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Induction of maturation gonads the Asian swamp eel *Monopterus albus* at different sizes with the hormones PMSG + antidopamine and hCG

Induksi maturasi gonad belut sawah *Monopterus albus* pada ukuran yang berbeda dengan hormon PMSG + antidopamine dan hCG

Aan Aryanti Sandra[1], Agus Oman Sudrajat[2*], Harton Arfah[2], Dinar Tri Soelistyowati[2]

[1]Aquaculture Science, Post Graduate Study Program, Faculty of Fishery and Marine Sciences, IPB University, Bogor, West Java 16680, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia
*Corresponding author: agusom@apps.ipb.ac.id

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ABSTRACT

Monopterus albus is a protogynous hermaphrodite species that undergoes a unidirectional sex change from female to male. Asian swamp eel cultivation activities face constraints in seeding due to limited mature gonad broodstock. This study aims to evaluate the effect of administering a combination of PMSG + Antidopamine (Oodev®) and hCG on the induction of gonad maturation of Asian swamp eels at various body sizes. The research design used was a factorial design consisting of control treatment (without hormone injection), P1 = hCG (dose 20 IU/kg), P2 = Oodev® (dose 0.5 ml/kg), and P3 = hCG+Oodev® (dose 20 IU+0.5 ml/kg) with body length groups, namely K1 (15–25 cm), K2 (30–40 cm), and K3 (45–55 cm). The results showed that eels in group K1 had the highest body length growth (3.63 ± 0.96 cm), while the highest body weight gain (5.30 ± 1.30 grams) in K3 ($P<0.05$). Administration of oodev® showed the highest values for GSI = 8.13 ± 0.72% and HSI = 4.36 ± 0.80% in K1 compared to K2 (GSI = 6.95 ± 2.86%; HSI = 3.26 ± 0.68%) with female sex. In contrast, group K3 experienced a decrease in GSI = 0.81 ± 0.11% and HSI = 2.48 ± 1.06% accompanied by masculinization. Administration of oodev® also increased the concentration of estradiol-17β in K1 and K2, while testosterone increased in K3. The conclusion of this study is that administering oodev® to 15-40 cm sized Asian swamp eels can accelerate gonad maturity with female sex status, while at sizes >45 cm it can accelerate masculinization and maturation of male gonads.

Keywords: body length size, gonad maturation, masculinization, *Monopterus albus*, oodev®

ABSTRAK

Monopterus albus adalah spesies hermaprodit protogini yang mengalami perubahan jenis kelamin dari betina ke jantan secara searah. Kegiatan budidaya belut sawah menghadapi kendala dalam pembenihan karena keterbatasan induk matang gonad. Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian kombinasi PMSG + Antidopamine (Oodev®) dan hCG terhadap induksi pematangan gonad belut sawah pada berbagai ukuran tubuh. Desain penelitian yang digunakan adalah rancangan faktorial yang terdiri dari perlakuan kontrol (tanpa injeksi hormon), P1 = hCG (dosis 20 IU/kg), P2 = Oodev® (dosis 0,5 ml/kg), dan P3 = hCG+Oodev® (dosis 20 IU+0,5 ml/kg) dengan kelompok ukuran panjang tubuh yaitu K1 (15–25 cm), K2 (30–40 cm), dan K3 (45–55 cm). Hasil penelitian menunjukkan bahwa belut kelompok K1 memiliki pertumbuhan panjang tubuh tertinggi (3,63 ± 0,96 cm), sementara pertambahan bobot tubuh tertinggi (5,30 ± 1,30 gram) pada K3 ($P<0,05$). Pemberian oodev® menunjukkan nilai tertinggi untuk GSI = 8,13 ± 0,72% dan HSI = 4,36 ± 0,80% pada K1 dibandingkan dengan K2 (GSI = 6,95 ± 2,86%; HSI = 3,26 ± 0,68%) dengan jenis kelamin betina. Sebaliknya, kelompok K3 mengalami penurunan nilai GSI = 0,81 ± 0,11% dan HSI = 2,48 ± 1,06% disertai dengan maskulinisasi. Pemberian oodev® juga meningkatkan konsentrasi estradiol-17β pada K1 dan K2, sedangkan testosterone meningkat pada K3. Kesimpulan penelitian ini adalah pemberian oodev® pada belut sawah ukuran 15-40 cm dapat mempercepat kematangan gonad dengan status kelamin betina sedangkan pada ukuran >45 cm dapat mempercepat maskulinisasi dan pematangan gonad jantan.

Kata kunci: maskulinisasi, *Monopterus albus*, oodev®, pematangan gonad, ukuran panjang tubuh

INTRODUCTION

The Asian swamp eel (*Monopterus albus*) is widely distributed across Asia, including the Indo-Malayan Archipelago, Japan, Asian Russia, Burma, China, and northern India (Supiwong *et al.,* 2019). *Monopterus albus* is one of the high-value fish species in the Chinese seafood market (Newton *et al.,* 2021), making it a potential export commodity. For instance, Indonesia's fishery export volume to China reached 428,056 tons, with an average annual growth rate of 9.65% from 2016 to 2021 (KKP, 2022). In 2022, the export volume of *M. albus* reached 8,197,296 kg with a free on board (FOB) value of 13,473,820 USD (BPS, 2022). Moreover, demand for *M. albus* continues to rise, while domestic production remains limited, resulting in local market prices of live eels reaching IDR 70,000 per kilogram (Diatin *et al.,* 2019). Due to its high economic potential, *M. albus* has become an important aquaculture commodity. However, one of the major challenges in its cultivation is the limited availability of sexually mature broodstock.

Monopterus albus is a protogynous hermaphroditic fish species, undergoing sex reversal from female to male through an intersex stage during its life cycle (Cai *et al.,* 2017). Previous studies have shown that sex reversal in *M. albus* is associated with body length and weight (Chen *et al.,* 2021), and may be influenced by both environmental and genetic factors (Feng *et al.,* 2017). The sequence of sex differentiation in *M. albus* is female→intersex→male, with body lengths ≤47 cm corresponding to the female stage, 47–55 cm to the intersex stage, and ≥55 cm to the male stage (Chen *et al.,* 2021). In addition, endogenous steroid hormones such as estrogen and androgen play crucial roles in gonadal differentiation; estrogen is involved in ovarian differentiation and maintenance, while androgen is associated with testicular maintenance (Li *et al.,* 2019).

