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Reproductive performances of wild male tiger shrimp *Penaeus monodon* post-injection of oocyte developer without eyestalk ablation

Performa reproduksi udang windu *Penaeus monodon* jantan alam pascainjeksi hormon oocyte developer tanpa ablasi tangkai mata

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ABSTRACT

The aim of this study was to evaluate the effect of oodev on the gonadal maturation and characteristic of spermatophore and spermatozoa produced by the wild male black tiger shrimp against eyestalk ablation. The treatments were two doses of oodev injection at 0.5 (OD0.5) and 1 (OD1.0) mL/kg of body weight and a control was eyestalk ablation (AB). The male stock of tiger shrimp used was from wild with body weight ranged from 55−85 g, stocked into three of 10 tonnages concrete tanks with density of 25 males/tank. Oodev injection was applied for two times with one week interval. Tiger shrimp of OD0.5 group produced the highest number of gonadal maturing which was 84% followed by AB (68%) and OD1.0 (64%). Oodev injection was able to shorten the time required for spermatophore maturation at maturation phase which happened simultaneously within 7 days post injection compared to that of ablated males. Positive correlation (R2=0.612) was detected between the shrimp weight and weight of spermatophore of the tiger shrimp while correlation between spermatophore weight and number of spermatozoa was relatively low (R2=0.415). Total fatty acid tented to be higher in males injected with oodev compared to ablated males. Concentration of arachidonic acid in the muscle of male stock in OD1.0 group was extremely low of 0.0037% of total lipid compared to AB (0.3190%) and OD0.5 (0.2806%). Oodev injection at the dose of 0.5 mL/kg of tiger shrimp could improve the number of males stock producing spermatophore compared to eyestalk ablation. Simultaneously matured-spermatophore of wild male tiger shrimp within short time could be achieved through oodev injection.

Keywords: oodev, spermatophore, reproduction, tiger shrimp

ABSTRAK

Tujuan penelitian ini adalah mengevaluasi efek hormon oodev terhadap pematangan gonad dan karakter spermatofor dan spermatozoa yang dihasilkan oleh induk udang windu jantan alam dibandingkan dengan teknik ablasi tangkai mata. Perlakuan yang dicobakan adalah dua dosis injeksi oodev yaitu 0,5 (OD0,5) dan 1 (OD1,0) mL/kg bobot tubuh dan kontrol yaitu ablasi tangkai mata (AB). Induk udang windu jantan yang digunakan berasal dari alam berbobot antara 55−85 g, ditebar dalam tiga bak pematangan gonad berkapasitas 10 ton dengan kepadatan 25 ekor/bak. Injeksi oodev dilakukan dua kali dengan interval satu minggu. Induk udang windu pada OD0,5 menghasilkan jumlah induk matang gonad tertinggi yaitu 84%, diikuti oleh AB (68%) dan OD1 (64%). Injeksi oodev mampu mempersingkat masa pematangan spermatofor pada fase maturasi dan pematangan tersebut terjadi secara simultan setelah 7 hari pascainjeksi dibandingkan dengan induk yang di ablasi. Korelasi positif (R2=0,612) dideteksi antara bobot tubuh dan bobot spermatofor induk udang windu, sedangkan korelasi antara bobot spermatofor dan jumlah spermatozoa relatif lemah (R2=0,415). Total asam lemak daging cenderung lebih besar pada induk udang windu jantan yang diinjeksi oodev dibandingkan yang diablasi. Konsentrasi arachidonic acid dalam daging induk yang diinjeksi OD1 sangat rendah sebesar 0,0037% dari lemak dibandingkan AB (0,3190%) dan OD0,5 (0,2806%). Injeksi oodev pada dosis 0,5 mL/kg udang windu dapat meningkatkan jumlah induk yang menghasilkan spermatofor dibandingkan ablasi tangkai mata. Pematangan gonad udang windu jantan alam secara simultan dan singkat dapat dilakukan melalui injeksi oodev.

Kata kunci: oodev, spermatofor, reproduksi, udang windu

INTRODUCTION

Demand on broodstocks of tiger shrimp for commercial hatcheries so far still relies on capture from the wild. Wild broodstocks in general have several good attributes, however their quality vary and they are claimed to be pathogenic carrier which transmitte diseases into aquaculture system (Coman *et al.,* 2006). Production of tiger shrimp broodstock from aquaculture still has several constrains including low number of maturing spawner, the final stage of maturation is not as good as the wild spawner, un-sincronized gonad maturation between male and female stocks, low mating rate, delayed spawning time, low hatching rate and low survival of post-larvae (Marsden *et al*., 2008; Uawisetwathana *et al.,* 2011; 2013; Laining *et al.,* 2014; Laining *et al.,* 2015).

In commercial hatcheries, the common technique used to stimulate the development of gonadal maturation of crustaceans including tiger shrimp is eyestalk ablation. Ablation is a technique to cut one of the spawner's eye in order to reduce production of gonadal inhitibing hormones. Although ablation can accelerate the process of gonadal development of tiger shrimp, it has several disadvantageous such as stimulating a strong stress even mortality of the spawner, destroying physiological function, decreasing gamet quality, and against to the animal welfare (Wongprasert *et al.,* 2006; Sainz-Hernández *et al.,* 2008; Uawisetwathana *et al*., 2011). Furthemore, the use of ablated tiger shrimp generally only for 3-4 maturation cycles before being discarded. One alternative to ablation which has been developed for tiger shrimp gonadal maturation is through hormonal manipulation (Laining *et al*., 2015).

