Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 10 (6): 512-522, 2015
ISSN 1816-4927 / DOI: 10.3923/jfas.2015.512.522
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In vitro Fertilization Technique in Banana Shrimp, *Fenneropenaeus merguiensis* (De Man, 1888)

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ABSTRACT

The aim of the study was to develop an appropriate basis for the optimization of *in vitro* fertilization of *Fenneropenaeus merguiensis* using three different culture medium including Natural Sea Water (NSW) as control medium, Artificial Sea Water (ASW) and Calcium Free saline (Ca-F saline). The unfertilized mature eggs were collected from the broodstock ovaries during spawning. The non-motile sperm of *F. merguiensis* activated as natural spawning. In NSW medium, ASW medium and Ca-F saline medium, cortical rods were released and hatching envelope formation took place in which the eggs activation events were reported. The Ca-F saline and ASW solution induced a slow egg activation contradict with the sequence of event for natural spawning of *F. merguiensis*. Fertilization was successfully obtained in all treatments with 8.67±4.04% in ASW, 19.67±7.38 and 4.33±4.04 in Ca-F saline. Although, the hatching rate were not successfully obtained by ASW and NSW culture medium treatment, hatching yield in Ca-F saline medium was obtained with 3.00±2.65. Overall, these findings will contribute to the development of *F. merguiensis* breeding technology and further understanding on sperm biology, cryobiology and reproductive biology in shrimp.

Key words: *Fenneropenaeus merguiensis*, *in vitro* fertilization, egg activation, fertilization, hatching rate

INTRODUCTION

Penaeid species are gonochoric and presently external fertilization (Malecha and Hedgecock, 1989) can be established by a few breeding techniques such as *in vitro* Fertilization (IVF) and artificial insemination. Now-a-days, the aquaculture sector is going through a delicate situation and improvements in some issues with regards to whether or not IVF should be carried out (Gallego *et al*., 2012). Manipulation of penaeid gametes was first reported by Clark *et al*. (1973), who performed IVF in brown shrimp, *Penaeus aztecus*. Then, of the IVF study on *F. merguiensis* also had been done by Nair (1987) using natural sea water as fertilization medium. Study by Browdy (1998) showed 2.48% fertilization occurred in *Litopenaeus setiferus* and 3.88% for *L. vannamei*.

It is well known that marine species that release gametes into the water rely on sperm-egg surface recognition events to mediate fertilization (Galindo *et al*., 2003). This event is known as egg activation. Egg activation in penaeid shrimps depends on sea water contact (Clark *et al*., 1980; Pillai and Clark Jr., 1988). The morphological changes involved in activation of decapods eggs had been described in many crustaceans *Homarus americanus* and *H. gammarus, Tranchypenaeus similes*, *Sicyonia ingentis*, *P. japonicus*, *P. monocerus*, *P. monodon*, *Farfantepenaeus aztecus* and *Litopenaeus setiferus* (Pongtippatee-Taweepreda *et al*., 2004).

A study shown that in zebra fish (*Danio rerio*), their egg's activation was influenced by the spawning medium (Hart and Yu, 1980; Hart and Fluck, 1995; Westerfield, 1995). It was found that the use of Natural Sea Water (NSW) at 21-22°C improved the interaction between an intact vital line envelope and sperm cells in the open the lycum shrimp, *Litopenaeus* (Rojas and Alfaro, 2007).

A better understanding of egg activation and *In vitro* Fertilization (IVF) technique is required to improve breeding programs in *F. merguiensis*. In this study, *F. merguiensis* was chosen due to its important commercial value in the Indo-Pacific region (Hoang *et al*., 2002). The large scale and high density of *P. monodon* and *L. vannamei* farming had caused the increased of disease outbreak (Chansela *et al*., 2012) and now more attention should be given to alternative species, *F. merguiensis* which is also favoured by consumers (Phongdara *et al*., 1999; Hoang *et al*., 2002).

This study was able to develop the IVF technique in *F. merguiensis* using different fertilization medium of (1) Natural sea water, (2) Artificial sea water and (3) Calcium free saline. The success of this IVF was assessed through (1) Egg activation, (2) Fertilization rate and (3) Hatching rate of IVF eggs.

