2026 Volume: 02 Issue: 06 www.ijrpa.com ISSN 2456-9995 Research Article

International Journal Research Publication Analysis

Page: 01-13

THE *TESTOSTERONE PROPIONATE* EFFECT ON TESTES MATURATION AND SPERM QUALITY OF GOLDFISH (*CARRASIUS AURATUS*)

***Tristiana Yuniarti, Nadya Rahma Dita, David Paskalucky Siahaan, Istiyanto Samidjan, Dan Alfabetian Harjuno Condro Haditomo**

Departement of Aquaculture, Fisheries and Marine Science Faculty, Diponegoro University, Jl. Jacub Rais, Semarang, Indonesia.

Article Received: 25 April 2026
Article Revised: 15 May 2026
Published on: 04 June 2026

***Corresponding Author: Tristiana Yuniarti**
Departement of Aquaculture, Fisheries and Marine Science Faculty, Diponegoro University, Jl. Jacub Rais, Semarang, Indonesia.

DOI: https://doi-doi.org/101555/ijrpa.8534

ABSTRACT

Testosterone propionate administration into male goldfish (*C. auratus*) will accelerate testicular maturation in fish so that they can produce seeds by shortening the time of gonad maturation. The study aims to determine the best effect and dose of *testosterone propionate* on testicular maturation and sperm quality of goldfish (*C. auratus*). The research took place on March 15, 2023 - April 15, 2023 at BBI Mijen, Animal Health Laboratory Type B Semarang and Chemistry Laboratory FMIPA UNNES. This study used an experimental method with a completely randomized design (CRD) consisting of 4 treatments and 3 repetitions, namely treatment A (no injection), treatment B (0.3 µl/g), treatment C (0.5 µl/g), and treatment D (0.7 µl/g). A total of 12 broodstock that have never spawned before with an age of ± 6 months and body weight of 104.25 ± 20.81 grams were used as test fish. The results showed that the injection of *testosterone propionate* had a significant effect on gonadal maturation of male goldfish (*C. auratus*) with the best dose of 0.5 µl/gram, but had no significant effect on sperm motility of goldfish (*C. auratus*). Sperm quality showed characteristics of milky white color, distinctive fishy odor, sperm volume 0.8 ml, and sperm pH 7.4, motility percentage 66.7 ± 4.71. The results showed that the injection of *testosterone propionate* can have a significant effect on the maturation of male goldfish (*C. auratus*) gonads with the best dose of 0.5 µl/g, but no significant effect on the sperm motility of goldfish (*C. auratus*).

KEYWORDS: Goldfish, Gonadal Maturation, Reproduction, Sperm, Testosterone.

1. INTRODUCTION

The goldfish (*C. auratus*), has a high export value. According to data from the Badan Pusat Statistik (2021), the export value of goldfish in 2021 has risen by 12.33% to USD 34.55 million compared to the previous year, which was only USD 30.76 million. When providing seeds, it is important to consider the spawning time span and the quality of the parent sperm. High-quality eggs and sperm result in superior seeds (Ibrahim *et al*., 2019).

To expedite the spawning cycle, semi-natural spawning can be employed through hormonal stimulation of the fish or alteration of the environment. Goldfish (*C. auratus*) have a relatively infrequent spawning period, with a spawning cycle lasting up to 2 months, resulting in 5 spawning for one year (Saragi *et al*., 2021). Injecting *testosterone propionate* into male fish broodstock accelerates primary and secondary gonad maturation (Wang *et al*., 2022). The study aims to determine the optimal *testosterone propionate* dose and its effect on gonad maturation and sperm quality in male goldfish (*C. auratus*).

2. MATERIALS AND METHODS

2.1. Materials

The study utilized 12 male goldfish (*C. auratus*), aged at approximately six months old and had never spawned before, with body weight of 104.25 ± 20.81 g. The goldfish were procured from a farmer in Bantul, Yogyakarta. Gonadally immature male goldfish broodstock were identified by their smooth pectoral fins and hard abdominal texture.

