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Effect of 17α-Hydroxyprogesterone and 17α-Hydroxypregnenolone on Sperm Quality and Sperm Quantity in Male Mud Spiny Lobster (*Panulirus polyphagus*)

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Abstract: The present study aimed to determine the effect of 17α-hydroxyprogesterone (17α-OHP) and 17α-hydroxypregnenolone (17α-OHPL) on sperm quality and sperm quantity in male mud spiny lobster (*Panulirus polyphagus*). The mean of sperm quality was increased in 17α-OHP and 17α-OHPL treated hormones. In 17α-OHP injected animals, the mean of sperm quantity of dose 0.01 $\mu g\ g^{-1}$ b.wt. was increased than 17α-OHPL. Meanwhile, 17α-OHP and 17α-OHPL concentrations were lower when injected with the hormones but 17α-OHP was higher at only day 15 (dose 0.01 and 0.1 $\mu g\ g^{-1}$ b.wt.). For 17α-OHPL, the hormone was a prohormone in the body of *P. polyphagus* and only required smaller to increase the sperm quantity. Besides, when the higher dose of 17α-OHPL (0.1 $\mu g\ g^{-1}$ b.wt.) was used in *P. polyphagus*, the development of *P. polyphagus* was inhibited and decreased the sperm quantity and 17α-OHPL concentration in hemolymph was lower. Injection of 17α-OHP in *P. polyphagus* has increased the sperm quality and quantity for both 17α-OHP dosage of 0.01 and 0.1 $\mu g\ g^{-1}$ b.wt. However, injection of 17α-OHPL in *P. polyphagus* has decreased the sperm quantity only, also for both dosage of 0.01 and 0.1 $\mu g\ g^{-1}$ b.wt. and lower on hormone concentration.

Key words: 17α-hydroxyprogesterone (17α-OHP), 17α-hydroxypregnenolone (17α-OHPL), mud spiny lobster, *Panulirus polyphagus*

INTRODUCTION

Spiny lobsters are amongst the largest-bodied and longest-lived present marine Arthropods (Wolff, 1978; Sheehy, 2001). Besides, spiny lobsters also make up a main part of the Niche Live Reef Fish Trade (LRFT) markets based in Hong Kong, Taiwan and Singapore. In Malaysia, the culture of spiny lobsters is still in its early years with activities based in the State of Sabah mostly concentrated in Lahad Datu, Semporna, Kudat and Kota Kinabalu. Spiny lobster culture occupied both grow-out of juveniles in marine cages and holding of adults in cement tanks and marine cages in the state of Sabah (Biusing and Lin, 2004). The major complexity in *P. polyphagus* aquaculture is the incapability of males and females to complete their life cycle under culture conditions. There are low survival rates in the larvae rearing of pueruli stage, difficult to maintain from pueruli until juvenile stages and very difficult to obtained the mature broodstock especially the male *P. polyphagus*. The accessibility of the mature males is important for induced mating trials in captivity or further trials on artificial insemination using ready sperm of *P. polyphagus*.

Vertebrates and invertebrate hormones with molecules that carry messages and their structures, have been quite conserved during development (Lafont, 1991). Vertebrate-type steroids have been documented in invertebrate groups including crustaceans (Lehoux and Sandor, 1970; Lafont, 1991; Cardoso *et al*., 1997). A number of steroids such as progesterone, 17α-hydroxyprogesterone (17α-OHP), 6β-hydroxyprogesterone, 20α-hydroxyprogesterone, pregnenolone, 17α-hydroxypregnenolone (17α-OHPL), 17β-estradiol, estrone, testosterone and others have been recognized in diverse crustaceans (Fingerman *et al*., 1993; Subramoniam, 2000; Tsukimura, 2001; Wilder *et al*., 2002; Oetken *et al*., 2004). In sea hare (*Aplysia depilans*), the steroids such as pregnenolone, dehydroepiandrosterone, progesterone, 17α-hydroxyprogesterone, testosterone, oestradiol and oestrone have been identified in the gonad while pregnenolone and 17α-hydroxypregnenolone have been identified in hepatic tissue (Lupo di Prisco *et al*., 1973).