MATERIALS AND METHODS

Preparation of fertilization media for IVF: The study used three different fertilization medium, UV-treated Natural Sea Water (NSW), Artificial Sea Water (ASW) and Calcium Free saline (Ca-F saline). The NSW was obtained from the hatchery NSW supply. The NSW was treated with UV prior to the experimentation to avoid any infections such as bacteria or parasite during the incubation of the fertilized eggs. In this study, NSW acted as the control medium to compare the egg activation events that occur naturally in sea water with ASW and Ca-F saline solution. The major ions means that those elements in sea water concentration larger than 1 ppm. Therefore, the major ions could be defined as the ions that significantly contribute to the salinity of sea water (Murray, 2004). There are 11 major ions according to the table above that represent the content of natural sea water that was used in this study (Dittmar, 1884). The ASW pH 8.0 was prepared according to Cavanaugh (1956) and Rojas and Alfaro (2007), with the following composition in gram per liter, 24.72 g NaCl, 0.67 g KCl, 1.03 g $CaCl_2$, 4.66 g $MgCl_2 \cdot 6(H_2O)$ and 0.18 g $NaHCO_3$. Ca-F saline pH 7.6 was prepared with the following composition: 21.63 g NaCl, 1.12 g KCl, 0.53 g H_3BO_3, 0.19 g NaOH, 4.93 g $MgSO_4 \cdot 7H_2O$ in 1 L sterile distilled water (adjusted water pH 7.4 with 1 N HCl) (Vuthiphandchai *et al*., 2007; Memon *et al*., 2012a, b). The Ca-F saline is also used as a good extender for other crustacean sperms such as orange mud crab (*Scylla olivacea*) and mud spiny lobster (*Panulirus polyphagus*) (Muhd-Farouk *et al*., 2014; Fatihah *et al*., 2014).

Shrimp handling and gametes collection for natural spawning: Matured and ready to spawn females (n = 40) of *F. merguiensis*, were collected from coastal water of Kedah, Malaysia (5°39"N, 100°19"E) by trawling net then were brought to the PulauSayak Fisheries Research Institute (FRI).

All matured females were kept in broodstock tank. Only healthy female without injury were selected. Oocytes were obtained by natural ovulation after isolating individual females in spawning tanks with 30 L of filtered and UV-treated Natural Sea Water (NSW) at 30°C.

Ten matured females were selected for natural spawning. Spawning activities were determined by the behaviour of the females indicated by the backward and forward movement in the tank as well as vigorous legs movement. At this moment, eggs were released slowly. Samples (100 mL) of spawn eggs were collected from the tank after 15, 30, 45 and 60 min and were fixed with 5% formalin for embryonic development observation under light microscope until hatching took place.

Fertilization rate was calculated by determining the number of fertilized eggs and unfertilized eggs. To determine the fertilization rate, the number of fertilized eggs were counted and divided by total eggs in treatment. To determine the hatching rate, the number of nauplii were counted and divided by total numbers of fertilize eggs.

In vitro model for primary binding

Fertilization and hatching trials: Spermatophores were removed with forceps (Memon *et al*., 2012a). The sperms were obtained by gentle grinding of the spermatophores using mortar and pastel in 1 mL Calcium Free (Ca-F) saline until sperms suspension was released. The sperms suspension was transferred to a 3 cm diameter glass petri dish. Sixty attempts had been done for IVF experiments. Nearly spawning female (n = 30) of ripe stage (Stage IV) of ovarian maturation stage were transferred into spawning tank and observed for spawning activity.

Observation for spawning activity was done every 5 min. Females that started spawning were collected immediately and placed on a dissecting tray without anesthetic to obtain the unfertilized eggs in the ovary. The carapace was cut opened, exposing the ripe, green ovary that was cut into pieces. The teased ovary portions were agitated in sea water to release ova or eggs. Within 5 min, the individual eggs were collected and place into a disinfected, clean Petri dish and covered with different fertilization medium: NSW, ASW and Ca-F saline. Thick sperms suspension (20,000 sperm mL^{-1}) were added to 2,000 eggs. Petri dish containing eggs were then shaken vigorously for about five minutes to allow eggs-sperm fertilization. The eggs were then transferred into incubation aquarium containing 6 L UV-treated NSW. The sequence of changes in eggs was evaluated under light microscopy with 10X magnification. The egg development observation was done until hatching occurred.

Fertilization rate was calculated by counting the number of fertilized eggs and unfertilized eggs. To determine the fertilization rate, the number of fertilized eggs were counted and divided by total eggs in treatment. Hatching rate was counted by counting the number hatched larvae and non-hatched larvae after 12-16 h of treatments. To determine the hatching rate, the number of nauplii were counted and divided by total numbers of eggs in treatment.

Experimental design: The summary of the experiment design work flows of the present study are as described in the Fig. 1.

Statistical analysis: One-way analysis of variance (ANOVA) was used to determine the significance between the fertilization and hatching rate. Significant differences were detected using the Tukey's multiple range test ($p<0.05$).