Several hormones functioning in reproductive system of vertebrate have also been detected in crustacean. Huang *et al.* (2008) stated that FSH-LH-likesubstances was detected in brain and thoracic ganglion of swimming crab, *Portunus trituberculatus* and GTH-*like substances* was also detected in the same organs of mud crab, *Scylla paramamosain* (Ye *et al*., 2009). Moreover, dopamine has been identified in eyestalk of giant prawn, *Macrobrachium rosenbergii* (Tinikul *et al.,* 2008) and shrimp (Chang *et al.,* 2007; Babu *et al.,* 2013; Sukthaworn *et al.,* 2013). Based on these findings, it is suspected that crustacean including tiger shrimp will response to hormone stimulation such as gonadotropin and antidopamine.

Oocyte developer (oodev) is a hormone premix containing PMSG (pregnant mare serum gonadotropin) and antidopamin (AD). PMSG is a glicoprotein hormone secreted by trophoblast cells of horse containing FSH and LH (Hafez & Hafez, 2000). Antidopamine is a neurotrasmitter which inhibits activity of dopamine receptor (Dufour *et al.,* 2010). Oodev has been applied to stimulate gonadal development of several species such as eel (Sudrajat *et al.,* 2014), and catfish (Nainggolan *et al*., 2014). The positive effect of this hormone has been also observed on vaname shrimp (Akbar *et al*., 2015) and female stock of tiger shrimp (Laining *et al*., 2015).

Sudrajat *et al.* (2014) has injected eel, *Anguilla bicolor* using combination of oodev and recombinant growth hormone (rGH) at combined dose of 20 IU PMSG/kg, 100 mg AD/kg and 10 μg rGH/kg to improve gonadal maturation up to 100% with a more matured gamet stage. Combination oodev at 15 IU and supplementation of 3% *Spirulina* sp in diet fed to catfish could faster the oocite development and viability of egg and larvae (Nainggolan *et al.,* 2014). Akbar *et al.* (2015) applied oodev to inject male vaname shrimp at dose of in order to 0.5 mL/kg enahnced the gonadal maturation with a improved reproductive performances compared to ablation. Application of oodev on female tiger shrimp has been also carried-out through three times injection at dose of 3 mL/kg resulting a higher percentage of maturing broodstock than that of ablation, but still with a lower hatching rate Laining *et al.* (2015).

The objective of this study was to evaluate the effects of oodev hormone on gonadal maturation and characteristic of spermatophore and spermatozoa produced by wild tiger shrimp, *Penaeus monodon* male against eyestalk ablation.

MATERIALS AND METHODS

Quarratine and acclimatization of wild male tiger shrimp

Male tiger shrimp were obtained from Makassar Strait of South Sulawesi. As much as 75 male stocks were selected with weight ranged from 50−70 g without morphological abnormality. The male stock were acclimatized in quarratine room for 2−4 weeks before being transferred to maturation tank. During culture period, males stock were fed freshfeed four times a day with squid and bivalve *Anadara granosa* at rate of 2−3% of dry basis shrimp biomass. Water supply was a filtrated and sterilized sea water.

Water quality was measured in every three

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days with temperature at 28.3±0.2 °C (mean±SD), salinity at 30.1±0.3 g/L, and dissolved oxygen at 5.63±0.17 mg/L. Every day all tanks were syphoned and exchanged the water at level of 60%. After four weeks acclimatization, the male stocks were transferred into three of 10 tonnages maturation concrete tanks. The tanks were set with aeration system and flow-through water supply at rate of 10 L/minutes. Photoperiod was arranged with 12-h light and 12-h dark.

Injection of oodev hormone and eyestalk ablation

The treatments were three doses of oodev hormone at 0 (AB); 0.5 (OD0.5); and 1.0 (OD1.0) mL/kg of shrimp weight. This experiment was designed without replication due to the limited maturation tanks. Application of oodev was through injection following the protocol applied by Laining *et al*. (2015) and shrimp without injection but ablated was the control (AB). Prior to treatments, male was individually eye-tagged for recognition and further randomly stocked into three of 10 tonnages concrete tanks with density of 25 males/tank for each treatment. Injection was performed twice with one week interval (H-0 nd H-7, respectively). Male shrimp were injected using 0.5 mL syringe at the 2nd or 3th segment of dorsal. At the same time of 2nd injection, other bacth of the males were ablated for control group.

Observation on spermatophore and spermatozoa

Unlike tiger shrimp female stock, gonadal development of male stock including testes and spermatophore is not easy to evaluate based on external appeareance. A technique to assess spermatophore development has been developed by artificially releasing the spermatophore out of ampulla terminale. The males which released ther spermatophore through electrical shock are assumed as a matured stock (Laining *et al.,* 2016).