Progesterone (P4) and its derivatives are sex steroid hormones that play main roles in gametogenesis (Fingerman *et al*., 1993; Rodriguez *et al*., 2002; Miura *et al*., 2006). Induced ovarian maturation and

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spawning in greasyback shrimp, (*Metapenaeus ensis*) by P4 and 17α-OHP have been shown by Yano (1985, 1987). Besides, pregnenolone (3β-Hydroxypregn-5-en-20-one) is an endogenous steroid hormone and also a prohormone that usually having minimal hormonal achieve by itself. The function of a prohormone was to develop the strength of the hormone that already occurs in the body. As we know, the metabolism and expected roles of other sex steroids such as pregnenolone was much less known which is commonly regarded as a steroid intermediate (Petkam *et al*., 2003).

17α-OHP and 17α-OHPL concentration of male *P. polyphagus* measured using ELISA. To ascertain vitellogenin (VTG) levels in crustaceans, there are a number of diverse techniques have been recognized including immunodiffusion (Quinitio *et al*., 1990; Tom *et al*., 1987), rocket immunoelectrophoresis (Yano, 1987) and ELISA (Sagi *et al*., 1999; Tsukimura *et al*., 2000; Vincent *et al*., 2001). As we know, an ELISA kit is a high sensitivity assay and has excellent specificity for detection. Furthermore, the advantage of the ELISA is the hemolymph samples can be assayed directly not with extraction or hormone improvement procedures (Chang *et al*., 1998).

There is no information on the roles of 17α-OHP and 17α-OHPL in male spiny lobster (*P. polyphagus*). Therefore, 17α-OHP and 17α-OHPL were used for enhancing sperm in mature male *P. polyphagus*. The objectives of the present study were to determine the effect of 17α-OHP and 17α-OHPL on the sperm quality and quantity and these two hormones concentrations in the hemolymph of *P. polyphagus*.

MATERIALS AND METHODS

Brood stocks: Total 25 healthy and sexually matured *P. polyphagus* male were used in the experiment. Total 10 males were treated with 17α-OHP, 10 males with 17α-OHPL and five males for control treatments. Each treatment used two fiberglass tanks (5 tonnes) for (1) Dose 0.01 $\mu g\ g^{-1}$ body weight (b.wt.) (5 males) and (2) Dose 0.1 $\mu g\ g^{-1}$ b.wt. (5 males) and one fiberglass tank (5 tonnes) were used for control treatment (5 males). One week acclimatization was adopted in order to reduce stress associated with handling and laboratory environment. The brood stocks were fed daily at 10% biomass with fresh squid (*Loligo* sp.).

Experimental designs: Male *P. polyphagus* was injected laterally in fifth abdominal segment with 0.5 mL dilution of pure hormone, 17α-OHP, 17α-OHPL and ethanol (control) at day 1, 8, 15, 22, 29 and 39. Hemolymph of *P. polyphagus* was taken every two weeks and analyzed using enzyme-linked immunosorbent assay (ELISA) kit (Cusabio Biotech Co., Ltd) to measure hormone level (Fatihah *et al*., 2013a, b, 2014). At day 42, hemolymph was taken and *P. polyphagus* was dissected to check sperms. Hormone concentrations were analysed using ELISA assay according to the manufacturer instruction.

Determination of live sperm: Sperm quality or viable sperm (percentages) were determined by counting the live sperm. Percentages of viable sperm were calculated by following equation:

$$\text{Percentage of live sperm} = \frac{\text{Observed No. of live sperm}}{\text{Total No. of sperm observed}} \times 100$$

Sperm quantity or sperm counts were conducted on a Neubauer hemocytometer. The surface of hemocytometer and coverslip was cleaned with 70% ethanol before used the hemacytometer. Total 5 square of hemocytometer was counted, averaged and divided 25 square of hemocytometer. The averaged sperm count in the 25-square grid (0.1 µL) was expressed as counts×10^4 cells/mL and divided the dilution of sperm:

$$\text{Sperm count (sperm mL}^{-1}) = \text{Average of total 5 square} \times 25\ (25\ \text{square}) \times 10^4\ \text{cells/mL} \times \text{Dilution of sperm}$$

Data analysis: Sperm quality, sperm quantity and hormone concentration were analyzed using one way ANOVA (Bonferroni) for the statistical evaluation of mean values. Significance was accepted at $p<0.05$.

