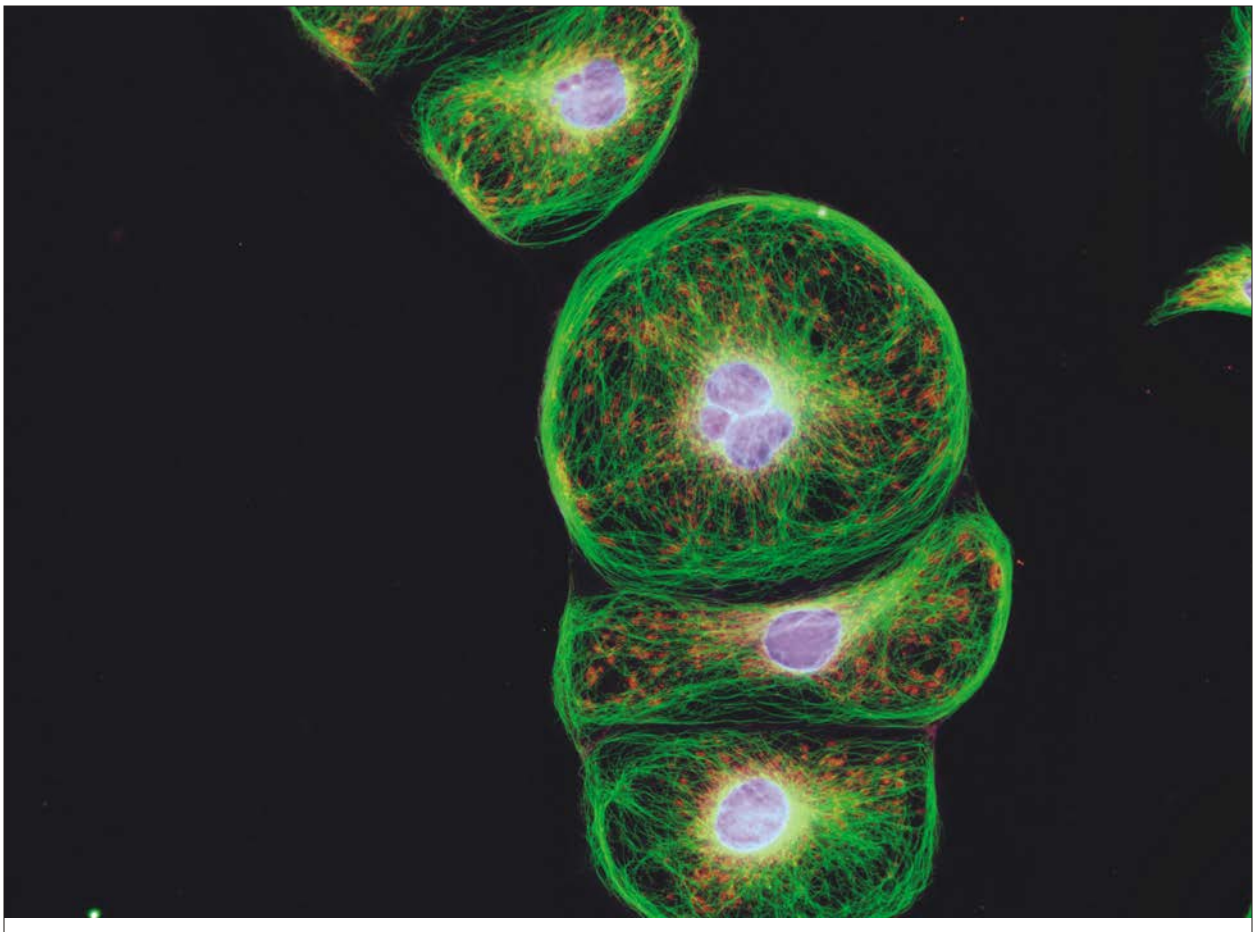

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Effect of Steroid Hormones 17α-Hydroxyprogesterone and 17α-Hydroxypregnenolone on Ovary External Morphology of Orange Mud Crab, *Scylla olivacea*

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ABSTRACT

Inducing ovary to mature using hormones known to be a great tool to enhance the production of larvae. This study shows the effect of reproductive steroid hormones, 17α-hydroxyprogesterone and 17α-hydroxypregnenolone with different doses on the ovarian maturation stages of orange mud crab, *Scylla olivacea*. This study period was 60 days. Total of 90 immature female orange mud crab *S. olivacea* were used in this study where 18 immature female *S. olivacea* acted as a control without the injection, while the other four treatments T1D1, T1D2, T2D1 and T2D2 each having 18 immature female *S. olivacea* and injected laterally in fifth abdominal segment with different dose of 17α-hydroxyprogesterone and 17α-hydroxypregnenolone with each hormone have two treatments with the dosage of 0.01 and 0.1 μg g^{-1} b.wt. at a day 0, 10, 20, 30, 40, 50 and 60th. Every ten days, 3 individual were sacrificed from the control and the treatment. The findings in this study indicate that 17α-hydroxyprogesterone and 17α-hydroxypregnenolone injection should be evaluating more dosage before being use as a practical way of improving ovarian development in commercial operations.

