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A novel technique for mass induction of propagation in small fish species: Hormone immersion

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ABSTRACT

Due to the lack of environmental stimuli found in their natural habitat, not all species of tiny fish can reproduce naturally in the conditions of captivity. Silver rasbora (*Rasbora argyrotaenia*) was used as the model organism in this study, which is the first to successfully use the hormonal technique of immersion to mass induce spawning and spermiation in small fish species. In this study, in order to find out the effect of immersion hormone treatment, two separate experiments in which commercial hormone Ovaprim™ was used for spawning induction and Ovagold™ for spermiation induction were conducted. Four doses of hormones (0.0 mL/L, 0.7 mL/L, 1.4 mL/L, and 2.1 mL/L) were evaluated on 24 females and 40 males in total. According to the experimental findings of female breeders, 1.4 mL/L dose of Ovaprim™ showed best results on latency period (8.67 h) with a total number of eggs (6994 egg) and fertilization rate (99.22 %). In addition, hatching and larval survival rate have also increased. Milt volume and sperm concentration have also seen an increase to 4.25 µL/g bw and 42.71 10^6 cell/µL respectively at 2.1 mL/L hormone concentration. The best results or responses were obtained by immersion treatments using Ovaprim™ doses of 1.4 mL/L on female and Ovagold™ concentrations of 2.1 mL/L on male. This new technique is a beneficial for breeding small fish species for either commercial or conservational culture condition.

1. Introduction

Fish spawning is commonly artificially induced with the widespread commercial hormone containing of synthetic sGnRH-a (salmon gonadotropin hormone-analog) and domperidone like Ovaprim™ (Syndel Laboratory Ltd., USA) throughout the stimulation of ovulation and spermiation (Acharjee et al., 2017; Cejko et al., 2018) which supports spawning. Ovaprim™ has been shown to be successful in inducing spawning in many fish species such as *Garra rufa* (Vazirzadeh et al., 2014), climbing perch (*Anabas testudineus*) (Bhattacharyya and Homechaudhuri, 2009), *Clarias batrachus* (Sahoo et al., 2008), and sturgeon (*Acipenser fulvescens*) (Anderson et al., 2013). After successful effect of Ovaprim™ on fish reproduction, some other companies started to produce equivalent products with different names; such as Ovopel (Hungary) (Jamróz et al., 2008) and Ovagold™ (Polaris Aqua Ltd., Indonesia), which has the same or

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similar content Ovaprim™ (sGnRH-a with domperidone).

Up to now, the development of induce breeding technology has often focused on large fish consumption with the economic aims of increasing hatchery productivity. Meanwhile, the development of spawning induction in small fish species is less worked out even though these species also have economic value both as food and ornamental fish. On the other hand, there are also many small endangered fish species due to over-exploitation and the destruction of their habitat. Not all species of small fish can spawn naturally in captivity due to the unavailability of environmental stimuli found in their natural habitat. Based on these matters, it is necessary to develop a spawning induction method that is applicable to small fish species.