The study utilized various equipment including four pieces of styrofoam blocks, aerators, filters, seser, buckets, scales, micropipette, 1 ml syringes, EDTA tubes, microtubes, water heaters, microscopes, cameras, surgical scissors, and a Water Quality Checker (WQC).

The materials utilized in this study consisted of commercial *testosterone propionate* hormone trademarked as Testohormon and manufactured by PT Wonderindo Pharmatama, along with physiological NaCl solution, Na EDTA, and artificial feed presented as pellets with a 39% protein content.

The study employed a Completely Randomized Design (CRD) with 4 treatment groups and 3 repetitions. The treatment dosage was determined according to prior research, as documented by Anto *et al.* in 2022. In this study, the treatment doses administered were as follows:

Treatment A: served as the control which did not involve injection

Treatment B: was given a 0.3 μL/g body weight injection of *testosterone propionate* hormone

Treatment C: was given 0.5 µL/g body weight injection of *testosterone propionate* hormone, and Treatment D: was given 0.7 µL/g body weight injection of *testosterone propionate* hormone.

2.2. Methods

The process of adjusting the test fish to the new culture medium is the initial stage of maintenance. The syringe is adjusted to the required dose. The body weight of the goldfish broodstock is measured initially, followed by hormone injection according to the treatment dose. Given that hormone doses are in microliter units, their adjustment is performed with a micropipette.

The fish is placed on a flat styrofoam and its head is covered with a damp cloth to maintain its calmness. Injections are administered into its intramuscular, and the syringe is cautiously removed after the hormone is released into the fish's body. The injection site is then gently pressed with a finger to keep the hormone from escaping. Thereafter, the fish is returned to its rearing environment. Injections are then to be repeated every five days until appropriate characteristics for gonad maturity.

Prospective male goldfish (*C. auratus*) are provided with pellets containing 39% protein for artificial feeding twice a day at 9:00 AM and 3:00 PM. Fish placement is determined based on a uniform injection dose. To evaluate gonad development, male fish gonads (testes) were sampled twice from prospective broodstock before injection and after gonad maturity. Histology preparations were conducted to provide a clearer determination of gonad development.

Sperm retrieval is conducted after the mature gonads of male goldfish (*C. auratus*) have been developed. Sperm is extracted until the fish has expelled all of it. The collection of fish sperm is accomplished through the use of an injection syringe. Subsequently, the sperm's characteristics, including colour, odor, volume, and pH are observed. The remaining sperm is transferred into a microtube container that has been filled with diluent solution. Sperm samples are diluted with physiological NaCl in order to observe sperm motility.

Fish intended for blood extraction are placed on a flat styrofoam surface and covered with a damp cloth over their head to preserve their tranquillity and ensure normal blood condition. The blood sample is then transferred to a vacutainer tube, previously filled with 10% EDTA, employing the same syringe used to extract the blood. The syringe pre-filled with EDTA in the barrel is used to withdraw blood from the lateral vein located at the back of the dorsal fin. The blood samples are subsequently analyzed with the UV-Vis spectrophotometry method.

3. RESULT AND DISCUSSION

3.1. Gonadal Maturity Speed

The process of gonadal maturation in male goldfish (*C. auratus*) starts when *testosterone propionate* is injected into the first fish, followed by visible signs of gonadal maturity such as serrations on the pectoral fins, an enlarged abdomen, and the release of white liquid from the genital. The number of days before these signs appear is used to calculate the speed of gonad maturation.

Before the injection, the male goldfish broodstock appeared to lack serrated pectoral fins and exhibited a hard abdomen without sperm emission. After injection with doses of 0.3 µl/g, 0.5 µl/g, and 0.7 µl/g, the fish demonstrated characteristics of gonad maturity, as shown in Table 1. These results indicate a successful treatment for inducing gonad maturation.

Table 1. Gonad maturity speed of male goldfish (*C. auratus*)

		Treatment		
Repetitions				
	Control	0,3 µl/g	0,5 µl/g	0,7 µl/g
1	-	22 days	16 days	20 days
2	-	23 days	17 days	21 days
3	-	24 days	18 days	22 days
Average±SD	,00±,00[d]	23±0,81[c]	17±0,81[a]	21±0,81[b]

From the start of the rearing period until its completion (day 30), the control group of fish did not yield significant findings, as evidenced by the absence of any discernible changes in the texture of the fish's abdomen or the presence of white liquid upon examination of the urogenital and pectoral fins for serrations. These findings suggest that the fish were not yet fully mature in terms of the gonads.