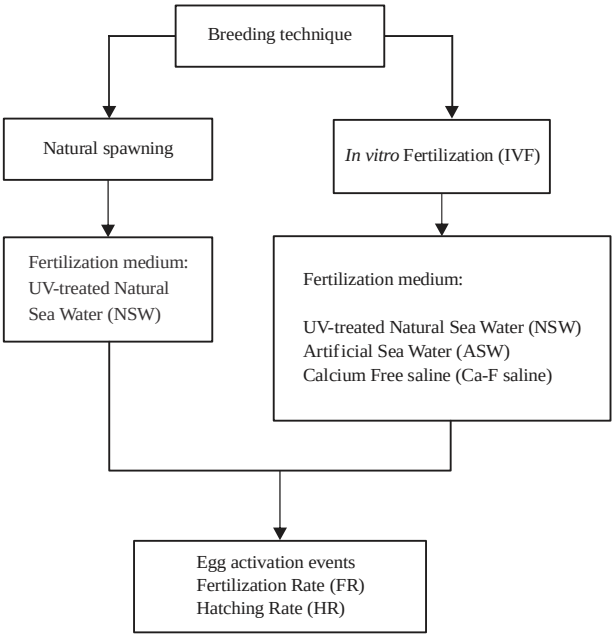


Fig. 1: Experimental design work flow summary of the present study to develop the *in vitro* fertilization technique in *Fenneropenaeus merguiensis*

RESULT

Egg activation of *Fenneropenaeus merguiensis* from natural spawning: Unreacted eggs of *F. merguiensis* were observed after spawning. The sequence of events in egg development from the time of spawning to the first mitotic stage were visualized under stereozoom microscope on the prepared mount of the egg. The eggs were not absolutely round shape and a bit elongated. Table 1 shows the sequence of egg activation took place in *F. merguiensis* during natural spawning. The formation of hatching envelope begins at early 10 min after spawning and thick hatching envelope was fully developed at 20 min after spawning.

Egg activation of *Fenneropenaeus merguiensis* from *in vitro* fertilization: Table 2 shows the egg activation for IVF of *F. merguiensis* using three different fertilization medium. However, the timing was not similar between natural spawning and IVF. Spawning eggs of IVF trials induced a slow release of cortical rods as well as slow jelly dissipation and hatching envelope formation. In NSW medium, the formation of hatching envelope started 30 min after spawning time. Meanwhile, in ASW and Ca-F saline medium, the formation of hatching envelope begins at 40-45 min after spawning due to Ca^{2+} ion action within the fertilization medium in ASW and in nursery tank of Ca-F saline treatment. Within 60 min, eggs cell cleavage started in all treatments until hatched.

Fertilization and hatching rates: Natural spawning showed the highest percentage of fertilization rate which was 98.33±1.28%, while the percentage of the nauplii hatched was 60.53±45.67% (Table 3). For IVF, NSW recorded the highest fertilization rate among the three

mediums which is 60.53±45.67% followed by ASW, 8.67±4.04% and Ca-F saline which is 4.33±4.04%. However, the only Ca-F saline medium was able to produce nauplii 3.0±2.65% hatching rate.

Table 1: Egg activation events in *Fenneropenaeus merguiensis* for natural spawning

