frontiers in Marine Science

TYPE Original Research
PUBLISHED 30 June 2026
DOI 10.3389/fmars.2026.1869628

OPEN ACCESS

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RECEIVED 30 April 2026
REVISED 22 May 2026
ACCEPTED 10 June 2026
PUBLISHED 30 June 2026

CITATION
Khalil HS, Saleh HHE, Attia E-SI, El-Agri AM, Abouel Azm FR, Hussein GHG and Abdel-Wahed RK (2026) Artificial spawning of African catfish (*Clarias gariepinus*) with different forms and sources of pituitary gland extract: influence on fecundity, sex hormones, and reproductive performance. *Front. Mar. Sci.* 13:1869628. doi: 10.3389/fmars.2026.1869628

Artificial spawning of African catfish (*Clarias gariepinus*) with different forms and sources of pituitary gland extract: influence on fecundity, sex hormones, and reproductive performance

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Introduction: The investigation was performed to compare the influence of using different pituitary gland extract types (dried and fresh) from common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) on fecundity, sex hormones, and artificial spawning performance of African catfish.

Methods: Pituitary glands were collected from sixty-four male catfish and sixty-four carp broodstocks to formulate four treatments: dried catfish pituitary gland extract ($DPG_{catfish}$), fresh catfish pituitary gland extract ($FPG_{catfish}$), dried carp pituitary gland extract (DPG_{carp}), and fresh carp pituitary gland extract (FPG_{carp}). Sixteen ripe females (920 to 1190 g) and sixteen ripe males (1060 to 1150 g) with a sex ratio (1:1 male ♂: female ♀) were chosen for each treatment.

Results and discussion: The highest fecundity and reproductive performance were observed in the $DPG_{catfish}$-treated group, followed by the DPG_{carp}-treated group. The latency periods were recorded as 12 h for $DPG_{catfish}$, 17 h for $FPG_{catfish}$, 12.5 h for DPG_{carp}, and 21 h for FPG_{carp}. $DPG_{catfish}$ treatment significantly improved ($P \leq 0.05$) ovulation performance, reproductive output, fertilization and hatching success, and circulating estrogen, progesterone, and testosterone levels. In contrast, both fresh pituitary treatments (catfish and carp) resulted in noticeably lower outcomes. The results indicate that $DPG_{catfish}$ extract consistently outperformed other treatments across all measured spawning parameters, suggesting its effectiveness in enhancing seed production in African catfish aquaculture.

KEYWORDS
dried vs. fresh pituitary gland extracts, artificial spawning, hormonal induction, fecundity, gonadal steroids

1 Introduction

In 2023, global production of aquatic animals reached a new record, representing a 2.1% increase over the peak recorded in 2022. This increase was mainly attributed to aquaculture, which grew by 4.2 percent, whereas capture fisheries recorded a slight decline of 0.2 percent. Out of the aquatic animals produced in 2023, 52 percent came from aquaculture, while 48 percent was supplied by capture fisheries, highlighting the increasing significance of aquaculture (Khalil and Nasr-Allah, 2025).

Egypt's aquaculture industry ranks seventh globally and first in Africa, providing 78% of the country's total fish production (Abdel-Hady et al., 2025). In recent years, African catfish culture in Egypt has experienced significant growth and obtained notable importance, evolving from being considered an unwanted fish in ponds of tilapia or just a controlling fish employed to limit overpopulation in mixed-sex tilapia earthen pond farms to becoming a valuable and promising species for aquaculture (El-Hawarry et al., 2016). Lately, fish farmers have increasingly recognized the several benefits of catfish, including its rapid growth, satisfactory taste, low feed demands, competitive market value, and strong disease resistance (Shourbela et al., 2020). This shift in perception and practice has heightened the importance of catfish aquaculture as a promising opportunity to secure food and drive economic growth in Egypt's aquaculture industry.

On the other hand, achieving large quantities of high-quality hatchlings remains a main challenge, mainly due to the shortage of natural spawning under captive conditions, the presence of diseases affecting various phases of the production cycle, and several other obstacles (Mylonas et al., 2010; Hasimuna et al., 2025). Consequently, the inducement of spawning in African catfish has attracted considerable global research interest (Brzuska et al., 1999; Brzuska, 2004), with growing attention in Egypt recently (Shourbela et al., 2020).