Technique of artificially ejaculation applied in the present study was by electrical shock using transformer set up at 5 mA and 8−12 V following the protocol applied by Lante and Laining (2016). The transformer was connected with an electrode placed near the gonophores at the base of the 5th pereiopodsa applied usually for two seconds. The eletrical shock stimulates contraction surrounding the terminal ampullae expelling a single spermatophore from each gonophore. The electrical shock to release the spermatophore was applied three times at 7, 14, and 21.days from the last injection. If a male released spermatophore at the first electrical shock, it was claimed to be at maturation stage and if the same male again released spermatophore at the second and third electrical shock, it was categorized to be at 1st re-maturation and 2nd re-maturation, respectively (Laining *et al*., 2016). Percentage of male stock maturing is calculated based on number of males releasing spermatophore in each of the maturation stage divided by number of injected males. Furthemore, it was also observed the treatment effect on the time required by the male to release the spermatophore or to mature post-oodev injection. If a male matured at the first electrical shock or at 7 days post-oodev injection then the male required 7-days (H-7) for spermatophore maturation. Accordingly, if a male matured at second and third electrical shock, then the male required 14 (H-14) and 21 (H-21) days for spermatophore maturation.

To assess the characteristic of spermatophore, the released spermatophore was weight using electrical balance (Sartorius, Germany), homogenized with micro-homogenizer (Radnoti, USA) and then added with 0.9 mL calcium-free saline solution (Jiang *et al.,* 2009). Trypan blue of 0.1% was added at level of 0.1 mL to improve the contrast between the cell and dilution. Number of spermatozoa was calculated using haemocytometer under microscope at 400× (Olympus DP21, Japan). The spermatozoa cells were snapped and counted the number using ImageJ software. Characterization of the sperm cell was observed for the number of total sperm cell and percentage of normal cell. Criteria for abnormal spermatozoa was evaluated according to Jiang *et al.* (2009) and Leelatanawit *et al.* (2014).

Histological and bio-chemical analysis

For histological assessment, three male stocks representing each groups was randomly selected to be dissected and taken their reproductive organ including testes, vas deferens and ampulla terminale. Samples were taken at H-14, H-21, and H-28. All organ samples was preserved in Davidson solution and after 24-h the preservative solution was changed with 70% alcohol prior to histology. Hematoxilin-eosin was used for staining and observed under microscope (Olympus DP21, Japan).

Proximate analysis and analysis of fatty acid content in muscle was carried out to determine the effect of eye stalk ablation on physiological

metabolism besides on reproduction. The muscle was dried in oven at 60 °C (Memmert, Germany) and stored in -20 °C before being analyzed. Proximate analysis was performed according to AOAC (1999) and profile of fatty acid was detected using gas chromatografi (GC, FID Perkin Elmer Clarus 680, USA).

Statistical analysis

Single data on percentage of maturing male was descriptively analyzed. Effect of treatments on spermatophore weight, number of spermatozoa and percentage of normal sperm cell was analyzed by one-way ANOVA *single factor* with multiple comparisons evaluated using LSD test at 0.05 of the level of significance using software SPSS version 21 (SPSS, IBM, US). Relationship between body weight, spermatophore weight and number of sperm cell was determined linear regression linier using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Results

Number of male tiger shrimps releasing spermatophore

Mean weight and number of males releasing spermatophore after electrical shock post-oodev injection is presented in Table 1. Number of males releasing their spermatophores for the fisrt time (maturation) was the highest at OD0.5 of 21 males or 68% of the oodev injected males. At OD1.0, males releasing spermatophore was 16 males (64%) relatively similar to AB which was 68%. At the 1st re-maturation, number of males releasing spermatophore was extremely different from maturation stage where at AB group was found 9 males (36%) and at the two dosages of the oodev injection were found only 12%. Furthermore, at the 2nd re-maturation, the males releasing spermatophore was only observed one male in AB group.

Table 2 presents the number of males releasing their spermatophores for the first times (maturation) after oodev injection based on the time required for maturation of the spermatophore. All male stoks injected with OD0.5 matured at H-7 or 100% of 21 males required 7 days to release their spermatophores, followed by OD1.0 of 94% (15% of 16 maturing males). For AB group, number of maturing males at H-7 was 8 males or 47% of 17 maturing males). Males injected with oodev generally could mature at H-7 however for the following days of H-21 only one male re-matured. In contrast, it was still found maturing males at H-14 and H-21 for AB group which was 29 and 24%, respectively (Table 2).

Characteristic of spermatophore and spermatozoa cell of tiger shrimp

Spermatophore weight, number of spermatozoa cell and percentage of normal spermatozoa in two spermatophore sacs of each individual male tiger shrimp were found indentic between the right and left side. Therefore, the data provided in Table 3 were only for one sac of the spermatophore of individual male from all groups. Mean spermatophore weight, number of spermatozoa and percentage of normal sperm at maturation stage in AB group was not significantly different ($P>0.05$) from other two groups.

