GYNOGENESIS APPLICATION IN BANANA SHRIMP, *Fenneropenaeus merguiensis* (de Man, 1888) THROUGH IN-VITRO FERTILIZATION

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MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2015

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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the Institute of Tropical Aquaculture
Universiti Malaysia Terengganu

October 2015

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

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Institute : **Institute of Tropical Aquaculture**

This research was conducted to discover gynogenesis application in banana shrimp, *Fenneropenaeus merguiensis* aided with In-Vitro Fertilization technique (IVF). Gynogenesis is the most suitable method to be applied in penaeid shrimp monosex production since the sperm are easily maintained compared to eggs, penaeid shrimp exhibit external fertilization, and the larvae produce are fertile.

Experiment was covered by three main objectives which were; i) to develop the In-vitro fertilization (IVF) technique with different fertilization medium which are Natural Seawater (NSW), Artificial Seawater (ASW) and an extender Calcium Free Saline (Ca-F saline); ii) to determine the effect of different UV light irradiation on *F. merguiensis* sperms quality for initial development of gynogenesis protocol; iii) to carry out the In-vitro assay assessment from irradiated sperms and eggs of gynogenesis. A total of 30 fully mature males and 40 females of Stage IV of the ovarian maturation stage were

used to develop In-vitro technique in *F. merguiensis*. Sperms suspension was added to mature eggs in different fertilization medium and agitated in stimulate as natural spawning. Eggs morphological changes were recorded. Another 90 males were used for sperm viability assessment, external morphology, and comet assay analysis. Fresh sperms taken from male's broodstock were diluted 1:10 with Ringer's solution and UV irradiated at 254nm and 365nm doses from 20-80 seconds. Morphology changes were detected by using Advance Microscope and Scanning Electron Microscope. DNA damages were detected by using comet assay analysis. For gynogenesis larvae production, 10 males and 20 females were used. Optimization of UV irradiated sperm from the second objective was used to produce gynogenesis larvae aided with IVF technique developed from the first objective. Chromosome sets manipulation had been identified by counting the number of chromosomes during egg development stages.

Fertilization of IVF was obtained in all treatments with 8.67±4.04% in ASW, 19.67±7.38% in NSW and 4.33±4.04% in Ca-F saline. Although the hatching rate were not successfully obtained by ASW and NSW treatments, hatching yield in Ca-F saline medium was achieved with 3.00±2.65%. Treatment with exposure 60s 365nm gave the best result for UV irradiated sperm with 76.2% of mean viability percentage, produced 76% of normal sperm morphology, and obtained high scores (score 3 and 4) of irradiated sperm DNA damage. The fertilization rate of gynogenesis production was very low (<3%) and no hatched larva was detected in the treatment aquarium. Based on chromosome counting, the percentage of the diploid cells was 16.67% out of 30 cells.

Results from this present study are still scanty to produce gynogenesis shrimp in *F. merguiensis*. Therefore, more research and pioneering approach should be carried out

using large number of high quality gametes, with suitable fertilization media on IVF, different shock induction of diploid gynogenetic *F. merguiensis* in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi kepeluan untuk Ijazah Master Sains.

APLIKASI GINOGENESIS DALAM UDANG PUTIH, *Fenneropenaeus merguiensis* (de Man, 1888) MELALUI PERSENYAWAAN TABUNG UJI

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Kajian ini telah direka untuk mengkaji aplikasi ginogenesis dalam udang putih, *Fenneropenaeus merguiensis* dengan bantuan teknik persenyawaan tabung uji. Ginogenesis adalah kaedah yang paling sesuai digunakan dalam penaeid pengeluaran udang monosex kerana sperma mudah didapati berbanding dengan telur, penaeid mempamerkan persenyawaan luaran, dan yang paling menarik dapat hasilkan larva yang subur.

Eksperimen merangkumi tiga objektif utama iaitu (i) untuk membangunkan teknik persenyawaan tabung uji (IVF) dengan media persenyawaan yang berbeza iaitu Air Laut Asli (NSW), Air Laut Buatan (ASW) dan Kalsium Bebas Saline (Ca-F saline); (ii) menentukan kesan radiasi cahaya ultraungu (UV) yang berbeza ke atas kualiti sperma *F. merguiensis* untuk pembangunan awal protokol gynogenesis; dan (iii) untuk menjalankan persenyawaan sperma dan telur bagi penghasilan ginogenesis melalui kaedah tabung uji. Sebanyak 30 ekor udang jantan matang dan udang betina matang

tahap 4 kematangan ovari digunakan untuk membangunkan teknik persenyawaan tabung uji *F. merguiensis*. Sperma dicampur bersama telur di dalam medium persenyawaan yang berbeza dan digoncang sedikit bagi menyamai kaedah persenyawaan normal. Perubahan telur direkodkan. Sebanyak 90 ekor udang jantan digunakan untuk kajian viabiliti udang, morfologi luaran, dan analisis 'comet assay'. Sperma segar diambil dari induk jantan dicairkan 1:10 dengan cecair Ringer dan radiasi UV dikenakan ke atas sperma dengan dos 254nm dan 365nm pada saat 20-80. Sperma segar diambil dari induk jantan dicairkan 1:10 dengan cecair Ringer dan radiasi UV dikenakan ke atas sperma dengan dos 254nm dan 365nm pada saat 20-80. Perubahan morfologi luaran dikesan menggunakan Mikroskop Advance dan Mikroskop Elekrton Pengimbas. Bagi penghasilan larva ginogenesis, 10 ekor udang jantan dan 20 ekor udang betina digunakan. Pengoptimumam UV ke atas sperma yang dilakukan dalam objektif kedua digunakan bagi membangunkan kaedah penghasilan larva ginogenesis dibantu oleh teknik persenyawaan tabung uji yang dibangunkan dalam objektif satu. Manipulasi set kromosom dikenalpasti dengan mengira jumlah kromosom telur.

Persenyawaan telah berjaya diperolehi dalam semua rawatan media dengan 8.67 ± 4.04% dalam ASW, 19.67 ± 7.38% dalam NSW dan 4.33 ± 4.04% di Ca-F saline. Walaupun kadar penetasan tidak berjaya diperolehi apabila disenyawakan di dalam media ASW dan NSW, hasil penetasan di dalam media Ca-F telah diperolehi dengan 3.00 ± 2.65%. Rawatan pada pendedahan 60s 365nm memberikan hasil yang terbaik untuk sperma yang diriadiasi dengan dos 76.2% min viabiliti, menghasilkan 76% morfologi sperma normal, mencapai skor tertinggi (skor 3 dan 4) tahap kemusnahan DNA. Kadar persenyawaan pengeluaran ginogenesis amat rendah <3 % dan tiada larva menetas dikesan dalam

akuarium rawatan. Berdasarkan pengiraan kromosom, peratusan sel diploid adalah 16.67 % daripada 30 sel.

Hasil daripada kajian ini masih tidak mencukupi untuk penghasilan ginogenesis *F. merguiensis*. Jadi, lebih banyak kajian perlu dilakukan dengan menggunakan sejumlah besar gamet yang berkualiti, yang sesuai dengan medium persenyawaan untuk persenyawaan tabung uji, induksi kejutan yang berlainan bagi penghasilan ginogenetik *F. merguiensis* pada masa akan dating.