To guarantee a consistent supply of high-quality fish seed, several methods have been established to breed pond fish in controlled environments (Shourbela et al., 2020; Khalil et al., 2021). Spawning induction methods have played a crucial role in increasing the growth and progress of the aquaculture sector (Xu et al., 2023). While some techniques focus on simple environmental adjustments, others rely on isolating mature males and females or providing substrates for egg attachment (Shourbela et al., 2020). Among these, hormonal induction remains the most advanced and effective approach currently available (Kucharczyk et al., 2020; Kucska et al., 2022), which has been employed to effectively induce ovulation and enable the successful collection of eggs in fish (Marimuthu et al., 2009; Marimuthu and Haniffa, 2010). Consequently, it is considered the optimal solution for the successful culture of Clarias spp (El-Hawarry et al., 2016).

As reported by Otoh, Ekanem (Otoh et al., 2024), hormones are biochemical transmitters synthesized by the body's endocrine glands and released directly into the circulation, where they act on specific target cells to regulate their anticipated function. Therefore, hormonal preparations for induced spawning influence the axis of hypothalamic-pituitary, leading to elevated gonadotropin levels in the bloodstream (Kucska et al., 2022). This is commonly achieved through the administration of exogenous hormones, including fish pituitary extracts, human chorionic gonadotropin (hCG), gonadotropin hormone (GTH), luteinizing hormone-releasing hormone (LHRH) and its analogues (LHRHa), and gonadotropin-releasing hormone (GnRH) and its analogues (El-Hawarry et al., 2016). It is important to note that the most effective hormone formulations and doses differ per species (Yaron et al., 2009; Mylonas et al., 2010; Mylonas et al., 2017). Ovaprim, human chorionic gonadotropin, frog pituitary extracts, carp pituitary tissue, and other hormonal substances have been used to accelerate fish spawning in African aquaculture, with different degrees of effectiveness (Yusuf et al., 2023).

According to Sahoo, Giri (Sahoo et al., 2008), reproductive performance under controlled conditions serves as a key indicator of breeding success, which is affected by several factors, such as the type and efficacy of the selected hormone, the administered dosage, and the gonadal maturity stage of the broodstock. Ruaza Jr (Ruaza, 2018), further noted that pituitary glands are frequently employed as spawning inducers due to their vital role in motivating growth, maturation, and ovulation processes. The pituitary gland is the principal source of the critical hormones that regulate reproductive processes in animals (Mosha et al., 2016). The use of pituitary extracts seems to have been notably successful in stimulating ovulation of different fish species, including *Clarias* species (Hossain et al., 2012; Dhara and Saha, 2013).

In this way, Houssay (1931) was the first to explain that crude pituitary extracts could successfully stimulate induced spawning. Additionally, Britz (1991) reported that hypophysation is the most efficient method for inducing spawning in African catfish, making it highly beneficial for commercial catfish farmers across Africa. Previous research has documented the efficacy of stimulating spawning in *C. gariepinus* using both homoplastic and heteroplastic pituitary extracts (Hecht, 1985). Carp pituitary extract (CPE) has proven to be one of the most reliable agents for accurately predicting ovulation timing (Liu et al., 1997). It has been successfully used in triggering spawning in *Ictalurus punctatus* females (Lambert et al., 1999). Furthermore, *C. gariepinus* pituitary extract, which is widely available and more cost-effective than other hormones (Adebayo and Fagbenro, 2004), has shown comparable effectiveness to both CPE and luteinizing hormone-releasing hormone analogues (LHRHa) in stimulating ovulation in channel catfish females (Bosworth, 2005). Although fish pituitary extracts remain one of the most commonly used and cost-effective methods in developing aquaculture systems, limited information is available regarding the impact of preserved natural pituitary extracts on fish reproductive performance (Okomoda et al., 2017).

Hence, this study aims to compare the effects of different forms of *C. gariepinus* and *C. carpio* pituitary gland extracts on fecundity, sex hormones, and artificial spawning performance in African catfish. Identifying the most effective form (dry versus fresh) and source (species-specific versus non-species-specific) of pituitary gland extracts is essential for improving artificial reproduction in African catfish. In this context, increasing seed availability is expected to enhance the growth and improvement of the aquaculture industry.

2 Materials and methods

2.1 Ethical statement

This study and the experimental protocol were performed in accordance with the stringent guidelines, authorization and approved by the National Institute of Oceanography and Fisheries (NIOF-IACUC, Egypt) committee for the ethical treatment of animals/aquatic animals. All methods were performed in accordance with the relevant guidelines and regulations (Approval no.: NIOF-AQ5-F-25-R-018). The authors confirm that the study was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org).

