Induction of Spermiation Using Ovaprim ™ with Topical Gill Method In The Silver Rasbora (Rasbora argyrotaenia)

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Induction of spermiation using Ovaprim™ with topical gill method in the silver rasbora (Rasbora argyrotaenia)

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ABSTRACT

The main obstacles on silver rasbora (*Rasbora argyrotaenia*) culture are having the limited number of broodstock and spawning depending on the season. The purpose of this study was to determine the effect of different dosage of Ovaprim™ induction by topical gill method to silver rasbora spermiation in order to continue the production out of its reproduction season with an optimum dose. A total of 30 male fish with a weight of 7.78 ± 0.20 g and length 4.11 ± 0.31 cm was used in this research. Topical gill treatments of Ovaprim™ were administered with following doses; 0.15 μl/g, 0.25 μl/g, 0.35 μl/g, 0.45 μl/g and 0.55 μl/g body weight. Milt volume, sperm concentration, sperm motility, and sperm viability parameters were observed in this study to understand the optimum dose of Ovaprim™ for male silver rasbora breeders. Spermiation induction of silver rasbora using Ovaprim™ with topical gill method has been successfully carried out, indicating an increase ($P < 0.05$) in milt volume, sperm concentration, sperm motility, and sperm viability. According to results a dose of Ovaprim™ is recommended to be used the 0.25 μl/g body weight in the spermiation induction of silver rasbora.

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1. Introduction

Silver rasbora (*Rasbora argyrotaenia*) belongs to Rasbora genus distributed naturally in the South East Asian countries (i.e. Indonesia, Thailand, Cambodia, Malaysia, and Philippines) [1,2] and this species has become one of the potential freshwater aquaculture products which have economic value, either as consumption [3] or as ornamental fish in these regions. In local fish market and restaurants, it has been served as dried or fried for human consumption, which increases the economic value of Rasbora species. Constraints on the fulfillment of demand due to high economic value in silver rasbora only depend on wild fishing and limited cultivation [4]. The spawning season of silver rasbora alleged that the peak spawning season occurs between September and December at temperature between 25.5 °C and 31.6 °C [4]. One of the obstacles in the production of silver rasbora is having limited number of broodstock [5] and spawning depending on the season [6,7]. In order to overcome these problems breeding methods of silver rasbora need to be improved. The availability of cultured silver rasbora can decrease the fishing pressure on wild stocks and this can be ensured by manipulating spawning out of spawning season through spawning induction [8,9].

Ovaprim™ is a commercial product which is a combination of sGnRH-a (salmon gonadotropin hormone-analogue) and domperidone (antidopamine) that is often used in induction of spawning fish [10] through excitation ovulation and spermiation [11,12]. Spermiation is a development of the spermatid into mature sperm [13]. The use of Ovaprim™ for induction of spermiation has shown to improve the quality and quantity of sperm production in various types of fish such as Levantine scraper (*Capoeta damascina*) [14], Eurasian perch (*Perca fluviatillis*) [15], tench (*Tinca tinca*) [16] and many ornamental fish species [17]. Topical gill induction method is also a potential utility as a spawning induction technique for small fish species [19]. Previously, spawning induction with topical gill induction of Ovaprim™ in rainbow shark (*Epalzeorhynchos erythrurus*) was done successfully. Ovaprim™ application in fish can be done by injection (intramuscular and intraperitoneal) and topical gill method [18,19]. Topical gill is an obvious route for topical application of inducing hormone through the gill [19]. It is an

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application to induce hormone which is different from the other methods such as intramuscular injection and intraperitoneal injection having the side effects on the fish. The advantage of the topical gill method is not to cause scarring and thus hormones can be absorbed directly through the gills [20]. In topical gill method, the success of hormone absorption partly related to nature of the gill barrier between the environment and blood through the gill lamellae that the hormone passes from only 1–5 μm thickness [21].