RESULTS

There was 72.11±5.38% viable sperm in the control animals. In 17α-OHP injected group, the viable sperm were 89.72±2.91% in dose 0.01 $\mu g\ g^{-1}$ b.wt. and 90.30±2.96% in dose 0.1 $\mu g\ g^{-1}$ b.wt. While, in 17α-OHPL injected group were 75.34±6.03% in dose 0.01 $\mu g\ g^{-1}$ b.wt. and 83.79±2.50% in dose 0.1 $\mu g\ g^{-1}$ b.wt. (Fig. 1). The means of sperm quantity were 38.84×10^6 cells mL^{-1} (control), 49.71×10^6 cells mL^{-1} (dose 0.01 $\mu g\ g^{-1}$ b.wt. in 17α-OHP), 49.67×10^6 cells mL^{-1} (dose 0.1 $\mu g\ g^{-1}$ b.wt. in 17α-OHP), 35.93×10^6 cells mL^{-1} (dose 0.01 $\mu g\ g^{-1}$ b.wt. in 17α-OHPL) and 11×10^6 cells mL^{-1} (dose 0.1 $\mu g\ g^{-1}$ b.wt. in 17α-OHPL) (Fig. 2). The sperm quality for both hormones (17α-OHP and 17α-OHPL) were significant different ($p<0.05$) each others.

The sperm quantity of 17α-OHPL was significant different ($p<0.05$) while no significant difference ($p>0.05$)

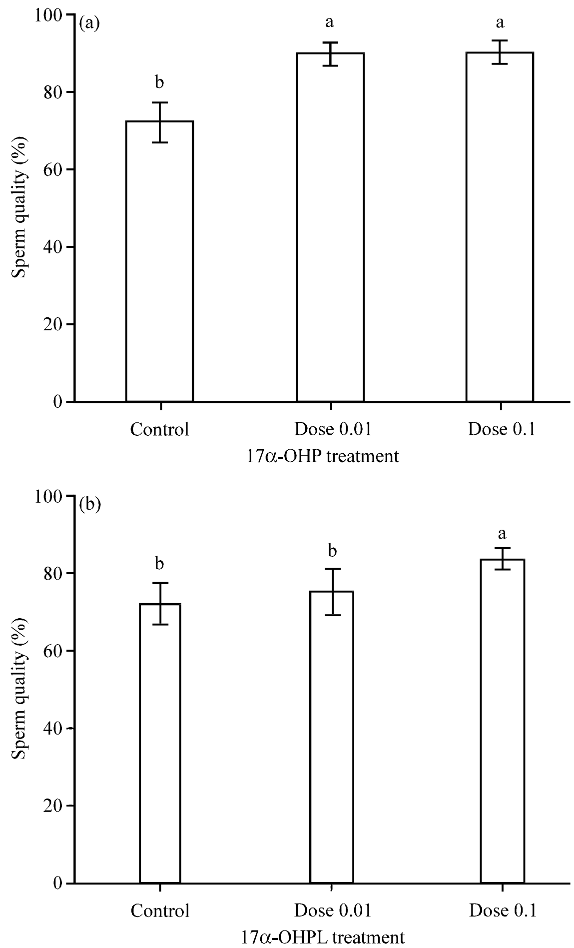

Fig. 1(a-b): Mean of sperm quality (%) (a) 17α-hydroxyprogesterone (17α-OHP) and (b) 17α-hydroxypregnenolone (17α-OHPL) injected group. Bars with different letters were significantly different ($p<0.05$)

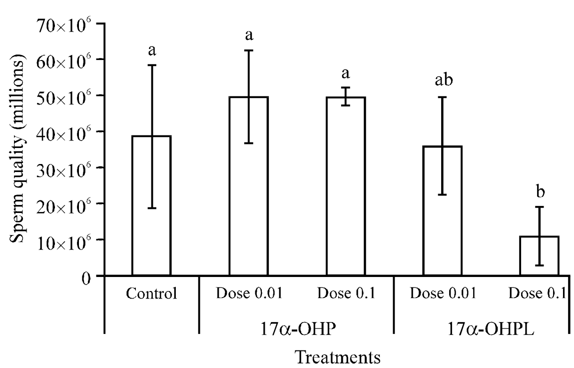

Fig. 2: Mean of sperm quantity for control, 17α-hydroxyprogesterone (17α-OHP) (dose 0.01 and 0.1) and 17α-hydroxypregnenolone (17α-OHPL) (dose 0.01 and 0.1)