2.2 Broodstocks- rearing conditions

Two hundred and thirty healthy catfish broodstocks (80 female and 150 male) and seventy-five male carp of reproductive age were acquired from the private fish farm in Kafer El-Shakh Governorate, Egypt. Broodstocks were transported to a commercial hatchery in Kafer El-Shakh Governorate and sterilized by being placed in plastic tanks filled with 15 ml of formalin, i.e., 150 ppm. For a month, the female was kept apart from the male and catfish were kept apart from the carpin an earthen pond (10 × 20 × 1.5 m). This allowed the fish to acclimate to the hatchery's water environment and prepare for spawning. During this study, broodstock were provisioned twice daily, six days per week, with a marketable floating diet at a rate equivalent to 2% of their biomass. The feed composition included 30% crude protein, 4.33% crude fiber, 5.73% crude fat, and 4.00 kcal/g gross energy (Grand Co., Damietta, Egypt, for fish feed manufacturing). Water quality parameters were monitored every 7 days to detect dissolved oxygen (DO) concentration and pH via a Tintometer group® (pH/OPR, DO, CD/TDS, Nr: 00724200; Germany 01/16). Water temperature was measured by using a centigrade thermometer. The values for water temperature ranged from 26 to 32 °C, DO values ranged from 5.34 to 6.22 mg/L, and pH ranged from 8.11 to 8.35.

2.3 Catfish broodstock selection

After a month, 66 mature females and 66 mature males were selected from the brood fish in the earthen pond for study of their external morphological features. The females exhibited a soft, inflated belly, rounded, enlarged vaginal papillae, and were ready to spawn. To accurately select the female broodfish, a catheter was used to extract a small egg sample from their ovaries. Over 90% of the mature ovaries had egg diameters above 900 µm when assessed using a calibrated ocular micrometer. The discoloration of the genital papilla was used as a criterion for assessing male maturity (Shourbela et al., 2020; Attia et al., 2025).

2.4 Experimental design

Experimental catfish brood fish were divided into four treatments as follows:

The first treatment: brood fish received injections with dried catfish pituitary gland extract ($DPG_{catfish}$).

The second treatment: brood fish received injections of fresh catfish pituitary gland extract ($FPG_{catfish}$).

The third treatment: brood fish received injections with dried carp pituitary gland extract (DPG_{carp}).

The fourth treatment: brood fish received injections with fresh carp pituitary gland extract (FPG_{carp}).

For each treatment, a sex ratio of 1:1 (male ♂: female ♀) was used to select sixteen mature female and sixteen mature male catfish. The weight of the females varied between 920 and 1190 g, while that of the males was 1060 to 1150 g.

2.5 Preparation of pituitary gland extracts (PG)

Thirty-two male catfish and thirty-two carp broodstock were first anesthetized with a 50 µl/L clove oil solution (Saleh et al., 2025) and then beheaded to excise the pituitary gland. The procedure included severing the fish's head to eliminate its lower jaw and expose the brain cavity. The pituitary gland was visible after the ventral part of the brain was cut out. Next, a set of forceps was used to gather the pituitary glands. After the removal of the pituitary gland from catfish and carp broodstock, it was transferred into a dark-colored bottle with 20 ml of absolute alcohol (97%) for 15 minutes. Following this period, alcohol concentrations were subsequently re-evaluated at 30 minutes, 8 hours, and 24 hours from the initiation of the procedure, consistent with the methodology described by (Britz, 1991). The bottle was refrigerated throughout this period. The pituitary glands were then dried on tissue paper for a full day at room temperature before being stored in a desiccator until needed. After 10 days, another set of broodstock (32 male catfish and 32 carp) was decapitated to get the fresh pituitary gland. Follow the same steps above, but after 24 hours, keep them in the refrigerator; the pituitary glands are ready for injection and not dried. Both fresh and dried pituitary gland were extracted with 1ml of physiological saline solution (sodium chloride 0.9%) after being crushed in a pestle, and the leftover fluid was then drawn into an injectable syringe by centrifuging the solution.