Based on the description of the Ovaprim™ induction of fish spermiation by topical gill method, it is expected to increase the milt volume, sperm concentration, sperm motility and sperm viability of silver rasbora. In addition, through the use of different doses, optimum dose is expected to be determined on induction of silver rasbora spermiation. The purpose of this study was to determine the optimum dosage of Ovaprim™ induction by topical gill method to silver rasbora spermiation in order to extend the production season in commercial hatcheries.

2. Materials and methods

The study was conducted under the oversight and approved by the Institute for Research and Innovation of Airlangga University (based on the decision letter from the Rector of Airlangga University, 886/UN3/2018).

2.1. Fish origin and husbandry

This research was carried out at the laboratory of Airlangga University in Banyuwangi Campus, East Java, Indonesia in May 2018. A total of 50 male fish with a weight of 7.78 ± 0.20 g and a length of 4.11 ± 0.31 cm was obtained from Technical Implementation of Unit Development of Freshwater Aquaculture of Umbulan (Pasuruan, East Java, Indonesia) in the early May 2018. Sexual maturation of fish was determined by gentle stripping the abdomen to see a little drop of milt. The selected 30 mature males were stocked in the experiment containers (70 × 50 × 50 cm) for one week before the experiment started to minimize stress on fish during treatment. The water temperature was kept at 27–28 °C and dissolved oxygen level was measured 4–5 ppm. Fish were fed with a commercial fish feed (PF-500, Prima Feed™; Indonesia).

2.2. Experimental design and Ovaprim™ induction

Five different doses of Ovaprim™ (Syndel Laboratories Ltd., Canada) were administered under anesthesia (300 ppm MS-222). The Ovaprim™ doses were 0.15 μl/g (n = 5), 0.25 μl/g (n = 5), 0.35 μl/g (n = 5), 0.45 μl/g (n = 5) and 0.55 μl/g (n = 5) and 153 mM NaCl was used in the control group with the dose of 0.2 μl/g on five male fish. The applied amount of Ovaprim™ was calculated considering fish body weight.

The treatment methods were modified from Hill et al. [19]. After anesthesia, fish were taken out of the water by dip net and the experimental doses were applied by a micropipette into fish mouth one by one. The opercula were held close during the application in order to avoid the solution leaking out. Afterwards, each fish was placed on a plastic tray and covered with a damp paper towel for 4 min. After the application finished, each fish were placed into the glass aquarium (40 × 30 × 30 cm). The water temperature was kept at 27–28 °C and dissolved oxygen was measured 4–5 ppm. After 10 h, the time needed for spermiation (based on latency period in common carp with GnRH + domperidone treatment [22]), semen was collected by gentle stripping from abdomen under the anesthesia (300 ppm MS-222) and stocked in 1.5 ml centrifuge tube. Contamination of samples with feces, urine, and water was carefully avoided.

2.3. Semen analysis

Milt was taken with micropipette with a sterile micro tip, calibrated every 0.01 μl [23] and total expressible milt volume was recorded. Milt volume was calculated by dividing expressible milt to total body weight of fish.

To avoid sperm aggregation and achieve an appropriate concentration for counting, milt was diluted 1:1000 times with an immobilizing solution containing 153 mM NaCl. Sperm concentrations were counted by a hemocytometer according to Zadmajid et al. [14]. Cell counting was conducted using a compound Eclipse E200-LED light microscope connected to a video monitor (100x and 400x magnification).

Sperm motility assessments were done by taking 1 μl of the sperm and placing it on the slide glass. The assessments were carried out at a room temperature of 26 °C. Object glass was then covered with a cover glass after adding distillated water for sperm activation. A video recorder attached to tight microscope (Eclipse E200-LED, Nikon, Japan) was used to measure sperm motility (100x and 400x magnification). The sperm motility was estimated by semi quantitative method based on Rurangwa et al. [24]. The measurement of the duration of sperm motility was done by observing the duration of the movement of sperm until it stopped.

