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Enhancement of ovarian maturation in bronze featherback *Notopterus notopterus* through pregnant mare serum gonadotropin for sustainable aquaculture and conservation

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Abstract. *Muslim, Pitriani E, Oktarina S, Khotimah NN. 2026. Enhancement of ovarian maturation in bronze featherback* Notopterus notopterus *through pregnant mare serum gonadotropin for sustainable aquaculture and conservation. Biodiversitas 27 (1): d270121. https://doi.org/10.13057/biodiv/d270121.* The bronze featherback *Notopterus notopterus* is a freshwater fish of high economic value whose population is declining due to overfishing and habitat destruction. This study evaluated the effectiveness of pregnant mare serum gonadotropin in inducing ovarian maturation in *N. notopterus*, addressing reproductive challenges that hinder its domestication and conservation. A total of 45 female fish (mean weight 50-55 g and total length 18-20 cm) at the second stage of ovarian maturity were injected intramuscularly with pregnant mare serum gonadotropin doses of 0, 1.0, 1.25, and 1.5 mL kg^{-1} body weight. Observations were conducted for nine days with parameters including Ovi-Somatic Index (IOS), Hepato-Somatic Index (IHS), oocyte diameter, fecundity, cholesterol levels, and ovarian maturity level. The results indicated that pregnant mare serum gonadotropin significantly influenced IOS, oocyte diameter, fecundity, and ovarian maturity level (ANOVA, $P<0.05$; post-hoc LSD test), whereas it showed no significant effect on IHS and cholesterol levels ($p>0.05$). A dose of 1.5 mL kg^{-1} produced the highest IOS, oocyte diameter, and fecundity values (378 eggs), as well as ovarian maturity levels up to stage V (mature). This study provides that hormonal induction using pregnant mare serum gonadotropin effectively stimulates ovarian development in *N. notopterus*, offering a biological solution to reproductive challenges that limit domestication success. By enhancing spawning success rates, reducing dependence on wild-caught broodstock, and enabling the establishment of captive breeding protocols, this hormonal induction technique offers practical tools for ex-situ conservation and directly contributes to biodiversity protection by supporting the long-term sustainability of *N. notopterus* populations. These findings show that aquaculture-based reproductive management supports SDG 14 by promoting sustainable use of aquatic resources. Hormonal induction serves as a bridge between reproductive biotechnology and conservation, helping restore freshwater biodiversity through domestication-based recovery strategies.

Keywords: Fecundity, *Notopterus notopterus*, oocyte, ovarian maturation, pregnant mare serum gonadotropin

INTRODUCTION

The *Notopterus notopterus* (Pallas, 1769) is a species of freshwater fish native to Southeast Asia, including Indonesia, India, Malaysia, Thailand, and Vietnam (Khansa et al. 2023; Wibowo et al. 2024). In Indonesia, *N. notopterus* is commonly found in various water systems, such as the Sei Gesek Reservoir in Bintan (Rapita et al. 2021), the Sei Pulai Reservoir in the Riau Islands (Susiana et al. 2021), the Kelekar River in Ogan Ilir (Muslim and Syaifudin 2022), and the Brantas River in East Java (Khansa et al. 2023). Belonging to the Notopteridae family, the *N. notopterus* holds not only economic value but also ecological importance within freshwater ecosystems (Sukendi et al. 2020; Muslim 2023; Wibowo et al. 2024). The *N. notopterus* regulates lower trophic organisms and supports food web stability. Its ecological role as a filter-feeding benthic organism is important for maintaining sediment quality and nutrient dynamics (Pelletier et al. 2020; Gupta and Kumar 2024). *Notopterus notopterus* is widely distributed across South and Southeast Asia, and although it is currently categorized as least concern, the species may still experience localized pressures from overfishing, habitat modification, and pollution, which could affect its population dynamics at the regional level (Memon et al. 2021; Muslim et al. 2023; Priyadi et al. 2025). However, reproductive performance of *N. notopterus* remains low even under cultured or semi-captive conditions, indicating physiological and endocrine constraints during domestication. As a key trophic regulator and bioindicator, its decline may destabilize ecosystems. Domestication offers a viable strategy to prevent extinction and ensure sustainable use of aquatic genetic resources (Hutagalung et al. 2024; Tave 2025). Preliminary domestication studies of the bronze featherback show high survival and growth under cultured conditions (Muslim and Simanjuntak 2023; Muslim et al. 2023). Aquaculture provides an alternative fish protein source while supporting ex-situ conservation by reducing pressure on wild stocks and preserving genetic resources (Froehlich et al. 2017; Mohd and Mushtaq 2025). Aquaculture is increasingly emphasized in global conservation strategies, especially for threatened freshwater species (Little et al. 2022; Guerra-García et al. 2023).