The three primary ways of hormonal application to encourage fish spawning and spermiation are by injection (Ningrum et al., 2019; Zadmajid et al., 2018), by application of implant (Rainis et al., 2003; Sink et al., 2010), and topical gill application (Adawiyah et al., 2019). The injection technique requires specialized skill and involves a stress risk for fish that may have an impact on spawning outcomes. Additionally, using the injection procedure on small fish presents difficulties. Small fish spawning is commonly induced with the topical gill method which may also cause stress during treatment. Furthermore, large number of fishes cannot be induced simultaneously using these two methods. A potential method that might be used for mass spawning induction in small fish is hormone immersion. Hormone immersion applications have been widely used in the administration of growth hormone (Gollas-Galvan et al., 2017), thyroxine hormone (Alinezhad et al., 2020), and sex reversal applications (Pandian and Kirankumar, 2003) in fish. Application of growth hormone by using immersion method is known to lead to an increase in the growth of rainbow trout (*Salmo gairdneri*) (Schulte et al., 1989) and tilapia (*Oreochromis* sp.) (Acosta et al., 2007). Thyroxine hormone immersion has been reported to improve development and survival of fish larvae in Sterlet sturgeon (*Acipenser ruthenus*) (Alinezhad et al., 2020), milkfish (*Chanos chanos*) (Lam et al., 1985), and *Heteropneustes fossilis* (Nayak et al., 2004). Steroid hormone immersion has been widely used for masculinization and feminization in fish sex reversal (Pandian and Kirankumar, 2003). The exact route of the hormone molecules from solution to fish endocrine system is unclear; however, a prior investigation found that the gonadotropin-releasing hormone (GnRH) of goldfish was promptly detected in their plasma after being applied to the gill (Sherwood and Harvey, 1986). Additionally, radiolabeled bovine serum albumin (BSA) was found on rainbow trout's gill and epidermis after immersion in the fluid, suggesting that the gill pillar cells may function as entrance points (Smith, 1982). In addition to gill pillar cells, lateral line and oral suction to absorption had also been considered (McLean, Donaldson, 1993). Consequently, a mechanism that enables the absorption of hormone at effective levels might start from the gills, lateral line and/or oral suction, which ultimately enhances spermiation and spawning.

In this study, silver rasbora (*Rasbora argyrotaenia*) that is small fish was used as a model organism to find out the succession of new hormone application method. Lack of broodstock (Araki et al., 2008) and spawning dependent on the season (Rouxel et al., 2008) are two challenges in the production of fish. The breeding practices for small fish species need to be enhanced in order to address these issues. By artificially inducing spawning and spermiation out of the spawning season, the availability of cultured small fish which are used as a food or ornamental source can reduce the fishing pressure on natural populations (Mylonas et al., 2010).

The use of the immersion method for spawning and spermiation induction in fish has not been reported. The purpose of this study was to determine the effect of the hormone immersion method on small size freshwater fish species and determine the optimal dose. This method will be useful in breeding of small fish species (< 10 g bw and/or < 10 cm length) both for commercial and/or conservation purposes.

2. Materials and methods

The study was carried out with the School of Health and Life Sciences Universitas Airlangga monitoring and ethical approval (based on the letter of assignment from the vice director of academics School of Health and Life Sciences Universitas Airlangga, 467/UN3.1.16/2022). This study consists of two experiments induction by immersion method. The first is Ovaprim™ (Syndel Laboratory Ltd., USA) spawning induction and the second is Ovagold™ (Polaris Aqua Ltd., Indonesia) spermiation induction. This research was conducted between April and May 2022, the first experiment was conducted at Technical Implementation Unit for Fish and Environmental Health Laboratory of Pasuruan (UFEHLP, East Java, Indonesia), and the second was conducted at School of Health and Life Sciences Universitas Airlangga (Banyuwangi, East Java, Indonesia).

2.1. Spawning induction

2.1.1. Fish origin and husbandry

A total of 24 females (6.62 ± 0.14 g bw and 7.81 ± 0.15 cm length) and 48 males (5.24 ± 0.45 g bw and 8.02 ± 0.77 cm length) were obtained from UFEHLP. In order to optimize maturity and reduce stress on fish during treatment, the male and female were stocked separately in the aerated containers (70 × 50 × 50 cm^3) for three weeks before the start of the experiment. The experimental tank water parameters were tested daily (pH 6.5–7.5, DO 5–8 ppm, T 25 °C). Fish were fed at satiation twice a day with a commercial feed (39–41 % crude protein and min 5% lipid, PF-500, Prima Feed™, Indonesia).

2.1.2. Spawning experiment

For female, applied hormone doses were 0.0 mL/L (Control Group: CF), 0.7 mL/L (Low Hormone Group: LHF), 1.4 mL/L (Medium Hormone Groups: MHF), and 2.1 mL/L (High Hormone Groups: HHF). For male, half of these experimental doses were applied and this group of male was used only for propagation. The dose arranged according to previous studies and experience (Ningrum et al., 2019). First, six female and 12 male fish were placed in separate bowls filled with 1 L unrefined salt water (15 ppt) for two minutes (modified from Alimuddin et al., 2011) to apply osmotic shock before hormone application. After osmotic shock, four different doses of hormone

(Ovaprim™) were applied to each experimental groups separately for two hours at 25 °C.