The sperm viability was observed by staining method using 1 μl milt and 1 μl eosin 2% staining solution (1:1 ratio) under the light microscope (Eclipse E200-LED, Nikon, Japan) connected to a video monitor (1000x magnification). The process was checked for 15 s.

2.4. Statistical analysis

The observed parameters were analyzed using variance analysis (ANOVA) with 95% confidence level. Significance between applied doses was identified using the Duncan's test with SPSS version 7.0. Statistical software. Results are presented as means ± SEM.

3. Results

Treatment of Ovaprim™ using topical gill method with different doses gave a more significant effect ($P < 0.05$) on the volume of silver rasbora milt than control group (Fig. 1a). The highest volume of milt was obtained 0.77 ± 0.03 μl/g at a dose of 0.45 μl/g. The results were not significantly different with a dose of 0.35 μl/g and

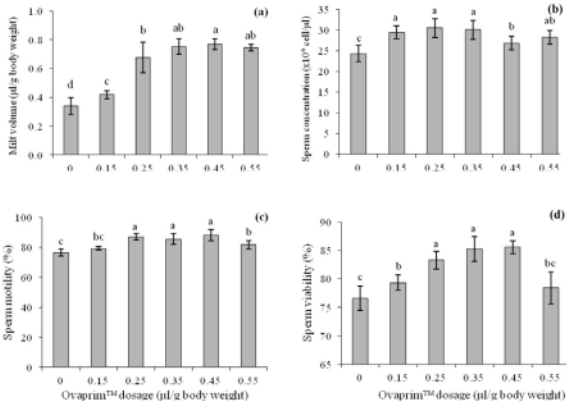

Fig. 1. The milt volume (a), sperm concentration (b), individual sperm motility (c), and sperm viability (d) of silver rasbora (R. argyrotaenia) which were given different doses of the Ovaprim™ with topical gill method (n = 5, mean ± SEM). Different letters above the bars showed a significant difference ($P < 0.05$).

0.55 µl treatments which resulted in milt volume of 0.75 ± 0.05 µl/g and 0.74 ± 0.02 µl/g respectively. However, a dose of 0.25 µl/g treatment resulted in a milt volume of 0.67 ± 0.1 µl/g body weight, less than a dose of 0.45 µl treatment ($P < 0.05$) but not significantly different from a dose of 0.35 µl/g and 0.55 µl/g treatments ($P > 0.05$) (Fig. 1a). A dose of 0.15 µl/g treatment resulted in the lowest milt volume of 0.41 ± 0.02 µl/g body weight (Fig. 1a).

All Ovaprim™ treatments with different doses gave a significantly higher ($P < 0.05$) spermatozoa concentration of silver rasbora than control group (Fig. 1b). The highest spermatozoa concentration was obtained 30.54 ± 2.28 × 10^6 cells/µl at a dose of 0.25 µl/g treatments. The results were not significantly different ($P > 0.05$) from a dose of 0.15 µl/g, 0.35 µl/g, and 0.55 µl/g treatments which resulted in spermatozoa concentration of 29.5 ± 1.54 × 10^6 cells/µl, 30.12 ± 2.27 × 10^6 cells/µl, and 28.34 ± 1.65 × 10^6 cells/µl respectively (Fig. 1b). However, spermatozoa concentration with a dose of 0.45 µl/g treatment resulted in 26.86 ± 0.6 × 10^6 cells/µl which was significantly less than treatment of a dose of 0.15 µl/g, 0.25 µl/g, and 0.35 µl/g ($P < 0.05$) but not significantly different from a dose of 0.55 µl/g ($P > 0.05$).

