Effect of different doses of 17α-methyl testosterone (17-α MT) hormone on male broodstock of tiger shrimp *Penaeus monodon* sperm quality

S R H Mulyaningrum*, A Parenrengi, S Lante, A Tenriulo, R Daud and Usman

Research Institute for Brackish Water Aquaculture and Fisheries Extension
Jl. Makmur Dg. Sitakka No. 129, Maros 90512, South Sulawesi

*E-mail : mulyaningrum@kkp.go.id

Abstract. This study aimed to determine the optimum dose of 17α-MT hormone to improve male shrimp *P. monodon* sperm quality. Male tiger shrimp *P. monodon* originated from pond with an average weight (57.56 ± 12.79) g were transferred to hatchery and acclimated for 1 week prior treatments. Shrimps were set up in controlled tanks in 5 shrimps/tank density. Treatments were 17α-MT hormone induction in different doses, i.e. A = control (ablation); B = 200 ng/100 g of broodstock body weight (BW); C = 300 ng/100 g BW; and D = 400 ng/100 g BW. The 17-α MT hormone was given using injection method every 7 days in 3 times frequency. Research was completely randomized designed with 4 treatments and 2 replications. Observed variables were: the amount of shrimps which carried spermatophores, weight of spermatophores, quantity of spermatozoa, spermatophores histology and water quality. Data of the amount of shrimps which carried spermatophores, weight of spermatophores and the quantity of spermatozoa were analyzed using analysis of variance (ANOVA), while data of spermatophores histology and water quality were analyzed descriptively. The amount of shrimps which carried spermatophores and weight of spermatophores were not significantly different (P>0.05), but spermatozoa quantity was significantly different (P<0.05). The highest spermatozoa quantity was obtained at 300 ng/100 g BW dose in fourth gonad maturity stage. The 17α-MT in 300 ng/100 g BW was the optimum dose for *P. monodon* sperm quality improvement and it could be applied to replace ablation method.

1. Introduction
The reproduction performance of tiger shrimp (*Penaeus monodon*) broodstock originated from ponds was lower than that of natural broodstock, tiger shrimp (*P. monodon*) broodstock in ponds could spawn, but the eggs couldn’t hatch [1]. The eggs that did not hatch were thought to be caused by the low quality of male tiger shrimp (*P. monodon*) sperm. An underdeveloped spermatophore caused the male shrimps to be unable to mate naturally both in ponds and in controlled tanks [2]. Low rate of natural mating caused a reduction in eggs fertilized by male shrimp sperm [3]. One of the efforts to improve the quality of male tiger shrimps originated from ponds was by doing hormonal induction.

Hormone induction on shrimps has been carried out by several researchers. Previous study have reported the maturation of *Penaeus japonicus* gonads which was carried out through 17α-hydroxyprogesterone hormonal stimulation [4]. Serotonin hormone injection to stimulate the maturation and spawning of *Penaeus vannamei* [5]. Combination of gonadotropin and antidopamine in *Litopenaeus vannamei* shrimp [6] and combination of gonadotropin (GTH) and anti-dopamine (AD) at a concentration of 0.3 mL/100g broodstock, resulted in 92% higher spawning rate than the spawning rate with eye ablation (60%) [7]. The used of 17-α MT hormone to obtain male gonads in fish and crustaceans [8]. This hormone is a synthetic androgen hormone that functions to affect sperm production. The 17α-MT hormone will also affect neurons through the preoptic hypothalamus [9]. Furthermore, the injection
of 17α-MT hormone to unproductive natural giant tiger shrimp, showed higher number of sperm than uninfected control [10]. Based on this result, the hormone will be used to inject the male tiger shrimps (P. monodon) to improve the quality of the sperm. The information about 17α-MT hormone induction in male broodstock of tiger shrimps originated from ponds was still limited. Therefore, it is necessary to improve the quality of male tiger shrimp broodstock originated from ponds through the induction of 17α-MT hormone. The aimed of this study is to evaluate the optimal dose of 17α-MT that can increase the sperm quality of tiger shrimps originated from ponds.