Data on spermatophore weight and number of spermatozoa produced by male tiger shrimps at three maturation cycles are presented in Table 4. Mean spermatophore weight released by males at H-7 did not significantly ($P>0.05$) differ among three treatments. Similarly, number of spermatozoa within spermatophore was not influenced by both oodev injection and ablation. Since the spermatophore produced by male stocks at H-14 was only occurred at AB group the statistical analysis was not performed for maturation stage. Accordingly, males injected with OD0.5 did not release spermatophore at H-21. From Table 4, it is showed that ablated males produced bigger spermatophore at H-7 and H-14 than that of produced at H-21. However, number of the normal spermatozoa did not differ based on the time required for spermatophore maturation.

Based on linear regression between body weight and spermatophore weight released by tiger shrimp at maturation and 1st re-maturation, there was a positive relationship between body weight and spermatophore weight with the coefficient determination (R^2) of the treatments ranged from 0.57–0.60. The same R^2 in all groups indicated that the relationship between shrimp weight and spermatophore weight of tiger shrimp was not influenced by both ablation and oodev injection. When all data were pooled, the R^2 value become higher of 0.612 (Figure 1).

Relationship between spermatophore weight and number of spermatozoa was also carried-out for each treatment and pooled data. Figure 2 showed that there was no positive relationship between those two reproductive parameters with R^2 value below 0.5 and the values ranged from

0.4–0.48 implying that the relationship was not affected by the neither abalation nor oodev injection. Even when data for all groups were pooled the R^2 value only 0.4147.

Proximate composition profile of fatty acid in muscle of male tiger shrimp

Proximates composition of muscle of the males at the end of assessment are presented in Table 5. There were no differences on proximate composition of the muscle among the three groups, except for crude protein in which shrimp injected with OD1.0 was likely to have higher protein content in the muscle compared to AB and OD0.5. Fatty acid (FA) profile of male tiger shrimp before injection (H-0) and at the end of assessment (H-21) is presented in Table 6. Total fatty acid in muscle of tiger shrimp prior to treatments was generally lower than those after treatments. Ablated male stocks contained lower total fatty acid of 2.2600% of lipid compared to OD0.5 and OD1.0 which were 2.6344% and 2.6449%, respectively. Injection of OD1.0 resulted in an extremely low arachidonic acid (ARA) content in muscle which was 0.0037% of lipid than that of 0.2632% for OD0.5 and 0.3190% for control AB.

Reproductive organ of male tiger shrimp

Reproductive organ of male tiger shrimp was identified to be relatively similar to reproductive organ of other penaeoidens in general consisting of five pairs lobul of testes connected to vas deferens and it terminate in ampulla terminale. Spermatophore developed inside the ampulla terminale (Figure 3A). Based on histological analysis, it was indicated that each of the testes lobul consisting of seminiferous tubules where germinal cell exist (Figure 3B and 4C). Figure B is the light micrograph of testes lobul consisting

Table 1. Mean weight and number (percentage) of males releasing the spermatophores post-oodev injection

Treatment*	∑ Male stock (shrimp)	Mean weight (g)	No. of male releasing spermatophore (shrimp)		
			Maturation	1st re-maturation	2nd re-maturation
AB	25	74.40 ± 10.22	17 (68%)	9 (36%)	1 (4%)
OD0.5	25	63.84 ± 12.20	21 (84%)	3 (12%)	0
OD1.0	25	66.08 ± 11.73	16 (64%)	3 (12%)	0

*AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg

Table 2. Number of male tiger shrimps maturing post-oodev injection based on the time required to relesease the spermatophore

Treatment*	∑ Male stock (shrimp)	No of male maturing based on the time required to release spermatophore (shrimp)		
		H-7	H-14	H-21
AB	17	8 (47%)	5 (29%)	4 (24%)
OD0.5	21	21 (100%)	0	0
OD1.0	16	15 (94%)	0	1 (6%)

*AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg

Table 3. Spermatophore weight, number of spermatozoa (per spermatophore sac) and number of normal sperm cells of tiger shrimp post-injection of oodev at maturation stage

Maturation stage	Parameter	Treatment*		
		AB	OD0.5	OD1.0
Maturation	Spermatophore weight (g)	0.0533 ± 0.0114a	0.046 ± 0.016a	0.0439 ± 0.016a
	No of spermatozoa (10^6)	85.194 ± 39.278a	69.236 ± 27.251a	74.502 ± 28.848a
	Normal spermatozoa (%)	48.90 ± 7.533a	57.00 ± 11.46a	53.373 ± 9.796a

*AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg. All values are mean±SD. The same letters across columns indicate no significant different mean values (P>0.05) on spermatophore weight data transformed $\sqrt{(x+1)}$ and number of spermatozoa transformed $\log_{10}(x)$.