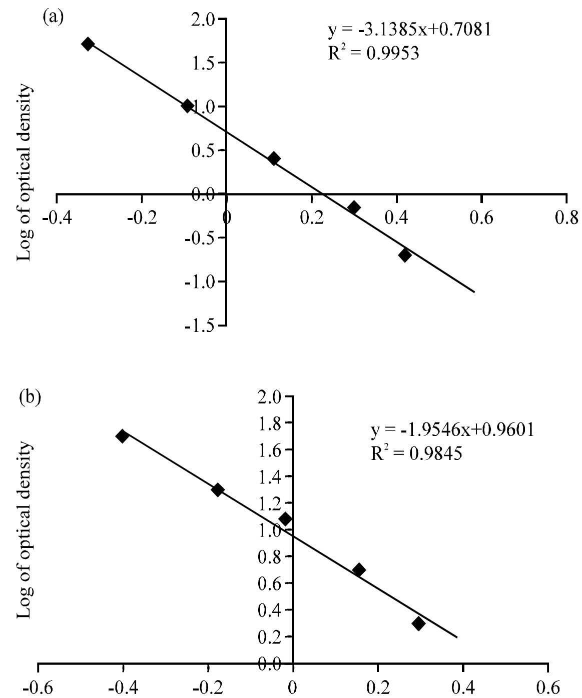

Fig. 3(a-b): ELISA standard curve (a) 17α-hydroxyprogesterone (17α-OHP) and (b) 17α-hydroxypregnenolone (17α-OHPL) concentration (ng mL^{-1})

in sperm quantity of 17α-OHP. In injection of 17α-OHPL, the sperm quantity in control treatment was higher than the dose 0.01 and 0.1 μg g^{-1} b.wt. Figure 3a and b shows a standard curve of 17α-OHP and 17α-OHPL, respectively. The highest of the means concentration of 17α-OHP was 72.84×10^{-4} ng mL^{-1} and the lowest of the mean concentration of 17α-OHP was 0.04×10^{-4} ng mL^{-1} (Fig. 4). The highest of the means concentration of 17α-OHPL was 111.39×10^{-4} ng mL^{-1} and the lowest of the means concentration of 17α-OHPL was 3.55×10^{-4} ng mL^{-1} (Fig. 5). The means control concentration of 17α-OHP was increased in day 1 until day 42 but for dose 0.01 and 0.1 μg g^{-1} b.wt., the means of concentration only increased in day 15. Besides, the concentration of 17α-OHPL in control animals were higher than dose 0.01 and 0.1 μg g^{-1} b.wt. every two weeks until day 42. The concentration of 17α-OHPL in dose 0.1 μg g^{-1} b.wt. was lower than dose 0.01 μg g^{-1} b.wt. There was no significantly different ($p>0.05$) in 17α-OHP concentration between control, dose 0.01 and 0.1 μg g^{-1} b.wt. However, there was significantly different ($p<0.05$) in the 17α-OHPL concentration between control, dose 0.01 and 0.1 μg g^{-1} b.wt.

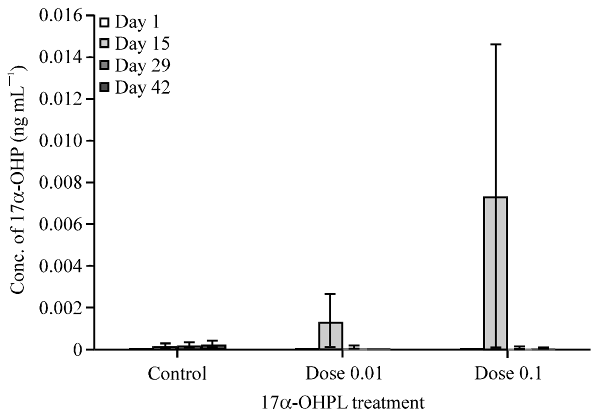

Fig. 4: Concentration of 17α-hydroxyprogesterone (17α-OHP) (ng mL^{-1}) in control, dose 0.01 and 0.1 μg g^{-1} b.wt. in days 1, 15, 29 and 42

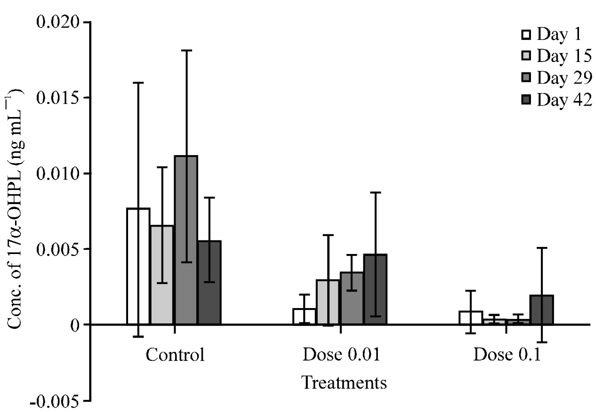

Fig. 5: Concentration of 17α-hydroxypregnenolone (17α-OHPL) in control, dose 0.01 and 0.1 μg g^{-1} b.wt. during the experiment of *P. polyphagus* at different culture periods