The highest individual motility of spermatozoa was obtained at a dose of 0.45 µl/g treatment which was 88.2 ± 3.70%. The results were not significantly different from a dose of 0.25 µl/g and 0.35 µl/g treatments which resulted in motility of individual spermatozoa 86.5 ± 2.16% and 85.4 ± 3.50% respectively (Fig. 1c). The dose of 0.15 µl/g treatment resulted in motility of individual spermatozoa of 79.2 ± 1.30%, not significantly different from The dose of 0.55 µl/g treatment ($P > 0.05$). The control treatment gave the smallest spermatozoa motility than all other treatments but not significantly different from a dose of 0.15 µl/g treatment ($P > 0.05$). No significant effect of Ovaprim™ induction on motility duration of spermatozoa was found ($P > 0.05$) but the duration of motility of spermatozoa of silver rasbora on all doses of treatment showed a longer duration than control group (Table 1).

The highest viability of spermatozoa was obtained 85.58 ± 1.15% at the dose of 0.45 µl/g treatment (Fig. 1d). The sperm viabilities were not significantly different between the doses of 0.25 µl/g, 0.35 µl/g and 0.45 µl/g which resulted in 83.32 ± 1.56%, 85.26 ± 2.14% and 85.58 ± 2.14% of viability respectively ($P > 0.05$). Treatments of a dose of 0.25 µl/g and 0.35 µl/g gave no significant difference. The control treatment gave the smallest spermatozoa viability compared with the other treatments except for a dose of 0.55 µl/g treatment (Fig. 1d).

4. Discussion

Inducing an increased dose of Ovaprim™ gave the tendency of an increase in the volume of milt and also motility and viability of spermatozoa including the dose of 0.45 µl/g and it tended to decrease at the highest dose of 0.55 µl/g (Fig. 1). Similar results on volume of milts, motility, and viability of spermatozoa were found in different species such as chub (*Leuciscus leuciscus*) [25], barbel (*Barbus barbus*) [26] and longspine scraper (*Capoeta trutta*) [27] in previous studies. However, the concentration of spermatozoa in sperm suspension is inversely proportional to the volume of the sperm suspension [27] and in this study, it tended to decrease at the dose of 0.45 µl/g.

Table 1
Sperm motility (individual (%) and duration (s)) of silver rasbora (*R. argyrotaenia*) which were given different doses of the Ovaprim™ with topical gill method (n = 5).

Treatment (µl/g body weight)	The duration of sperm motility (s)
0 (Control)	103.6 ± 8.02
0.15	118.2 ± 25.12
0.25	112 ± 29.97
0.35	111.8 ± 20.87
0.45	110 ± 12.90
0.55	109.6 ± 13.10

The hormone sGnRHa present in Ovaprim™ is a hormone analogous to gonadotropin releasing hormone (GnRH) that directly stimulates the pituitary gland to secrete luteinizing hormone (LH) [28]. Krol et al. [29] explains that GnRH is able to stimulate the pituitary gland directly or indirectly to produce hormones that accelerate spermiation and increase sperm production while antidopamine that includes in Ovaprim™ serves to block dopamine that inhibits LH secretion [28] which acts indirectly in the process of spermiation and hydration of spermatozoa [18]. Therefore, the increase of the volume of milt due to the inducing Ovaprim™ up to a particular dose can be explained by an increase in hydration processes that cause an increase in number of testicular and seminal fluid, and increased rate of spermiation or spermiogenesis [30]. Moreover, LH stimulates the Leydig cells that are found in the testes to produce steroid hormones 11-ketotestosterone (11-KT) and 17α,20β-dihydroxy-4pregnen-3-one (DHP) that play a role in the process of spermiogenesis [31].

However, the highest dose of Ovaprim™ (0.55 µl/g) in this study led to a decrease in the number of milt volume, which is probably because of the nature of homeostasis in the body causing the lack of body's response to stimulation from the outside [30]. It is known that high doses of Ovaprim™ might have a negative effect to the spermiation activity; therefore, milt volume can be low [32]. It is also suspected that this study has the similar trend on decreasing concentration, motility and sperm viability of silver rasbora sperm at a dose of 0.55 µl/g.