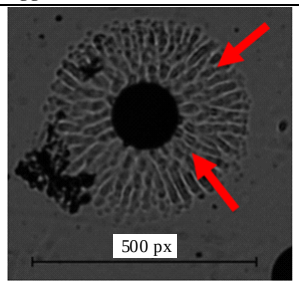
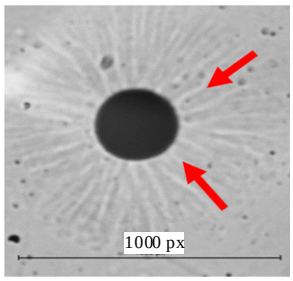
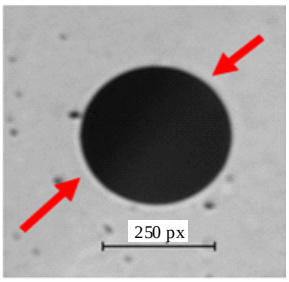
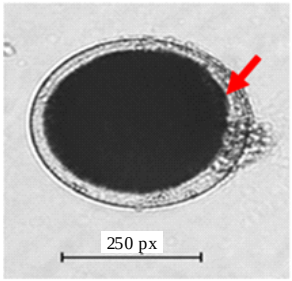
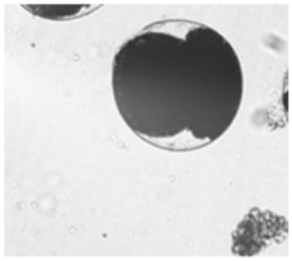
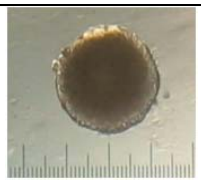
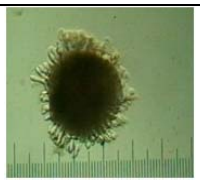
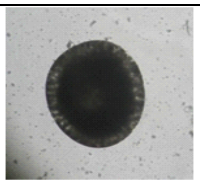
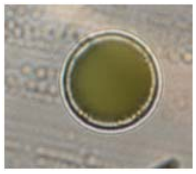
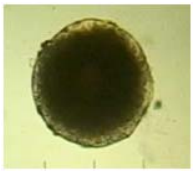
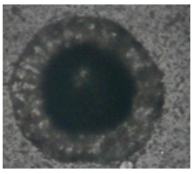
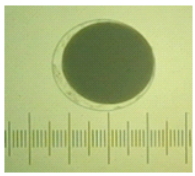
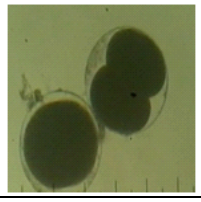
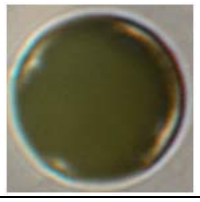
Time (min)	Egg activation	Description of egg stage
1		Formation of corona (arrow) around the egg and become dissipated for about 45-60 sec after natural sea water contact
5		Cortical rods begin to disappear, followed by the formation of jelly coat stage (arrow)
10		A transparent hatching envelope (arrow) begins to form around the egg surface
20		A thick hatching envelope was fully developed During this phase, the second polar body was extruded closed to the first polar body
60		First mitotic division took place at 60 min of post-spawning First cell cleavage formation

Table 2: Egg activation events and changes for *in-vitro* fertilization of *Fenneropenaeus merguiensis* using three different fertilization medium

Egg activation Time (min)	Fertilization medium (treatment) NSW	ASW	Ca-F saline	Description of egg activation
15				Formation of cortical crypts in treatment Ca-F saline occur faster than in treatment NSW Whereby, cortical rods begin to emerge from the eggs
30				Presence of natural Ca^{2+} ion in NSW, assist faster eggs activation performance compared to ASW which consist artificial salt water However, in Ca-F saline, absence of Ca^{2+} ion slowed down the eggs activation
45				Egg activation sequence in Ca-F saline treatment occurred faster when Ca^{2+} ion were obtained from culture water
60				Within 60 min, the eggs cells started to divide and undergo embryonic development until hatching

NSW: Natural sea water, ASW: Artificial sea water, Ca-F saline: Calcium free saline solution

Table 3: Fertilization and hatching of natural spawning and *in vitro* fertilization of *Fenneropenaeus merguiensis*

Breeding techniques	Fertilization rate (%)	Hatching rate (%)
Natural spawning	98.33±1.28	60.53±45.67
IVF (NSW)	19.67±7.38	-
IVF (ASW)	8.67±4.04	-
IVF (Ca-F saline)	4.33±4.04	3.00±2.65

IVF: *In vitro* fertilization, NSW: Natural sea water, ASW: Artificial sea water

DISCUSSION

Egg activation of *Fenneropenaeus merguiensis* from natural spawning: The egg activation is the starting point of a developmental program leading to the formation of offsprings (Ciapa and Chiri, 2000). The timing of egg activation events in shrimps with closed and open the lycum followed the same sequence of changes after in contact with sea water (Rojas and Alfaro, 2007). The egg activation sequence involves the released of jelly precursor from the cortical crypts, transformation of the precursor material into a jelly layer, exocytosis of cortical vesicles and formation of hatching envelope (Lynn *et al*., 1992).

In *F. merguiensis*, the egg activation comprised of the following sequence of events (1) Unreacted egg, (2) Cortical rod extrusion and (3) Formation of hatching envelope. Once the eggs were in contact with sea water surface, the eggs were unreacted and not in a round shape. Many round pits or cortical crypts appeared on the eggs surface. Within the egg cytoplasm, the cortical rods are packed in the cortical crypts, which are located in the periphery (Ciapa and Chiri, 2000).

Cortical rods expelled and form corona around the eggs. The cortical rod was composed of numerous, tightly packed, bottle-brush like elements embedded within the electron-dense matrix (Pongtippatee-Taweepreda *et al*., 2004). The bottle-brush structure in the cortical rods of *P. monodon* has also been observed in other crustacean species of penaeid shrimp (Duronslet *et al*., 1975; Clark *et al*., 1990) and American lobster (*Homarus* sp.) (Talbot and Goudeau, 1988).