Table 4. Weight of spermatophore, no.of spermatozoa per spermatophore sac and percentage of normal spermatozoa of tiger shrimp stock post-oodev injection at different times required for spermatophore maturation

Time required for spermatophore maturation	Parameter	Treatment*		
		AB	OD0.5	OD1.0
H-7	Spermatophore weight (g)	0.0523 ± 0.009ab	0.0449 ± 0.0154a	0.043 ± 0.0168a
	No of spermatozoa (10^6)	93.962 ± 35.329a	70.983 ± 29.817a	72.425 ± 31.166a
	Normal sperma (%)	46.482 ± 10.591a	53.111 ± 17.202a	51.918 ± 10.343a
H-14	Spermatophore weight (g)	0.0587 ± 0.01a	-	-
	No. of spermatozoa (10^6)	102.613 ± 23.439	-	-
	Normal sperma (%)	51.87 ± 6.224a	-	-
H-21	Spermatophore weight (g)	0.0416 ± 0.0104b	-	0.0469
	No. of spermatozoa (10^6)	43.238 ± 30.719	-	81.158
	Normal sperma (%)	47.200 ± 4.603a	-	53.210

*AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg. All values are mean±SD. The same letters across columns indicate no significant different mean values (P>0.05) on spermatophore weight data transformed $\sqrt{(x+1)}$ and number of spermatozoa transformed log10 (x).

of immature sperm cell whereas figure 3C and 3D is the testes with matured spermatozoa.

The histological illustration revealed that the five pairs lobul testes have different stages of seminiferous tubules development, while one pair lobul contained relatively similar tubules (Figure 3C). Stage of these development could be identified by comparing the proportion of the germinal cell and the size of the sperm duct and the number of sperm cell inside the duct (Picture 3B, C, and D). Furthermore, sperm cell inside the testes commonly without spike whereas those existing in MDV have partly completed with spike (Figure 3F) similar to those existed in the spermatophore (Picture 3H).

Discussion

Ablation and oodev injection are basically to manipulate the hormonal system in shrimp. Ablated shrimp lost the sinus gland and organ X which secrets the gonadal inhibiting hormone (GIH) (Uawisetwathana *et al.,* 2011; Urtgam *et al*., 2015). Shrimp without GIH due to the ablation lead to the continuously maturation process. As the consequent of the over matured is the slow growth, depleted energy for cell regeneration and health healing, decreased gamet quality and the discarded broodstock making a short time of their usage (Uawisetwathana *et al.,* 2011).

The present study indicated that shrimp injected with OD0.5 produced higher number of males releasing spermatophore compared to AB and OD1.0. The effect of oodev injection was entirely happened at the maturation stage. On the other word, stimulation of gonadal development through oodev injection resulted in a lower re-maturing which was only 14% and 19% for OD 0.5 and OD1.0, respectively. Meanwhile, in the AB group percentage of male did the 1st re-maturing was realtively similar to the 2nd re-maturing. However, number of male maturing was lower than that of oodev injected males. The low maturing males after ablation compared to oodev injection was also observed in pond-reared tiger shrimp males (Laining *et al*., 2016). Similarly, it was also reported in wild female tiger shrimp injected with oodev which stimulated spermatophore up to 93% compared to 60% in ablated female (Laining *et al*., 2015). Re-maturation occurred at AB group found in this experiment showed that the lost of eye stalk caused an inhibiting production of GIH which lead to a continuously maturation process. This phenomenon supported the previous study by Wongprasert *et al.* (2006) who reported that ablated tiger shrimp can mature and spawn three times continously with a trend of decreasing significantly the hatching rate and survival of nauplii.

Oodev injected males tended to mature once and stimulated a simultaneously spermatophore maturation within 7 days post-injection. This is indicated that the effect of oodev on