Fish reproductive regulation is a complex process influenced by multiple factors, including environmental, social, neural, endocrine, and metabolic cues (Gabilondo *et al.,* 2022). One of the key mechanisms involved in gonadal maturation is the synthesis of gonadotropin-releasing hormone (GnRH) in the hypothalamus, which regulates the secretion of two pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones stimulate gonadal development and the secretion of sex steroid hormones (Zohar *et al.,* 2010; Biran & Levavi-Sivan, 2018). One of the hormones that can be used to stimulate gonadal maturation in fish is pregnant mare serum gonadotropin (PMSG) combined with an antidopaminergic agent (AD), as well as human chorionic gonadotropin (hCG). PMSG has a higher follicle-stimulating hormone (FSH) content relative to luteinizing hormone (LH), making it more effective in accelerating gonadal maturation in fish (Mellisa *et al.,* 2022).

The antidopaminergic agent (AD) functions by blocking the dopaminergic inhibitory system to enhance GnRH secretion (Zohar *et al.,* 2010). In addition to PMSG + AD, hCG can also be used in the gonadal maturation process. As a gonadotropin, hCG plays a significant role in fish reproduction by stimulating gonadal maturation and triggering ovulation and spawning (Elakkanai *et al.,* 2015; Murugananthkumar *et al.,* 2017; Kucharczyk *et al.,* 2020). hCG acts directly on the gonads (ovaries and testes), and thus its effects may be more rapid due to its direct induction at the gonadal level, stimulating the synthesis and release of sex steroid hormones, which in turn play a key role in gonadal maturation, spermiation, and ovulation (Saleh *et al.,* 2020). Research on hormonal reproductive manipulation in *Monopterus albus*, particularly in relation to body length, remains limited. Therefore, this study aims to evaluate the effects of PMSG + AD (Oodev®) and hCG combinations on the induction of gonadal maturation in Asian swamp eels across various body size categories.

MATERIALS AND METHODS

Materials and experimental subjects

The hormones used in this study were Pregnant Mare Serum Gonadotropin (PMSG) + Antidopaminergic agent (AD), marketed under the trade name Oodev®, and human Chorionic Gonadotropin (hCG), marketed under the trade name *Pregnyl*®. The experimental subjects were Asian swamp eels (*Monopterus albus*) categorized into three size groups: 15–25 cm, 30–40 cm, and 45–55 cm, with 60 individuals per size group. These eels were wild-caught from rice fields in Kerinci Regency using traditional bamboo traps locally known as *bubu*, *posong*, or *lukah* (in the Kerinci language).

Experimental design

The study employed a factorial design with two tested factors: hormone treatment and eel size group. The hormone treatments consisted of control (no hormone injection), treatment P1(hCG at a dose of 20 IU/kg), treatment P2 (PMSG + AD (Oodev®) at a dose of 0.5 mL/kg), treatment P3 (hCG + Oodev® at a combined dose of 20 IU + 0.5 mL/kg). The size groups were K1 (female, 15–25 cm), K2 (female, 30–40 cm), K3 (intersex, 45–55 cm), based on the classification by Chen *et al.* (2021).

Preparation of rearing media and experimental procedures

The rearing tanks used in this study were made of wood lined with plastic tarpaulin, each measuring 120×80×40 cm³. Tanks were thoroughly cleaned with water and filled with 5–15 cm of water, followed by continuous aeration. Environmental enrichment was provided using shredded raffia string and bamboo. Oxytetracycline (OTC) was applied to the water at a concentration of 0.02 g/L. The water temperature during the study ranged from 22.66 to 22.90°C.

Prior to hormone injection, the eels were anesthetized using *Arowana*® brand stabilizer at a dosage of 1 mL/L for three minutes. Hormone administration was performed via intramuscular injection according to the specified treatment doses. Injections were administered every 10 days over a 70-day study period, for a total of seven injections. Throughout the study, eels were fed daily during both the acclimatization and experimental phases (morning, afternoon, and evening) with *Tubifex* worms at 3% of their body weight. Water in the rearing tanks was replaced as needed when water quality declined (e.g. due to turbidity or organic buildup).

Study parameters

Body weight gain

Body weight was measured before the initial hormone injection and every 10 days prior to each subsequent injection. Measurements were taken using a digital scale with a precision of 0.1 g. Final body weight gain was calculated at the end of the study using the following formula.

$$BWG = B_f - B_i$$

Note:
BWG = Body weight gain (g)
Bf = Final body weight after the rearing period (g)
Bi = Initial body weight before the rearing period (g)

Body length gain

Total body length of the Asian swamp eels was measured at the beginning and end of the study using a 100 cm measuring ruler. Body length gain was calculated using the formula.

$$BLG = P_f - P_i$$

Note:
BLG = Body length gain (cm)
Pf = Final body length (cm)
Pi = Initial body length (cm)

Gonadosomatic index (GSI)

The gonadosomatic index was assessed on day 0 and at the end of the study on day 70. GSI was calculated using the formula adapted from Ghaedi *et al.* (2016) as it shown below.

$$GSI = \frac{G_w\,(g)}{B_w\,(g)} \times 100$$

Note:
GSI = Gonadosomatic index (%)
Gw = Gonad weight (g)
Bw = Body weight (g)

Hepatosomatic index (HSI)

The hepatosomatic index was measured at the beginning (day 0) and end of the study (day 70), and calculated following Wang *et al.* (2016) by using the formula below.

$$HSI\,(\%) = \frac{L_w\,(g)}{B_w\,(g)} \times 100$$

Note:
HSI = Hepatosomatic Index (%)
Lw = Liver weight (g)
Bw = Body weight (g)

Survival rate (SR)

The survival rate of the Asian swamp eels was calculated at the end of the experiment. Survival Rate (SR) was determined using the formula from Yu *et al.* (2020).

$$SR\,(\%) = \frac{N_f}{N_i} \times 100$$

Note:
SR = Survival Rate (%)
Nf = Final number of surviving eels
Ni = Initial number of eels

Sex identification

Sex identification in Asian swamp eels was determined through morphological observations including body length, head shape, abdominal coloration, tail morphology, and the presence and coloration of gametes in the gonads. All experimental subjects were classified into three sex categories: female, intersex, and male (Chen *et al.,* 2021). Sex identification was conducted at both the beginning and the end of the study (Day 70).