According to Pongtippatee-Taweepreda *et al*. (2004), sperm penetration into eggs occurred up to 1 min post-spawning in *P. monodon* and once the thick hatching envelope had developed, other sperm penetration were not allowed. Formation of additional embryonic development are formed after fertilization as a protective covering for developing embryo, in lobster (Talbot and Goudeau, 1988), penaeid shrimp (Lynn *et al*., 1993) and palaemonid shrimp (Glas *et al*., 1997).

This study showed that *F. merguiensis* as well as *P. monodon* have a hatching envelope that is similarly strengthened by cortical vesicular contents and eventually released from the egg surface. However, the timing of the event is the only difference where the hatching envelope in *F. merguiensis* were completed within 20 min while in *P. monodon*, within 15 min.

Egg activation of *Fenneropenaeus merguiensis* from *in vitro* fertilization: Recently, remarkable achievement has been achieved in reproduction of penaeid shrimp under laboratory including artificial insemination and IVF (Browdy, 1998; Pongtippatee-Taweepreda *et al*., 2004; Rojas and Alfaro, 2007). During natural spawning, the female appears to release eggs while the seminal plasm containing sperm simultaneously and vigorously mixing the two with her pleopods (Clark *et al*., 1973). In developing IVF technique, the present study has attempted to spare this mixing action.

In this study, the events of egg activation were similar for both natural spawning and IVF technique but not the timing Fertilization process were much slower in IVF compared to natural spawning. The present study found that dissecting the ovaries from fully mature females (Stage IV) produced non-ovulated oocytes, thus showing small amount of binding pattern for sperms and eggs. According to Rojas and Alfaro (2007), the dissection of ovarian tissue at the time of spawning which was released individually after rupturing the tissue with fine tweezers, allowed the expulsion of cortical rods to take place in a normal way. Similar technique was applied during this experiment by taking out the ovary tissue at the time of spawning. Under the light microscope, sperm cells were observed in the surrounding environment but not bound to the oocyte surface (Rojas and Alfaro, 2007).

The Mg^{2+} and Na^{+} (naturally found in sea water) have been shown to be required for egg activation of the marine shrimp (*Sicyonia ingentis*) and starfish (*Asterina pectinifera*), respectively (Lindsay *et al*., 1992; Harada *et al*., 2003). In Penaeid shrimp, the cortical rods is initiated once the eggs are in contact with sea water requiring only external Mg^{2+} concentration when it is fertilized (Pillai and Clark Jr., 1987) thus, supporting the fast activity of IVF using NSW medium in this study. Stimulation by Mg^{2+} may lead to the changes in electrical properties on egg membrane (Lindsay and Clark Jr., 1994). The Ca^{2+} and Mg^{2+} ions have the ability to induce membrane fusion or adhesion (Ahkong *et al*., 1975; Ohki and Ohshima, 1985; Takeda and Kasamo, 2002). Study by Yasui *et al*. (2012), demonstrated that addition the of Ca^{2+}, Mg^{2+} and DMSO affected sperm motility. Ca^{2+} had been reported to exhibit negative effects upon the sperm of some fish species (Christen *et al*., 1987; Cosson *et al*., 1991).

Some of the first evidence suggested that Ca^{2+} might be required for fertilization in fish eggs as reported by Yamamoto (1944) when he demonstrated in medaka fish, *Oryzias latipes* and IVF

did not occur in eggs that were bathed in Ca^{2+} free Ringer's solution. He showed that the sperm still keep their swimming activity in Ca^{2+} free medium and thus proposed that Ca^{2+} is required for some other feature of fertilization, such as the fusion of the sperm with the eggs cortex (Yamamoto, 1944). The same situation occurred in egg activation of Ca-F saline where the eggs were activated in the absence of Ca^{2+} during sperm-egg fertilization activity. Ca^{2+} content were already available in the eggs. The intracellular Ca^{2+} in unfertilized eggs may help with the fertilization process in Ca-F saline solution. Once the eggs were transferred into the incubation tank, the eggs will obtained the Ca^{2+} from extracellular environment of natural sea water in the incubation tank. Cortical granules are situated in the cytoplasm of unfertilized eggs in many species (Webb and Miller, 2013). Theses cortical granules will be released into the interchorionic space (Vacquier, 1976; Schalkoff and Hart, 1986). The Ca^{2+} might diffuse out of the chorion in the time taken (~30 min) between obtaining the unfertilized eggs and performing the IVF experiments (Yamamoto, 1944) thus in this situation, successful fertilization required external Ca^{2+}.