Based on research, *testosterone propionate* hormone injections into male goldfish broodstock significantly impact gonad maturation speed. Among the four treatments tested, the 0.5 µl/g treatment produced the fastest maturation on days 16, 17, and 18. The treatment of 0.7 µl/g resulted in mature gonads on days 20, 21, and 22, while 0.3 µl/g led to mature gonads on days 22, 23, and 24. The control treatment did not mature gonads until the end of the study. Injecting a dose of 0.5 µl/g body weight increased testosterone levels in male goldfish broodstock, subsequently accelerating the process of spermatogenesis. Testosterone is an essential ingredient in spermatogenesis. According to Delbes et al. (2022), spermatogenesis in fish highly relies on steroid hormones, progestins, and androgens (including testosterone).

3.2. Gonadal Maturity Stages

Morphological observations were made by dissecting the fish gonads. Gonads were observed morphologically based on the shape, colour, development of gonad contents, and gonad weight. Histological gonad observations were made by making histology preparations.

In the treatment of 0.3 µl/g, 0.5 µl/g, and 0.7 µl/g which were previously in the GMS I phase or immature gonads can reach GMS IV or mature gonads after *testosterone propionate* injection. Gonads change in size from 1/8 of the abdominal cavity and have not been filled with sperm to become large up to 1/3 of the abdominal cavity and have been fully filled with sperm, showing a milky white colour.

In treatment A (control) male goldfish broodstock with treatment without injection after maintenance reaches GMS III on day 30. Indicated by changes that initially at the beginning of maintenance at GMS I visible gonads are small filling 1/8 of the abdominal cavity and have not been filled with sperm, after day 30 maintenance shows results.

Based on the study, the optimal dosage for promoting gonad maturation in male goldfish (*C. auratus*) is achieved through a 0.5 µl/g weight treatment, requiring 4 injections of *testosterone propionate* hormone to reach GMS IV. It is noteworthy to mention that the technical abbreviations were explained accordingly upon first use. A treatment consisting of 0.7 µl/g necessitates 5 injections of the hormone to reach GMS IV. Finally, a dosage of 0.3 µl/ g requires 5 injections of *testosterone propionate* hormone to achieve GMS IV. During the 30-day maintenance period, the results of the control treatment showed GMS III had been achieved, indicating that the treatments of 0.7 µl/g, 0.5 µl/g, and 0.3 µl/g are prepared for spawning, while the control treatment cannot be utilized for spawning. This is supported by Mukti et al. (2020), which asserts that male fish broodstock that have entered the GMS IV phase exhibit denser, white testicular characteristics, signifying readiness for spawning.

3.3. Gonadal Somatic Index

The development of gonadal maturity corresponds to the value of the Gonadal Maturity Index. The value of the gonadal somatic index increases with changes in gonadal maturity. The gonadal somatic index is calculated by weighing the body weight of the fish and weighing the gonads of the fish using an analytical balance. According to Farhadi and Harlıoğlu (2019), the gonadal maturity index can be calculated as follows:

$$GSI\ (\%) = \frac{Gonadal\ weight\ (g)}{Body\ weight\ (g)}\ x\ 100\%$$

The study found significant variation in Gonad Somatic Index (GSI) among male goldfish (*C. auratus*) that received *testosterone propionate* hormone injections. Treatment C (0.5 µl/g body weight) showed the highest GSI value of 4.23±0.69^{b}, followed by treatment D (0.7 µl/g body weight) with a value of 3.98±0.23^{b} and treatment B (0.3 µl/g body weight) with a value of 3.78±0.32^{b}. Treatment A (control) resulted in the lowest GSI value of 2.52±0.23^{a}.