2. Materials and Method

2.1. Field work
The research was conducted at the hatchery installation in Barru, South Sulawesi. The study used male tiger shrimp (P. monodon), originated from ponds with an average weight of 57.56 ± 12.79 g and an average length of 17.95 ± 1.31 cm. The broodstock was transferred to the hatchery and acclimatized for 1 week prior to treatment. The shrimps were kept in a 3-ton-volume main tank equipped with water intake and discharge channels as well as oxygen networks with male broodstocks density of 5 males/tank.

2.2. Induction of 17α-MT hormone
The 17α-MT hormone induction was performed by injection method. The treatment was done with different doses of hormones, namely: A = control (ablation); B = 200 ng/100 g BW; C = 300 ng/100 g BW, and D = 400 ng/100 g BW. The research design was completely randomized designed consisting of 4 treatments and 2 replications for each treatment.

The broodstocks were labeled by inserting a number on one of its eyestalks. Prior to the injection, morphological observations were made on the male broodstock, the broodstock carrying the spermatophore was removed by electric shock. Hormone induction was done every 7 days with a frequency of 3 injections. Hormone-induced male broodstock were restored and adapted in an extruded polystyrene foam box filled with sea water that had been equipped with pure oxygen for 10-15 minutes then returned to the rearing tank.

The combination of fresh feed and pellets was given as much as 15% of the total weight of shrimps (45% squid, 20% shellfish, 15% sea worms, and 20% commercial semi-moist pellets). Feeding was done in 3 times/day in the morning, afternoon and evening.

Observation of the response of hormone induction to the sperm production was carried out 10 days after the last injection. The spermatophore was released by electric shock. The observed variables included: the percentage of male broodstock carrying spermatophore, the weight of spermatophore, the number of spermatozoa and the histology of spermatophore. The observation on the number of the sperm was carried out under a microscope using a physiological solution as a sperm medium. The obtained spermatophores were then preserved in formalin buffer for histological analysis.

2.3. Water quality monitoring
As supporting data, water quality observations were made including: temperature, salinity, pH, and dissolved oxygen.

2.4. Data analysis
The research data were analyzed using analysis of variance (ANOVA), while the histological data of the spermatophore and water quality were analyzed descriptively.
3. Result and Discussion

3.1. Percentage of ale shrimp carrying spermatophores

Induction of 17α-MT hormone at a dose of 200 ng/100 g resulted in 30% male shrimp carrying spermatophores; 45% at a dose of 300 ng/100 g; 67.50% a dose of 400 ng/100 g, and 55% at the control (Figure 1).

Figure 1. Effect of 17-α MT induction on percentage of male shrimp carrying spermatophore

The results of analysis of variance (ANOVA) showed that the administration of 17α-MT hormone at different doses did not have a significant effect on the percentage of broodstock carrying spermatophores (P > 0.05).

Several factors that influence sperm production in male tiger shrimp *P. monodon* were the weight of shrimp, the age and the environmental conditions of cultivation [11]. The maturation of male shrimp reproductive organs (testes, vas deferens, terminal ampoules, and spermatophore) is influenced by the shrimp age, while the body weight explains differences in growth conditions [12].

3.2. Spermatophore weight

The weight of the spermatophore from each treatment was presented in Figure 2. The dose of 17α MT hormone of 200 ng/100 g resulted in an average spermatophore weight of 21.0 mg. 300 ng/100 g dose resulted in a sperm weight of 14.50 mg. The dose of 400 ng/100 g resulted in a sperm weight of 23.50 mg. Meanwhile, the control treatment resulted in a sperm weight of 26.50 mg (Figure 2).

Injection of 17α-MT hormone at different doses did not have a significant effect on the spermatophore weight (P > 0.05). The same result was obtained previous study, the stimulation of the spermatophore with different diets did not provide significant results on the weight of the spermatophore [13]. The weight of the spermatophore is related to the body weight of the shrimp and is not related to the number of spermatozoa produced [14].
3.3. The amount of spermatozoa

Giving hormone at a dose of 300 ng/100 g resulted in the highest spermatozoa density ($16.83 \times 10^6$ cells/mL). The lowest of spermatozoa density ($8.47 \times 10^6$ cells/mL) was resulted at a dose of 200 ng/100 g. Hormone injection at a dose of 400 ng/100 g and the control treatment resulted in the spermatozoa density of ($13 \times 10^6$ cells/mL) and ($11 \times 10^6$ cells/mL), respectively (Figure 3). Administration of 17α-MT hormone at different doses had a significant effect on sperm density ($P < 0.05$).