In this study, the increase in the number of milt volume is directly proportional to the increase of sperm concentration (Fig. 1a and b). Increased sperm concentration occurred when Ovaprim™ was applied up to a dose of 0.35 µl/g and tended to decrease at the doses of 0.45 and 0.55 µl/g. Similar results were found in *Etroplus suratensis* [33] and some other species [14,34,35] that the hormonal treatment using Ovaprim™/sGnRHa increased sperm density coinciding with increases in milt volume. Contrary to these, in another study, sperm concentration of chub (*Leuciscus chepalus*) was inversely proportional to the volume of sperm produced, due to a slower spermiation rate than its sperm hydration rate [25]. These findings show that each species has a different sperm hydration response to Ovaprim™ treatment.

In teleost, the sperm motility is an important factor in the evaluation of sperm quality [35,36]. It was confirmed that exogenous hormonal treatment in fish affects sperm motility, but differences between the control group and fish subjected to hormonal treatment have not always been significant [30] which was seen in this study in the duration of sperm motility. In contrast, the individual sperm motility was significantly higher than control group except at the dose of 0.15 µl/g treatment. Similar results were found in spermatozoa motility of Northern pike (*Esox lucius*) due to the administration of Ovaprim™ [12]. Increased individual sperm motility is thought to be associated with an increase in seminal plasma osmotic pressure. The seminal plasma environment affected not only sperm motility but also sperm production and maturation [12]. To understand the reason of this increase clearly, further research supposed to be carried out on seminal plasma osmotic pressure.

The viability of spermatozoa on the results of studies showed the same tendency with the motility of the spermatozoa. The similar viability of spermatozoa obtained in three doses of Ovaprim™ 0.45, 0.35 and 0.25 µl/g (Fig. 1d). In this study, it was found that the value of the viability of spermatozoa was directly proportional to the volume of the milt and sperm concentration. In the

previous study [38] it was indicated that a large volume of milt on the availability of plasma protein in seminal plasma served to maintain sperm motility and this statement might be the reason of the high spermatozoa viability in this study.

5. Conclusions

In this study, spermiation induction of silver rasbora using Ovaprim™ with topical gill method has been successfully carried out for the first time and an increase in milt volume, sperm concentration, sperm motility, and sperm viability were observed. In summary, this study demonstrates that doses of 0.25 μl/g, 0.35 μl/g and 0.45 μl/g are treatments that produce the best performance or response to improve milt quality and quantity. Therefore, it can be recommended that farmers should use the dose of 0.25 μl/g Ovaprim™ in spermiation induction as a part of artificial breeding of silver rasbora production because it is the smallest and most cost effective dose among the three doses. These results can lead the silver rasbora hatcheries to apply out of season production in order to increase production capacity which might increase the production and decrease overfishing pressure on the wild stocks of silver rasbora.

Conflicts of interest

Authors have no competing interests to declare.

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References

[1] Kusuma WE, Samuel PD, Wiadnya DGR, Hariati AM, Kumazawa Y. Complete mitogenome sequence of *Rasbora argyrotaenia* (Actinopterygii: Cyprinidae). Mitochondrial DNA Part B Resour 2017;2:373–4. https://doi.org/10.1080/23802359.2017.1347835.

[2] Aryani N. Native species in Kampar Kanan river, Riau province Indonesia. Int J Fish Aquat Sci 2015;2:213–6.

[3] Herawati T, Yustiati A, Nurhayati A, Mustikawati R. Proximate composition of several fish from jatigede Reservoir in sumedang district, west Java. IOP Conf Ser Earth Environ Sci 2018. https://doi.org/10.1088/1755-1315/137/1/012055. 137-7.

[4] Rosadi E, Herawati EY, Setyohadi D, Bintoro G. Distribution, composition, and abiotic environment of Silver Rasbora (*Rasbora argyrotaenia* Blkr) fish in upstream areas of Barito watershed, South Kalimantan. J Environ Ecol 2014;5:117–31. https://doi.org/10.5296/jee.v5i1.5880.

[5] Araki H, Berejikian BA, Ford MJ, Blouin MS. Fitness of hatchery-reared salmonids in the wild. Evol Appl 2008;1:342–55. https://doi.org/10.1111/j.1752-4571.2008.00026.x.