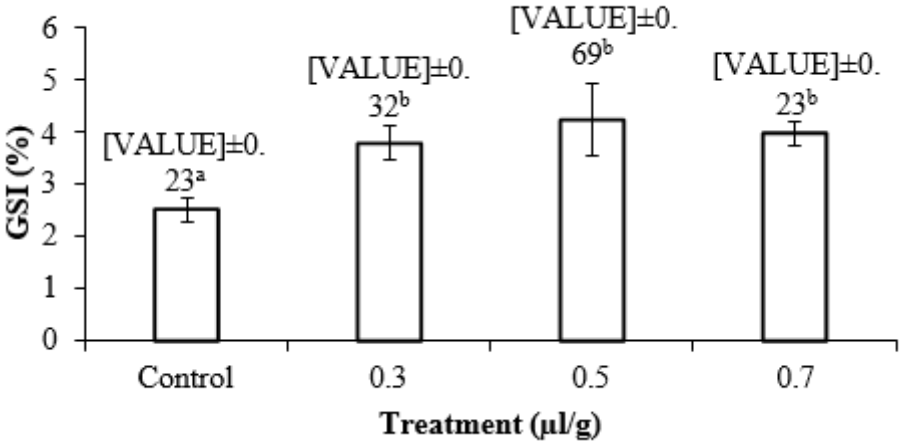

Figure 1. Gonadal Somatic Index (GSI) goldfish (*C. auratus*)

Before the injection of male goldfish broodstock that were not yet gonadally mature, the GSI value was 1.67%, indicating a low GSI condition. The treatment of 0.7 µl/g resulted in gonadally mature male goldfish with GSI values of 3.98%, 3.69%, and 4.26%. The best dose of 0.5 µl/g showed that male goldfish broodstock with mature gonads had GSI values of 4.26%, 3.37%, and 5.08%. In the treatment which received 0.3 µl per gram, the gonadally mature male goldfish broodstock displayed GSI values of 3.79%, 3.38%, and 4.17%. Meanwhile, the control treatment that has reached the GMS III phase exhibited GSI values of 2.31%, 2.85%, and 2.41%. An elevated index of gonadal maturity (GSI) value indicates that the fish has undergone the process of gonadal maturity, implying that it is prepared for spawning. The index of gonadal maturity (GSI) is a dependable tool for assessing fish sexual maturity level and productivity (Bouzzammit et al., 2022).

3.4. Male gonadal Histology

Observations of male testes involve examining their shape, size, location within the body cavity, colour, fluid discharge, or by slicing them to observe their histological structure. Preparations were photographed for observation purposes. Histological observations were conducted before and after injection-induced gonad maturation.