Gonadal histology

Gonadal histology was examined to evaluate structural differences in the gonads of eels subjected to various hormonal treatments across different size groups. Observations were made descriptively, focusing on the histological structures of gonadal tissue, including oogonia, nucleus, yolk granules, yolk, degraded oocytes, spermatogonia, spermatocytes, spermatids, and spermatozoa. Gonadal histology was observed at the end of the experiment (Day 70). The histological assessment aimed to determine the stage of gonadal development, with all samples categorized into three developmental stages: female, intersex, and male. The histological procedure followed the method described by Gunarso (1989), with tissue staining performed using hematoxylin and eosin (H&E) staining.

Estradiol and testosterone hormone analysis

The concentrations of estradiol and testosterone in the blood plasma were measured on Day 40 and Day 70 of the study. Hormone quantification was conducted using the enzyme-linked immunosorbent assay (ELISA) method with the VIDAS ELISA kit.

RESULTS AND DISCUSSION

Results

Body weight and length gain

This study analyzed the effects of hormonal treatments and size groupings on the body weight and length gain of Asian swamp eels (*Monopterus albus*) over a 70-day rearing period. The results of the analysis are presented graphically in Figures 1 and 2. Body weight gain showed no significant differences across all factors (P>0.05) (Figure 1a, b, c). Body length gain did not differ significantly among treatment groups (P>0.05), but showed a significant difference among size groups (P<0.05), and no significant interaction effect was observed (P>0.05) (Figure 2a, b, c). Based on the observations, both body weight and length gain

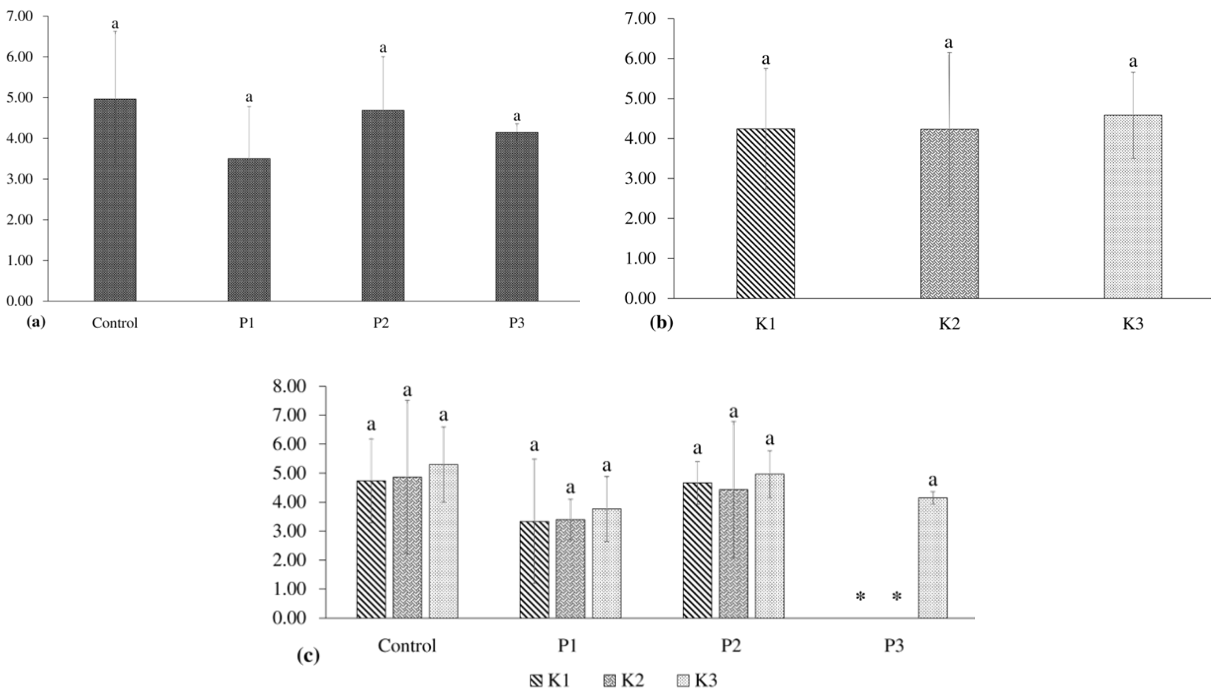

Figure 1. Body weight gain of Asian swamp eel (*Monopterus albus*) over a 70-day rearing period. (a) effect of hormonal treatment; (b) effect of size group; (c) interaction effect. P1 = hCG treatment; P2 = PMSG+AD or Oodev® treatment; P3 = hCG + Oodev® combination treatment. K1 = size group 15–25 cm; K2 = size group 30–40 cm; K3 = size group 45–55 cm. Different superscripts above the bars within each factor indicate statistically significant differences (P<0.05). *All eels died, data excluded from statistical analysis.

parameters were lower in hormonally treated eels compared to the control group.

Gonadosomatic index (GSI)

Administration of PMSG+AD (Oodev®) for 70 days was effective in increasing the gonadosomatic index (GSI) in Asian swamp eels of size groups K1 and K2 with female sex. However, a decrease in GSI was observed in size group K3, which exhibited male sex characteristics (Figure 3). Based on observations of the gonadosomatic index, the highest values for each factor were recorded