2.6 Spawning of catfish brood fish

African catfish broodstock were transported from an earthen pond to the hatchery and placed in sixteen concrete ponds (2 ×2 ×1 m); each pond has 8 broodstock. There is a total of 128 fish in this brood, with 64 males and 64 females distributed among the four groups (each group has 16 females and 16 males). Sixteen concrete ponds were used to house the brood fish, with each treatment corresponding to a distinct 2 pond for the males and females (each treatment has 4 ponds; 2 ponds for male and 2 ponds for female, 2 repeated ponds (n=2)). Eight broodstock were placed in each pond (16 females per treatment). Three females broodstock from each pond (6 females per treatment, in addition to two fish before injection (0 time)) were used to estimate blood hormones levels, and fecundity and ovarian measurements, data from the remaining

five females broodstock in each pond (10 females per treatment) were used to assess stripping response parameters and spawning performance. Two days before the injection, the feed was not provided. One or two liters of water per minute were the flow rate.

Before the injection, brood fish were tranquilized with a 50 µl/L solution of clove oil (Saleh et al., 2025). To calculate the hormone dosage, the trial's brood fish (male and female) were weighed before being injected. Every 20 pituitary glands were extracted with 20 ml of physiological saline solution; the brood fish were injected with 1 ml (one pituitary gland) per kilogram of brood fish. The hormonal injection was done in a single dose in the morning (7-9 AM), and brood fish were injected intramuscularly using a 3 ml syringe. After hormonal injection, the broodfish were returned to the ponds. One pituitary gland per kg of fish weight was used, regardless of whether the fish were dried or fresh. The weights of the dried glands before drying were equal to the weight of the fresh glands, thus standardizing the hormonal dose.

2.7 Checking for ovulation

The ovulation status of the female catfish was monitored every hour for the next 10 hours. When the females created greenish-brown eggs, it was determined that they had ovulated because the eggs oozed out readily when the abdomen was softly pulled towards the vaginal hole (El-Hawarry et al., 2016). The ovulation rates and latency period of each group were assessed directly.

2.8 Stripping and fertilization of catfish eggs

To remove the eggs from the shell, we gently pressed down on the belly toward the tail with our thumbs, then transferred them to a plastic dish that had been pre-weighed. After each female egg was removed, around one gram of the stripped egg was added to the formalin solution (5% concentration) and allowed to sit until the total number of eggs removed from each female was calculated. Simultaneously, males were dissected into little pieces to extract their milt, which was obtained by opening their abdomens and extracting their testes. The eggs were prepared by applying mild soap to the shelled eggs, adding 150 ml of fresh water to activate the semen, and then gently shaking the bowl to mix the ingredients. Following this, the eggs were washed in a fertilizing solution containing 4 grams of sodium chloride and 3 grams of urea per liter of water (Saadony et al., 2014). The fertilization process took five minutes. After that, the eggs were rinsed with water, and more fresh water was introduced. After letting the mixture sit for 5–10 minutes, the eggs were transferred to the incubation tanks. After 12 hours of incubation, a sample of eggs was collected from the middle of each group's incubator and analyzed under a microscope to find out what proportion of the eggs were fertilized. After 35 to 40 hours of incubation, the rate of hatching was calculated. The water temperature of incubation aquaria was between 27 and 30 °C, DO was 6.54 to 7.25 mg/L, and pH was 8.08 to 8.31.

2.9 Evaluation for sexual hormones in blood

Progesterone and estrogen levels were estimated from blood samples collected from female catfish, whereas testosterone levels were estimated from blood samples taken from male catfish. Without the use of an anticoagulant, blood specimens were drawn from the caudal vein at 0, 6, 12, and 24 hours after the injection, followed by being placed in Wasserman tubes. Blood specimens were collected from six female and six male subjects within each treatment group (two fish per time point). After 30 minutes of blood clotting at ambient temperature, the sample was spun at 3000 rpm for 10 minutes to extract serum. The serum was then stored at -20 °C for hormone assessment. An enzyme-immunoassay method using commercially available ELISA kits was used to assess sex steroid hormones. Using the techniques outlined by Alvarado, Serrano (Alvarado et al., 2015), progesterone was quantified. The radioimmunoassay technique was outlined by Foster and Dunn (1974) for the measurement of estrogen and testosterone.

2.10 Reproductive indices

To evaluate fish fertility (absolute fecundity, or AF, and relative fecundity, or RF), a one-gram specimen of eggs was removed from each female and preserved in 5% formalin. Egg diameter was measured on a slide using an eye-piece micrometre in the binocular microscope at a power magnification of 10 X. Then, using the procedure outlined by Budi, Hartono (Budi et al., 2020), the measurement was transformed into millimeters.

Absolute fecundity = the average quantity of eggs produced per female during each spawning event (Murua et al., 2003).

Relative fecundity = the average number of eggs produced per female body weight during each spawning event (Qadri et al., 2015).

Gonad-somatic index (GSI %) = [gonad weight/fish weight] × 100.