However, a key constraint in early-stage domestication is reproductive failure, often due to endocrine system disruption in an artificial environment (Chong 2022; Lu et al. 2022; Jaillardon and Kaiser 2023; Socha et al. 2025). Hormonal stimulation is commonly used to overcome reproductive failure, with Pregnant Mare Serum Gonadotropin (PMSG), a glycoprotein hormone with FSH- and LH-like activity, proven to induce follicular growth and oocyte maturation in teleosts (Pamungkas et al. 2019; Ath-Thar et al. 2021; Byambaragchaa et al. 2021). Pregnant mare serum gonadotropin is a glycoprotein hormone produced by placental trophoblastic cells in pregnant mares and extracted from their blood (Vilanova et al. 2019; Lösle et al. 2025). This hormone is detected in serum during the 40 to 120 days of pregnancy (Somanjaya et al. 2021; Thach et al. 2022). Structurally, pregnant mare serum gonadotropin consists of two subunits, namely α (16.96 kDa) and β (43.72 kDa) (Combarnous and Nguyen 2019; Lösle et al. 2025), with a half-life of about 2-3 days (Tirpan et al. 2019). The use of pregnant mare serum gonadotropin has been reported to induce gonadal maturation in various fish species, including *Channa striata* (Ath-Thar et al. 2021), *Rasbora maninjau* (Zulfadhli et al. 2024), *Trachinotus blochii* (Cahyono et al. 2019), *Tor douronensis* (Mellisa et al. 2019), *Rasbora lateristriata* (Ein et al. 2024), *Pangasianodon hypophthalmus* (Pamungkas et al. 2021), and *Puntius orphoides* (Iskandar et al. 2023).

Although hormonal induction using pregnant mare serum gonadotropin is widely applied in freshwater fish, its specific application and physiological response in *N. notopterus* remain unclear. Ongoing population decline due to overfishing and habitat degradation underscores the urgent need for effective reproductive management. This study evaluated the effectiveness of pregnant mare serum gonadotropin in inducing ovarian maturation in *N. notopterus*, addressing reproductive challenges that hinder its domestication and conservation. Linking pregnant mare serum gonadotropin application with the reproductive physiology of *N. notopterus* introduces a novel dimension to reproductive biotechnology research in this endemic species. Ultimately, this effort aligns with SDG 14 and the Convention on Biological Diversity by promoting freshwater biodiversity conservation through species-level reproductive innovation.

MATERIALS AND METHODS

Experimental setup: Aquaria and test fish preparation

Twelve aquarium units ($34 \times 39 \times 25$ cm^3) were thoroughly cleaned with freshwater and air-dried before use. The outer surfaces of the aquaria were wrapped in black plastic sheets. Each unit was filled Water quality was monitored daily, including temperature (26.0-28.8°C), pH (6.5-7.8), dissolved oxygen (4.5-7.5 mg L^{-1}), and ammonia (0.023-0.031 mg L^{-1}). Ammonia and dissolved oxygen were measured on the first and last days of the experiment. A total of 45 female bronze featherback with homogeneous characteristics (mean weight 50-55 g and total length 18-20 cm), were used to ensure consistency. Fish were collected from the Kelekar River, South Sumatra, acclimated for 7 days in laboratory conditions, and then transferred randomly to experimental aquaria**.** The fish were randomly assigned to four treatment groups: control (0 mL kg^{-1}), 1.0, 1.25, and 1.5 mL kg^{-1} of pregnant mare serum gonadotropin (Oodev®), with three replicates per treatment and three fish per replicate (n = 3 aquaria/treatment, 3 fish/aquarium). Randomization was performed by assigning fish numbers and drawing lots to allocate them to aquaria**.** The sample size was determined with reference to previous similar studies (Zulfadhli et al. 2024, 2025) and represents the minimum replication required to achieve sufficient statistical power for one-way ANOVA, as commonly applied in aquaculture reproductive trials**.** The sample size was determined based on previous similar studies and the minimum number required to perform ANOVA effectively. Prior to hormonal injection, the fish were acclimatized for 24 h.

Injection of pregnant mare serum gonadotropin and rearing conditions

The pregnant mare serum gonadotropin dosages (1.0, 1.25, and 1.5 mL kg^{-1}) were selected based on the literature to evaluate dose-dependent reproductive responses (Hutagalung et al. 2015; Tomasoa et al. 2015; Hutagalung 2019). These doses were chosen as they represent a range around the effective threshold reported for cyprinid and featherback species, allowing the identification of both optimal and supra-optimal responses. The injection was performed intramuscularly and only once. To minimize operator bias, all injections and dissections were conducted by the same trained researcher, and measurements (e.g., OSI, HSI, oocyte diameter) were cross-checked by a second operator blinded to the treatment groups. After injection, the fish were kept in individual aquaria (3 fish per unit) under constant aeration. Uneaten food and feces were siphoned daily to maintain water quality. The fish were fed natural food *Caridina gracilirostris* at 5% body weight, twice daily. All rearing conditions (water quality) were kept uniform across all treatment groups to control for environmental variables (Jenkins et al. 2014).