Fertilization and hatching rates: Success or failure of fertilization is associated with the molecular fitness of the receptor-ligand presents on the gametes (Hirohashi *et al*., 2008). Result shows that the most suitable fertilization medium for the eggs of *F. merguiensis* was natural sea water as the number of eggs fertilized over the total of egg samples was much higher compared to ASW and Ca-F saline medium with 60.53±45.67% fertilization rate. This shows that the chemicals component in NSW triggered the egg activation as soon as the eggs were released into the sea water and contribute to the early stage of egg development. However, Ca-F saline solution were able to produce nauplii.

The delay in Ca-F saline treatment did not influence the egg activation which they were capable to continue the egg activation sequence until developing hatching envelope. Hatched nauplii were found (<5%) actively swimming after 16 h of Ca-F saline treatment. The present study was able to obtained nauplii and zoea larvae at the end of the experiment.

In marine species which transmit gametes into water depend on sperm-egg surface recognition events to mediate fertilization (Galindo *et al*., 2003). The use of high quality gametes both from males and females during this process is essential in order to achieve suitable fertilization and hatching rates for both aquaculture and scientific purposes (Gallego *et al*., 2012). Spermatozoa have great influence both on fertilization success and the subsequent development of the embryo and larvae (Butts *et al*., 2011; Ottesen and Babiak, 2007). Sperm-egg ratio is also important to optimize fertilization and hatching rate. The sperm suspensions used in this study (20,000 sperm mL^{-1}) were added to 2,000 eggs for each treatment. Excess sperm sticking to the chorion of adhesive eggs can also serve as a substrate for microbial activity.

CONCLUSION

The fertilization of the treatments showed the present study was able to develop the basic protocols for *in vitro* fertilization technique for *F. merguiensis* under controlled experimental conditions and the best medium is Natural Sea Water (NSW) treated with ultraviolet. The NSW showed that, it had the fastest egg activation of *F. merguiensis* compared to artificial sea water and calcium free saline. Knowledge of the chemical nature of the culture medium for the egg activation will be very important in understanding to improve the production of this economically important species. The present study served as a basis for improved efficiency in broodstock management of further steps in perfecting the *in vitro* techniques in the future for shrimp hatchery productions.

ACKNOWLEDGMENTS

The authors would like to thank Institute of Tropical Aquaculture Hatchery of Universiti Malaysia Terengganu, Malaysia and Fisheries Research Institute, PulauSayak, Department of Fisheries Malaysia for their facilities. This study was supported by Exploratory Research Grant Scheme (ERGS) ERGS/1/2013/STG07/UMT/02/01 from the Ministry of Education of Malaysia.

REFERENCES

Ahkong, Q.F., W. Tampion and J.A. Lucy, 1975. Promotion of cell fusion by divalent cation ionophores. Nature, 256: 208-209.

Browdy, C.L., 1998. Recent developments in penaeid broodstock and seed production technologies: Improving the outlook for superior captive stocks. Aquaculture, 164: 3-21.

Butts, I.A.E., I. Babiak, A. Ciereszko, M.K. Litvak, M. Slowinska, C. Soler and E.A. Trippel, 2011. Semen characteristics and their ability to predict sperm cryopreservation potential of Atlantic cod, *Gadus morhua* L. Theriogenelogy, 75: 1290-1300.

Cavanaugh, G.M., 1956. Formulae and Methods of the Marine Biological Laboratory Chemical Room: Marine Biological Laboratory. Woods Hole, Massachusetts, USA.

Chansela, P., N. Goto-Inoue, N. Zaima, T. Hayasaka and M. Sroyraya *et al*., 2012. Composition and localization of lipids in *Penaeus merguiensis* ovaries during the ovarian maturation cycle as revealed by imaging mass spectrometry. PLoS One, Vol. 7. 10.1371/journal.pone.0033154

Christen, R., J.L. Gatti and R. Billard, 1987. Trout sperm motility: The transient movement of trout sperm is related to changes in the concentration of ATP following the activation of the flagellar movement. Eur. J. Biochem., 166: 667-671.

Ciapa, B. and S. Chiri, 2000. Egg activation: Upstream of the fertilization calcium signal. Biol. Cell, 92: 215-233.

Clark, Jr. W.H., P. Talbot, R.A. Neal, C.R. Mock and B.R. Salser, 1973. *In vitro* fertilization with non-motile spermatozoa of the brown shrimp *Penaeus aztecus*. Mar. Biol., 22: 353-354.

Clark, Jr. W.H., J.W. Lynn, A.L. Yudin and H.O. Persyn, 1980. Morphology of the cortical reaction in the eggs of *Penaeus aztecus*. Biol. Bull., 158: 175-186.

