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DEVELOPMENT / EGG-LAYING HORMONE

MALEE CHANPOO : DEVELOPMENT OF THE REPRODUCTIVE SYSTEM  
AND LOCALIZATION OF EGG-LAYING HORMONE (ELH) IN THE GONAD  
OF A TROPICAL ABALONE, HALIOTIS ASININA LINNAEUS. THESIS  
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The aims of this thesis were to classify the germ cells and study the development of gonad and localization of egg-laying hormone in *Haliotis asinina*. The gonadal histology and germ cells were studied by light microscopy using paraffin and semithin methods. Connective tissue frameworks of the gonad consist of the outer and inner gonadal capsules linked by flat sheets of connective tissue called trabeculae. Trabeculae partition the gonad into compartments and each trabecula acts as the axis on which growing germ cells are attached and proliferate to form oogenetic or spermatogenic unit. Each trabecula contains small capillaries in the center, surrounded by muscle cells, collagen fibers intermingled with fibroblasts, and a substantial number of granulated cells which have many processes. Germ cells in oogenetic unit could be classified into six stages according to their histological characteristics: oogonium and five stages of oocytes, *i.e.*, Oc<sub>1</sub> with intense basophilic cytoplasm, Oc<sub>2</sub> with light basophilic cytoplasm and lipid droplets, Oc<sub>3</sub> with a few yolk granules, Oc<sub>4</sub> with increasing number of yolk granules and thin jelly coat, Oc<sub>5</sub> with numerous yolk granules and fully formed jelly coat. Germ cells in spermatogenic process could be classified according to the appearance of chromatin and the presence or absence of nucleolus into thirteen stages: spermatogonium, five stages of spermatocytes, secondary spermatocyte, four stages of spermatids and two stages of spermatozoa.

Definitive gonad appears to be clearly separated from the hepatopancreas at 2 months. Gonial cells are found at 2 months; early spermatocytes, spermatids and immature spermatozoa appear at 4 months, early oocytes (Oc<sub>1</sub>, Oc<sub>2</sub>) are later observed at 6 to 7 months. While completely mature spermatozoa could arise in the gonad as early as 7 months; mature oocytes (Oc<sub>4</sub>, Oc<sub>5</sub>) occur much later at 10 to 11 months. The male animals tend to reach full sexual maturity and start normal reproductive cycle as early as 7 to 8 month, while female animals reach maturity and start reproductive cycle around 11 to 12 months.

Localization of egg-laying hormone (ELH) was performed by immunofluorescence, immunogold with silver enhancement and immunoperoxidase techniques using polyclonal antibody to recombinant abalone egg-laying hormone (aELH) of *H. rubra* as a probe. Anti-aELH exhibits strong bindings, which implies the presence of aELH, to muscle cells and granulated cells within trabeculae and capsules. The cytoplasm of immature oocytes (stages 1, 2, 3) are moderately stained, while that of mature oocytes (stages 4, 5) are only weakly stained. It is possible that, aELH may be synthesized and released by granulated cells. This hormone may act directly on muscle cells to induce their contraction, which cause the expulsion of ripe oocytes or spermatozoa from the gonad. This study will apply to increase the number of abalone.