Fish dissection

The fish were anesthetized using MS222 (Arwana®) at a dose of 1 mL per 3 L of water. Blood samples were collected from the gill lamella vein using a syringe. Cholesterol levels were measured using an enzymatic colorimetric assay with Easy Touch® strips, which has been validated for fish plasma cholesterol analysis. The fish was then dissected using surgical tools. After dissection, the liver and ovaries were separated from other internal organs. The ovaries were weighed to calculate the Ovary-Somatic Index (OSI) using the formula OSI =

[ovary weight (g)/body weight (g)] × 100 (Sharma and Ram 2020), The liver was weighed to calculate the Hepato-Somatic Index (HSI): HSI = [liver weight (g)/body weight (g)] × 100 (Htun-Han 1978). Oocyte diameters were measured using a micrometer microscope (accuracy 0.1 µm). Fecundity was calculated using the formula: fecundity = [ovary weight (g) × number of eggs in sub-sample/sub-sample weight (g)] (Kant et al. 2016). Ovarian maturation stages were determined according to (Gupta et al. 2021). All experimental procedures involving fish were conducted following standard aquaculture practices to minimize stress and discomfort, and in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Data analysis

All data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to analysis. Statistical comparisons among treatment groups were performed using one-way Analysis of Variance (ANOVA) at a significance level of $p<0.05$. If significant differences were detected, post hoc comparisons were carried out using the Least Significant Difference (LSD) test. Effect sizes (η^2) were reported alongside p-values to indicate the magnitude of treatment effects. Confidence intervals (95% CI) were calculated for key reproductive metrics. Analyses were conducted using SPSS software version 28. These statistical tests were chosen to compare mean values among multiple treatment groups under the assumption of normal distribution and equal variances.

RESULTS AND DISCUSSION

Ovisomatic and hepatosomatic indices

The results indicated that pregnant mare serum gonadotropin treatment significantly affected Ovi-Somatic Index (IOS) values ($p<0.05$), while no significant effect was observed on IHS values ($p>0.05$) (Figure 1). Different letters indicate significant differences between treatments on the same observation day ($p<0.05$).

At day 0 (prior to injection), OSI values were low and comparable across treatments, indicating a uniform baseline condition. By day 3 post-injection, OSI values had increased in all pregnant mare serum gonadotropin-treated groups, with a more pronounced effect at higher doses, suggesting early stimulation of ovarian development. This trend continued through day 6, where OSI values were significantly higher in the medium- and high-dose groups compared to the control, reflecting progressive ovarian growth and yolk accumulation. The highest OSI values were recorded on day 9, particularly in the group receiving 1.5 mL kg^{-1} pregnant mare serum gonadotropin, which exhibited more than a twofold increase from baseline. The dose-dependent pattern and temporal progression indicate that pregnant mare serum gonadotropin effectively accelerates vitellogenesis in *N. notopterus*, likely due to its gonadotropic activity in promoting follicular growth and maturation. In contrast, HSI values showed minimal variation throughout the observation period, with no statistically significant differences among treatments at any time point ($p>0.05$). This stability indicates that pregnant mare serum gonadotropin treatment did not significantly influence hepatic size in relation to body weight during the nine-day experiment.

Oocyte diameter

The results of measuring the oocyte diameter of the *N. notopterus* in the pregnant mare serum gonadotropin dose treatment of 0 mL kg^{-1} fish were significantly different ($P<0.05$) from all treatments (Table 1).

At day 3 post-injection, oocyte diameters in the control group (0 mL kg^{-1}) predominantly ranged between 2.3-3.2 mm, with no presence of large oocytes (>3.3 mm). In contrast, pregnant mare serum gonadotropin-treated groups (1.0, 1.25, and 1.5 mL kg^{-1}) exhibited a broader distribution, including larger size classes, indicating earlier stimulation of oocyte growth. By day 6, the frequency of larger oocytes increased markedly in the 1.25 and 1.5 mL kg^{-1} groups, with the appearance of oocytes exceeding 3.3 mm in diameter. The high-dose group (1.5 mL kg^{-1}) demonstrated a substantial shift in distribution, with a reduced proportion of small oocytes (<2.8 mm) and a corresponding increase in intermediate (3.0-3.2 mm) and large (>3.3 mm) oocytes. At day 9, the differences among treatments became more pronounced. The 1.5 mL kg^{-1} group recorded the largest oocytes, averaging 3.1±0.4 mm, compared to only 2.3-3.2 mm in the control. This group also had the highest proportion of oocytes exceeding 3.3 mm, while lower doses (1.0 and 1.25 mL kg^{-1}) displayed intermediate size distributions. These findings demonstrate that pregnant mare serum gonadotropin induces a dose-dependent enhancement of oocyte growth, with the most pronounced effect observed at 1.5 mL kg^{-1}.