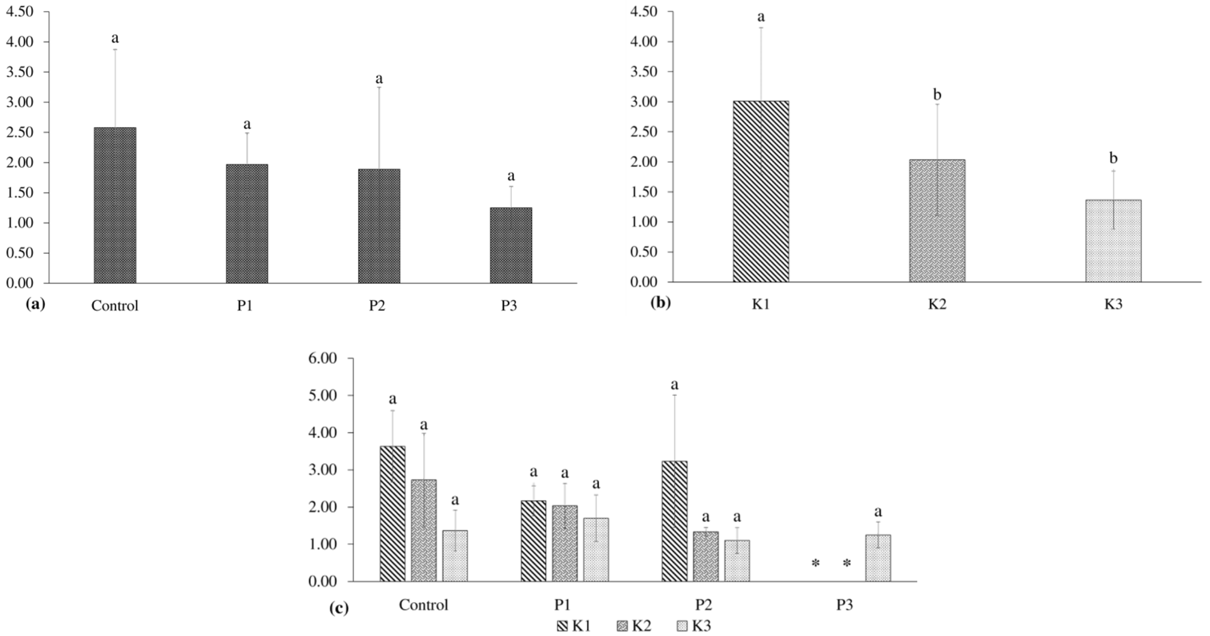

Figure 2. Body length gain of Asian swamp eel (*Monopterus albus*) over a 70-day rearing period. (a) effect of hormonal treatment; (b) effect of size group; (c) interaction effect. P1 = hCG treatment; P2 = PMSG+AD or Oodev® treatment; P3 = hCG + Oodev® combination treatment. K1 = size group 15–25 cm; K2 = size group 30–40 cm; K3 = size group 45–55 cm. Different superscript above the bars within each factor indicate statistically significant differences (P<0.05). *All eels died, data excluded from statistical analysis.

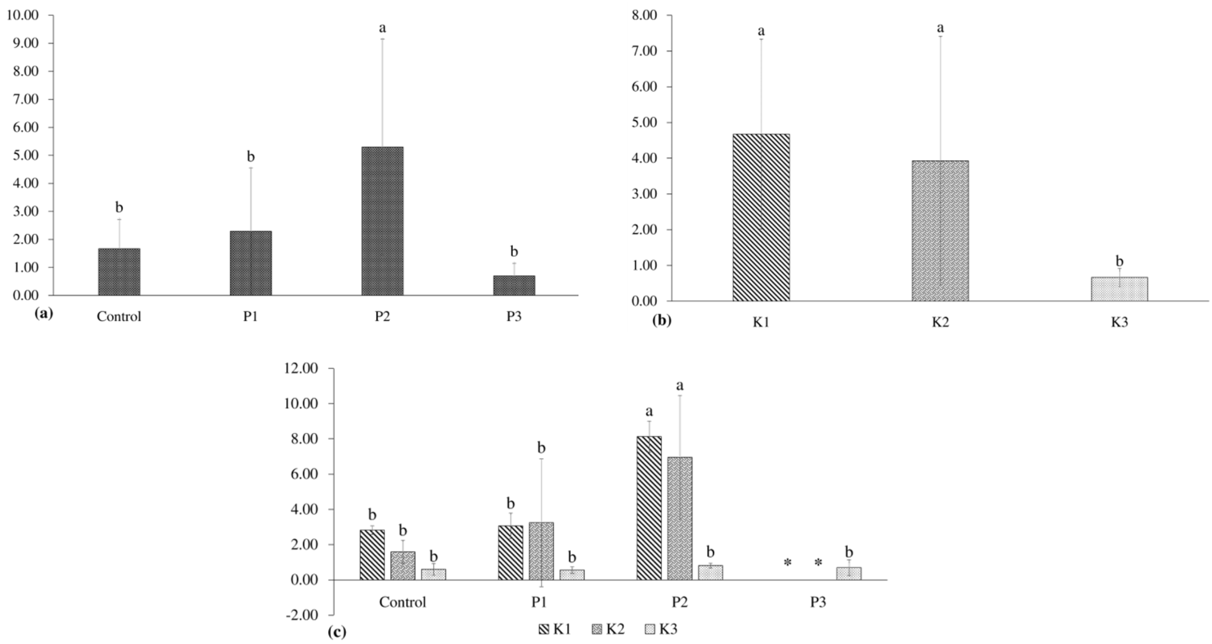

Figure 3. Gonadosomatic index (GSI) of Asian swamp eel (*Monopterus albus*) over a 70-day rearing period. (a) effect of hormonal treatment; (b) effect of size group; (c) interaction effect. P1 = hCG treatment; P2 = PMSG+AD or Oodev® treatment; P3 = hCG + Oodev® combination treatment. K1 = size group 15–25 cm; K2 = size group 30–40 cm; K3 = size group 45–55 cm. Different superscript above the bars within each factor indicate statistically significant differences (P<0.05). *All eels died, data excluded from statistical analysis.

as follows: treatment factor P2 (PMSG+AD or Oodev®) at 5.29 ± 3.85%, size group factor K1 at 4.67 ± 2.65%, and the interaction factor (P2 × K1) at 8.13 ± 0.87%. However, in size group K3, all treatments showed a decrease in gonadosomatic index (GSI). Statistical analysis using ANOVA revealed significant differences in the hormone injection factor, size group factor, and their interaction ($P<0.05$).

Hepatosomatic index (HSI)

Observation of the hepatosomatic index parameter showed that administration of PMSG+AD (Oodev®) for 70 days increased HSI values in Asian swamp eels of size groups K1 and K2 (female sex), while all treatments in size group K3 (male sex) exhibited decreased HSI values (Figure 4). The highest HSI values recorded for each factor were: treatment factor P2 at 3.37 ± 1.10%, size group factor K1 at 3.24 ± 1.08%, and interaction factor (P2 × K1) at 4.36 ± 0.79%. However, in size group K3, all treatments showed a decline in hepatosomatic index (HSI). Statistical analysis using ANOVA showed that the hepatosomatic index values differed significantly across hormone injection factors, size group factors, and their interaction ($P<0.05$).

Sex status

The results of the sex status parameter for Asian swamp eel after 70 days of maintenance, analyzed descriptively, showed that Asian swamp eels in groups K1 and K2 remained female under all treatments. Meanwhile, group K3 treated with P2 and P3 experienced sex reversal from the intersex stage to male. The sex status and characteristics of female, intersex, and male Asian swamp eels after hormonal induction on day 70 of rearing can be seen in Figures 5 and 6.