Assessing the male sexual maturity, a drop of milt was taken by a gentle strip of abdomen after anesthesia applied (300 ppm MS-222). Female sexual maturity was assessed using a catheter (FR 06, OneMed Indonesia) (Kucharczyk et al., 2022) to look for transparent greenish yellow eggs from the urogenital hole after fish were anesthetized with 300 ppm MS-222.

After induction treatment, one female and two males were mated and expected to lay fertilized eggs on palm tree fibers as spawning substrate in a 10 L cylinder plastic container. Mating was obtained at 04:00 p.m. and spawning occurred during night time.

2.1.3. Spawning parameters

The spawning parameters observed in this study are latency period, total produced egg, fertilization rate, hatching rate, and survival rate of larvae. Latency period, which was checked by direct observation every one hour, is the time between end of hormonal immersion and beginning of spawning. Total produced egg was counted in the spawning substrate. Using a light microscope (× 40) connected to a video monitor, the diameters of eggs (n = 60) were measured. The diameters of the ovoid-shaped egg were determined using the formula:

$$\text{Egg diameter} = \sqrt{(D \times d)} \quad (1)$$

where D refers for the larger section and d for the smaller section of the egg. Fertilization rate (FR, %) was counted following formula:

$$\text{FR (\%)} = (\text{total fertilized eggs/total eggs}) \times 100 \quad (2)$$

Fertilized eggs are shown with a transparent color whereas unfertilized eggs will be cloudy white. Hatching rate (HR, %) was obtained using equation:

$$\text{HR (\%)} = (\text{total hatched eggs/total fertilized eggs}) \times 100 \quad (3)$$

Survival rate of larvae was observed 3 days after hatching (dah). Survival rate (SR, %) larvae was counted using formula:

$$\text{SR (\%)} = (\text{total larvae in 3 dah/total hatched larvae}) \times 100 \quad (4)$$

2.2. Spermiation induction

2.2.1. Fish origin and rearing

A total of 40 adult male of silver rasbora (4.91 ± 0.57 g body weight and 7.49 ± 0.45 cm length) was imported from UFEHLP and placed in the experiment containers (70 × 50 × 50 cm^3) one week before the start of the experiment to reduce fish stress during treatment. The temperature of the water was observed at 26–29 °C, the dissolved oxygen level was kept approximately at 7 ppm, and pH was fixed around 7. Fish were fed at satiation twice a day with a commercial feed (39–41 % crude protein and min 5 % lipid, PF-500, Prima Feed™; Indonesia).

2.2.2. Spermiation experiment

The experiment doses of Ovagold™ and induction procedures in this treatment were the same with female Ovaprim™ doses and treatment methods in the spawning experiment; 0.0 mL/L (Control Group: CM), 0.7 mL/L (Low Hormone Group: LHM), 1.4 mL/L (Medium Hormone Groups: MHM), and 2.1 mL/L (High Hormone Groups: HHM). Ten male were used in each experimental group and hormone induction time was two hours, after applied two minutes osmotic shock in 15 ppt salty water as explained in spawning experiment. Ten hours after hormone induction, the required time for spermiation, all the experimental specimen milt was stripped by slightly pressing the abdomen under anesthesia (300 ppm MS-222) and stored in a 1.5 mL centrifuge tube for sperm analysis.

2.2.3. Milt analysis

Milt was collected using a micropipette with a sterile micro tip that was adjusted every 0.01 mL (Cejko et al., 2011), and the total volume of expressible milt was noted. The volume of milt was determined by dividing the expressible milt by the total body weight of the fish.

Milt was diluted 1000-fold with an immobilizing solution containing 153 mM NaCl in order to prevent sperm aggregation and achieve the proper concentration for counting. Sperm concentrations were measured by a hemocytometer (Zadmajid et al., 2018). A compound Eclipse E200-LED light microscope connected to a video display (× 100 and × 400 magnification) was used for cell counting.