Quantification of fecundity

The fecundity increased over time following pregnant mare serum gonadotropin injection, especially at higher doses. The highest fecundity was recorded on day 9 with a dose of 1.5 mL kg^{-1}, amounting to 378 eggs per individual. Although not significantly different compared to doses of 1.0 and 1.25 mL kg^{-1}, this result was statistically significantly different from the control treatment (0 mL kg^{-1}), which did not receive hormone injection (Figure 2). Although the control fish did not undergo hormonal induction, oocytes at early vitellogenic stages were still observed, explaining the presence of measurable egg diameters in Table 1. However, no spawning or egg release was recorded in this group.

Physiological responses to hormone administration appeared to be more effective at doses of 1.25 and 1.5 mL kg^{-1}, which may reflect the optimal dose for induction of gonadal maturation in this species. The lowest fecundity on day 3 was recorded at a dose of 1.0 mL kg^{-1} (approximately 210 eggs). However, significant increases were observed on days 6 and 9 in all treatment groups.

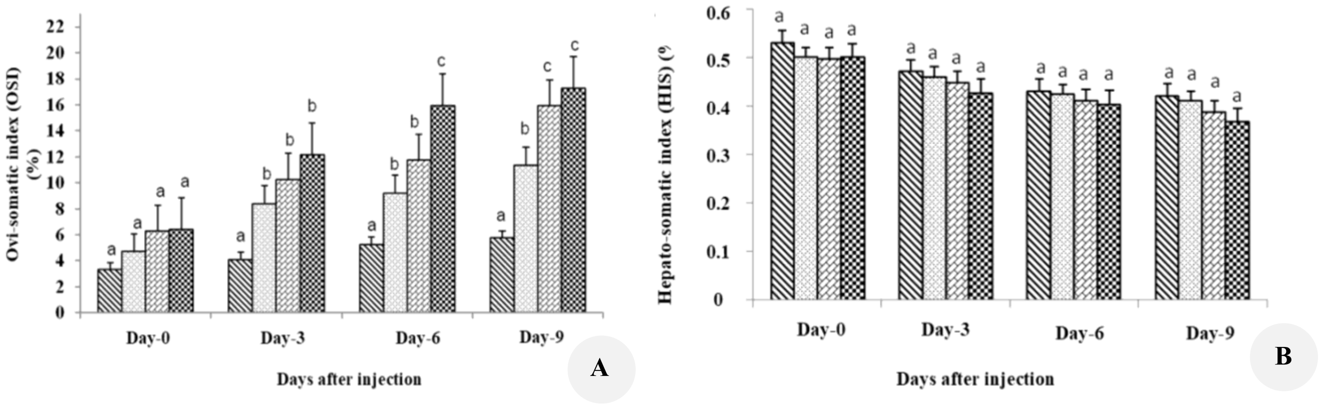
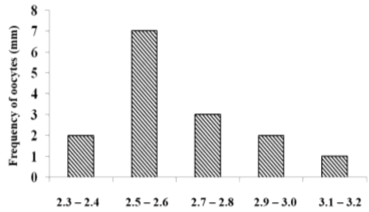
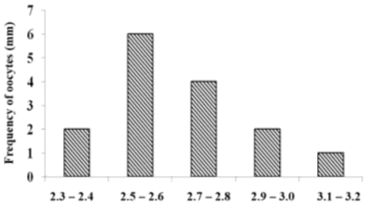
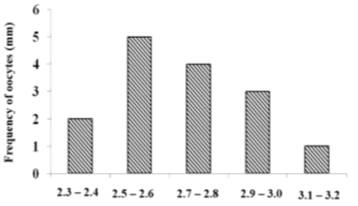
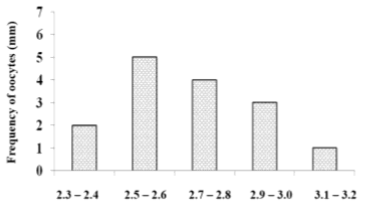
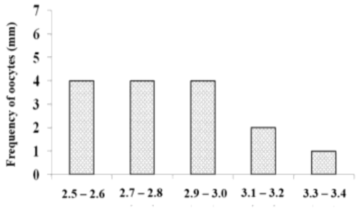
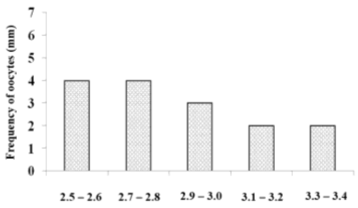
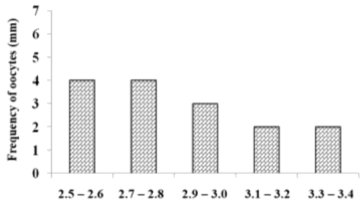
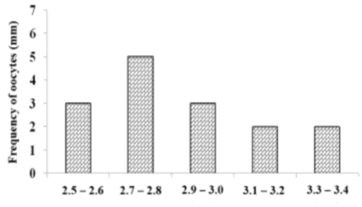
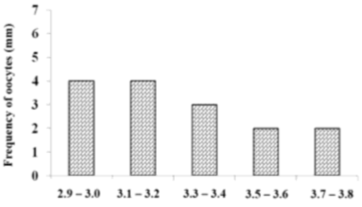
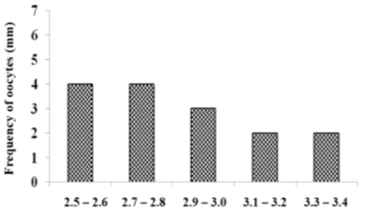
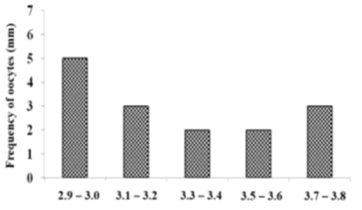
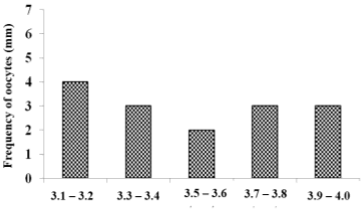