Apart from using hormones, sperm stimulation in shrimp could also be done through feed [15]. Some of the hormones commonly used to improve sperm performance were steroid hormones [16], pituitary hormones [17], thyroid hormones [18] and testosterone [19].

Figure 2. Male tiger shrimp *P. monodon* spermetophore weight in different doses of 17α-MT hormone

Figure 3. Number of spermatozoa of male tiger shrimp *P. monodon* in different doses of 17α-MT hormone. Values with the same superscript after means value were not significantly different ($P > 0.05$) in Duncan test.
3.4. Spermatophore histology

The results of histological analysis showed that there were differences in the level of gonad maturity in the administration of 17-α-methyl testosterone hormone with different concentrations as shown in Figure 4.

Gonad maturity level of giant tiger shrimps of the control treatment were at maturity stage V which was indicated by the presence of spermatozoa remains. The male broodstock of tiger shrimps in the treatment of a hormone dose of 200 ng/100 g were at maturity stage III. Its histological analysis showed that there were fewer spermatozoa compared to spermatocyte. The broodstock treated by 300 ng/100 g dose of hormone were at maturity stage IV with indication of spermatozoa dominance. Meanwhile, the treatment of hormone doses of 400 ng/100 g resulted in tiger shrimps at maturity stage III.

The gonad maturity level in the ablated shrimp was higher than the hormone injection treatment. It was suspected that the regulation of the hormone injected in the shrimp body takes longer than the ablation method, so the gonad maturity level was lower than that of ablation method.

![Figure 4](image)

**Figure 4.** Tiger shrimp *P. monodon* gonad maturity stage in different doses of 17α-MT hormone induction: A. gonad maturity stage V: spermatozoa remains (black arrow); B. gonad maturity stage III: the amount of spermatozoa (black arrow) less than spermatocyte (red arrow); C. gonad maturity stage IV: dominant spermatozoa (black arrow) and D. gonad maturity stage III: the amount of spermatozoa (black arrow) less than spermatocyte (red arrow).

At maturity stage III, spermatids have a slightly stained nucleus and medium-sized acrosome space, an oval anterior acrosome body with moderate electron density formed on the lateral internal wall of the acrosome space [20]. The cytoplasm was filled with many small pre-acrosome vesicles. The nucleus of the spermatids was slightly less dense and the cytoplasm was reduced to the thin cytoplasm surrounding the nucleus. In addition, the body of the anterior acrosome had become more rounded and have been moved from the lateral to the more central region. At maturity stage IV, the spermatids had a nucleus with more stains and larger acrosome space. The spermatid acrosome space at maturity stage IV and V was as large as the core. The cellular shape at maturity stage V was rounder and larger than that of maturity stage IV. At maturity stage V, the anterior acrosomal body was denser in the enlarged acrosome space. There was a residual plasma from syncytia that was degenerated. The cytoplasm was greatly reduced, and there was a remnant of body resembling mitochondria in it.
3.5. Water quality

There was no difference in water quality in each treatment of this study (Table 1). In general, water quality during the maintenance of giant tiger shrimps was in a suitable condition for cultivation. Water quality was one of the factors that determine the success of shrimp farming. Salinity was closely related to the osmotic process and the regulation of shrimp ions to the environmental fluid. High salinity could result in the inhibition of shrimp growth [25]. Temperature greatly affects the growth of aquaculture organisms. The common temperature for the environment of tropical species ranges from 29–30°C [26]. pH was one of the vital environmental characteristics that affect metabolism and physiological processes in shrimp [27]. The optimum pH ranging from 6.8 to 8.7 could increase growth and maximum production. The minimum value of dissolved oxygen was 3.9 mg/L and the maximum value was 4.2 mg/L for the rearing of tiger shrimps (P. monodon) in ponds [28].

