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CHARACTERISATION AND RELATIVE EXPRESSION OF A GENE ENCODING vWD-KAZAL IN DIFFERENT DEVELOPMENTAL STAGES OF OOCYTE MATURATION IN GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii* (de Man, 1879)

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ABSTRACT

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science

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Institute	**:**	**Institute of Tropical Aquaculture**

Breeding of giant freshwater prawn, *Macrobrachium rosenbergii* is dependent on the availability of matured female broodstocks. The insufficient supply of matured female broodstocks has become a major constraint for sustainable production of larvae. To date, the induction of ovarian maturations of *M. rosenbergii* is based on eyestalk ablation technique which may cause high mortality rate and lower seed ratio of the broodstock. Molecular approaches based on ovarian maturation related genes, vWD domain and three Kazal-type domains gene (vWD-Kazal) that can be potentially used for the induction of ovarian maturations in broodstock were investigate. This research aims to achieve the following objectives: 1) To identify

and characterize vWD-Kazal in the ovary of *M. rosenbergii* 2) To determine the expression of vWD-Kazal in different ovarian maturation stages of *M. rosenbergii*; and 3) To determine the expression of vWD-Kazal in various tissues (hepatopancreas, stomach, intestine and gill) at different ovarian maturation stages of *M. rosenbergii*. vWD-Kazal in the ovary of *M. rosenbergii* were isolated using rapid amplification of cDNA end (RACE) method. Following that, the expression levels of vWD-Kazal in various tissues were determined by using quantitative PCR technique. The gene segment of *M. rosenbergii* vWD- Kazal (*MrvWD-Kazal*) comprises of 2,194 bp with an open reading frame of 1,998 bp encoding 667 amino acids with a predicted molecular mass of 72.5 kDA and had high similarity to *M. nipponense* vWD-Kazal (90%). qPCR analyses showed that the level of *MrvWD-Kazal* mRNA expression varied in the ovary ($p<0.05$) but no differences in the expression levels were observed in the hepatopancreas, stomach, intestine and gill ($p>0.05$). In the ovary, the level of *MrvWD-Kazal* expression increased from ovarian maturations of Stage 1 (Spent; 1.00-fold), Stage 2 (Proliferative; 3.47-fold) to Stage 3 (Premature; 6.18-fold) and decreased at Stage 4 (Mature; 1.31-fold). The expressions of *MrvWD-Kazal* suggested that *MrvWD-Kazal* plays a critical role in ovarian maturation of *M. rosenbergii* as *MrvWD-Kazal* is involved in the binding of oocyte membrane receptor to vitellogenin and lead to an uptake of vitellogenin in oocyte. In conclusion, *MrvWD-Kazal* is not associated with hepatopancreas, the digestive system and respiratory system based on the insignificant differences in *MrvWD-Kazal* expressions in these tissues.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN DAN EKSPRESI RELATIF GEN PENGEKODAN vWD-KAZAL PADA PERINGKAT PERKEMBANGAN YANG BERBEZA PADA KEMATANGAN OOSIT UDANG GALAH, *Macrobrachium rosenbergii* (de Man, 1879)

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Pembiakan udang galah, *Macrobrachium rosenbergii* bergantung kepada sumber induk betina yang matang. Kekurangan bekalan induk betina yang matang merupakan penghalang bagi pengeluaran larva yang mampan. Sehingga kini, rangsangan kematangan ovari *M. rosenbergii* adalah menggunakan teknik pemotongan mata yang akan menyebabkan peningkatan kadar kematian dan merendahkan kadar pembenihan. Kaedah molekular menggunakan gen yang berkaitan dengan kematangan ovari iaitu gen domain vWD dan tiga domain jenis-Kazal (vWD-Kazal) berpotensi meransangkan kematangan induk betina telah dikaji. Kajian ini bertujuan bagi mencapai objektif berikut: 1) Untuk mengenalpasti dan

mencirikan vWD-Kazal dalam ovari *M. rosenbergii*; 2) Untuk menentukan pengekspresan vWD-Kazal di dalam fasa kematangan ovari *M. rosenbergii* yang berbeza; dan 3) Untuk menentukan pengekspresan vWD-Kazal dalam pelbagai tisu (hepatopankreas, perut, usus dan insang) pada fasa kematangan ovari *M. rosenbergii* yang berbeza. vWD-Kazal telah dikenalpasti menggunakan teknik *rapid amplification of cDNA end* (RACE) daripada sampel ovari. Paras pengekspresan vWD-Kazal dalam pelbagai tisu telah ditentukan menggunakan teknik kuantitatif PCR (qPCR). Segmen gen *M. rosenbergii* vWD-Kazal (*MrvWD-Kazal*) terdiri daripada 2,194 bp dengan 1,998 bp Open Reading Frame, 667 asid amino dengan ramalan berat molekul 72.5 kDA dan mempunyai persamaan yang tinggi dengan *M. nipponense* vWD-Kazal (90%). Analisis qPCR menunjukkan paras pengekspresan *MrvWD-Kazal* berbeza di dalam ovari ($p<0.05$) dan tiada perbezaan pengekspresan dalam hepatopankreas, perut, usus dan insang ($p>0.05$). Di dalam ovari, paras pengekspresan *MrvWD-Kazal* meningkat dari Fasa 1 kematangan ovari (*Spent*; 1.00-*fold*), Fasa 2 (*Proliferative*; 3.47-*fold*) hingga Fasa 3 (*Premature*; 6.18-*fold*) dan menurun di Fasa 4 (*Mature*; 1.31-*fold*). Perbezaan pengekspresan *MrvWD-Kazal* mencadangkan bahawa *MrvWD-Kazal* memainkan peranan penting dalam kematangan oosit *M. rosenbergii* dimana *MrvWD-Kazal* terlibat dengan mengikat reseptor membran oosit kepada vitellogenin dan menyebabkan pengambilan vitellogenin oleh oosit. Kesimpulannya, *MrvWD-Kazal* tidak terlibat di dalam hepatopankreas, sistem pencernaan dan sistem pernafasan berdasarkan tiada perbezaan pengekspresan *MrvWD-Kazal* yang ketara dalam tisu-tisu ini.