Figure 1. Changes in somatic indices of *Notopterus notopterus* after injection with PMSG at various doses: A. Ovisomatic Index (OSI, %), representing the proportion of ovary weight relative to body weight, B. Hepatosomatic Index (HSI, %), representing the proportion of liver weight relative to body weight. Bars represent mean±95% Confidence Intervals (CI). Different letters indicate statistically significant differences among treatment groups (ANOVA, p<0.05)

Table 1. Distribution and frequency of oocyte diameter of *Notopterus notopterus* after injection of pregnant mare serum gonadotropin at doses of 0-1.5 mL kg⁻¹ fish, observed on Day-0, Day-3, Day-6, and Day-9

Treatment	Observation day		
	Day-3	**Day-6**	**Day-9**
0 mL kg^{-1} fish			
1.0 mL kg^{-1} fish			
1.25 mL kg^{-1} fish			
1.5 mL kg^{-1} fish			

Cholesterol concentration

Statistical analysis revealed no significant differences ($p>0.05$) in plasma cholesterol levels among treatments following pregnant mare serum gonadotropin administration (day 0: P = 0.80, day 3: P = 0.12, day 6: P = 0.26, day 9: P = 0.47); however, distinct temporal trends were observed throughout the experimental period (Figure 3).

At day 0 (pre-injection), cholesterol concentrations were relatively high and comparable across all treatments (270-340 mg L^{-1}). By day 3, cholesterol levels showed a slight decline across all treatments. The highest concentration recorded during the experiment occurred on day 6 in the 1.25 mL kg^{-1} group (265 mg L^{-1}), whereas the lowest value at this stage was observed in the 1.5 mL kg^{-1} group (187 mg L^{-1}). This pattern indicates that moderate pregnant mare serum gonadotropin doses maintained relatively elevated cholesterol levels during mid-vitellogenesis. By day 9, cholesterol concentrations decreased further in all treatments, with the most pronounced reduction observed in the 1.5 mL kg^{-1} group (180 mg L^{-1}). In contrast, the control group retained the highest concentration at this stage (218 mg L^{-1}).

Ovarian maturation stage

The treatment of pregnant mare serum gonadotropin significantly accelerated ovarian maturation in *N. notopterus*, as evidenced by morphological changes in the gonads and the progression of oocyte developmental stages (Table 2).