The sperm motility was analyzed by depositing 1 mL of sperm on a slide glass. The assessments were conducted at a room temperature of 26 °C. Sperm motility was measured using a video recorder connected to a threenocular microscope (Eclipse E200-LED, Nikon, Japan) (100 × and 400 × magnification). Based on Rurangwa et al. (2004), a semi-quantitative technique was used to quantify the sperm motility.

For staining procedure, a mixture of 1 mL milt and 1 mL eosin 2 % was utilized to assess sperm viability using a light microscope (Eclipse E200-LED, Nikon, Japan) connected to a video monitor (× 1000 magnification). The process was examined for 15 s.

2.3. Statistical analysis

Both experiments were carried out in duplicate and arithmetic means of all the data of all the parameters were used in statistical analysis. Variance analysis (ANOVA) was used to assess the observed parameters with a 95 % confidence level. The Duncan's test was done with the statistical program SPSS version 7.0 to determine the significance between applied dosages. The results are displayed as means ± standard deviation.

3. Results

3.1. Spawning induction

Based on the results, it was known that the CF with a time of 17.67 ± 0.51 h is the longest latency time, but it is not significantly different from the LHF (with a latency time of 17.33 ± 1.75 h) ($P > 0.05$). The fastest latency time occurred in MHF which was 8.67 ± 0.51 h. Meanwhile, in HHF spawning was not occurred (Fig. 1).

Ovaprim™ treatment using the immersion method with different doses produced more significant effect on the number of eggs produced by silver rasbora ovulation ($P < 0.05$). Considering the results, it is known that the CF produced 2076.17 ± 200.56 eggs was the lowest number. The highest number of eggs was collected in the MHF which was 6994 ± 450.42 eggs. Meanwhile, HHF there was no spawning because no eggs were produced (Fig. 2). There was no significant difference on egg diameter in all experimental groups ($P > 0.05$).

Different doses of Ovaprim™ treatment applied by immersion method gave a significant impact on the fertilization rate (FR) of silver rasbora ($P < 0.05$). The results illustrate that an FR of 94.82 ± 0.97 %, the lowest result, was obtained from CF. The MHF resulted in the highest FR value, which was 99.22 ± 0.13 % (Fig. 3).

MHF dose had significant effect ($P < 0.05$) on hatching rate (HR), which was 99.06 ± 0.44 %. The LHF, which resulted in a HR of 96.57 ± 0.79 % was not significantly different from the CF ($P > 0.05$) (Fig. 3).

Survival rate (SR) of silver rasbora larvae in CF was calculated 97.74 ± 0.87 % which is a lower value than other experimental groups and was not significantly different from the LHF ($P > 0.05$) which was SR of 98.31 ± 0.68 %. The highest survival rate was obtained in MHF 1.4 mL/L, which was 99.07 ± 0.15 % (Fig. 3).

3.2. Spermiation induction

pH of the milt for each male experimental groups was recorded 7.8 and also fishy smell and milky color in all group were the same. In contrast to hormone application on female, HHM dose showed the best results on male of silver rasbora both on milt volume (Fig. 4) and sperm concentration (Fig. 5).

The shortest duration of sperm motility was observed at Control group (120 s) and the longest at MHM group (124.6 s) but no significant differences were seen among them (Fig. 6). The percentages of motility and viability of sperm showed similar tendency. While the dose increased, both motility and viability had increased slightly till MHM which was followed by a negligible decrease (Fig. 6).