Latency period: The duration between administration and the initiation of ovulation, measured in hours (Zidan et al., 2020).

Ovulation rate = (number of ovulated females/numbers of injected females) × 100.

Ovulation index = (weight of the stripped egg mass)/(weight of eggs mass + remnant ovaries) × 100 (Szabó et al., 2002).

Fertilization rate (FR) = (number of fertilized eggs/total number of incubated eggs) × 100;

Hatching rate = (number of hatched eggs (larvae)/total number of fertilized eggs) × 100.

2.11 Statistical analysis

The Levene's test was used to determine the homogeneity of variances, and the Kolmogorov-Smirnov test was used to determine if the data were normal before analysis. The mean ± standard error was used to examine sex hormones, fecundity, and reproductive performance metrics (Hussein et al., 2020). A one-way analysis of

TABLE 1 Fecundity and ovarian measurements of African catfish.

Items	Source and form of the pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
Female weight, g	980.00± 20.00	1030.00± 70.0	1090± 100.0	985.00± 65.0	0.683
Ovary's weight, g	139.89± 5.37	127.90± 2.55	135.24± 2.69	133.99± 3.54	0.291
Female GSI, %	14.29± 0.84	12.49± 1.10	12.54± 1.39	13.64± 0.55	0.580
Absolute fecundity	151650± 1870[a]	136780± 330[b]	145000± 2450[a]	135075± 2025[b]	0.009
Relative fecundity	154.77± 1.25	133.44± 9.39	134.37± 14.58	137.59± 7.03	0.436
Egg diameter, mm	1.15± 0.06	1.11± 0.02	1.21± 0.03	1.18± 0.03	0.359

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

variance (ANOVA test) was used to statistically evaluate the data via the Statistical Package Program (SPSS, 2015) version 23. When the differences were substantial, the Duncan multiple range test was used to compare the means of the treatments (Duncan, 1955). Statistical significance was established at P≤ 0.05 for all analyses.

3 Results

3.1 Fecundity and ovarian measurements

The study assessed the impact of pituitary gland source (catfish vs. carp) and preparation type (dried vs. fresh) on the reproductive performance of African catfish. The reproductive parameters measured included female body weight, ovary weight, gonadosomatic index (GSI), absolute and relative fecundity, and egg diameter, as shown in Table 1. There were no substantial variations in either female body or ovary weights amongst study groups (P > 0.05). Similarly, the GSI exhibited no substantial variation amongst study groups (P > 0.05). Absolute fecundity exhibited substantial changes amongst study groups (P ≤ 0.05). The peak fecundity was noted in the group treated with $DPG_{catfish}$ extract, followed by DPG_{carp}. Fresh preparations resulted in lower fecundity. Relative fecundity did not substantially change between trial groups (P > 0.05). Likewise, Egg diameter measurements showed no substantial change between study groups (P > 0.05). The results of GSI in males (Figure 1) showed that the highest value was detected in the group treated with $DPG_{catfish}$ extract, followed by DPG_{carp}, while fresh preparations resulted in lower values.

3.2 Estrogen hormone

Estrogen levels in female blood are presented in Table 2. At the time of injection, estrogen levels in females were similar across all treatment groups. However, after 6 hours, a slight rise in estrogen was detected in all treatments, though the differences remained insignificant (P > 0.05). The maximum concentration at this time was found in the $DPG_{catfish}$ group, while the minimum was in the FPG_{carp} group. At 12 hours post-injection, significant differences appeared among treatments (P ≤ 0.05). Fish induced with $DPG_{catfish}$ extract exhibited the highest estrogen concentration, followed by those injected with DPG_{carp}. In contrast, both fresh pituitary treatments (catfish and carp) resulted in noticeably lower estrogen levels. By 24 hours, estrogen concentrations had decreased across all trial groups, and the differences were no longer statistically substantial (P > 0.05). Nevertheless, fish treated with $DPG_{catfish}$ maintained the highest mean level.