Clark, Jr. W.H., A.I. Yudin, J.W. Lynn, F.J. Griffin and M.C. Pillai, 1990. Jelly layer formation in penaeoidean shrimp eggs. Biol. Bull., 178: 295-299.

Cosson, M.P., J. Cosson and R. Billard, 1991. Synchronous triggering of trout sperm is followed by an invariable set sequence of movement parameters whatever the incubation medium. Cell Motility Cytoskeleton, 20: 55-68.

Dittmar, W., 1884. Report on researches into the composition of ocean-water, collected by H.M.S. Challenger: During the years 1873-1876. H.M. Stationery Office, New Scotland, pp: 251.

Duronslet, M.J., A.I. Yudin, R.S. Wheeler and W.H. Clark Jr., 1975. Light and fine structural studies of natural and artificially induced egg growth of penaeid shrimp. Proc. Ann. Meet. World Maricult. Soc., 6: 103-122.

Fatihah, S.N., J. Safiah, A.B. Abol-Munafi and M. Ikhwanuddin, 2014. Effect of 17α-hydroxyprogesterone and 17α-hydroxypregnenolone on sperm quality and sperm quantity in male mud spiny lobster (*Panulirus polyphagus*). Pak. J. Biol. Sci., 17: 1124-1129.

Galindo, B.E., V.D. Vacquier and W.J. Swanson, 2003. Positive selection in the egg receptor for abalone sperm lysin. Proc. Natl. Acad. Sci., 100: 4639-4643.

Gallego, V., I. Mazzeo, M.C. Vilchez, D.S. Penaranda, P.C.F. Carneiro, L. Perez and J.F. Asturiano, 2012. Study of the effects of thermal regime and alternative hormonal treatments on the reproductive performance of European eel males (*Anguilla anguilla*) during induced sexual maturation. Aquaculture, 354-355: 7-16.

Glas, P.S., L.A. Courtney, J.R. Rayburn and W.S. Fisher, 1997. Embryonic coat of the grass shrimp *Palaemonetes pugio*. Biol. Bull., 192: 231-242.

Harada, K., E. Oita and K. Chiba, 2003. Metaphase I arrest of starfish oocytes induced via the MAP kinase pathway is released by an increase of intracellular pH. Development, 130: 4581-4586.

Hart, N.H. and R.A. Fluck, 1995. Cytoskeleton in teleost eggs and early embryos: Contributions to cytoarchitecture and motile events. Curr. Top. Dev. Biol., 31: 343-381.

Hart, N.H. and S.F. Yu, 1980. Cortical granule exocytosis and cell surface reorganization in eggs of *Brachydanio*. J. Exp. Zool., 213: 137-159.

Hirohashi, N., N. Kamel, H. Kubo, H. Sawada, M. Matsumoto and M. Hoshi, 2008. Egg and sperm recognition systems during fertilization. Dev. Growth Differentiat., 50: S221-S238.

Hoang, T., S.Y. Lee, C.P. Keenan and G.E. Marsden, 2002. Effect of temperature on spawning of *Fenneropenaeus merguiensis*. J. Thermal Biol., 27: 433-437.

Lindsay, L.L. and W.H. Clark Jr., 1994. Signal transduction during shrimp oocyte activation by extracellular Mg^{2+}: Roles of inositol 1,4,5- trisphosphate, tyrosine kinases and G-proteins. Development, 120: 3463-3472.

Lindsay, L.L., P.L. Hertzler and W.H. Clark Jr., 1992. Extracellular Mg^{2+} induces an intracellular Ca^{2+} wave during oocyte activation in the marine shrimp *Sicyonia ingentis*. Dev. Biol., 152: 94-102.

Lynn, J.W., P.S. Glas and J.D. Green, 1992. Assembly of the hatching envelope around the eggs of *Trachypenaeus similis* and *Sicyonia ingentis* in a low sodium environment. Biol. Bull., 183: 84-93.

Lynn, J.W., P.S. Glas, Q. Lin and J.D. Green, 1993. Assembly of extracellular envelopes around the eggs and embryos of the marine shrimp, *Sicyoni ingentis*. J. Reprod. Dev., 39: 90-91.

Malecha, S.R. and D. Hedgecock, 1989. Prospects for domestication and breeding of marine shrimp. Sea Grant Technical Report No. UNIHI-SEAGRANT-TR-89-01, University of Hawaii Sea Grant College Program, Honolulu, USA.

Memon, A.J., A.D. Talpur, M.I. Khan, M.O. Fariddudin, J. Safiah, A.B. Abol-Munafi and M. Ikhwanuddin, 2012a. Morphology of fresh and cryopreserved sperms of banana shrimp *Penaeus merguiensis* (De Man, 1888). Res. J. Appl. Sci., 7: 54-59.

