CHAPTER V

CONCLUSIONS

1. The full length cDNAs of *PmCOMT*, *PmFAMeT*, *PmBr-cZ1* and *PmBr-cZ4* were successfully characterized. *PmCOMT* and *PmBr-cZ4* was 1176 and 1879 bp in length containing the ORFs of 666 and 1329 bp corresponding to the polypeptides of 221 and 443 amino acids, respectively.

2. Two isoforms of *PmFAMeT* and *PmBr-cZ1* were identified. The full length cDNA of *PmFAMeT-l* and *PmFAMeT-s* were 1312 and 1297 and bp in length containing the ORFs of 843 and 828 bp corresponding to a protein of 280 and 275 amino acids, respectively. The full length cDNAs of *PmBr-CZ1-l* and *PmBr-CZ1-s* were 2119 and 1897 bp in length and contained the ORFs of 1443 and 1329 bp corresponding to a polypeptide of 481 and 443 amino acids, respectively.

3. Genomic organization of *PmCOMT* was characterized by genome walk analysis and overlapping PCR. The *PmCOMT* gene contained 3 exons (194, 111 and 361 bp) and 2 introns (143 and 147 bp).

4. Quantitative real-time PCR revealed differential expression of *PmFAMeT*, *PmBr-cZ1* and *PmBr-cZ4* but comparable expression of *PmCOMT* during ovarian development of intact broodstock of wild *P. monodon*. Eyestalk ablation resulted in up-regulation of *PmBr-cZ1*, down-regulation of *PmFAMeT* but had no effect on the expression level of *PmCOMT* and *PmFAMeT*.

5. Serotonin administration promoted the expression level of *PmFAMeT* in ovaries of 18-month-old shrimp approximately 50 fold at 1 hpi. Administration of 20E in commercially cultured juveniles resulted in the reduced expression level of *PmCOMT* but promoted the expression levels of *PmFAMeT*, *PmBr-cZ1* and *PmBr-cZ4*.

6. *In situ* hybridization indicated that *PmFAMeT, PmBr-C Z1* and *PmBr-C Z4* was localized only in the cytoplasm of oogonia and previtellogenic oocytes while *PmCOMT* was localized in the cytoplasm of oogonia, previtellogenic oocytes and follicular cells in both intact and eyestalk-ablated broodstock.

7. Recombinant proteins of PmCOMT, PmFAMeT-l and PmFAMeT-s were successfully expressed *in vitro*. Polyclonal antibodies against these recombinant proteins were produced in rabbit. Western blot analysis indicated that the PmCOMT protein was more preferentially expressed in earlier stages (previtellogenic and vitellogenic ovaries) than that of late stages (early cortical rod and mature ovaries) of ovarian development of *P. monodon*.

8. Immunohistochemistry suggested the translocations of both PmCOMT and PmFAMeT proteins during oogenesis *in P. monodon*. These proteins seemed to be translocated to the cortical rod during the maturation of oocytes.

9. The information suggested that *PmCOMT*, *PmPmFAMeT*, *PmBr-cZ1* and *PmBr-cZ4* play important roles on reproduction of *P. monodon*. In addition, the expression profiles of *PmPmFAMeT* , *PmBr-cZ1* and *PmBr-cZ4* can be used as the biomarker to monitor the reduced degree of ovarian maturation of *P. monodon* as a consequence effects of neurotransmitters, hormones and maturation diets.