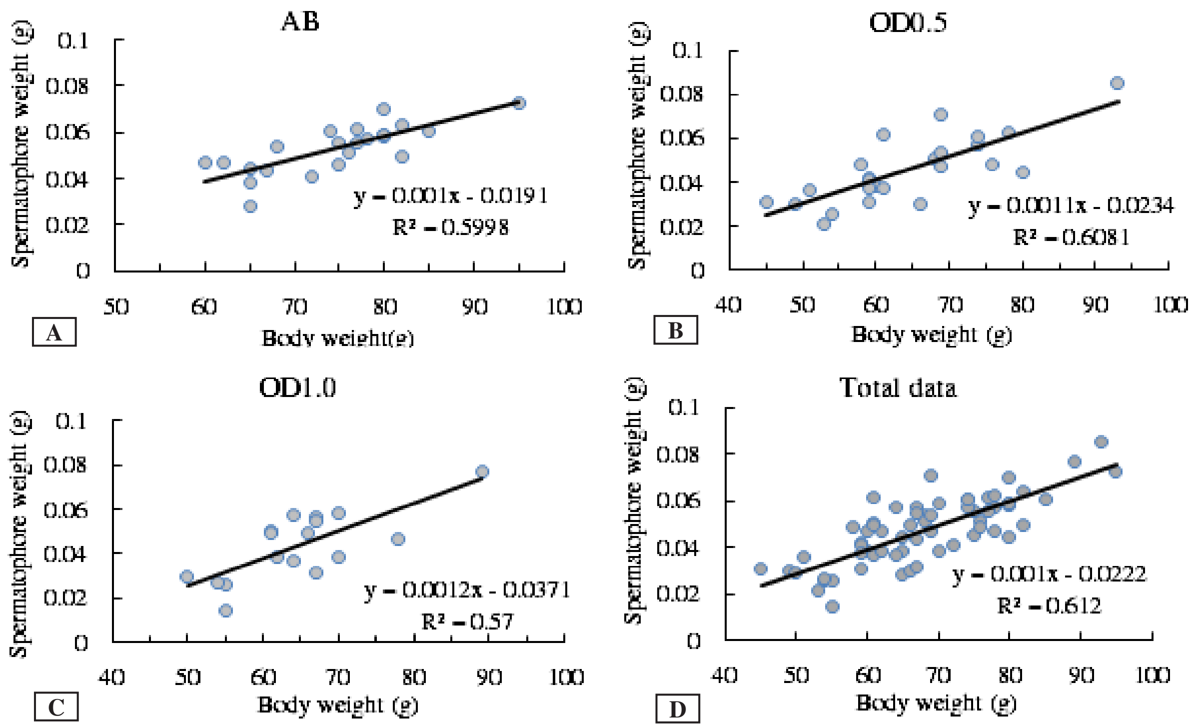

Figure 1. Relationship between body weight and spermatophore weight of tiger shrimp post-injection and ablation and pooled data of all treatments.

Table 5. Proximate composition (%, air dry) of muscle of males tiger shrimp prior to tretament (H0) and at the end of assessment (H-21)

Nutrient*	T0	Treatment**		
		AB	OD0.5	OD1.0
Moisture	6.2	9.0	5.9	6.6
Lipid	3.1	4.3	5.8	5.5
Crude protein	78.1	73.1	72.8	76.0
Crude fibre	0.7	0.1	0.1	0.1

* All analysis were carried-out as simplo (n=1). **AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg

spermatophore maturation occurred within a short time, therefore further application of this hormone is required to stimulate the male to re-mature. Simultaneously spermatophore maturation through oodev injection observed in the present study could support the artificial insemination to increase the fertilization rate of cultured tiger shrimp broodstock. The use of pond-reared tiger broodstock for nauplii production still has several contraints particularly the low mating rate so that application of artificial insemination become very important (Coman *et al*., 2007; Lante & Laining, 2016). Since duration of spermatophore to naturally maturate is different between individu, sincronization between spermatophore maturation and the application of artificial insemination can be achieved through oodev injection in particular when a large number of female stocks need to be inseminated.

For the AB group at the maturation stage, male tiger shrimp required different times for spermatophore maturation (Table 2). This is indicated that hormonal change caused by ablation has different effects on each individual male tiger shrimp. Optic lobe located at eye stalk which was not completely destroyed during ablation was suspected to still produce GIH to some extend so that inhibition of gonadal maturation still occurred (Wongprasert *et al.,* 2006). In contrast, spermatophore maturation in OD0.5 and OD1.0 happened simultaneously and required shorter period within 7 days. This phenomenon showed

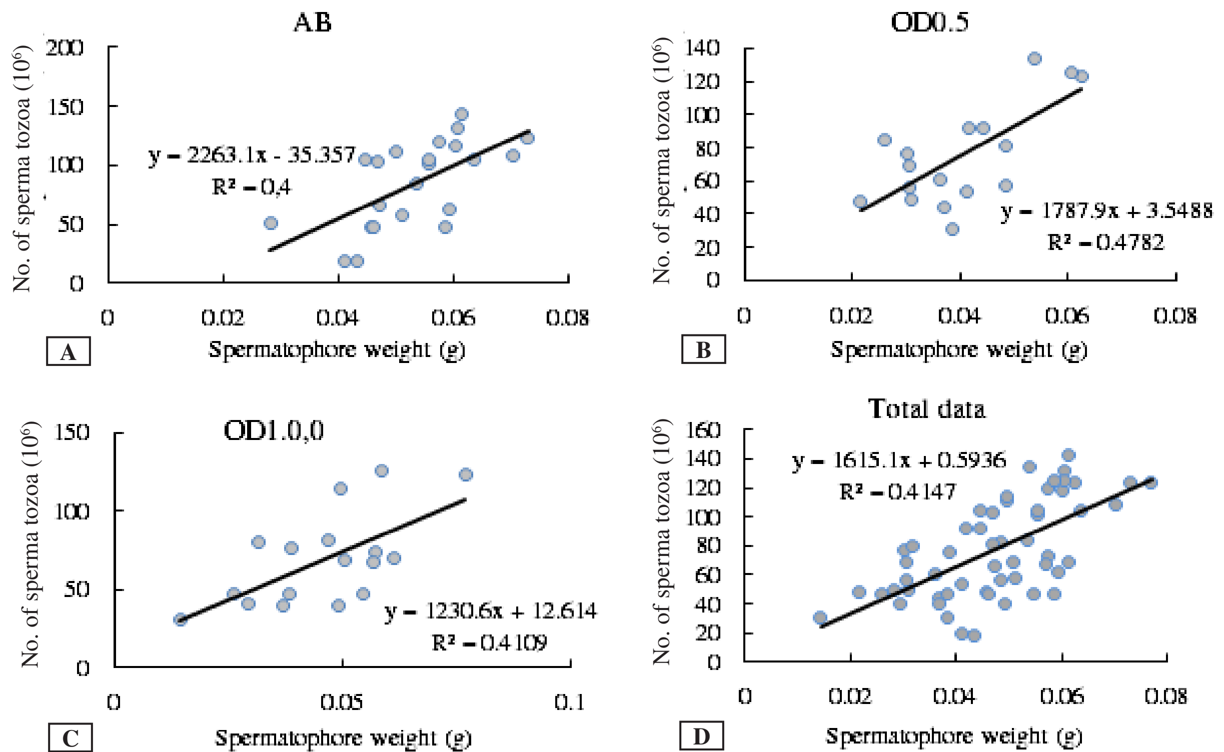

Figure 2. Relationship between spermatophore weight and number of spermatozoa of tiger shrimp post-injection and ablation and pooled data of all treatments.

