

Abstract (บทคัดย่อ)

In this study, we aimed to investigate sperm modifications that are related to sperm maturation and capacitation in two shrimp species including the giant freshwater prawn, *Macrobrachium rosenbergii*, and the black tiger shrimp (*Penaeus monodon*). In both species, spermatogenesis takes place in the testis and the testicular sperm are then emptied into male reproductive tract, i.e., vas deferens, and the terminal ampoule containing spermatophores. During mating, the spermatophores are deposited into female thelycum which is sperm storage organ in female. We have shown that modification of *M. rosenbergii* sperm proteins occurred during their storage in male reproductive tract and also in female thelycum. However, sperm protein profiles of sperm taken from the thelycum at 1 h- and 6 h-post insemination were not different, suggesting that modification of sperm proteins in female thelycum may occur rapidly. Moreover, we showed the accumulation of actin in *M. rosenbergii* sperm in the spike and the base of spike of sperm during maturation process, especially when the sperm were stored in the spermatophore. We also showed changes of tyrosine phosphorylation pattern in sperm during translocation from testis into vas deferens, spermatophores and finally into thelycum of both species. The localization pattern of *P. monodon* sperm could be used as one of the markers for sperm capacitation in this shrimp species. Proteolytic activities of sperm were also studied. The results showed that *P. monodon* sperm contained high trypsin-like activities and they gained these activities during storage in female thelycum. The presence of trypsin-like enzymes in sperm of both species was also demonstrated by mean of immunofluorescence staining using soybean trypsin inhibitor. The results suggested the presence of sperm trypsin-like proteases in the subacrosome region of *P. monodon* and the acrosome region of *M. rosenbergii* sperm. The sperm protein modifications and proteolytic activities may be important for sperm maturation and capacitation which is the process that make the sperm ready for fertilization.

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Introduction

Because of increasing domestic and foreign demands, the fresh water prawn *Macrobrachium rosenbergii* is becoming more important economic aquatic animal, and their production has become more depended on the development of efficient aquaculture system. There are many factors that contribute to the successful aquaculture and one way to increase the commercial production of quality prawn is to promote the maturation of viable gametes and to obtain the high quality gametes for production of high quality offsprings. However, it has been shown that spawning success and the number of offsprings produced per spawn are decreased after prolonged captivity of the parent broodstocks. One problem of this decreased fecundity that has not been adequately addressed is the male fertility and sperm quality.

Reproduction of *M. rosenbergii* is different from most other aquatic animals, i.e., male and female mate prior to the female spawns when male deposits its spermatophores on the female abdomen; and then, at the time of spawning, the female releases the eggs to her abdomen and brood fertilized eggs until the eggs hatch as free-swimming larvae in brackish water. It has been shown in the black tiger shrimp *Penaeus monodon*, whose is the close-type thelycum, that the sperm gain their fertilizing ability in the female thelycum and possess high level of sperm protein tyrosine phosphorylation; this process resembles "capacitation" in mammalian sperm. It was also found that *P. monodon* sperm need to reside at least 3 days in thelycum to gain full ability to undergo acrosome reaction which is another marker for sperm capacitation. However, in *M. rosenbergii*, which has the open-type thelycum, little is known about how sperm become functionally developed, and how they achieve fertilizing ability during their production in the testis and transit in male reproductive tract, and while they are in the thelycum. Therefore, in this study, we aimed at investigating the sperm modifications during their production in the testis, transit in spermatid duct and spermatophore, and after their deposit in the female thelycum. Furthermore, as a comparison between close- and open-type thelycum shrimp species, we will determine whether capacitation process occurs in the sperm of *M. rosenbergii* as that happen in *P. monodon*. We also expect to find out some markers denoting sperm with high fertilizing competency which could be applied as markers for selecting high quality male broodstock for aquaculture. Moreover, understanding mechanisms that make the sperm ready to fertilize in this species may lead to the development of an *in vitro* capacitating

method to improve the fertilization and production of larvae for aquaculture of this species.

The project was aimed at investigating sperm modifications during their production in the testis, the transit in the male reproductive tract, and during deposition in female open thelycum which could be used as markers for the sperm maturation and capacitation processes in *M. rosenbergii*. In addition, some biochemical changes in sperm during capacitation in *P. monodon* were also studied.