[6] Rouxel C, Suquet M, Cosson J, Severe A, Quemener L, Fauvel C. Changes in Atlantic cod (*Gadus morhua* L .) sperm quality during the spawning season. Aquacult Res 2008;39:434–40. https://doi.org/10.1111/j.1365-2109.2007.01852.x.

[7] Alavi SMH, Pšenička M, Policar T, Rodina M, Hamáčková J, Kozák P, et al. Sperm quality in male *Barbus barbus* L . fed different diets during the spawning season. Fish Physiol Biochem 2009;35:683–93. https://doi.org/10.1007/s10695-009-9325-7.

[8] Mylonas CC, Fostier A, Zanuy S. Broodstock management and hormonal manipulations of fish reproduction. Gen Comp Endocrinol 2010;165:516–34. https://doi.org/10.1002/9781444392210.ch4.

[9] Yaron Z, Bogomolnaya A, Drori S, Biton I, Aizen J, Kulikovzky Z, et al. Spawning induction in the carp: past experience and future prospects - a review. Isr J Aquac 2009;61:5–26.

[10] Arfah H, Maftucha L, Carman O. Induced spawning of giant gouramy *Osphronemus gouramy* Lac. by Ovaprim. J Akuakultur Indones 2006;5:103–12.

[11] Acharjee A, Chaube R, Joy KP. Ovaprim, a commercial spawning inducer, stimulates gonadotropin subunit gene transcriptional activity: a study correlated with plasma steroid profile, ovulation and fertilization in the catfish *Heteropneustes fossilis*. Gen Comp Endocrinol 2017;251:66–73. https://doi.org/10.1016/j.ygcen.2016.10.001.

[12] Cejko BI, Krejszef S, Żarski D, Judycka S, Targońska K, Kucharczyk D. Effect of carp pituitary homogenate (CPH) and sGnRHa (Ovaprim) on northern pike (*Esox lucius*) spermiation stimulation and its effect on quantity and quality of sperm. Anim Reprod Sci 2018;193:217–25. https://doi.org/10.1016/j.anireprosci.2018.04.073.

[13] Mañanós E, Duncan N, Mylonas CC. Reproduction and control of ovulation, spermiation and spawning in cultured fish. In: Cabrita E, Robles V, Herráez P, editors. Methods reprod. Aquac. Mar. Fresh water species. Boca Raton, London, Newyork: CRC Press, Taylor and Francis Group; 2009. p. 3–80. https://doi.org/10.1533/9781845696474.1.109.

[14] Zadmajid V, Bashiri S, Shara N, Butts AIE. Effect of hCG and Ovaprim™ on reproductive characteristics of male Levantine scraper, *Capoeta damascina* (Valenciennes, 1842). Theriogenology 2018;115:45–56. https://doi.org/10.1016/j.theriogenology.2018.04.017.

[15] Żarski DŻ, Bernáth G, Król J, Cejko BI, Bokor Z, Pallińska-Żarka K, et al. Effects of hCG and salmon gonadoliberine analogue on spermiation in the Eurasian perch (*Perca fluviatilis*). Theriogenology 2017;104:179–85. https://doi.org/10.1016/j.theriogenology.2017.08.022.

[16] Podhorec P, Gosiewski G, Ammar I Ben, Sokolova-Mikolajczyk M, Chyb J, Milla S, et al. The effect of GnRHa with or without dopamine inhibitor on reproductive hormone levels and sperm quality in tench *Tinca tinca*. Aquaculture 2017;470:91–4. https://doi.org/10.1016/j.aquaculture.2016.12.023.

[17] Hill JE, Kilgore KH, Pouder DB, Powell JFF, Watson CA, Yanong RPE. Survey of Ovaprim use as a spawning aid in ornamental fishes in the United States as administered through the University of Florida Tropical Aquaculture Laboratory. N Am J Aquacult 2009;71:206–9. https://doi.org/10.1577/A08-020.1.