The comparison of estrogen levels in female blood over time after injection (Figure 2) shows a significant peak (P ≤ 0.05) at 12

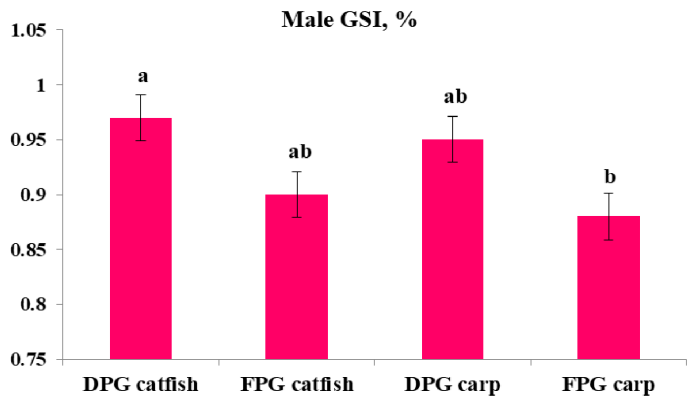

FIGURE 1
Gonadosomatic index (GSI) in males. Dissimilar superscript letters in columns are substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

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TABLE 2 Blood estrogen hormone (pg/ml) of African catfish females.

Items	Source and form of pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
At injection	2915± 45	2915± 45	2915± 45	2915± 45	1.000
6 hours	3210± 60	3180± 30	3105± 55	3090± 10	0.293
12 hours	4215± 65^{a}	3500± 10^{c}	3819± 39^{b}	3452± 67^{c}	0.001
24 hours	3755± 65	3590± 30	3642± 62	3582± 27	0.190

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

hours, followed by a slight decline in the $DPG_{catfish}$ and DPG_{carp}, while the $FPG_{catfish}$ and FPG_{carp} exhibited only a slight increase over time.

3.3 Progesterone hormone

Progesterone concentrations in *C. gariepinus* females following administration of pituitary extracts from catfish and carp in either dried or fresh form are displayed in Table 3. The progesterone concentrations followed the same dynamic changes as the estrogen level in female blood. At the point of injection (0 hours), progesterone levels were similar across all treatment groups. Females treated with $DPG_{catfish}$ (6 hours post-injection) exhibited a significantly higher progesterone concentration in comparison with other trial groups (P ≤ 0.05). This trend intensified at 12 hours post-injection, where $DPG_{catfish}$ again yielded the highest progesterone level (P ≤ 0.05). At 24 hours, a gradual decline in progesterone concentrations was observed across all treatments. Yet, $DPG_{catfish}$ still maintained the highest hormone levels, followed by DPG_{carp}, whereas both fresh extracts resulted in significantly lower values (P ≤ 0.05).

Progesterone levels increased over time following injection, peaking significantly (P ≤ 0.05) at 12 hours and declining 24 hours in the dry pituitary preparations group, whereas the fresh pituitary extract group showed a more gradual increase (Figure 3).

3.4 Testosterone hormone

The impact of pituitary gland source and preparation type on blood testosterone concentrations in male *C. gariepinus* is presented in Table 4. At the time of hormone administration (0 hours), testosterone levels were identical across all treatment groups. At 6 hours post-injection, males treated with both dried catfish and carp pituitary extract exhibited a substantially higher testosterone level (P ≤ 0.05). At 12 hours post-treatment, males receiving dried catfish pituitary exhibited the highest testosterone concentration, followed by dried carp, while both fresh pituitary preparations yielded significantly lower testosterone levels (P ≤ 0.05). By 24 hours, the testosterone levels remained higher in the dried catfish group, followed by the dried carp. The fresh catfish and carp treatments resulted in considerably lower hormone levels with substantial alterations between treatments (P ≤ 0.05). Analysis of hormone levels in male blood over time (Figure 4) revealed a statistically significant increase following injection across all treatments.

3.5 Stripping response

The study evaluated the influence of pituitary gland source (catfish vs. carp) and type (dried vs. fresh) on the stripping response parameters of *C. gariepinus* (Table 5). Our results showed that the latency duration was 12 and 17 h for dried and fresh catfish