At day 0 (pre-injection), ovaries in all treatment groups were at stage II (developing), indicating an early vitellogenic phase. By day 3, morphological changes became evident in the 1.25 and 1.5 mL kg^{-1} groups, with ovaries exhibiting a denser texture and a transition in coloration from pale yellow to a more intense yellow, reflecting increased vitellogenin deposition. By day 6, the 1.25 and 1.5 mL kg^{-1} treatments exhibited marked advancement in maturation, with ovaries displaying a bright yellow-orange coloration and prominent blood vessels, indicating active vitellogenesis. In these groups, a shift from stage II to stage III (maturing I) and even stage IV (maturing II) was observed, whereas the control and 1.0 mL kg^{-1} groups remained predominantly at stage II, with only limited progression to stage III. By day 9, ovaries in the 1.5 mL kg^{-1} group reached stage V (mature), characterized by orange coloration, increased vascularization, and the presence of fully developed, hydrated oocytes filling the ovarian cavity. The 1.25 mL kg^{-1} group primarily reached stage IV, while the 1.0 mL kg^{-1} and control groups largely remained in earlier stages, indicating slower gonadal development.

Discussion

The increase of IOS reflects the progression of oocytes containing vitellogenin, a yolk precursor protein that plays a key role in oocyte development (Ferdinand et al. 2023; Sibiya and Baskaralingam 2023). Pregnant mare serum gonadotropin contains both Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH plays a dominant role in regulating vitellogenic follicle growth by stimulating the production of estradiol-17β (E2) in ovarian follicles (Oduwole et al. 2021; Kuokkanen and Pal 2023). In teleosts, as in other vertebrates, E2 acts as a systemic endocrine signal that induces hepatic synthesis of vitellogenin during the oocyte growth phase (Dahlia et al. 2023; Takahashi and Ogiwara 2023). This process is known as vitellogenesis, represents a central stage in gonadal maturation and is tightly integrated with the Hypothalamic Pituitary Gonadal (HPG) axis, which is conserved across vertebrate reproductive systems (Acevedo-Rodriguez et al. 2018; Gupta et al. 2021; Ferdinand et al. 2023). Thus, the enhancement of vitellogenesis observed here demonstrates that exogenous hormonal induction using pregnant mare serum gonadotropin can compensate for the endocrine limitations that normally hinder domestication in freshwater species. These findings highlight how exogenous hormonal induction can successfully accelerate vitellogenesis in declining freshwater fish species, supporting both aquaculture productivity and biodiversity conservation.

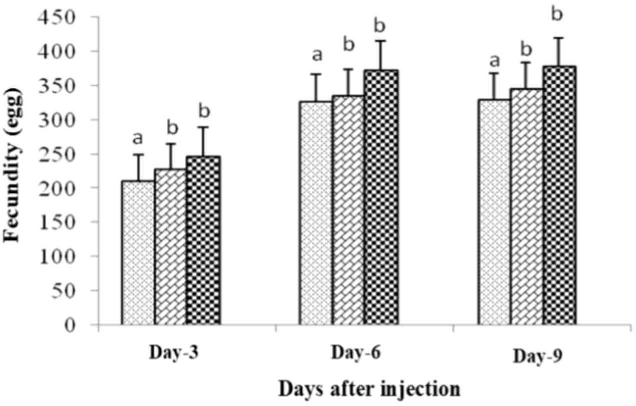


Figure 2. Fecundity (eggs/fish) of *Notopterus notopterus* following pregnant mare serum gonadotropin administration. Bars represent mean±95% Confidence Intervals (CI). Different letters indicate statistically significant differences among treatment groups (ANOVA, $p<0.05$). The control group (0 mL kg^{-1}, non-injected) is not shown because no eggs were released during the observation period

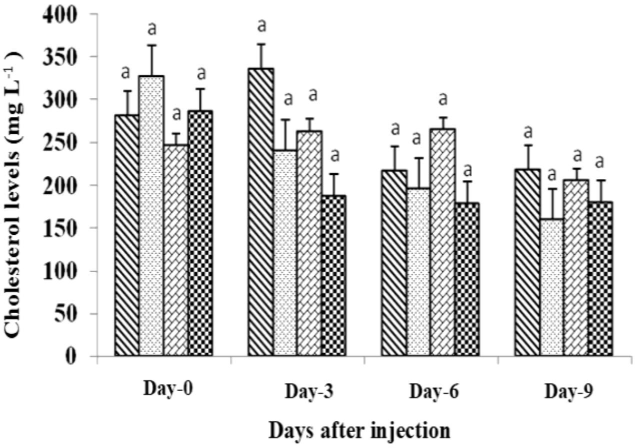
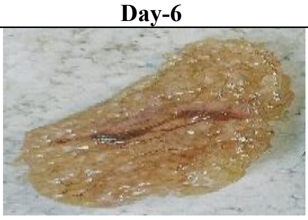


Figure 3. Cholesterol levels (mg/dL) in *Notopterus notopterus* after hormonal induction using pregnant mare serum gonadotropin. Bars represent mean±95% Confidence Intervals (CI). Different letters indicate statistically significant differences among treatment groups (ANOVA, $p<0.05$)