Memon, A.J., A.D. Talpur, M.I. Khan, M.O. Fariddudin, J. Safiah, A.B. Abol-Munafi and M. Ikhwanuddin, 2012b. Optimization of spermatophores cryopreservation protocol of banana shrimp (*Penaeus merguiensis*). J. Anim. Vet. Adv., 11: 1688-1704.

Muhd-Farouk, H., A.B. Abol-Munafi, S. Jasmani and M. Ikhwanuddin, 2014. Effect of steroid hormones 17α-hydroxyprogesterone and 17α-hydroxypregnenolone on ovary external morphology of orange mud crab, *Scylla olivacea*. Asian J. Cell Biol., 9: 23-28.

Murray, J., 2004. Major ions of seawater. http://www.ocean.washington.edu/courses/oc400/Lecture_Notes/CHPT4.pdf

Nair, S.R.S., 1987. *In vitro* fertilization of banana prawn *Penaeus merguiensis* de man. Mahasagar: Bull. Natl. Inst. Oceanogr., 20: 187-190.

Ohki, S. and H. Ohshima, 1985. Divalent cation-induced phosphatidic acid membrane fusion. Effect of ion binding and membrane surface tension. Biochimica Biophysica Acta (BBA)-Biomembranes, 812: 147-154.

Ottesen, O.H. and I. Babiak, 2007. Parental effects on fertilization and hatching success and development of atlantic halibut (*Hippoglossus hippoglossus* L.) embryos and larvae. Theriogenology, 68: 1219-1227.

Phongdara, A., W. Chotigeat, A. Chandumpai, C. Tanthana and P. Duangtong, 1999. Identification of *Penaeus merguiensis* and *Penaeus indicus* by RAPD-PCR derived DNA markers. Sci. Asia, 25: 143-151.

Pillai, M.C. and W.H. Clark Jr., 1987. Oocyte activation in the marine shrimp, *Sicyonia ingentis*. J. Exp. Zool., 244: 325-329.

Pillai, M.C. and W.H. Clark Jr., 1988. Hatching envelope formation in shrimp (*Sicyonia ingentis*) ova: Origin and sequential exocytosis of cortical vesicles. Tissue Cell, 20: 941-952.

Pongtippatee-Taweepreda, P., J. Chavadej, P. Plodpai, B. Pratoomchart, P. Sobhon, W. Weerachatyanukul and B. Withyachumnarnkul, 2004. Egg activation in the black tiger shrimp *Penaeus monodon*. Aquaculture, 234: 183-198.

Rojas, E. and J. Alfaro, 2007. *In vitro* manipulation of egg activation in the open thelycum shrimp *Litopenaeus*. Aquaculture, 264: 469-474.

Schalkoff, M.E. and N.H. Hart, 1986. Effects of A23187 upon cortical granule exocytosis in eggs of *Brachydanio*. Roux's Arch. Dev. Biol., 195: 39-48.

Takeda, Y. and K. Kasamo, 2002. *In vitro* fusion of plant golgi membranes can be influenced by divalent cations. J. Biol. Chem., 277: 47756-47764.

Talbot, P. and M. Goudeau, 1988. A complex cortical reaction leads to formation of the fertilization envelope in the lobster, *Homarus*. Gamete Res., 19: 1-18.

Vacquier, V.D., 1976. Isolated cortical granules: A model system for studying membrane fusion and calcium-mediated exocytosis. J. Dupramolecular Struct., 5: 27-35.

Vuthiphandchai, V., S. Nimrat, S. Kotcharat and A.N. Bart, 2007. Development of a cryopreservation protocol for long-term storage of black tiger shrimp (*Penaeus monodon*) spermatophores. Theriogenology, 68: 1192-1199.

Webb, S.E. and A.L. Miller, 2013. Ca^{2+} signaling during activation and fertilization in the eggs of teleost fish. Cell Calcium, 53: 24-31.

Westerfield, M., 1995. The Zebrafish Book: A Guide for the Laboratory use of Zebrafish (Danio Rerio). University of Oregon Press, Eugene, OR, Pages: 385.

Yamamoto, T., 1944. Physiological studies on fertilization and activation of fish eggs. I. Response of the cortical layer of the egg of *Oryzias latipes* to insemination and to arti-ficial stimulation. Annotationes Zool. Jap., 22: 109-125.

Yasui, G.S., T. Fujimoto, L. Arias-Rodriguez, Y. Takagi and K. Arai, 2012. The effect of ions and cryoprotectants upon sperm motility and fertilization success in the loach *Misgurnus anguillicaudatus*. Aquaculture, 344-349: 147-152.