ウスエダミドリイシの卵形成に関する生理学的研究

Form 3

Abstract

Title

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

（ウスエダミドリイシの卵形成に関する生理学的研究）

Scleractinian corals undergo mass spawning and release their gametes synchronously once a year. Towards 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 as well as the proximate cue are not well established. The current study aimed to elucidate the physiological processes of oogenesis in *Acropora tenuis*.

The first study was to investigate oogenesis-related genes/proteins, which were germ cell markers (Vasa and Piwi), major yolk precorcors (vitellogenin; VG1 and VG2), and their precursor (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* hybridisation (ISH) of *AtVasa* and *AtLDLR1* genes and immunohistochemistry (IHC) of AtVasa and AtVG1. AtVasa immunoreactivity was detected in germline cells and ooplasm, whereas AtVG immunoreactivity was detected in ooplasm and putative ovarian tissues. *AtVasa* was localised in germline cells located in the retractor muscles of the mesentery, whereas *AtLDLR1* was localised in the putative ovarian and mesentery tissues. *AtVasa* and *AtPiwi* were expressed in all oocyte stages. Germline cells expressing *AtVasa* were present throughout the year. *AtVG* and *AtLDLR* 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, as it determines gonadal germline cells and ensures normal oocyte development. On the other hand, 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 was to investigate 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 in low seawater temperature (21°C) contained vitellogenic oocytes, while corals cultured in high seawater temperature (29°C) had immature oocytes. Transcript levels of *AtVG1* and *AtLDLR1* were significantly higher at 21°C than 29°C. Corals cultured under short photoperiod (10h light) had relatively larger oocytes than corals under long photoperiod (14h light). However, no significant differences were observed in mRNA expressions of *AtVG1*, *AtVG2*, *AtLDLR1*, *AtLDLR2*, and *AtLDLR3*. *AtVasa* and *AtPiwi* showed higher transcript level in the14h group. The results suggested that seawater temperature might be the proximate cue, and that low water temperature triggers vitellogenin synthesis in corals. Photoperiod is more likely to involve in synchronizing the oogenic cycle by initiating the formation of oocytes after spawning.

The third study was to focus on the endocrine system that takes part in the reproduction of corals. Attention was paid to detect steroid hormones – progesterone, testosterone, and estradiol-17β (E2) – in *A. tenuis* and study the relationships between vitellogenesis/vitellogenin synthesis and these steroids. This study also investigated the effect of E2 on vitellogenin synthesis in corals and identified steroidogenic enzymes in *A. tenuis* genome. Liquid chromatography-mass spectrometry (LCMS) revealed that E2 could be detected in coral branches in March, April, and May, but not in June. On the other hand, testosterone and progesterone did not fluctuate much in 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 may endogenously synthesize progestogens and androgens from cholesterol.

The present study clearly shows that several genes/proteins are involved in the process of oogenesis in corals, and that their expressions are regulated in part by steroid hormones. The present study also showed the importance of photoperiod and water temperature in regulating oogenesis in corals. In conclusion, it is possible that oogenesis in corals is, to some extend, manipulated by mimicking changes in such proximate factors under artificial conditions.

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