Table 2. Ovarian maturity level of the *Notopterus notopterus* during hormonal induction at different doses (0.5, 1.0, 1.5 IU/fish) observed on Day-0, Day-3, Day-6, and Day-9

T	Observation day			
	Day-0	Day-3	Day-6	Day-9
0 mL kg^{-1} fish	 Stage II (Developing)	 Stage II (Developing)	 Stage II (Developing)	 Stage II (Developing)
1.0 mL kg^{-1} fish	 Stage II (Developing)	 Stage II (Developing)	 Stage III (Maturing I)	 Stage III (Maturing I)
1.25 mL kg^{-1} fish	 Stage II (Developing)	 Stage III (Maturing I)	 Stage III (Maturing I)	 Stage IV (Maturing II)
1.5 mL kg^{-1} fish	 Stage II (Developing)	 Stage III (Maturing I)	 Stage IV (Maturing II)	 Stage V (Mature)

Note: T: Treatment

From an ecological perspective, vitellogenesis is highly sensitive to environmental cues such as photoperiod, temperature, salinity, and food availability, which can modulate gonadotropin release and reproductive timing (Kumar et al. 2022; Abinaya and Baskaralingam 2023). Such plasticity ensures reproductive success by synchronizing spawning with favorable conditions for larval survival (Cortese et al. 2024). The gonadotropin/mechanism accelerates this maturation by enabling FSH to act on theca cells, producing testosterone that is converted into estradiol-17β by the aromatase enzyme within granulosa cells (El Sayed et al. 2019; Liu et al. 2021; Takahashi and Ogiwara 2023). The resulting vitellogenin synthesized in the liver accumulates in oocytes, leading to a marked increase in ovarian size and IOS values (Jeng et al. 2020; Sato et al. 2021; Ferdinand et al. 2023), a response that integrates both hormonal control and environmental adaptability in reproductive ecology.

The statistically insignificant decrease in HSI values indicates that liver metabolic function remains robust and unaffected. The stability of hepatosomatic index suggests energy redistribution toward ovarian development during vitellogenesis without compromising liver physiology. The liver remains the primary site of vitellogenin synthesis under estradiol-17β regulation; this process does not significantly reduce liver mass, as vitellogenesis redirects energy and metabolic resources to support oocyte growth rather than impairing hepatic integrity (Mushirobira et al. 2020; Gupta et al. 2021; Ishwarya et al. 2023; Ramasubramanian and Priyadarisini 2023). The stable hepatosomatic index during vitellogenesis likely reflects an adaptive energy allocation strategy where the liver maintains its structural integrity while supporting reproductive protein synthesis (Leão et al. 2021). This stability further indicates that pregnant mare serum gonadotropin induction does not impose excessive metabolic stress on broodstock, an important factor when considering its application in long-term breeding and conservation programs. This is supported by studies in goldfish showing metabolic adjustments in the liver during reproduction of *Carassius auratus* (Ladisa et al. 2021, 2022), skipjack tuna indicating maintained hepatic lipid levels and HSI during ovarian development (Grande et al. 2016), and evidence from *Trisopterus luscus* demonstrating reliance on stored hepatic lipids as a capital-breeding strategy (Alonso-Fernández et al. 2012). Several previous studies reported a range of *N. notopterus* oocyte diameters that varied depending on the level of maturity and environmental conditions (Yanwirsal et al. 2017), reporting oocyte diameters of 3.8-4 mm, 1.1-3.9 mm (Muslim et al. 2024), and 3.10 mm (Azadi et al. 1995). Comparatively, studies on other species such as *C. striata* also demonstrated significant increases in OSI and fecundity following pregnant mare serum gonadotropin administration (Hutagalung 2019; Awal et al. 2025). In addition to *N. notopterus*, the use of pregnant mare serum gonadotropin has also been reported to be effective in stimulating gonad maturation in various other fish species (Tomasoa et al. 2015; Zulfadhli et al. 2024). This cross-species effectiveness confirms that the reproductive impairment of *N. notopterus* under captive conditions is physiological rather than genetic, and that exogenous gonadotropins can effectively stimulate ovarian development even in species with low natural spawning activity in captivity. The oocyte development observed at the highest pregnant mare serum gonadotropin dose in this study fell within the upper range of previous reports, confirming the role of hormonal treatment in accelerating gonadal maturation. Collectively, these findings reinforce that the responsiveness to pregnant mare serum gonadotropin is not species-specific but part of a broader reproductive endocrine capacity across teleosts, supporting its potential for cross-species applications in aquaculture and conservation breeding programs.