Key words: Steroid hormones, 17α-hydroxyprogesterone, 17α-hydroxypregnenolone, orange mud crab, *Scylla olivacea*, ovarian maturation stages

INTRODUCTION

Crustacean productions are getting more and more attention in developing countries both for domestic consumption as well as for export commodities including crab culture which gained its importance within the past few decades due to great demands of live crabs and crab products in the export market (Redzuari *et al*., 2012). Hence, inducing the ovarian maturation for reproduction in crustacean aquaculture is one of the approaches in meeting the market demands and one of the methods is using hormones. Hormones application in aquaculture is common for the past few years. This is because hormone injection is a practical alternative way in inducing gonad development that having less handling that gives less stress are place on the crustaceans (Ikhwanuddin *et al*., 2013). Most of the hormones are used for reproductive means and mostly are for the gonad development. Female gonad development is a process commonly referred as ovarian maturation stage. Ovarian maturation stages are a process which the ovary of the female develop into a

mature stage of ovary which could be observes through the external morphology on the crab ovary. External morphology observation is one of the practices in determining the ovarian maturation stage of an ovary where the ovary colour changes with further development of the ovarian maturation stage (Charniaux-Cotton and Payen, 1988). This process is circulated and affected by Vitellogenin Inhibitor Hormones (VIH) which inhibit the ovarian maturation stage (Wilder *et al*., 2002) and steroids hormones such as 17α-hydroxyprogesterone (17α-OHP) and 17α-hydroxypregnenolone (17α-OHPL) that inducing the ovarian maturation stage (Tsukimura, 2001). Some steroid hormones showed they are able to induce vitellogenesis which was demonstrated in the kuruma prawn *Penaeus japonicus* (Yano, 1987). Positive results from the correlation of circulatory estradiol-17α and progesterone with ovarian maturation stage were reported in the giant tiger shrimp, *Penaeus monodon* (Quinitio *et al*., 1994). However, relatively negative relationship between circulatory levels of steroid hormones and the ovarian development was detected in the kuruma prawn *Marsupenaeus japonicus* (Okumura and Sakiyama, 2004). In order to gain more understanding of this steroids hormone, this present study used 17α-hydroxyprogesterone and 17α-hydroxypregnenolone in order to determine their effects on ovarian maturation stages of orange mud crab, *Scylla olivacea* through ovary external morphology characteristics (Okumura and Sakiyama, 2004).

MATERIALS AND METHODS

Maintenance of animals: Sexually immature (determined by observing the abdominal flaps) female *S. olivacea* with a mean Body Weight (BW) of 80±15 g and Carapace Width (CW) of 75±10 mm were collected from coastal water of Kedah, Peninsula Malaysia, (Lat. 5°34´ 58 N; Long. 100°22´ 58 E). The crabs were maintained under controlled conditions (12-h light/12-h dark; temperature 26±1°C) in fiber glass tank at nine crabs per m^3 with the salinity of 29±1 ppt. They were acclimatized for 7 days before being used for experimentation. The crabs were fed daily twice once in 7.00 am and once in 7.00 pm with blood cockles, *Anadara granosa* and the water was changed every 10 days with 100% water changed.

Test hormone: Test hormone, 17α-hydroxyprogesterone and 17α-hydroxypregnenolone were obtained from the commercial drug store. They were in powder form and diluted with 95% alcohol to obtain the concentration of 0.01 and 0.1 μg μL^{-1} for each hormone.

Experimentation: The 90 immature female mud crabs (Fig. 1) were selected and divided into five equal groups. The crabs in the first group served as controls, did not receive any treatment and were sacrificed on day 0 of the experiment. The crabs in the second (T1D1) and third (T1D2) group were injected with 17α-hydroxyprogesterone with the dose of 0.01 and 0.1 μg BW^{-1}, respectively. The crabs in the fourth (T2D1) and fifth (T2D2) group were injected with 17α-hydroxypregnenolone with the dose of 0.01 and 0.1 μg BW^{-1}, respectively. All of the treatments received injection on day 0, 10, 20, 30, 40, 50 and 60th of the experiment. Three mud crabs were sacrificed with ice anesthetization on day 0 and every 10 days of the experiment till the 60th day of experiment before the hormone injections.