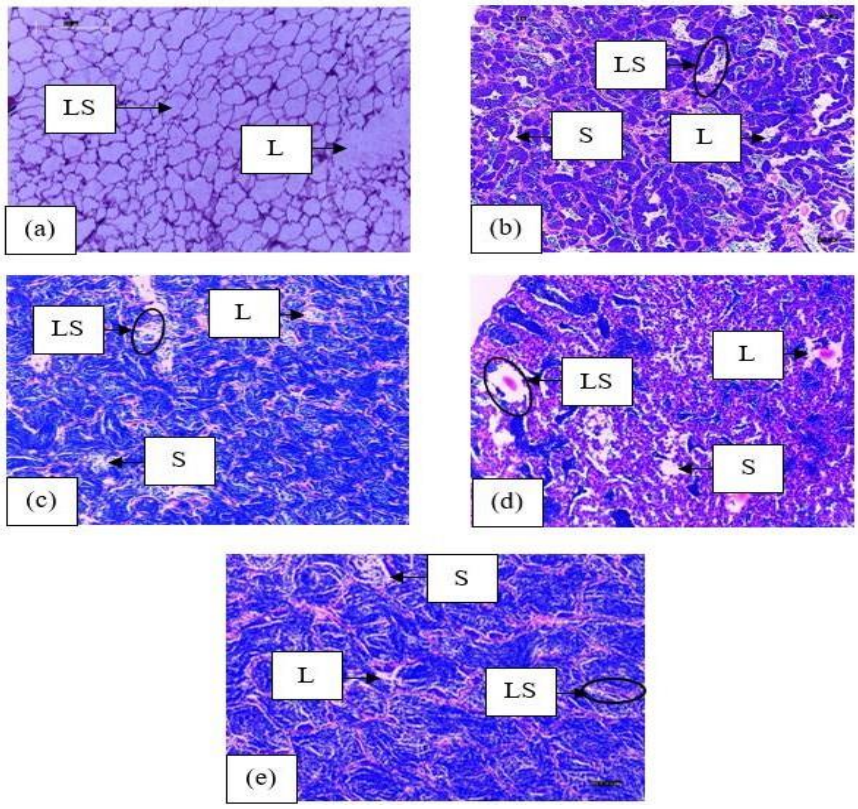

Figure 2. (a) Histology of testicles before injection (10x); (b) Histology of testicles following injection of 0.5 µl/g (10x); (c) Histology of testicles following injection of 0.7 µl/g (10x); (d) Histology of testicles following injection of 0.3 µl/g (10x); (e) Histology of testicles under control treatment. Structures: Seminiferous tubules (LS); Lumen (L); Spermatozoa (S)

The histological observations of male goldfish gonads prior to injection reveal that they are in GMS I, characterized by an unfilled seminiferous lobe with no spermatozoa, an empty lumen, and walls connected to spermatogonium created by Sertoli cells. Subsequent treatment injections of

0.3 µl/g, 0.5 µl/g, and 0.7 µl/g induce gonad maturation, as reflected in the gonads being in GMS IV, where spermatogenesis takes place and spermatids are transformed into spermatozoa. At the conclusion of spermiogenesis, spermatozoa are released and occupy the seminiferous tubules lumen. Use of technical term abbreviations, such as "lumen," will be explained upon first usage. Causal connections between statements are fundamental, and biased or subjective language is to be avoided.

The histological observations of male goldfish gonads in treatment A, conducted without injections throughout the 30-day study period, revealed changes in gonadal maturity. At the GMS

III stage, the seminiferous lobe's lumen was not fully filled with spermatozoa, but the lumen showed signs of being filled. Additionally, spermatogonium produced by Sertoli cells was present on the wall.

The results of histological observations after gonad maturation in the treatment of 0.7 µl/g, 0.5 µl/g, and 0.3 µl/g microscopically have shown entering GMS IV where the lumen containing spermatozoa can be seen. This indicates that sperm have been produced in the seminiferous tubules so that the parent is ready to be spawned. According to Demin et al., (2019) states that in the final stage of spermatogenesis fully formed mature spermatozoa that have filled the lumen of the lobule. In the control treatment on the 30th day of maintenance of histological observations of the gonads changed to the GMS III phase where the seminiferous lobes had begun to fill with primary spermatocytes which had partially developed into secondary spermatocytes and visible spermatids which were located spread out in the seminiferous tubules. This indicates that the gonads of male goldfish (C. auratus) are not ready for spawning.

3.5. Sperm Characteristic

Macroscopic examination of sperm can be conducted through direct observation, which involves assessing the sperm characteristics of volume, colour, pH, and odor. The criteria are used to assess the sperm volume, colour, pH, and odor. Colour and odor are evaluated through direct observation, while volume is measured with a syringe and pH is measured using a pH meter. Technical term abbreviations are defined when first used. The language is neutral, objective, and adheres to standard academic structure and formatting conventions, including consistent citation and footnote styles. At treatment levels of 0.3 µl/g, 0.5 µl/g, and 0.7 µl/g, the sperm appeared milky white and emitted a distinctive odor. Table 2 presents the sperm characteristics data obtained during the study.

Table 2. Volume and pH of fresh sperm of male goldfish. (*C. auratus*)

	Treatments							
Repetitions	Contro		0,3 µl/g		0,5 µl/g		0,7 µl/g	
	Volume (ml)	pH	Volume (ml)	pH	Volume (ml)	pH	Volume (ml)	pH
1	0	0	0,7	7,4	0,75	7,6	0,8	7,4
2	0	0	0,65	7,2	0,7	7,4	0,7	7,4
3	0	0	0,6	7,0	0,6	7,4	0,65	7,2
Average±SD	,0±,0	0±,0	0,65±0,04	7,2±0,16	0,68±0,06	7,4 ±0,09	0,71±0,06	7,3±0,09