The increase in fecundity has a positive effect on gonadal maturity and egg production. This is consistent with the function of pregnant mare serum gonadotropin, which contains gonadotropin activity (FSH and LH), which plays a role in stimulating follicle growth and ovulation in female fish (Somanjaya et al. 2021; Sun et al. 2024). The observed improvement in fecundity suggests that hormonal induction may directly overcome the reproductive limitation that has previously constrained domestication attempts in this species. These differences are likely due to variations in broodstock size and weight, as fecundity is closely correlated with body size (Barneche et al. 2018; Chen et al. 2022). Other contributing factors include broodstock age, physiological condition, nutritional status, and environmental quality (Evans-Powell et al. 2024; Hadj-Hammou et al. 2024). Stress factors during handling or during the hormone injection process may also have an effect on the quality and quantity of eggs produced (Murugananthkumar and Sudhakumari 2022; Islam et al. 2024). Pregnant mare serum gonadotropin can temporarily increase fecundity (Gupta et al. 2024); however, ultimate reproductive success still depends on internal energy reserves and environmental conditions (Lema et al. 2024). In ecological terms, hormone-induced gains in fecundity may boost short-term reproductive output but cannot fully offset poor broodstock conditions or suboptimal habitats. Therefore, to maximize the benefits of pregnant mare serum gonadotropin, broodstock management must be optimized. This includes selecting appropriately sized and healthy broodstock, providing targeted nutritional supplementation before induction, minimizing handling stress during spawning, and adjusting hormone dosage and timing according to species-specific requirements (Ganesh 2021; Engdaw and Geremew 2024; Torsabo et al. 2024; Wylie et al. 2025). A non-significant decline in cholesterol levels was also observed, reflecting its physiological utilization in steroid hormone synthesis, particularly estradiol and progesterone during gonadal maturation (Strauss 2019; Arias et al. 2022). This lack of significant statistical change may be explained by hepatic compensation, where the liver maintains cholesterol homeostasis despite increased utilization for steroidogenesis. In line with the opinions of Rajakumar and Senthilkumaran (2020) and Kocerha and Denslow (2025), cholesterol levels tend to be low during the highest gonadal activity phase because they are used for hormone biosynthesis. Cholesterol serves as a precursor for steroid hormones regulating oocyte development (Strauss 2019; Huff et al. 2023; Zhao et al. 2024). Therefore, extensive cholesterol utilization during reproductive activity may lead to a decrease in its concentration in the blood. According to several studies, blood cholesterol levels in fish are influenced not only by reproductive activity but also by factors such as diet, environmental conditions, stress status, and individual metabolism (Li et al. 2023; Du et al. 2025). Although no statistically significant changes were observed in cholesterol levels, the trend supports the hypothesis that pregnant mare serum gonadotropin stimulates steroidogenesis, further validating the hormonal pathway responsible for gonadal development in captive conditions. Thus, fluctuations in cholesterol levels may serve as an indirect indicator of steroidogenic activity during fish reproduction (Rajakumar and Senthilkumaran 2020; Bassi et al. 2022). Notopteridae are known to exhibit relatively slow natural reproductive development compared to fast-maturing species (Yanwirsal et al. 2017). Therefore, the 9-day observation period in this study reflects hormonally induced physiological responses rather than natural ovarian maturation. Pregnant mare serum gonadotropin can accelerate vitellogenesis and steroidogenesis by stimulating ovarian follicles, thereby shortening early maturation phases even in slow-maturing species. This is a crucial next step in completing the domestication cycle of *N. notopterus*.

In conclusion, pregnant mare serum gonadotropin treatment significantly enhanced gonadal maturation in *N. notopterus*. These findings demonstrate the potential application of pregnant mare serum gonadotropin in broodstock management programs to induce synchronous and predictable ovarian maturation. Implementing this hormonal treatment could enhance spawning success rates in hatchery operations, reduce reliance on wild-caught broodstock, and support the establishment of captive breeding protocols as part of ex-situ conservation strategies. Furthermore, the use of hormonal induction provides a practical tool for advancing the domestication process and developing sustainable aquaculture systems for this ecologically and economically valuable species. Importantly, the demonstrated efficacy of pregnant mare serum gonadotropin in inducing synchronous ovarian maturation also contributes to conservation goals by reducing dependence on wild-caught broodstock. By supporting consistent hatchery-based seed production, this approach alleviates fishing pressure on natural populations and strengthens captive breeding initiatives. Such outcomes directly align with SDGs 14, which emphasizes sustainable use of aquatic resources and biodiversity conservation. Ultimately, the successful application of hormonal induction in this study highlights the significant role of this approach in strengthening aquaculture-based conservation strategies and maintaining freshwater biodiversity.

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