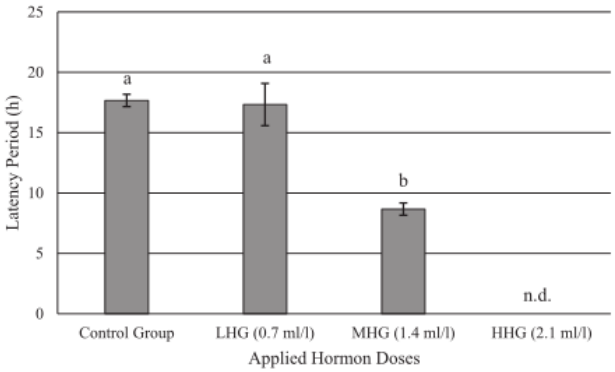

Fig. 1. Latency periods (h) under different dose of immersed hormone application on silver rasbora (*Rasbora argyrotaenia*) spawning induction (CF: Control Group of Female; LHF: low dose hormone group for female; MHF: medium dose hormone group for female; HHF: high dose hormone group for female; n.d.: no data) ($n = 6$). The columns represent mean values and the bars represent standard deviation (± stdev). Different letters above the bars indicated a significant difference ($P < 0.05$).

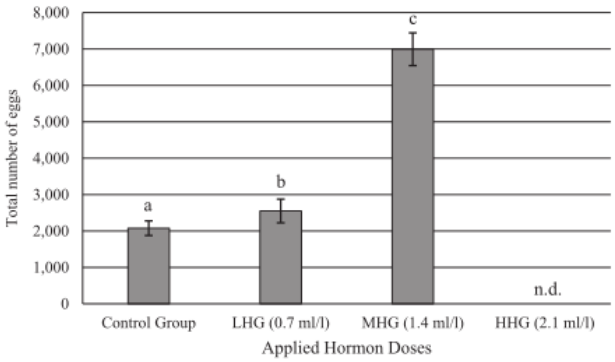

Fig. 2. Total number of eggs under different doses of immersed hormone application on female silver rasbora (*Rasbora argyrotaenia*) spawning induction (CF: Control Group of Female; LHF: low dose hormone group for female; MHF: medium dose hormone group for female; HHF: high dose hormone group for female; n.d.: no data) ($n = 6$). The columns represent mean values and the bars represent standard deviation (± stdev). Different letters above the bars indicated a significant difference ($P < 0.05$).

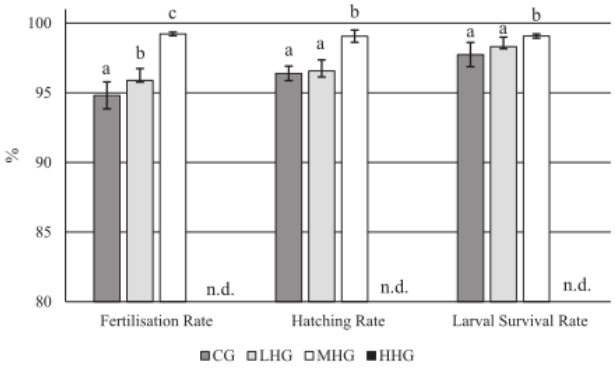

Fig. 3. Fertilization, hatching and larval survival rate under different doses of immersed hormone application on female silver rasbora (*Rasbora argyrotaenia*) spawning induction (CF: Control Group of Female; LHF: low dose hormone group for female; MHF: medium dose hormone group for female; HHF: high dose hormone group for female; n.d.: no data) ($n = 6$). The columns represent mean values and the bars represent standard deviation (± stdev). Different letters above the bars indicated a significant difference ($P < 0.05$).

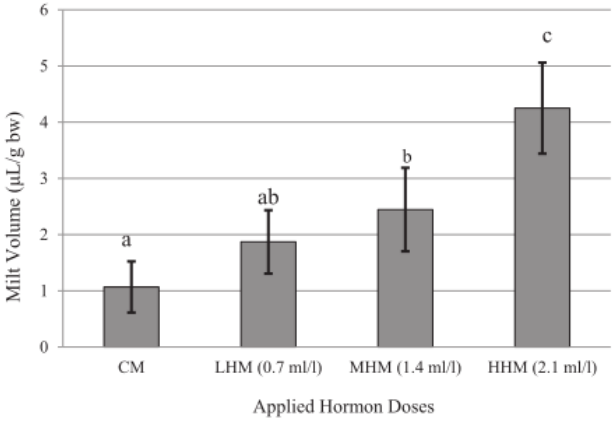

Fig. 4. Milt volume (µL/g bw) under different doses of immersed hormone application on male silver rasbora (*Rasbora argyrotaenia*) spermiation induction (CM: Control Group of male; LHM: low dose hormone group for male; MHM: medium dose hormone group for male; HHM: high dose hormone group for male) ($n = 10$). The columns represent mean values and the bars represent standard deviation (± stdev). Different letters above the bars indicated a significant difference ($P < 0.05$).

