ウスエダミドリイシの卵形成に関する生理学的研究

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Title : Physiological studies on oogenesis of the scleractinian coral, *Acropora tenuis*
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Abstract

Scleractinian corals undergo mass spawning and release their gametes synchronously once a year. Prior to this yearly event, the gametes differentiate and develop until they reach maturation. To date, little is known about the internal mechanisms of oogenesis in scleractinian corals and the proximate cues. The current study aimed to investigate the physiological processes of oogenesis in *Acropora tenuis*.

The first study investigated oogenesis-related genes/proteins, including germ cell markers (Vasa and Piwi), major yolk precursors (vitellogenin: VG1 and VG2), and their receptors (low-density lipoprotein receptors: LDLR1, LDLR2, and LDLR3). Coral branches were collected monthly from coral reefs around Sesoko Island (Okinawa, Japan) for real-time qPCR analysis (*AtVasa*, *AtPiwi*, *AtVG1*, *AtVG2*, *AtLDLR1*, *AtLDLR2*, and *AtLDLR3*) and for histological observation by *in situ* hybridization of *AtVasa* and *AtLDLR1* genes and immunohistochemistry of AtVasa and AtVG1 proteins. AtVasa immunoreactivity was detected in germline cells and ooplasm, whereas AtVG1 immunoreactivity was detected in ooplasm and putative ovarian tissues. *AtVasa* was localized in germline cells located in the retractor muscles of the mesentery, whereas *AtLDLR1* was localized in the putative ovarian and mesentery tissues. *AtVasa* and *AtPiwi* were expressed during all oocyte stages. Germline cells expressing *AtVasa* were present throughout the year. AtVG1 and *AtLDLR1* were expressed in oocytes and coral tissues during vitellogenic phases (Stage III and onwards), respectively. Vasa and Piwi play physiological and molecular roles throughout the oogenic cycle because they determine gonadal germline cells and ensure normal oocyte development. By contrast, the roles of VG and LDLR were limited to the vitellogenic stages because they act in coordination with lipoprotein transport, vitellogenin synthesis, and yolk incorporation into oocytes.

The second study investigated the effects of seawater temperature and photoperiod on oogenesis of *A. tenuis*. Immature coral branches were cultured in indoor tanks for 13 weeks under different seawater temperature and photoperiod conditions. Corals cultured at a low seawater temperature (21°C) contained vitellogenic oocytes, whereas corals cultured at a high seawater temperature (29°C) had immature oocytes. Transcript levels of *AtVG1* and *AtLDLR1* were significantly higher at 21°C than 29°C. Oocytes were larger from corals cultured under a short photoperiod (10 h light) than under a long photoperiod (14 h light). However, no significant differences were observed in the mRNA levels of *AtVG1*, *AtVG2*, *AtLDLR1*, *AtLDLR2*, or *AtLDLR3*. The transcript levels of *AtVasa* and *AtPiwi* were higher under the 14 h photoperiod. The results suggested that seawater temperature might be the proximate cue, and that low water temperature triggers vitellogenin synthesis in corals. The photoperiod is likely to involve synchronizing the oogenic cycle by initiating the formation of oocytes after spawning.

The third study focused on the endocrine system, which in involved in coral reproduction. The focus was steroid hormones—progesterone, testosterone, and estradiol-17β (E2)—in *A. tenuis* and their relationships with vitellogenesis/vitellogenin synthesis. This study also investigated the effect of E2 on vitellogenin synthesis in corals and identified steroidogenic enzymes expressed from the *A. tenuis* genome. Liquid chromatography–mass spectrometry detected E2 in coral branches in March, April, and May, but not in June. By contrast, testosterone and progesterone did not fluctuate much during the same months. Immersing branches in E2-containing seawater failed to increase vitellogenin transcription. The results indicate that E2 is involved in oogenesis but does not positively regulate vitellogenin synthesis. Steroidogenic enzymes (except CYP19A) were identified in *A. tenuis*, suggesting that corals endogenously synthesize progestogens and androgens from cholesterol.

The findings show that several genes/proteins are involved in oogenesis in corals, and that their expression is regulated in part by steroid hormones. Also, our results indicate the importance of photoperiod and water temperature in regulating oogenesis in corals. In conclusion, it is possible that oogenesis in corals is, at least in part, manipulated by mimicking changes in such proximate factors under artificial conditions.