Direct observations of sperm morphology were conducted, revealing distinct characteristics in treatments of 0.7 µl/g, 0.5 µl/g, and 0.3 µl/g including a milky white colour and a distinctive fishy odor. In the 0.7 µl/g treatment, volume measurements yielded 0.8 ml, 0.7 ml, and 0.65 ml, with sperm pH levels measuring 7.4, 7.4, and 7.2. The measurement of sperm volume and pH levels in the treatment of 0.5 µl/g demonstrated sperm volumes of 0.75, 0.7, and 0.6 ml and sperm pHs of 7.6, 7.4, and 7.4. Meanwhile, in the 0.3 µl/g treatment, sperm volumes were measured at 0.70, 0.65, and 0.6 ml, and sperm pH levels were 7.4, 7.2, and 7.0. According to Nurfitih et al. (2023), fresh sperm of *cyprinid* fish can be identified macroscopically by their distinct characteristics, including a fishy odor, milky white colour, and pH level around 7. It is not possible to observe these same traits in male goldfish within the control treatment who have underdeveloped gonads.

3.6. Sperm Motility

Sperm motility is measured via microscopic examination by diluting one drop of sperm in a solution on an object glass. Using a microscope with 100x magnification, the motility value of male goldfish (*C. auratus*) injected with *testosterone propionate* hormone was observed. Using a microscope with 100x magnification, the motility value of male goldfish (*C. auratus*) injected with *testosterone propionate* hormone was observed. Results show almost identical motility value amongst all treatments, with the 0.7 µl/g treatment yielding a motility value of 63.33 ± 4. Technical term abbreviations will be explained upon first use. The mean sperm motility result for the treatment of 0.5 µl/g is 66.67 ± 4.71, while for the treatment of 0.3 µl/g it is 63.33 ± 4.71. No sperm motility results are obtained in the control treatment, which can be attributed to the immature gonads of the fish broodstock in treatment A.

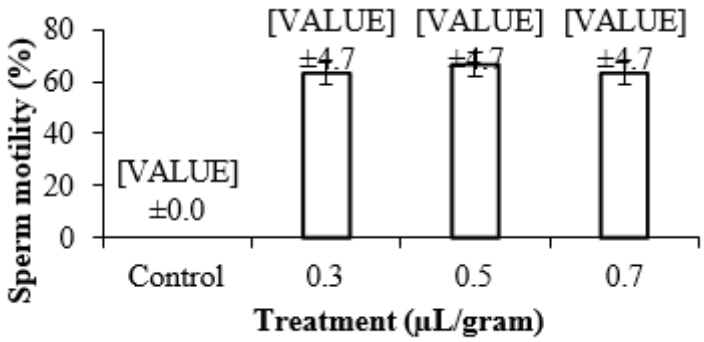

Figure 3. Histogram sperm motility male goldfish. (*C. auratus*)

Based on the study, the mean sperm motility outcomes were consistent across all treatments. Sperm motility of 60%, 60%, and 70% was observed in the 0.7 µl/g treatment. The 0.5 µl/g

treatment yielded sperm motility results of 70%, 70%, and 60%. The 0.3 µl/g treatment showed 60%, 70%, and 60% sperm motility. As the gonads are not mature in control treatment, sperm motility cannot be observed. Angsana (2022) reports that fish sperm diluted with physiological NaCl exhibits 70% motility. The administration of *testosterone propionate* hormone at different doses does not significantly impact male goldfish's sperm motility, and there is no correlation between the hormone administration and male goldfish sperm motility.

3.7. Total Plasma Protein (TPP)

Blood samples were collected from each treatment using 1 ml, then evaluated via the UV-vis spectrophotometric method, which measures light energy from a chemical substance at a specific maximum wavelength. The sample's absorbance is detected with a UV-vis spectrophotometer, and the outcomes are applied to the equation y = bx + a through interpolation.

The data were collected for each treatment: A (20.90 mg/mL), B (6.13 mg/mL), C (18.63 mg/mL), and D (67.27 mg/mL). Testing the Total Plasma Protein (TPP) levels in male goldfish (C. auratus) injected with testosterone propionate hormone was carried out. The results show a significant increase in TPP levels in the blood of male goldfish (*C. auratus*).