| Table 1. Water quality during experiment |
|------------------------------------------|
| Water quality variables                  | Doses of 17-α MT (ng/100 g) | References |
|                                          | Control | 200 | 300 | 400 |                                      |
| Salinity (ppt)                           |         |     |     |     | 10-35 [21]                           |
| Minimum                                  | 32.9    | 32.9| 32.9| 32.9|                                      |
| Maximum                                  | 33.4    | 33.4| 33.4| 33.4|                                      |
| Mean±std                                 | 33.15±0.29 | 33.15±0.29 | 33.15±0.29 | 33.15±0.29 |
| Temperature (°C)                         |         |     |     |     | 26-30 [22]                           |
| Minimum                                  | 29.4    | 29.4| 2.94| 29.4|                                      |
| Maximum                                  | 30.2    | 30.1| 30.2| 30.1|                                      |
| Mean±std                                 | 29.78±0.43 | 29.78±0.38 | 29.80±0.41 | 29.75±0.40 |
| pH                                       |         |     |     |     | 7.5-9.0 [23]                         |
| Minimum                                  | 7.91    | 7.91| 7.91| 7.93|                                      |
| Maximum                                  | 8.07    | 8.04| 8.03| 8.07|                                      |
| Mean±std                                 | 7.99±0.07 | 7.98±0.08 | 7.98±0.06 | 7.99±0.08 |
| DO (ppm)                                 |         |     |     |     | 4-7 [24]                             |
| Minimum                                  | 7.17    | 6.53| 6.53| 7.17|                                      |
| Maximum                                  | 7.92    | 7.68| 7.92| 7.68|                                      |
| Mean±std                                 | 7.44±0.34 | 7.19±0.53 | 7.22±0.59 | 7.41±0.24 |

4. Conclusion

The 17α- methyl testosterone hormone at a dose of 300 ng/100 g resulted in the best response of shrimp spermathopore the highest sperm amount and better level of gonad maturity than other doses. Therefore, this dose could be used to replace the ablation method.

Acknowledgement

This research was funded by the 2019 State Budget for the Research Institute for Brackish Water Aquaculture and Fisheries Extension. Thank you very much to drh. Wahyuni who has contributed in the histological analysis of the spermatophore, and technicians who have helped this research.

References

[1] Lante S and Haryanti 2005 The performance of spermatozoa of tiger shrimp (Penaeus monodon) originated from sea and ponds. Jurnal Penelitian Perikanan Indonesia 11(7): 10-5. (In Indonesia)
[2] Laining A, Usman, Muslimin and Palinggi N N 2014 Growth and reproductive performance of pond reared tiger shrimp fed different combination of maturation diet. *Jurnal Riset Akuakultur*, 9 (1): 67-77. (In Indonesia)

[3] Pontiopatake P, Vanichhviriyakit R, Chavadej J, Plodpai P, Pratoomchart B, Sobhon B and Withayachumnarkul, B. (2007). Acrosome reaction in the sperm of the black tiger shrimp, *Penaeus monodon* (decapoda, Penaidae). *Aquaculture Research*, 38:1635-44.

[4] Yano I 1988 Effect of 17 Alpha-Hydroxy-Progesteron on vitellogenin secretion in Kuruma prawn, *Penaeus japonicas*. *Aquaculture* 61:49-57.

[5] Vaca A A and Alfaro J 2000 Ovarian maturation and spawning the white shrimp, *Penaeus vannamei*, by serotonin injection. *Aquaculture* 182:373-85.

[6] Ramdani H 2013 Hormonal manipulation on vannamie shrimp for 28 days as a substitute of eyed ablation technique in realizing the appearance of the gonads. Thesis. Aquaculture Department of Fisheries and Marine Science, Bogor Agriculture Institute. Bogor. (In Indonesia)

[7] Laining A, Lante S and Usman 2015 Induce of gonadal maturation and improvement of egg fertilization rate of female broodstock tiger shrimp *Penaeus monodon* through hormonal induction without eyed ablation. *Jurnal Riset Akuakultur*, 10(1):61-8. (In Indonesia)

[8] Zairin M 2002 Sex reversal produce male or female nile tilapia fry (Jakarta: Penebar swadaya) (In Indonesia)

[9] Bary T P, Ashok, M and Marwah P 2007 Stability of 17α-methyltestosterone in fish feed. *Aquaculture* 271: 523-9.

[10] Haryanti Fahrudin and Sembiring S B M 2014 Induction of 17-a methyl testosterone on the spermatogenesis profile of male tiger shrimp *Penaeus monodon*. Annual report of the Research Center for Marine Aquaculture, Gondol, Bali. p 11 (In Indonesia)

[11] Jiang S, Huang J., Zhou F., Chen X., Yang Q., Wen W and Ma Z 2009 Observations of reproductive development and maturation of male *Penaeus monodon* reared in tidal and earthen ponds. *Aquaculture* 292: 121–28.