Table 6. Profile of fatty acid (% of lipid) in muscle of male tiger shrimp prior to treatments (H-0) and at the end of assessment (H-21)

Parameter	H-0	Treatment* (H-21)		
		AB	OD0.5	OD1.0
Omega 3	0.4398±0.014	0.4553±0.0143	0.4650±0.0003	0.4282±0.0029
Omega 6	0.5318±0.018	0.3826±0.0133	0.2020±0.0008	0.0964±0.0009
Omega 9	0.3034±0.009	0.4253±0.0139	0.3946±0.0004	0.5111±0.0018
Un-saturated fatty acid	1.3268±0.045	1.4333±0.6823	1.3325±0.0027	1.1869±0.0025
Saturated fatty acid	0.5482±0.019	0.8267±0.0238	1.3025±0.0044	1.4581±0.0045
Mono-unsaturated fatty acid (MUFA)	0.3366±0.012	0.5772±0.0190	0.5302±0.0018	0.6538±0.0013
Poly-unsaturated fatty acid (PUFA)	0.9902±0.033	0.8561±0.0279	0.8023±0.0008	0.5330±0.0012
Arachodonic acid (ARA)	0.1285±0.005	0.3190±0.0107	0.2632±0.0006	0.0037±0.0002
Docosahexanoic acid (DHA)	0.1720±0.006	0.2500±0.0081	0.2806±0.0005	0.2322±0.0018
Eicosapentanoic acid (EPA)	0.2551±0.008	0.1976±0.0060	0.1778±0.0001	0.1841±0.0000
Total fatty acid (C 4:0 - C 24:1)	1.8749	2.2600	2.6344	2.6449

*AB =eyestalk ablation; OD0.5 = injection of 0.5 mL oodev/kg; OD1.0 = injeksi 1.0 mL/kg

that oodev injection can stimulate the development of reproductive organ of male tiger shrimp faster than that of ablated males.

Weight of spermatophore, number of spermatozoa and percentage of normal spermatophore were not significantly different among treatments at maturation (Table 3). This is implied that spermatophore maturation through oodev injection can produce spermatozoa with similar quality with those produced by ablation regardless the difference times required for the maturation. For maturation stage, AB group