[18] Yanong RPE, Martinez C, Watson CA. Use of Ovaprim in ornamental fish aquaculture, vols. 1–8; 2009.

[19] Hill JE, Baldwin JD, Graves JS, Leonard R, Powell JFF, Watson CA. Preliminary observations of topical gill application of reproductive hormones for induced spawning of a tropical ornamental fish. N Am J Aquacult 2014;67:7–9. https://doi.org/10.1577/FA04-015.1.

[20] Podhorec P, Kouril J. Induction of final oocyte maturation in Cyprinidae fish by hypothalamic factors: a review. Vet Med 2009;54:97–110. https://doi.org/10.17221/50/2009-VETMED.

[21] Sherwood NM, Harvey B. Topical absorption of gonadotropin-releasing hormone in Goldfish. Gen Comp Endocrinol 1986;19:13–9. https://doi.org/10.1016/0016-6480(86)90244-3.

[22] Dorafshan S, Mostafavi H, Amiri BM. Induction of spawning in common carp *Cyprinus carpio*, using pituitary extract and GnRH analogue in combination with Domperidone. Iran J Biotechnol 2003;1:213–7.

[23] Cejko BI, Targońska K, Kowalski RK, Zarski D, Sarosiek B, Kucharczyk D, et al. The effectiveness of hormonal preparations (Ovopel, Ovaprim, LHRHa, hCG and CPE) in stimulating spermiation in dace *Leuciscus leuciscus* (L.). J Appl Ichthyol 2012;28:873–7. https://doi.org/10.1111/jai.12054.

[24] Rurangwa E, Kime DE, Ollevier F, Nash JP. The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture 2004;234:1–28. https://doi.org/10.1016/j.aquaculture.2003.12.006.

[25] Cejko BI, Krejszeff S. Sperm characteristics of chub *Leuciscus cephalus* (L.) collected in artificial condition after Ovopel and Ovaprim treatment. Aquacult Res 2016;47:847–56. https://doi.org/10.1111/are.12544.

[26] Cejko BI, Zarski D, Judycka S, Kucharczyk D, Sarosiek B, Kowlski KR. Effect of two commercial preparations containing different GnRH analogues with dopamine antagonists on barbel *Barbus barbus* (L.). sperm quantity and quality 2014:97–109. https://doi.org/10.1007/s10499-013-9657-2.

[27] Zadmajid V. Comparative effects of human chorionic gonadotropin (hCG) and Ovaprim™ (sGnRHa + domperidone) on the reproductive characteristics of wild-caught male *Longspine scraper*, Capoeta trutta (Heckel, 1843). Aquaculture 2016;463:7–15. https://doi.org/10.1016/j.aquaculture.2016.05.029.

[28] Anderson GW, Genz J, Mcdougall C. Using Ovaprim™ as a conservation tool for lake sturgeon, *Acipenser fulvescens*: the short and long term effects of endocrine manipulation during the reproductive cycle. Winnipeg; 2013.

[29] Krol J, Glogowski J, Demska-Zakes K, Hliwa P. Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period. Czech J Anim Sci 2006;51:220–6. https://doi.org/10.17221/3932-CJAS.

[30] Mylonas CC, Duncan NJ, Asturiano JF. Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. Aquaculture 2017;472:21–44. https://doi.org/10.1016/j.aquaculture.2016.04.021.

[31] King RH, Young G. Milt production by non-spermiating male Atlantic salmon (*Salmo salar*) after injection of a commercial gonadotropin releasing hormone analog preparation , 17 a -hydroxyprogesterone or alone or in combination. Aquaculture 2001;193:179–95. https://doi.org/10.1016/S0044-8486(00)00472-5.

[32] Vazirzadeh A, Zahedinejad S, Bahri A. Spawning induction in doctor fish, *Garra rufa* (Heckel, 1843) by Ovaprim and captive rearing of larvae. Iran J Ichthyol

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