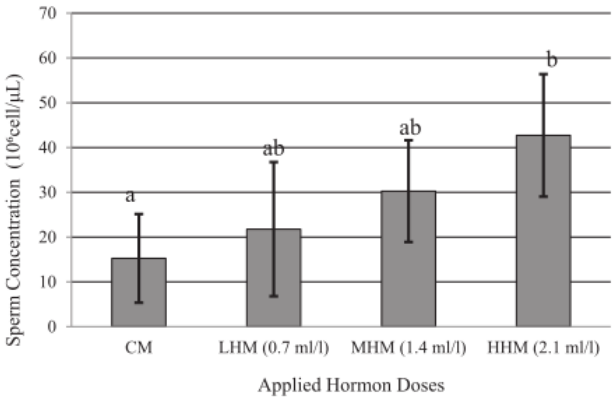

Fig. 5. Sperm concentration (10^6 cell/µL) under different doses of immersed hormone application on male silver rasbora (*Rasbora argyrotaenia*) spermiation induction (CM: Control Group of male; LHM: low dose hormone group for male; MHM: medium dose hormone group for male; HHM: high dose hormone group for male) ($n = 10$). The columns represent mean values and the bars represent standard deviation (± stdev). Different letters above the bars indicated a significant difference ($P < 0.05$).

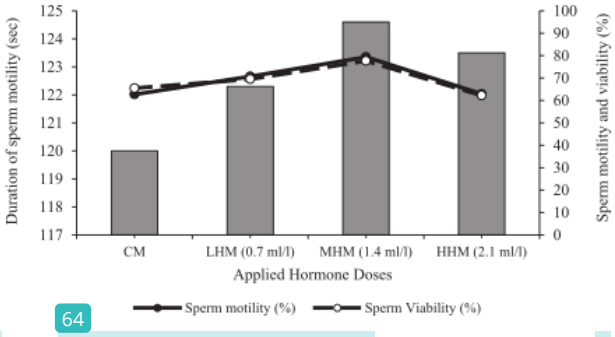

Fig. 6. Duration of sperm motility (sec), sperm motility (%) and sperm viability (%) under different doses of immersed hormone application on male silver rasbora (*Rasbora argyrotaenia*) spermiation induction (CM: Control Group of male; LHM: low dose hormone group for male; MHM: medium dose hormone group for male; HHM: high dose hormone group for male) ($n = 10$). The columns represent mean values.

4. Discussion

4.1. Spawning induction

Hormone immersion method successfully induced silver rasbora spawning. Increased dose of Ovaprim™ gave the tendency of a decrease in the latency period and an increase in the total number of eggs, fertilization rate, hatching rate, and survival rate of larvae. Meanwhile, no spawning was observed in the highest dose (2.1 mL/L) treatment. High dosages of Ovaprim™ are known to have a potential negative impact on the stimulation of fish spawning (Vazirzadeh et al., 2014), thus causing the fish not to be able to spawn, while the LHG treatment didn't lead to spawning around 17 h in this study. Ovaprim™ contains a combination of the hormone sGnRH-a and domperidone that has a hormonal role in fish spawning. The hormone sGnRH-a is a pure peptide found in teleostei fish and is useful in secreting gonadotropin hormone II (GTH-II) or luteinizing hormone (LH) that trigger final maturation and ovulation (Anderson et al., 2013). Domperidone is a commercial medicine that blocks the brain's dopamine system as a dopamine antagonist. An active dopamine system decreased GnRH activity and increased dopamine activity both contribute to the inhibition of ovulation and spermiation in captivity, whereas in fish species with a weak dopamine system, decreased GnRH activity contributes almost entirely to reproductive disorders. Similar to treatment with a dopamine antagonist alone, GnRH-a medication merely promotes LH secretion; however, the most effective treatment is the combination of GnRH-a and domperidone (Mañanós et al., 2009). Hyperosmotic baths as a part of treatment increase hormone intake through an osmoregulation mechanism (Schulte et al., 1989) so that it can improve the effects. Fish will lose body fluids when immersed in hyperosmotic media. Then when immersed in freshwater media containing hormones, the fish will absorb water to replace the previous loss, along with this the fish also absorb the hormones contained in the media.