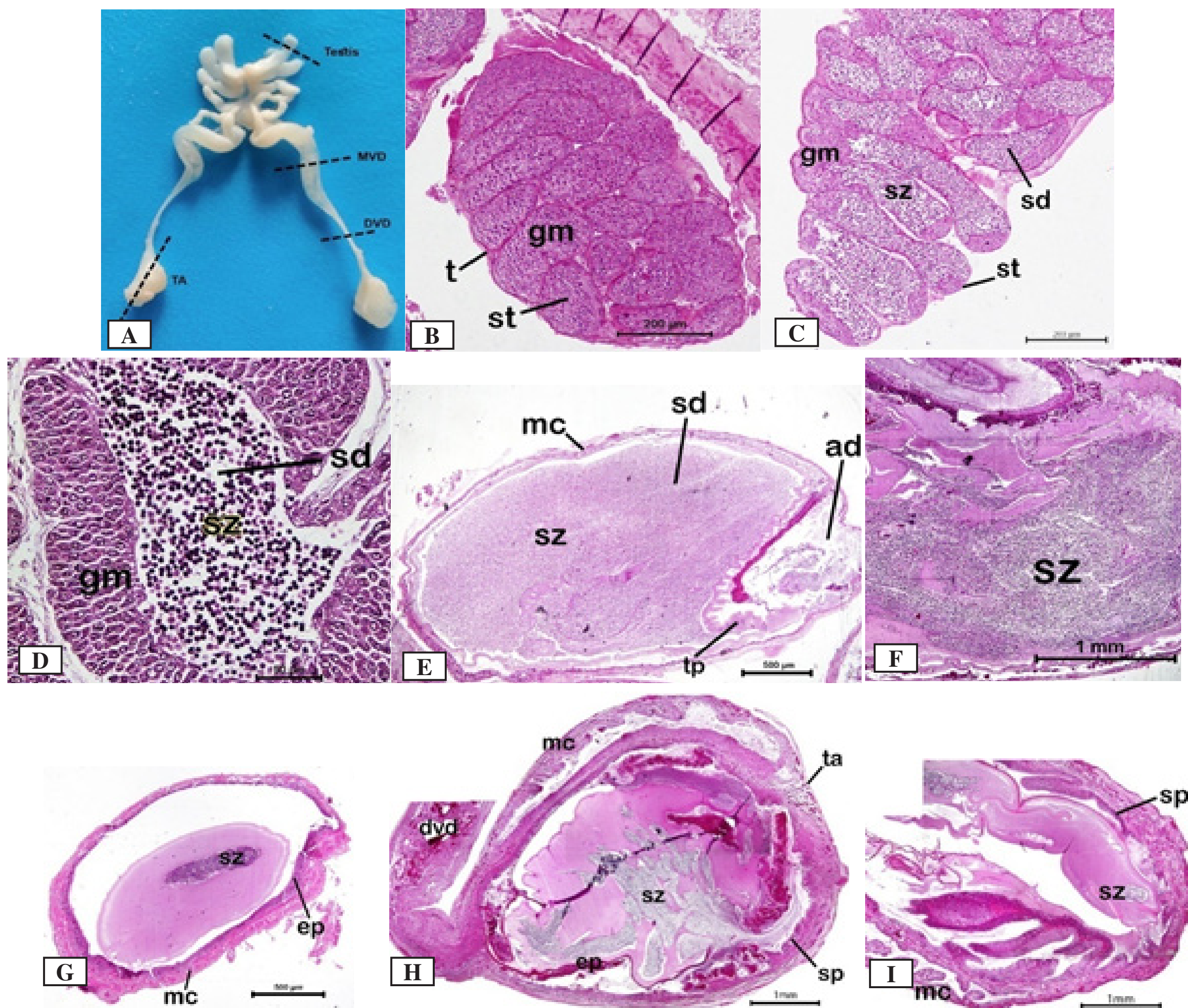

Figure 3. Repoductive organ of male tiger shrimp, *Penaeus monodon* (A). Light micrograph of ampulla terminale (B,C), testes (D,E,F) and vas deferens (G, H) and distal deferens (I). MVD = median vas deferens; DVD = distal vas deferens; TA = ampulla terminale; sz = spermatozoa; ep = epitelium; sp = spermatofor; mc = muscular; t = testes; st = seminiferous tubules; gm = germinal; sd = seminiferous duct; tp = typhlosole; ad = accessory duct.

produced heavier spermatophore at H-7 and H-14 compared to spermatophore released at H-2. However, number of normal spermatozoa was not different for the three maturation periods indicating that re-maturation in ablated males might reduce a certain aspect of the spermatophore quality

The R^2 values for the linear regression between body weight and spermatophore weight at the three groups were relatively similar and the R^2 for total data was 0.612. The R^2 value detected in the present study implied that dependent variable of male body weight affected the independent variable of spermatophore weight meaning that the bigger size of the male shrimp, the bigger its spermatophore. In contract, no positive relationship between spermatophore weight and number of spermatozoa produced with the low R^2 value which was 0.4147. Similarly, it was found by Meunpol *et al.* (2005) who concluded that no relationship between spermatophore weight and number of spermatozoa cell or correlation between these two variables was low (Jiang *et al.*, 2009). The low R^2 value also impiled that there is an independent variable influenced the number of spermatozoa such as nutrition (Braga *et al.,* 2013; Leelatanawit *et al.,* 2014). In addition, weight of spermatophore is not only afftected by the sperm cell mass but only by the intermediated acellular secreted by vas deferens (Diwan & Josep, 2009).

Ablation is a technique to destroy the optic lobe which can negatively affect the physiological process besides reproduction while hormonal injection does not largely affect the physiological process due to the specific targeted organ (Wongprasert *et al.,* 2006). Molecular assessment indicated the change of gen expression on

the metabolism pathway on ablated shrimp (Uawisetwathana *et al.,* 2011). Total fatty acid in muscle of AB group was lower than that of oodev injected males indicating the metabolism change due to the lost of several secretion glands and nervous system after ablation. The main effect of ablation on reproduction is the lipid transport from hepato-pancreas to gonad associated with depletion of GIH level in sinus gland. The lipid transport is also associated with the elevation of several lipid classes in hemolymph (Sainz-Hernández *et al.,* 2008). Increasing of total fatty acid in muscle has been also detected on cultured male tiger shrimp injected with sGnRH-a compared to ablated shrimp (Laining *et al.,* 2016).

Testes contained immature sperm cells indicated by undeveloped spike at the anterior of the spermatozoa. The pattern of spermatozoa development observed in the present tudy was relatively similar to that found on giant freshwater shrimp, *Macrobrachium rosenbergii* (Okumura, 2004), lobster, *Nephrops norvergicus* (Rotllant *et al.,* 2012) and white shrimp, *Litopenaeus schmitti* (Fransozo *et al*., 2016). Based on morphological observation on spermatozoa cell, it was also identified undeveloped spike-spermatozoa inside spermatophore. Leelatanawit *et al.* (2014) stated that spermatozoa of tiger shrimp has still developed in vas deferens before reaching spermatophore. Based on histological observation it showed that oodev stimulation did not negatively affect the anatomy of reproductive organ of male tiger shrimp.

CONCLUSION

Oodev injection at 0.5 mL/kg of wild male tiger shrimp produced higher number of male releasing spermatophore of 84% compared to ablated which was 68%. Simultaneously gonad maturation of male tiger shrimp could be stimulated by oodev injection which also shorten the time of the maturation.

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