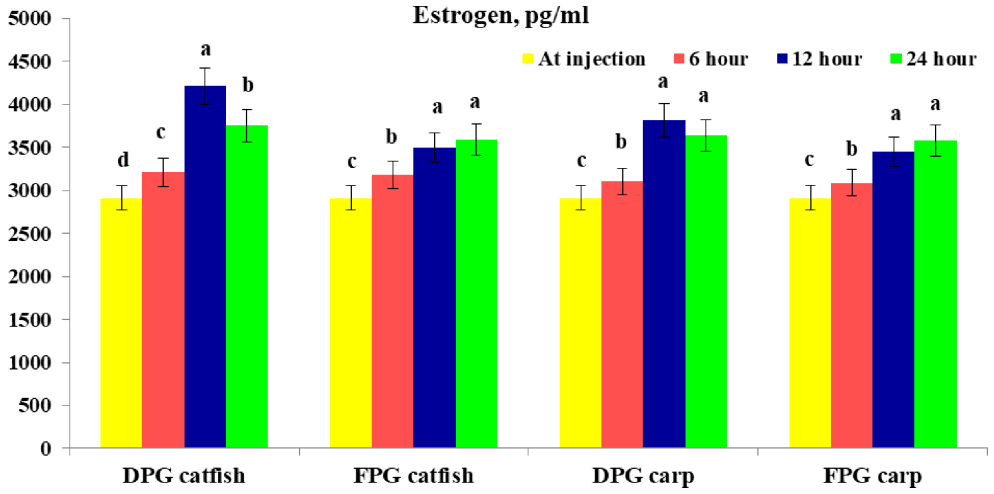

FIGURE 2
Changes in female estrogen concentrations over time after injection. Dissimilar superscript letters in columns are substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

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TABLE 3 Blood progesterone hormone (ng/ml) of African catfish females.

Items	Source and form of the pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
At injection	2.57± 0.06	2.57± 0.06	2.57± 0.06	2.57± 0.06	1.000
6 hours	4.74± 0.09^{a}	2.89± 0.06^{c}	3.91± 0.06^{b}	2.90± 0.07^{c}	0.000
12 hours	8.18± 0.36^{a}	3.18± 0.03^{b}	7.01± 0.52^{a}	3.23± 0.05^{b}	0.001
24 hours	5.57± 0.06^{a}	3.41± 0.16^{c}	4.55± 0.09^{b}	3.53± 0.05^{c}	0.000

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

pituitary glands, while it was 12.5 and 21 h for dried and fresh carp pituitary gland, respectively. The ovulation index and the weight of ovulated eggs per female were significantly superior in the $DPG_{catfish}$ group, followed by DPG_{carp}, while lower values were recorded in the fresh catfish and carp pituitary groups (P ≤ 0.05). Additionally, the percentage of ovulated females showed a similar trend, approaching statistical significance throughout treatments (P = 0.054).

3.6 Spawning performance

Females injected with $DPG_{catfish}$ produced the significantly highest number of ovulated and fertilized eggs as well as larvae per female followed by those treated with DPG_{carp}, whereas lower values were recorded with the fresh pituitary gland preparations (P ≤ 0.05) (Table 6). Moreover, fertilization rate was also highest in the $DPG_{catfish}$ group, significantly exceeding (P ≤ 0.05) that of the fresh carp group (Table 6). Additionally, hatching rate showed a similar trend, approaching statistical significance among treatments (P = 0.054). Furthermore, larval survival after 4 days was significantly higher in both dried catfish and carp groups than in both fresh pituitary treatments (P ≤ 0.05) (Table 6).

4 Discussion

Reproductive parameters measured included female body weight, ovary weight, gonadosomatic index (GSI), absolute and relative fecundity, as well as egg diameter. The results indicated that neither the source nor the preparation procedure of the pituitary extract influenced the female body weight, ovary weight, and GSI (P > 0.05), proposing constant ovarian development regardless of the hormonal treatment utilized.

Our findings are consistent with Mehrim, Abdelhamid (Mehrim et al., 2014), who concluded that the non-significant differences when using reproductive stimulating materials could be due to the relatively small proportion of ovarian weight in relation to the total body mass. De Graaf, Galemoni (De Graaf et al., 1995) reported comparable results in their study on *C. gariepinus*, applying artificial propagation methods. Additionally, Sharaf (2005) observed non-significant variations in the GSI among *C. gariepinus* females subjected to a single application of synthetic GnRHs or HCG. However, a previously recognized linear correlation was identified by Eyo and Mgbenka (1992) amongst fecundity, weight, and length of ovaries, GSI, and the body weight of *C. gariepinus*. In this context, Nguenga, Teugels (Nguenga et al.,

