



## **Morphological observations on the formation of brood pouch of pot-bellied seahorse *Hippocampus abdominalis***

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Seahorses have one of the most unique reproductive strategies. Male, not female, carries and cares embryos in a brood pouch formed on the ventral surface of the tail. Lumen of the brood pouch is surrounded by loose connective tissue called “pseudoplacenta” and dermis. How the brood pouch is formed during the development from juvenile to adult is not sufficiently understood. We tried to make brood pouch-forming processes of seahorse clearer by morphological analyses. First we characterized pseudoplacenta and dermis of mature male seahorse by several staining methods. Pseudoplacenta was mainly composed of reticular fibers, while dermis was mainly composed of collagenous fibers. Observation of various juvenile seahorses showed that formation of brood pouch was initiated from linear projections of epithelia on both ventro-lateral sides of the body. The projections elongated toward the ventral midline, fused together and finally formed a baggy structure composed of a single dermis layer with neither smooth muscle nor pseudoplacenta, and then, pseudoplacenta having two layers of dermis and smooth muscle was formed. Thus, the fully-developed brood pouch was established. Finally, we analyzed the localization and gene expression of C-type lectins (haCTL1 and haCTL2). haCTL1 was localized in both outer and luminal epithelia of brood pouch throughout the developmental stages, while haCTL2, which was not detected in early stage of development, became specifically localized in the luminal epithelium as development proceeded. Thus, the luminal epithelium is considered to be drastically and specifically changed so as to carry the embryos during the development of brood pouch.

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