Gonadal histology

The histological observations of the gonads of Asian swamp eels (*Monopterus albus*) over a 70-day maintenance period are presented in Figure 7. The histological analysis showed that in the control treatment groups K1 and K2, oogonia (og) and nuclei (N) were still present. In treatment P1 groups K1 and K2, yolk (Y) and yolk granules (YG) had begun to appear, although nuclei (N) and oogonia (og) were still present. Treatment P2 (PMSG + AD or Oodev®) showed that the gonads of groups K1 and K2 were filled with yolk (Y), indicating that administration of PMSG + AD (Oodev®) effectively induced gonadal maturation in Asian swamp eels of groups K1 and K2, resulting in female differentiation. In contrast, in group K3 (45–55 cm), sex reversal from intersex to male

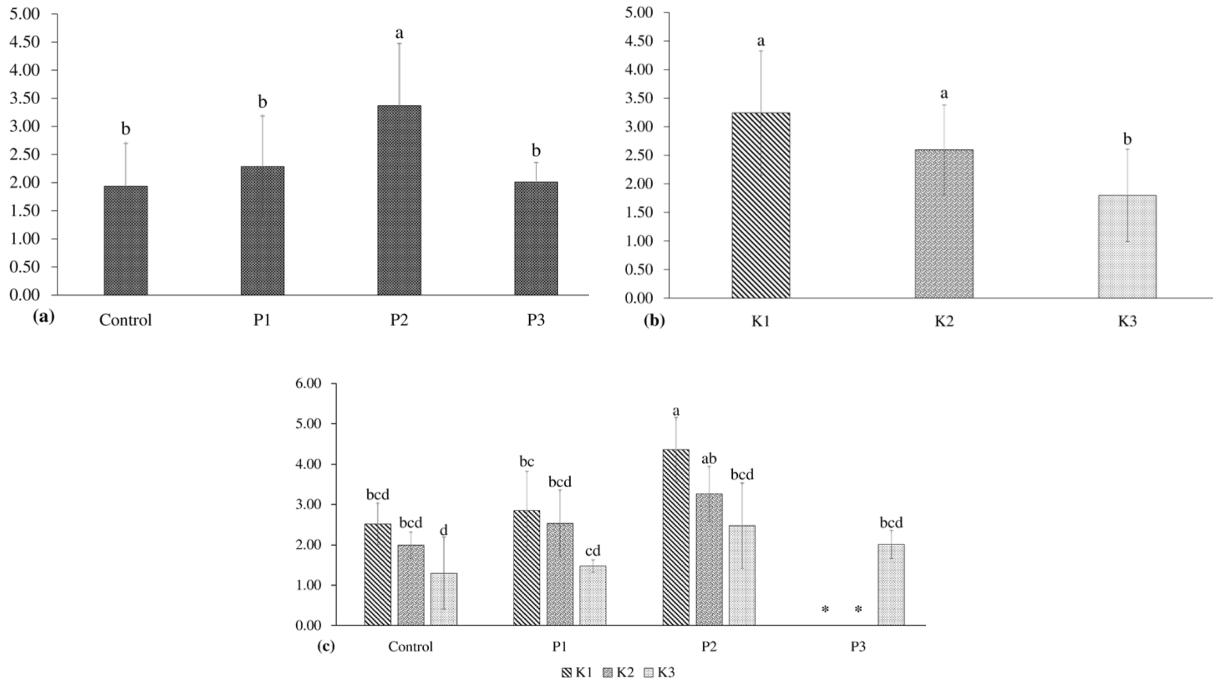

Figure 4. Hepatosomatic index (HSI) values of Asian swamp eel (*Monopterus albus*) over 70 days of rearing. (a) treatment factor; (b) size group factor; (c) interaction factor. P1 (hCG); P2 (PMSG+AD or Oodev®); P3 (hCG+Oodev®). K1 (size 15–25 cm); K2 (size 30–40 cm); K3 (size 45–55 cm). Different superscript above each bar indicate significant differences ($P<0.05$). *All eels died, data excluded from statistical analysis.

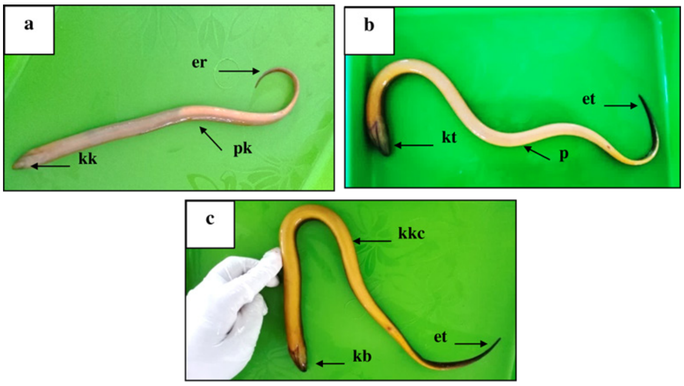

Figure 5. Sex differentiation status of Asian swamp eels (*Monopterus albus*) induced hormonally, visually or descriptively observed on day 70 based on body morphology. (a) female, (b) intersex, (c) male; kk = small head, kt = blunt head, kb = large head, p = white, pk = whitish-yellow, kkc = yellowish-brown, er = pointed tail, et = blunt tail.

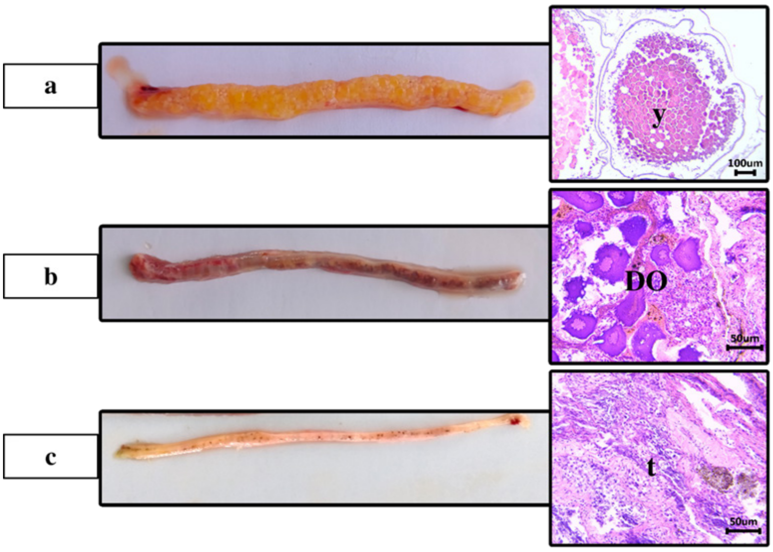

Figure 6. Sex differentiation status of Asian swamp eels (*Monopterus albus*) hormonally induced and observed descriptively on day 70 based on gonadal morphology and histology. (a) female, (b) intersex, (c) male, y = yolk, DO = degraded oocyte, t = testis.