Data collective and analysis: The data taken by physical observation of the external morphological characteristics based on ovary colouration and the Gonad Somatic Index (GSI) of stage 1, 2, 3 and 4 ovary (Islam *et al*., 2010; Azmie *et al*., 2012).

Fig. 1(a-b): Two types of the abdominal shape of *Scylla olivacea* female, (a) Sexually immature with narrow and triangular shaped (V-shape) and (b) Sexually matured with wide and globular shaped abdomen with darkened color (U-shape)

Table 1: Percentage of the ovary stage occurrences during the 60 days of experimental period for the ovarian maturation of orange mud crab, *Scylla olivacea* treated with steroid hormones 17α-hydroxyprogesterone and 17α-hydroxypregnenolone

	Ovary external morphology based on the ovary colouration			
	Immature stage ovary (%)	Mature stage ovary (%)		
Hormonal treatments	Creamy white (Stage 1)	Yellow (Stage 2)	Orange (Stage 3)	Red orange (Stage 4)
Control				
No injection	100	-	-	
17α-hydroxyprogesterone				
T1D1	83.36	5.55	5.55	5.55
T1D2	94.45	-	5.55	-
17α-hydroxy regnenolone				
T2D1	72.22	-	16.67	11.11
T1D2	83.36	-	11.11	5.55

RESULTS AND DISCUSSION

Ovary colouration observation: Observation was done on the ovary external morphology during the 60 days experiment period (Table 1) with control 100% having their ovary maintained at stage 1 with the characteristic of white translucent and creamy white with ribbon-like structure. For T1D1, 16.65% of the treatment had the occurrence of mature stage ovary of stage 2 having the yellow colour ovary and stage 3 that have light orange colour ovary while the other 83.36% of the treatments maintain with in stage 1 ovary. For T1D2, 11.11% of the treatment had the occurrence of mature stage ovary of stage 3 that have light orange colour ovary and stage 4 with red orange ovary while the other 89.89% maintain at stage 1. For T1D2, 5.55% of the treatment had the occurrence of mature stage ovary of stage 3 that have light orange colour ovary while the other 95.45% maintain at stage 1. For T2D1, 27.78% of the treatments developed into mature stage which were stage 3 and 4 while the other 72.22% maintained at stage 1. The treatments of T2D2 produced 16.67% matured ovary stage which were stage 3 and 4 while the other 83.33% were at

Table 2: Cycle of each treatments reaching mature stage ovary occurrences during the 60 days of experimental period for the ovarian maturation of orange mud crab, *S. olivacea* treated with steroid hormones 17α-hydroxyprogesterone and 17α-hydroxypregnenolone

Hormonal treatment	Days
Control	
No injection	Never reach mature ovary
17α-hydroxyprogesterone	
T1D1	20±14.14
T1D2	25±7.07
17α-hydroxy regnenolone	
T2D1	50±10.00
T1D2	60±0.00

Table 3: Mean of Gonad Somatic Index (GSI) from day 1 until day 60 for the ovarian maturation of orange mud crab, *S. olivacea* treated with steroid hormones 17α-hydroxyprogesterone (17α-OHP) and 17α-hydroxypregnenolone (17α-OHPL)

	Days						
Treatments	0	10	20	30	40	50	60
Control							
No injection	0.33±0.1	0.27±0.05	0.16±0.02	0.16±0.08	0.17±0.07	0.16±0.03	0.17±0.03
17α-OHP							
T1D1	0.33±0.1	0.58±0.72	0.14±0.07	0.79±0.95	0.15±0.08	0.29±0.21	0.17±0.07
T1D2	0.33±0.1	0.23±0.06	0.19±0.01	0.12±0.04	0.07±0.07	1.44±2.27	0.24±0.04
17α-OHPL							
T2D1	0.33±0.1	0.08±0.02	0.11±0.07	0.24±0.08	1.75±2.86	0.43±0.33	2.51±0.72
T2D2	0.33±0.1	0.09±0.07	0.15±0.1	0.19±0.01	0.17±0.09	0.37±0.38	1.74±0.68