Based on results, the shortest latency period of spawning occurred in 1.4 mL/L dose treatment (MHF). On the other hand, Ovaprim™ induction at optimal doses will accelerate the latency time of spawning (Sahoo et al., 2003). According to the comparison of various hormonal preparations, Ovaprim™ is one of the most effective preparations especially in cyprinid fish such as common dace

(*Leuciscus leuciscus*) (Kucharczyk et al., 2019), Caspian roach (*Rutilus caspicus*) (Nargesi et al., 2022), and ide (*Leuciscus idus*) (Kucharczyk et al., 2020). Previous studies also found the same tendency that spawning induction using exogenous hormonal treatment in optimal dose has accelerated spawning latency period in snow trout (*Schizothorax zarudnyi*) (Rahdari et al., 2014), *Labeo rohita* (Khan et al., 2006), and stripped snakehead (*Channa striatus*) (Marimuthu et al., 2007).

Ovaprim™ immersion also affected ($P < 0.05$) the total number of produced egg obtained after spawning significantly. It is found that the total number of egg tends to increase until optimum dose (1.4 mL/L). The number of eggs released is influenced by the level of LH in the body which functions to stimulate ovulation in females and spermiation in males (Cejko et al., 2018). In this case, the presence of LH is influenced by the interaction of sGnRH-a and domperidone contained in Ovaprim™ which is given as treatments. The administration of Ovaprim™ using immersion method on the induction of silver rasbora spawning did not affect the egg diameter. The size of egg diameter is influenced by the age of the broodstock, the source of nutrients eaten by the broodstock, and environmental conditions (Kohal et al., 2017; Peixoto et al., 2004). Ovaprim™ only works in the process of final maturation and ovulation. The process of egg maturation is regulated by the gonadotropin hormone processed and stored in the pituitary gland to go to the gonads. Gonadotropin secreted by the pituitary is gonadotropin I (GTH I) which acts to increase the secretion of 17-estradiol which stimulates the synthesis and secretion of vitellogenin, while LH triggers the final maturation process so that hormonal induction does not have the effect of increasing egg diameter (Nagahama, 1987). In previous studies, silver rasbora spawning induction with different doses of Ovaprim™ using the injection method resulted in no significant difference in egg diameter (Ningrum et al., 2019).

The degree of fertilization increased with increasing dose until the optimal dose (1.4 mL/L). LH levels in the body stimulate final maturation completely characterized by germinal vesicle break down (GVBD) in oocytes. Hormones will work normally (optimally) at a certain level, decreasing or increasing hormone levels will decrease their biological potential. In the low (suboptimal) dose, there is a possibility that the hormone given cannot stimulate the release of gonadotropins optimally so that imperfect egg maturation causes fertilization not to take place properly (Dewantoro et al., 2017). In addition, the process of egg fertilization is influenced by the quality of eggs and spermatozoa, and the ability of sperm to move spontaneously to enter the micropyle of the egg (Valdebenito et al., 2015). The treatment that has been carried out produces the same trend in the hatching rate and survival rate of larvae, where the treatment dose of 1.4 mL/L is better than the control treatment and the treatment dose of 0.7 mL/L. Hatching rate and survival rate of larvae are related to fertilization rate, which means an increase in fertilization rate will be followed by an increase in hatching rate and survival rate (Muchlisin et al., 2007).