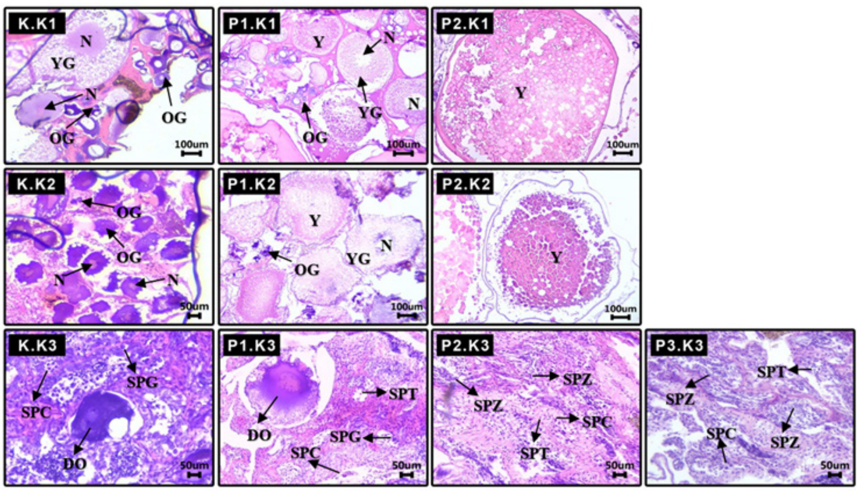

Figure 7. Histological development of gonads in Asian swamp field eels induced by hormonal treatments. K (Control), P1 (hCG treatment), P2 (PMSG + AD or Oodev®), P3 (hCG + Oodev®). K1 (Group 1), K2 (Group 2), K3 (Group 3). OG (oogonium), N (nucleus), YG (yolk granules), Y (yolk), DO (degraded oocyte), SPG (spermatogonia), SPC (spermatocyte), SPT (spermatid), SPZ (spermatozoa).

was observed in treatments P2 and P3, evidenced by the absence of oocytes in the degradation stage (DO) and the presence of male germ cells such as spermatocytes (SPC), spermatids (SPT), and spermatozoa (SPZ). However, in the control and P1 treatments of group K3, the gonads remained at the intersex stage, as indicated by the continued presence of degenerating oocytes (DO).

Estradiol-17β (E2) and testosterone (T) hormone levels

Descriptive observations revealed that the concentrations of estradiol-17β (E2) and testosterone (T) in the blood differed significantly between female and male groups of Asian swamp eels. Estradiol-17β levels were higher in Group K1 and K2 (predominantly female) compared to Group K3 (predominantly male). As shown in Figure 8, the highest concentration of estradiol-17β was recorded in eels treated with P2 in Group K2 on day 40, reaching 0.79 ng/mL, followed by a decline to 0.57 ng/mL by day 70. In contrast, Group K1 showed a continuous increase in estradiol-17β levels, peaking at 0.68 ng/mL on day 70. Meanwhile, testosterone levels in the larger-sized eels of Group K3 increased over the maintenance period, with the highest value observed in the P3 treatment group on day 70, reaching 0.82 ng/mL.

Survival rate

The survival rate (SR) of Asian swamp eels was assessed at the end of the study using a non-factorial design. The SR parameter over the 70-day rearing period is presented in percentage (%) in Figure 9. Based on Figure 9, the survival rate was observed to be lower in eels treated with P3 (hCG + Oodev®) and P1 (only hCG) compared to the control and P2 (Oodev®) treatments. Statistical analysis using ANOVA revealed a significant difference (P<0.05) among the treatments.

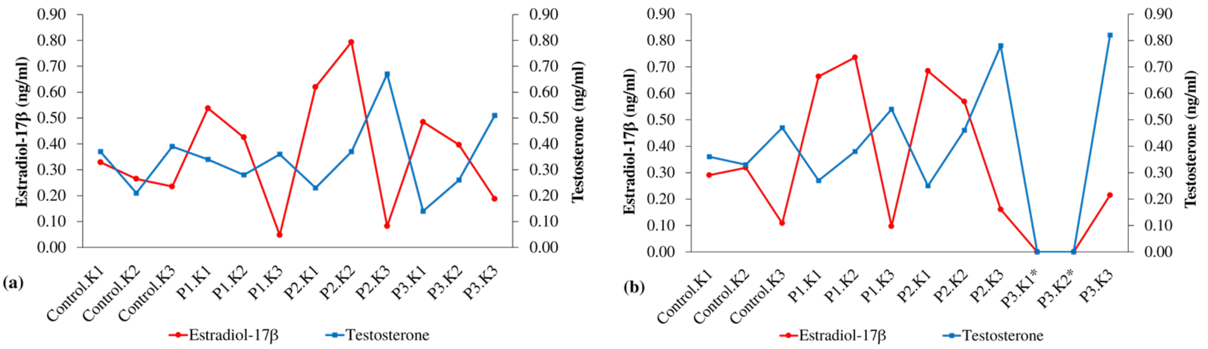

Figure 8. Graph of estradiol-17β and testosterone hormone levels in Asian swamp eels. (a) day 40; (b) day 70; K = control; P1 = hCG; P2 = PMSG+AD or Oodev®; P3 = hCG + Oodev®; K1 = Group 1; K2 = Group 2; K3 = Group 3. *All eels died, data excluded from statistical analysis.

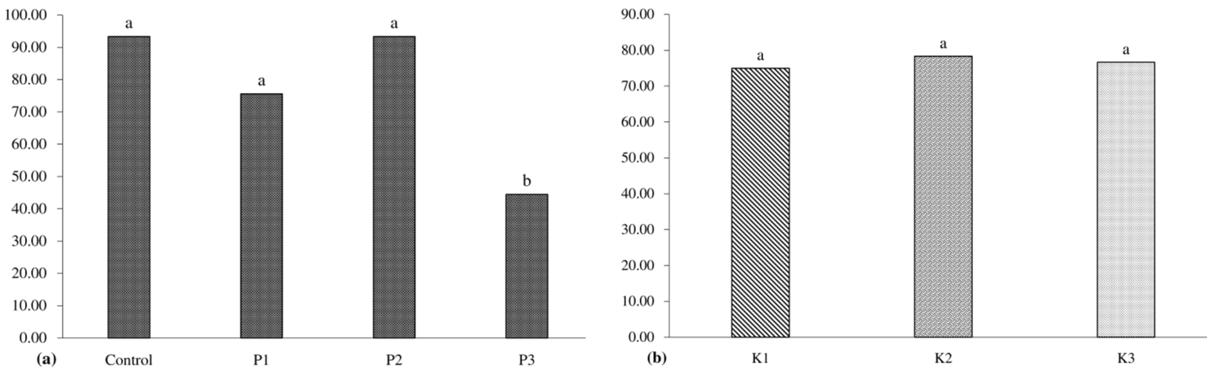

Figure 9. Survival rate graph of Asian swamp eel *(Monopterus albus)* during 70 days of rearing. (a) Treatment, (b) Size groups. K1 = size 15–25 cm, K2 = size 30–40 cm, K3 = size 45–55 cm, P1 = hCG, P2 = PMSG+AD or Oodev®, P3 = hCG + Oodev®. Different superscripts above the bars within each factor indicate significant differences (P<0.05).