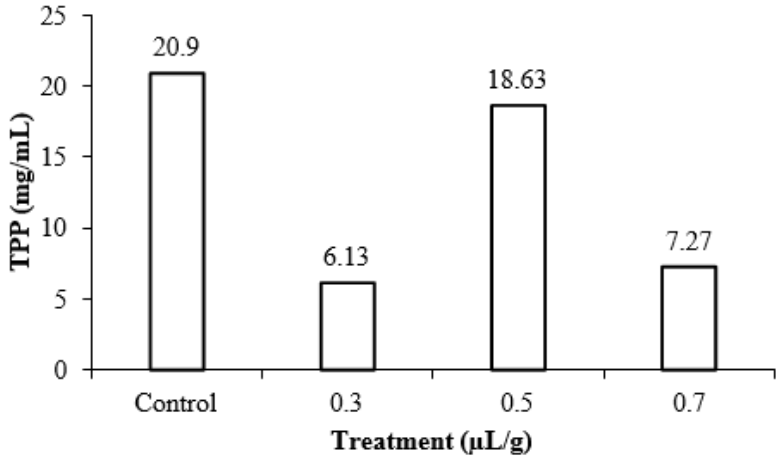

Figure 3. Histogram Total Plasma Protein male goldfish (*C. auratus*)

Before injection or on day 0, the total plasma protein level was 0.45 mg/mL across all treatments. On day - 22, after gonadal maturation, the treatment with 0.3 µl/g of total plasma protein showed an increase to 6.13 mg/mL. The treatment with 0.5 µl/g of total plasma protein, on day - 16 after gonadal maturation, showed an increase to 18.63 mg/mL. On day - 20 after gonadal maturation, the treatment with 0.7 µl/g of total plasma protein showed an increase to

7.27 mg/mL. Finally, the control treatment showed an increase in total plasma protein level to 20.9 mg/mL. The formation of plasma proteins through lipoprotein reactions is responsible for the elevation of plasma protein levels in the bloodstream. Fidianti (2022) reports that gonad maturation results in an increase in the total plasma protein value in fish.

3.8. Water Quality

Water quality parameters were monitored pre and post-spawning. These parameters comprised temperature measured through a thermometer, dissolved oxygen measured through a DO meter, and pH measured through a pH meter.

The outcomes of water quality measurements on the parent rearing media were attained based on conducted research. The parameters ascertained include temperature, pH, and dissolved oxygen. The water quality measurements collected during the study can be observed in Table 3, while Table 3 provides a detailed presentation of the results obtained from these measurements.

Table 3. Water quality parameter.

Parameters	Value range
Tempt (ºC)	26,5 – 28
pH	7,4 — 8
DO	4,1 — 5,4

An analysis was conducted on the water quality of the male carp broodstock rearing containers to ensure that the water met optimal requirements for male carp broodstock survival. Technical term abbreviations were explained on first use. The morning and evening measurements revealed data on temperature, pH, and DO.

4. CONCLUSION

The *testosterone propionate* hormones with varying doses demonstrated a significant impact on the maturation of male goldfish gonads (*C. auratus*), while there was no observed effect on the quality of sperm in goldfish (*C. auratus*). The most effective *testosterone propionate* hormone dose in this study was determined to be 0.5 µl/g, as indicated by gonadal maturation markers (GMS IV) such as changes in serrations on the pectoral fins and texture of the fastest abdomen. The timeframe for such markers to appear is 16 days, and the GMS achieved was 4. The sperm sample demonstrates an average motility percentage of 60% - 70%, with sperm having milky white characteristics and a distinctive smell. The volume of the sperm sample is 0.75 ml and the pH level is 7.6. Additionally, the total plasma protein levels exhibit an increase to 18.63 mg/mL.

