, little has been known about physiological and biochemical features on the process of egg production. This research mainly focus on physiological, molecular and biochemical features of egg formation, final oocyte maturation and sperm motility (circadian rhythm of sperm motility) for establishment of successful fertilization techniques in order to improve fish aquaculture system.

Vitellogenesis involves the production and incorporation of egg yolk precursor (Vitellogenin, Vg) into developing oocytes in oviparous animals. The teleost Vg is a large glycophospholipoprotein synthesized in the liver and transported to the ovary then incorporated into the growing oocytes. Vitellogenins plays a vital role in oocytes and embryo development in oviparous teleosts. In Chapter I, I cloned three complete cDNAs encoding different forms of Vg from maturing female liver of EJ and characterized as *EjVgAα, EjVgAβ* and *EjVgC* based on current Vg nomenclature and phylogeny. The cloned *EjVgAα, EjVgAβ* and *EjVgC* encoded 1631, 1634 and 1257 amino acid (AA) residues including expected signal peptides, respectively. The AA sequences of *EjVgAα* and *EjVgAβ* showed 73% sequence identity to each other and highly similarities with those of other teleost fishes. However, shorter cDNA sequence (*EjVgC*) that lacked phosvitin (Pv) domain and no β`-component (β'-c) region showed a close similarity to the phosvitin-less (PvL) Vg of other fish, was classified as type-C. Phylogenetic analyses indicated an evolutionary association of *EjVgAα* and *EjVgA*β are the homologous proteins. In addition, I cloned and characterized multiple Vg genes from kawakawa, *Euthynnus affinis* (a teleost representative from higher group), as *EaVgAa*,

EaVgAb and *EaVgC* and compared with corresponding Vgs in EJ. Furthermore, quantification of high Vg mRNA expression in liver during vitellogenic stages of EJ, demonstrate that *EjVgAα* and *EjVgAβ* are the main functional Vgs in mature female and the production initiated in liver of 26 weeks post hatched and Vg mRNA expression continuously increases until spawning (36 weeks post hatched).

In Chapter II, I clarify morphological and biochemical changes in oocytes during final oocyte maturation (FOM). Based on its morphological changes and observation of spawning, EJ completes FOM within eight hours, and the process can be classified into five stages: stage-I, post-vitellogenic oocyte (PVO); stage-II, germinal vesicle migration (GVM); stage-III, germinal vesicle breakdown (GVBD); stage-IV, late hydration; and stage-V, ovulation under photoperiod of 14L:10D with water temperature 19.5 to 20.9˚C. The average wet weight of Stage-I oocytes was 69 µg with a longitudinal diameter of 728 µm and 75% water content and then increased to 244 µg, 1,386 µm, and 94%, respectively, at Stage-V. Gel chromatographic analysis indicated a change in the peak position of native lipovitellin from 430 to 170 kDa. Moreover, the SDS-PAGE analysis indicated proteolytic degradation during the latter stages of FOM. The free amino acid (FAA) content in the Stage-I oocytes (2 nmol individual^{-1}) became approximately 11-fold greater in Stage-V eggs (22 nmol individual^{-1}), suggesting that the FAAs are produced from the proteolytic degradation of the yolk proteins and regulate the buoyancy of the eggs through osmotic effector generation in the EJ.

As EJ exhibits group synchronous and multiple spawning, the reproductive characteristics of the male in this species, especially sperm features and activation, are still largely unknown. In Chapter III, I confirmed that features of the anchovy sperm and characteristics of the activations, regarding sperm motility and moving velocity. The average size of the sperm was 51 ± 1.3 µm in total length and possessed a normal structure with clockwise, anticlockwise, and linear motion. The initial motility at one minute after activation in seawater was 75 ± 12% during spawning time (21:00–22:00) in this species, and the initial moving velocity (196 ± 26 µm/sec) remained constant for fifteen minutes post activation. While, comparatively low motility (30 ± 10%) was found at 17:00, and the sperm was almost immotile in the morning (08:00–09:00). This is the first time to demonstrate time specific activation, that is, circadian rhythm, in teleost males. Swimming ability was also confirmed with sperm that swam for more than one hour in seawater without an exogenous activating factor derived from the ovary in females. These results suggest that the characteristics in the EJ sperm motility, circadian rhythm and long moving period, are acquired for adaptation to their spawning characteristics of group synchronous spawning.