[12] Ceballos-Vázquez B P, Palacios E, Aguilar-Villavicencio J and Racotta I S 2010 Gonadal development in male and female domesticated whiteleg shrimp, *Litopenaeus vannamei*, in relation to age and weight. *Aquaculture* 308: 116–23.

[13] Braga A L, Nakayama C L, Martins J G, Colares E P and Wasielesky Jr, W 2010 Spermatophore quality of the pink shrimp *Farfantepenaeus paulensis* (Decapoda, Dendrobranchiata) broodstock fed with different maturation diets. *Aquaculture* 307: 44–8.

[14] Prasetyo D, Laining A and Sudrajat A O 2017 Reproductive performances of wild male tiger shrimp *Penaeus monodon* post-injection of oocyte developer without eyestalk ablation. *Jurnal Akualutur Indonesia* 16 (2): 193–204.

[15] Leelatanawit R, Uwaisetwathana U, Khudet J, Klaunchi A, Phomklad S, Wongtripop S, Anghthoung P, Jiravanichpaisal P, and Karoonuthaisiri N 2014 Effects of polychaetes (*Perinereis nuttia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*). *Aquaculture* 433: 266–75.

[16] Alfaro-Montoya J 1996 Effect of 17-alpha-methyltestosterone and 17 alpha hydroxyprogesterone on the quality of white shrimp *Penaeus vannamei* spermatophores. *Journal of the World Aquaculture Society* 27(4): 487–92.

[17] Schulz R W, de França L R, Lareyre J J, LeGac F, Chiarini-Garcia H., Nobrega R H and Miura T 2010 Spermatogenesis in fish. *Gen. Comp. Endocrinol*. 165: 390–411.

[18] Wagner M S, Wajner S M and Maia A L 2008 The role of thyroid hormone in testicular development and function. *J. Endocrinol*. 199: 351–65.

[19] Walker W H 2009 Molecular mechanisms of testosterone action in spermatogenesis. *Steroids* 74: 602–7.

[20] Feng T, Paterson B and Johnston S D 2017 New insights into the spermatogenesis of the black tiger prawn, *Penaeus monodon*. *Journal of Morphology* 278: 689–703.
[21] Murdjani Z, Arifin and Adiwijaya D 2007 Best management practices (BMP) in intensive tiger shrimp *Penaeus monodon* cultivation. Report of Brackish Water Aquaculture Development Center, Jepara. p 67 (In Indonesia)

[22] Atmomarsono M 2003 Some efforts to overcome tiger shrimp disease in a comprehensive and integrated manner. Paper was presented at a consultation meeting and dissemination of environmentally friendly aquaculture technology. Maros South Sulawesi. p 15 (In Indonesia)

[23] Tharavathy N C 2014 Water quality management in shrimp culture. *Acta biologica indica*, 3(1):536-40.

[24] Mangampa M T, Ahmad M, Atmomarsono M and Tjaronge M 2003 Business for hatchery and grow out of fishery commodities. Paper was presented at a consultation meeting and socialization of environmentally friendly aquaculture technology. Cooperation between Aquaculture Research Center and Research Institute for Brackish Water Aquaculture. Maros South Sulawesi. p 17 (In Indonesia).

[25] Tonnek S, Syafaat M N and Haryanti 2015 The growth of fast growing strain of tiger shrimp larvae and selection of broodstock originated from ponds. Proc., of Aquaculture Technology Innovation Forum. Center for Aquaculture Research and Development. Jakarta. pp 979-83 (In Indonesia)

[26] Boyd C.E 1990 Water quality in ponds for aquaculture (Alabama, USA: Auburn University) p 482.

[27] Ramanathan N P, Padmavathy T, Francis S, Athithian and Selvaranjitham N 2005 Manual on polyculture of tiger shrimp and carps in freshwater, Tamil Nadu Veterinary and Animal Sciences University, Fisheries College and Research Institute. Thoothukudi, India. pp 1-161

[28] Pushparajan N and Soundarapandian P 2010 Recent farming of marine black tiger shrimp, *Penaeus monodon* (Fabricius) in South India. *African J. of Basic and Applied Sciences* 2(1): 33-6.