REFERENCES

1. Angsana. (2022). Effect of Honey Supplementation in Physiological NaCl Sperm Diluent on Sperm Motility, Fertilization Rate, and Egg Hatching Rate of Nilem Fish (*Osteochilus hasselti*). [Undergraduate Thesis]. Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, 52 pp.
2. Anto. (2022). Effect of Testosterone Administration at Different Doses on the Reproductive Performance of Male African Catfish (*Clarias gariepinus* Burchell). [Undergraduate Thesis]. Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, 91 pp.
3. Badan Pusat Statistik. (2021). Indonesian Ornamental Fish Export Value 2021 [Statistical Report].
4. Statistics Indonesia. https://www.bps.go.id/subject/56/perikanan
5. Bouzzammit, N., El Habouz, H., Ben-Bani, A., & El Ouizgani, H. (2022). Spawning season, size at first maturity, and fecundity in chub mackerel (*Scomber colias* Gmelin, 1789) from the Atlantic coast of Morocco. Regional Studies in Marine Science, 53, 1–12.
6. Delbès, G., Blázquez, M., Fernandino, J. I., Grigorova, P., Hales, B. F., Metcalfe, C., Martin, L. N., Parent, L., Robaire, B., Rwigemera, A., Van Der Kraak, G., Wade, M., & Marlatt, V. (2022). Effects of endocrine disrupting chemicals on gonad development: Mechanistic insights from fish and mammals. Environmental Research, 204, 112126.
7. Demin, S. I., Stefanova, V. N., Granovitch, A. I., & Mikhailova, N. A. (2019). Spermatogenesis and lobular cyst type of testes organization in the marine gastropod *Littorina saxatilis* (Olivi, 1792). Cell and Tissue Research, 376, 457–470.
8. Farhadi, A., & Harlıoğlu, M. M. (2019). The annual cycle of spermatozoa content in the vas deferens and some reproductive parameters in the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823). Aquaculture Reports, 13, 168–180.
9. Fidianti, A. (2022). Effect of 17α-Methyltestosterone Injection at Different Doses on the Reproductive Performance of Male African Catfish Broodstock (*Clarias gariepinus* Burchell). [Undergraduate Thesis]. Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, 95 pp.
10. Hussain, T., Murtaza, G., Kalhoro, D. H., Kalhoro, M. S., Metwally, E., Chughtai, M. I., Mazhar, M. U., & Khan, S. A. (2021). Relationship between gut microbiota and host metabolism: Emphasis on hormones related to reproductive function. Animal Nutrition, 7(1), 1–10.

11. Ibrahim, Y., Fadhillah, R., & Karim, A. (2020). Supplementation of Inorganic Zinc ($ZnSO_4 \cdot 7H_2O$) in Feed on Sperm Motility and Viability of Serukan Fish (*Osteochilus* sp.). Journal of Aquaculture, Teuku Umar University, 3(1), 29–34.
12. Olapade, J., & Tarawallie, S. (2014). The length–weight relationship, condition factor, and reproductive biology of *Pseudotolithus senegalensis* (Valenciennes, 1833) (croakers) in Tombo, Western Rural District of Sierra Leone. African Journal of Food, Agriculture, Nutrition and Development, 14(6), 2176–2189.
13. Pourmohammad, M., Keivany, Y., & Paykan-Heyrati, F. (2021). Reproductive biology of oriental sole, *Brachirus orientalis*, in the northern Persian Gulf. Regional Studies in Marine Science, 47, 1–5.
14. Saragi, C. N. M., Gunawan, I., & Rozik, M. (2021). Effect of Thyroxine Hormone Immersion on Egg Hatching Rate, Growth, and Survival of Oranda Goldfish Larvae (*Carassius auratus* Linnaeus). Journal of Tropical Fisheries, 16(1), 19–26.
15. Sutapa, G. N., & Kasmawan, I. G. A. (2016). Effects of 60Co Gamma Radiation-Induced Mutation on the Physiological Growth of Tomato Plants (*Lycopersicon esculentum* L.). Journal of Radiation Safety and Environment, 1(2), 5–11.
16. Wang, J., Wang, W., Li, J., Luo, Z., Li, Z., Chai, M., Xiang, C., Luo, L., Zhao, R., & Liu, S. (2022). Effects of sex hormone rescue on gametogenesis in allotriploid crucian carp. Aquaculture, 516(7), 1–11.