Discussion

The results of the study on weight and length increment parameters showed that Asian swamp eels (*Monopterus albus*) subjected to hormonal treatments exhibited lower growth performance compared to the control group. This is likely attributed to the absence of injection-induced stress in the control group, allowing them to efficiently allocate dietary energy toward maintenance and somatic growth. Energy, defined as the chemical bonds in consumed feed, is released through oxidative reactions (Smith, 1989). During catabolic processes, the chemical energy from food is transformed, with 40–50% being captured in high-energy compounds such as adenosine triphosphate (ATP), while the remaining energy is lost as heat (Zonneveld *et al.,* 1991). Amin (1991) reported that during gonadal development in European eel (*Anguilla anguilla*), the ovary and testis utilized 25.08% and 20.28% of total energy intake, respectively. Fish growth tends to decline during gonadal development due to the reallocation of energy toward gonad production (Lugert *et al.,* 2016). Consequently, in *Monopterus albus*, gonadal maturation can inhibit somatic growth.

The gonadosomatic index (GSI) is a critical parameter reflecting reproductive development in fish and serves as an indicator of gonadal maturity (Kaur *et al.,* 2018). The GSI provides a reliable measure of gonadal development for a given species under analysis (Flores *et al.,* 2019). The present study demonstrated the highest GSI values in female eels from size groups K1 and K2, which received treatment P2 (PMSG+AD or Oodev®), with values reaching 8.13% and 6.95% respectively after 70 days of rearing. ANOVA statistical analysis revealed significant differences in treatment, body size group, and their interaction (P<0.05). These findings indicate that the administration of pregnant mare serum gonadotropin (PMSG) and antidopamine (AD) effectively stimulated vitellogenesis and gonadal development in *Monopterus albus*.

The use of PMSG+AD (Oodev®) to accelerate gonadal maturation has also been successfully applied to other fish species, including *Channa striata* (snakehead), *Osteochilus hasselti* (nilem carp), *Poropuntius tawarensis* (kawan fish), and *Rasbora lateristriata* (yellow rasbora) (Anwar *et al.,* 2018; Fitriatin *et al.,* 2018; Mellisa *et al.,* 2022; Rey *et al.,* 2023). A rapid increase in GSI typically signals the onset of vitellogenesis (Freitas *et al.,* 2024). Conversely, in intersex and male eels, a decline in GSI was observed. This decline is attributed to the degeneration of oocytes and the progression toward testicular development (Chen *et al.,* 2021). These findings are consistent with the current study, where a reduction in GSI was observed in intersex and male eels from size group K3 by day 70 of the rearing period.

The gonadosomatic index (GSI) and hepatosomatic index (HSI) are commonly used indicators of gonadal development, energy allocation for reproduction, gonadal maturation, and spawning processes (Hismayasari *et al.,* 2015; Saleh & Ali, 2017). The findings of this study showed that the HSI value progressively increased in female Asian swamp eels (*Monopterus albus*) in groups K1 and K2 receiving treatment P2 throughout the experimental period. The highest average HSI values were recorded at 4.36% for group K1 and 3.26% for group K2 after 70 days of rearing. An increase in HSI typically occurs when the ovary enters the vitellogenic phase, suggesting that vitellogenin production in the liver leads to increased liver mass, which subsequently elevates the HSI value (Pham & Nguyen, 2019; Kumar *et al.,* 2022). During vitellogenic growth, oocytes accumulate vitellogenin a yolk precursor protein rich in phospholipids synthesized by the liver and transported via the bloodstream to the ovaries. The oocytes uptake and convert it into yolk reserves. Upon completion of vitellogenesis, the ovary becomes filled with oocytes ready to undergo final maturation and ovulation (Reading *et al.,* 2017).

Nissling *et al.* (2015) noted that peak HSI values in female fish occur just before spawning, which correlates with intensified feeding activity, as observed in Baltic flounder. Likewise, the increase in HSI during ovarian maturation supports the liver's enhanced role in vitellogenin synthesis essential for oocyte development (Freitas *et al.,* 2024). In contrast, a decline in HSI is typically observed in male fish as they reach reproductive maturity (Freitas *et al.,* 2024). In the present study, male Asian swamp eels from group K3 treated with P2 and P3 showed a decrease in HSI values on day 70, recorded at 2.48% and 2.01%, respectively. These results were statistically validated via ANOVA, which revealed significant differences among treatment groups, body size groups, and their interaction (P<0.05).

The descriptive analysis of sex status over the 70-day rearing period revealed that eels in groups K1 and K2 remained female across all treatments.

In contrast, group K3 exhibited sex reversal from intersex to male under treatments P2 and P3 during the same period. Prior studies have indicated that sex reversal in *Monopterus albus* is influenced by body length or weight (Chen *et al.,* 2021) and environmental factors (Feng *et al.,* 2017). Additionally, endogenous steroid hormones play a critical role in sex differentiation (Li *et al.,* 2019). The mechanisms underlying sex reversal in this species are complex and likely influenced by a combination of genetic, environmental, nutritional, and hormonal factors (Feng *et al.,* 2017; Li *et al.,* 2019; Jiang *et al.,* 2024).

This study found that the administration of PMSG+AD (Oodev®) and hCG+Oodev® hormones accelerated the transition from intersex to male in *Monopterus albus* over a 70-day period. Although the precise mechanism of PMSG+AD in inducing sex reversal remains unclear, it is hypothesized that the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) components of PMSG, combined with the dopaminergic inhibition effect of AD, stimulate gonadotropin-releasing hormone (GnRH) secretion, contributing to the observed sex reversal. Several studies have reported that exogenous gonadotropin (FSH and LH) administration can induce sex changes in protogynous hermaphroditic fish species such as the orange-spotted grouper (*Epinephelus coioides*), tiger grouper (*Epinephelus fuscoguttatus*), and Asian swamp eel (*Monopterus albus*) (Tang *et al.,* 1974; Huang *et al.,* 2019; Palma *et al.,* 2019; Li *et al.,* 2023).