4.2. *Spermiation induction*

Ovagold™ immersion successfully induce silver rasbora spermiation. Milt volume and sperm concentration increased following raised Ovagold™ doses. Meanwhile, the percentage and duration of sperm motility and sperm viability were not significantly different. Ovagold™ contained sGnRH-a and domperidone same with Ovaprim™. In our previous study, silver rasbora spermiation was also successfully induced by topical gill and injection of Ovaprim™ (Adawiyah et al., 2019; Budi et al., 2020).

The pituitary gland can be stimulated by GnRH directly or indirectly to produce hormones that speed up spermiation and increase sperm production, while domperidone in Ovagold™ works to block dopamine that prevents LH secretion, which indirectly affects the process of spermiation and spermatozoa hydration (Anderson et al., 2013; Krol et al., 2006; Yanong et al., 2009). Consequently, an increase in hydration processes that results in an increase in testicular and seminal fluid, as well as an accelerated rate of spermiation or spermiogenesis, can be used to explain the rise in milt volume caused by inducing Ovagold™ up to a specific dose (Mylonas et al., 2017). Moreover, LH induces the production of the steroid hormones 11-ketotestosterone (11-KT) and 17α,20β-dihydroxy-4-pregnen-3-one (DHP) in the testes' Leydig cells, which are essential for the process of spermiogenesis (King and Young, 2001).

According to this study, an increase in sperm concentration is positively associated with an increase in the volume of milt (Fig. 4 and Fig. 5). Similar findings showed that the hormone therapy utilizing sGnRH-a boosted milt volume and sperm density in *Etroplus suratensis* (Dhas et al., 2017) and some other species (Garber et al., 2009; Kowalski et al., 2012; Zadmajid, 2016). When assessing the quality of sperm in teleost, sperm motility and viability are key considerations (Golpour et al., 2015; Kowalski et al., 2012). Exogenous hormonal treatment on fish has been claimed to affect the percentage and duration of sperm motility and sperm viability, but differences between the control group and fish receiving hormonal treatment have not always been significant (Mylonas et al., 2017), which was also evident in this study.

5. Conclusions

This study is the first to effectively induce spawning and spermiation using the hormonal technique of immersion. Results showed a decrease in the latency time, and a rise in spawning and hatching parameters such as the total number of produced eggs, fertilization rate, hatching rate, larval survival rate; also showed an increase in milt parameters such as milt volume and sperm concentration. In conclusion, this study indicates that immersion treatment with Ovaprim™ concentrations of 1.4 mL/L and Ovagold™ concentration of 2.1 mL/L generates remarkable results or responses. It is very common to use injection method for hormone application on big size fishes (ie: carp, sea bream, sea bass) but this newly discovered method might be better for small size fish since injection is very difficult to them and also the success of the hormone introduction to muscles is suspicious because of the body size. In addition, hormone injection is always stressful for the small size fish and may cause mortality. This new method for hormone application may not be suitable for big size fish because of the hormone consumption in big volume of water but gives good results for small fish such as silver rasbora or most probably on ornamental fish as well.

CRediT authorship contribution statement

Darmawan Setia Budi: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Supervision, Writing - original draft, Writing - review & editing. **Sherly Puspitasari:** doing spawning induction treatments, measurement and observation data, data analysis. **Rahma Pu Nuramalia Febrianti:** doing spermiation induction treatments, data measurement and observation, data analysis. **Türker Bodur:** Formal analysis, Visualization, Writing - review & editing. **Akhmad Taufiq Mukti:** Conceptualization, Methodology, Formal analysis, Supervision, Validation, Writing - original draft.

Declaration of Competing Interest

On behalf of my co-authors, I am submitting the enclosed manuscript entitled "**A novel technique for mass induction of spawning in small fish species: hormone immersion**" for possible publication Animal Reproduction Sciences journal. All persons listed as authors have read, contributed to preparing the manuscript and attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to Animal Reproduction Sciences. No person(s) more than the authors listed have contributed significantly to its preparation. The manuscript has not been submitted for publication nor has been published in whole or in part elsewhere. **There is no competing interest to declare by the authors.**

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