stage 1 ovary. Through these results, we can see that the control do not have any progress within the experimental periods. This might due to non-inducing gonad development approach was done on the ovarian maturation of the control *S. olivacea*. However, all the treatments using both reproductive hormones 17α-OHP and 17α-OHPL had positive response as they were able to produce mature ovary stage. Literature review shows that there are no studies were found on the effect of 17α-OHPL on the ovarian maturation of *S. olivacea*, but a study was done on the ovary with the injection of 17α-OHP on freshwater crabs, *Barytelphusa cunicularis* (Kale *et al*., 2011). The study by Kale *et al*. (2011) also resulted with the enhancement of the ovarian development, which support the current study results. The 17α-OHPL is a reproductive hormones same as 17α-OHP thus, the results can be relate to the study done on the freshwater crabs, *Barytelphusa cunicalaris*. Results of present study (Table 2) suggested control group were not able to develop its ovary to any of the mature ovary stage. However, each of the other treatment able had developed their ovaries to mature stage ovary. T1D1, T1D2, T2D1 and T2D2 had the mean maturation cycle of 20±14.14, 25±7.07, 50±10.00 and 60±0.00 days, respectively.

Gonad Somatic Index (GSI): From the results (Table 3) it shows that if the sexually immature mud crab is not induced in any manner or injected with reproductive hormones for the development of the ovarian maturation, the ovary would not be able to develop on its own within 60 days and maintain at stage 1 at the end of the experiment. For 17α-OHP (Table 3) it shows that for T1D1 it had the earliest reaction towards the ovarian maturation as on day 00 there is already a stage 3 and on day 30 the treatment again produced a stage 4 ovary and stage 2 ovary at day 50.

For T1D2, there's a little bit late than T1D1 as it only starts to show positive reaction on day 30 where by stage 4 ovary was obtained and on day 50 stage 3 ovary was obtained. As for T2D1, it shows a positive reaction on day 40 where the stage 3 ovary was obtained. On day 50 and 60, both treatments T2D1 and T2D2 obtain mature ovaries of stage 3 and 4.

Though all of the treatments are able to produce mature ovaries, the GSI of the mature ovaries does not have the same GSI with the study done by Azmie *et al.* (2012) on the ovarian maturation stage of *S. olivacea* with GSI at stage 1, 2, 3 and 4 are 0.66, 2.22, 7.96 and 10.20%, respectively. However, not all of the treatments produced all three mature ovary stages. For T1D1, it had all the ovarian maturation stages of stage 1, 2, 3 and 4 with the GSI of 0.25, 0.53, 1.41 and 1.88%, respectively. For T1D2, it only obtained stage 1 and 3 with the GSI of 0.25 and 4.06%, respectively. For T2D1, it only produced stage 1, 3 and 4 ovaries with the GSI of 0.24, 2.92 and 3.02%, respectively. For T2D2, it also able to produce only stage 1, 3 and 4 ovaries with the GSI of 0.24, 1.36 and 2.51%, respectively. Though all the treatments were able to develop the ovarian maturation stage, the increments of the GSI showed that they are much lower as compared to Azmie *et al.* (2012) on the *S. olivacea* ovarian maturation with different natural diets. The present study shows using these treatments to induce the ovarian maturation stage had caused a side effect which the GSI results of the present study were much smaller compared to the study done by Islam *et al.* (2010).

Based on the literature reviews this is the first report that demonstrates positive ovarian maturation development from injections effects of either 17α-OHP or 17α-OHPL on the ovary of *S. olivacea*. For 17α-OHPL, literature review shows that this is the first report regarding its positive effects on ovarian maturation stages in crustaceans. Using sexually immature female mud crab might be another cause of the smaller GSI. Naturally a non-mating or not yet mate female mud crab do not have the physiology which needed for it to develop its ovary. However, when induced with hormones, it able to produce mature stage ovary but lacks in GSI. The reason sexually immature female mud crabs were used in the present study because we want to prove if the hormone used in this experiment had any effects since an immature female mud crab would not reach sexually matured ovary unless it undergoes mating first. In the natural way of breeding, the *Scylla* spp. would undergo molting and while the female shell is still soft, the mating process continues which eventually leads to copulation that would trigger the ovarian maturation once the spermatophores are deposited into the sperm sacs of the female orange mud crab (Baiduri *et al.*, 2014). Thus, using sexually matured female have the possibility to increase the ovarian maturation much faster than using an immature female mud crab.

CONCLUSION

The study shows that T2D1 treatment of 17α-hydroxypregnenolone with 0.01 μg BW^{-1} dose are the best treatment which are able to produce the highest percentage, 27.78% mature ovary stages (27.78%) and having the highest gonad somatic index (2.51±0.72%). In addition, all of the treatments also able to develop the ovarian maturation stage of orange mud crab, *Scylla olivacea*.

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