Casas & Saborido-Rey (2021) reported that the hypothalamic-pituitary-gonadal (HPG) axis, also known as the endocrine axis, is the main signaling pathway that regulates sex change in hermaphroditic fish. The hypothalamus releases gonadotropin-releasing hormone (GnRH) in the brain, which stimulates the pituitary gland to synthesize and secrete gonadotropin hormones (GtHs), namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Zohar *et al.,* 2010). FSH plays a key role in regulating testicular development (spermatogenesis), whereas LH is involved in controlling testicular maturation (spermiation) (Mylonas *et al.,* 2017). In this study, estradiol-17β (E2) and testosterone (T) levels in the blood were found to vary significantly among female, intersex, and male Asian swamp eels (*Monopterus albus*).

Results indicated that in group K2 females treated with P2 (PMSG+AD or Oodev®), estradiol-17β levels increased on day 40 to 0.79 ng/ml but declined to 0.57 ng/ml by day 70. Conversely, females in group K1 exhibited a continuous increase, peaking at 0.68 ng/ml on day 70. In group K3, testosterone levels increased in intersex eels and peaked in males under treatment P3, reaching 0.82 ng/ml on day 70 of rearing. These patterns suggest that estradiol-17β levels rise during the female phase, while testosterone levels elevate during the intersex and male phases. Serum estradiol-17β levels significantly increase first during the female stage and then decline markedly during the sex reversal process in *M. albus* (Chen *et al.,* 2021).

Hwang *et al.* (2022) also observed high estradiol-17β concentrations in Japanese eel (*Anguilla japonica*) females during the previtellogenic or early vitellogenic phases. In general, estradiol-17β levels in many fish species increase during vitellogenesis and decline after reaching full maturation (Moreira *et al.,* 2015; Ghosh *et al.,* 2016; Pham & Nguyen, 2019; Mushirobira *et al.,* 2020). This study demonstrates that administration of PMSG+AD (Oodev®) elicited different hormonal responses across body size groups: groups K1 and K2 showed elevated estradiol-17β levels, while group K3 exhibited increased testosterone levels. The rise in estradiol-17β levels in K1 and K2 females is likely attributed to enhanced aromatase enzyme activity induced by PMSG+AD injections. Watanabe *et al.* (1999) stated that PMSG significantly increases the activity of P-450arom. Cytochrome P-450 aromatase (P-450arom), encoded by the *cyp19a1* gene, is the enzyme that catalyzes the conversion of testosterone into estradiol-17β and plays a critical role in gonadal differentiation and development (Watanabe *et al.,* 1999; Zhang *et al.,* 2008).

However, the mechanism by which PMSG+AD increases testosterone in intersex and male eels of group K3 (45–55 cm) remains unclear. This phenomenon may be linked to the ongoing sex reversal process in *Monopterus albus*, in which FSH and LH contained in pregnant mare serum gonadotropin (PMSG) play pivotal roles in sex differentiation. During the transitional stage from intersex to male, *M. albus* exhibits a significant increase in 11-ketotestosterone concentrations (Chen *et al.,* 2021). Furthermore, Meng *et al.* (2022) reported that testosterone levels in fully masculinized *M. albus* are significantly higher than in females.

The histological analysis of Asian swamp eel (*Monopterus albus*) gonads showed the most favorable results in treatment group P2 (PMSG+AD or Oodev®) for size groups K1 and K2. In these groups, gonads treated with P2 were fully filled with yolk, indicating advanced vitellogenesis. This confirms that PMSG+AD (Oodev®) enhances the vitellogenesis process, leading to gonadal maturation in K1 and K2 groups. Gonadal maturation in fish is characterized by enlarged ovaries that increase in weight and change color to yellowish due to yolk accumulation. The mature stage is further marked by high yolk granule density, thickening of the zona radiata, and a decreased nucleus-to-cytoplasm ratio (Esmaeili *et al.,* 2017).

Histological results in group K3 under treatment P2 indicated sex reversal from intersex to male. Fan *et al.* (2022) reported that intersex Asian swamp eels contain numerous male germ cells, including spermatogonia and spermatocytes, alongside a few early-stage or degenerating oocytes (degraded oocytes). In contrast, mature male eels are characterized by testes filled with spermatids and spermatozoa (Chakraborty, 2018). The study also found that survival rates were lower in treatment groups P3 and P1 compared to the control and P2 groups. This reduction in survival is likely due to paradoxical effects associated with suboptimal hormone dosage or duration.

Hunter & Donaldson (1983) noted that excessive hormone dosages can induce paradoxical phenomena, leading to increased mortality, reduced growth rates, and even sterility in fish. Other studies have confirmed that administering gonadotropin-releasing hormone (GnRH) in combination with LH can result in paradoxical effects in both mammalian and fish ovarian follicles (Habibi & Andreu-Vieyra, 2007; Tsafriri, 2012). The paradoxical effect of gonadotropin hormones has been observed in many species, including humans, and has puzzled researchers for decades (Fallah & Habibi, 2020).

This study confirmed that the effects of hCG, PMSG+AD (Oodev®), and hCG+Oodev® vary depending on body size. hCG injection in K1 and K2 eels promoted vitellogenesis but did not achieve full gonadal maturation. In K3, no sex reversal occurred during the 70-day maintenance period. Meanwhile, PMSG+AD (Oodev®) injection in K1 and K2 successfully stimulated vitellogenesis to full gonadal maturity, while in K3 it induced sex reversal from intersex to male by day 70. However, hCG+Oodev® injection in K1 and K2 caused mortality after 40 days of maintenance, whereas in K3, sex reversal occurred by day 70. Overall, the findings indicate that the most effective treatment was PMSG+AD (Oodev®), yielding optimal outcomes in groups K1 (15–25 cm), K2 (30–40 cm), and K3 (45–55 cm) over a 70-day maintenance period.

CONCLUSION

The administration of PMSG+AD (Oodev®) at a dose of 0.5 ml/kg in Asian swamp eels (*Monopterus albus*) with a body length ranging from 15–40 cm can accelerate gonadal maturation in individuals with a female sex status, whereas in eels with a body length >45 cm, it can promote masculinization and gonadal maturation in males.

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