DISCUSSION

17α-OHP and 17α-OHPL are sex steroids that present in male *P. polyphagus*. In the earlier studies, 17α-OHP was injected in White shrimp (*Penaeus vannamei*) (Alfaro, 1996) and Black tiger shrimp (*Penaeus monodon*) (Yashiro *et al*., 1998) to check the sperm counts. Thus, 17α-OHP also was injected in *P. polyphagus* to check the sperm quality and quantity and hormone concentration in the present study. This was the first study that demonstrated 17α-OHP affected sperm quality, sperm quantity and hormone concentrations in male *P. polyphagus*. It was possible to inject of 17α-OHPL because 17α-OHPL concentration was higher than 17α-OHP in male *P. polyphagus*. Besides, there was some fact that 7α-OHPL may have activity as a neurohormone in male Japanese red-bellied newts, *Cynops pyrrhogaster*, an aquatic amphibian. Acts as a neuronal activator, 7α-hydroxypregnenolone stimulates locomotor activity of newts through the dopaminergic system (Matsunaga *et al*., 2004). Thus is also the first record of using 17α-OHPL on *P. polyphagus* or any other crustaceans.

In the present study, the sperm quality improved by injecting 17α-OHP and 17α-OHPL. The sperm quantity increased of both doses (0.01 and 0.1 μg g^{-1} in 17α-OHP) but not for 17α-OHPL. Besides, 17α-OHPL concentration in dose 0.1 μg g^{-1} b.wt. was lower than control treatment and with injection of dose 0.01 μg g^{-1} b.wt., because in *P. polyphagus* was less of 17α-OHPL improved to enhance the hormone concentration. When more doses of injection of 17α-OHPL, the hormone was inhibiting the development of *P. polyphagus* and instantaneously the hormone concentration was decreased. In dose 0.1 μg g^{-1} b.wt. of 17α-OHPL, there was more descending compared dose 0.01 μg g^{-1} b.wt. Because dose 0.1 μg g^{-1} b.wt. was higher and hinders the growth or development of *P. polyphagus*. The response in sperm quantity indicates no significant improvement ($p>0.05$) for the variable at dose 0.01 and 0.1 μg g^{-1} b.wt. from control treatment in 17α-OHP. From Alfaro (1996), there was no significant difference between dose 0.01 and 0.1 μg g^{-1} b.wt. of 17α-OHP in sperm count in *P. vannamei*.

In addition, the present results indicate the presence of 17α-OHP and 17α-OHPL concentration in hemolymph of male *P. polyphagus*. It was higher of 17α-OHPL concentration than 17α-OHP concentration in male *P. polyphagus*. Progesterone and 17β-estradiol have been initiate in the mandibular organ, kidney, ovary and hemolymph in American lobster, *Homarus americanus* with mandibular organ showing the uppermost concentration (Couch *et al*., 1987). Besides, *Panulirus homarus* show a positive relationship among hemolymph 17β-estradiol and progesterone levels and ovarian cycle (Kirubagaran *et al*., 2005).

Furthermore, 17α-OHP had no significant effect on hemolymph hormone concentration of *P. polyphagus* but 17α-OHPL had significant effect. There was no significant effect on hemolymph vitelogenin (Vg) levels of ridgeback shrimp, *Sicyonia ingentis* when injected of progesterone, 17α-HP and 17β-estradiol (Tsukimura *et al*., 2000). In greasyback shrimp (*Metapenaeus ensis*) (Yano, 1985) and sword prawn (*Parapenaeopsis hardwicki*) (Kulkarni *et al*., 1979), injection of progesterone into shrimp also accelerated ovarian development.

CONCLUSION

17α-OHP and 17α-OHPL has affected the sperm quality in the study. In addition, the sperm quantity was increased with injection of 17α-OHP in dose 0.01 μg g^{-1} b.wt. and dose 0.1 μg g^{-1} b.wt. but decreased in sperm quantity and hormone concentrations was lower with injection of 17α-OHPL. The hormone concentration of 17α-OHP was lower than 17α-OHPL. Treatment with 17α-OHP shows increased of sperm quality and quantity but, treatment without 17α-OHPL shows increased of sperm quantity and higher of hormone concentration. For the further study recommendation, higher of 17α-OHP and 17α-OHPL doses should be done to see whether there is any significant on the sperm quality and quantity and hormone concentration higher accordingly. Besides, the study period should be extending to produce better or positive results.

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