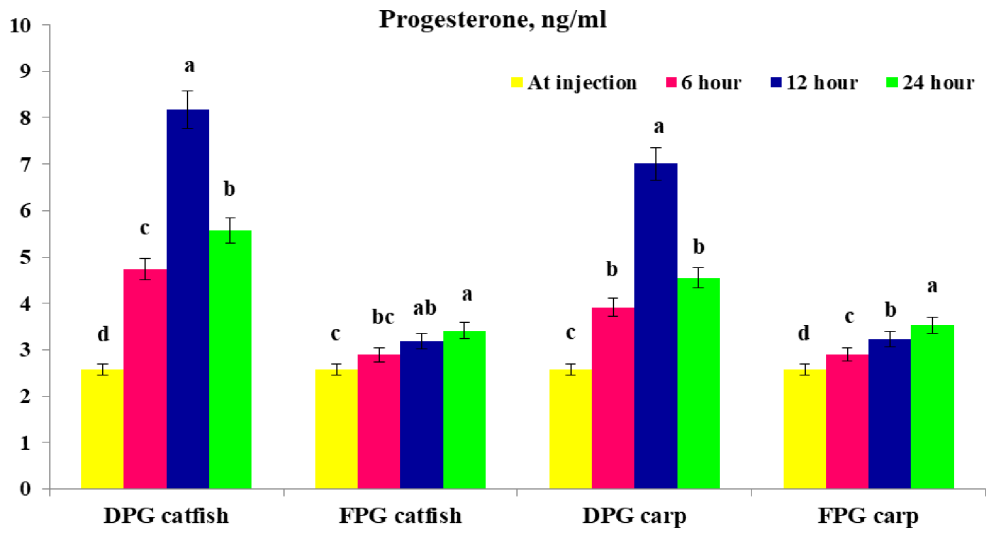

FIGURE 3
Changes in female progesterone concentrations over time after injection. Dissimilar superscript letters in columns are substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

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TABLE 4 Blood testosterone hormone (ng/ml) of African catfish male.

Items	Source and form of pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
At injection	4.39± 0.14	4.39± 0.14	4.39± 0.14	4.39± 0.14	1.000
6 hours	11.38± 0.88^{a}	7.09± 0.54^{b}	9.87± 0.64^{ab}	6.55± 1.28^{b}	0.048
12 hours	18.80± 0.46^{a}	8.74± 0.60^{c}	14.33± 0.88^{b}	10.07± 0.19^{c}	0.001
24 hours	18.09± 0.46^{a}	9.68± 0.47^{c}	15.38± 1.12^{b}	9.84± 0.32^{c}	0.002

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

2004) observed that both oocyte diameter and absolute fecundity elevated as the body weight of *Heterobranchus longifilis* increased.

Our results revealed no significant variation among treatments in relative fecundity, which align with Natea, Wakjira (Natea et al., 2017), who reported that treatment with African catfish pituitary extract in *C. gariepinus* resulted in a non-significant higher relative fecundity value, followed by treatment with pituitary extract from *C. carpio*. However, our results showed higher absolute fecundity valuesfor the dried catfish pituitary group followed by DPG_{carp}, while fresh preparations resulted in significantly lower fecundity. According to Haffray, Enright (Haffray et al., 2005), the use of suitable hormonal treatments are generally not associated with reduced egg quality; however, as noted by Mikolajczyk, Chyb (Mikolajczyk et al., 2004), itmay also enhance fish fecundity when compared to naturally maturing broodstock.

Egg diameter measurements showed no substantial changes between trial groups. Similarly, no substantial alterations (P > 0.05) were detected by Mehrim, Abdelhamid (Mehrim et al., 2014) in either the diameter of individual eggs or the egg weight (mg per egg) across all treatments involving various reproductive stimulants. Also, in *Heterobranchus bidorsalis*, egg diameter did not differ significantly among treatments (Nwokoye et al., 2007). Moreover, Carrillo, Zanuy (Carrillo et al., 1995) argued that egg diameter is an unreliable measure of egg or larval excellence.

Regarding the sex steroid hormone levels in blood, estrogen and progesterone concentrations in females increased with time. Fish treated with $DPG_{catfish}$ maintained the highest levels of estrogen and progesterone. As observed by Zaki and Abd El-Ghaffar (2020), the stimulatory hormones in their study (Ovaprim, pituitary gland extract, and human chorionic gonadotropin) led to elevated concentrations of sex steroid hormones in both female and male fish, including plasma 17β-estradiol, testosterone, and 11-ketotestosterone. They also indicated that sex steroid hormones in females perform a crucial function in regulating ovulation, oocyte maturation, and spawning processes specifically, 17β-estradiol is involved in regulating the synthesis of vitellogenin and promoting ovarian growth through the late stages of oocyte development. A similar pattern of rising 17β-estradiol levels over time, peaking at 12 hours, was reported by AdeBIyI, SIRaj (Adebiyi et al., 2013) following the application of gonadotropin-releasing hormone analogue (GnRHa) in River catfish *Hemibagrus nemurus*. They attributed this increase to the influence of GnRH on the endocrine system. Administering GnRHa with or without dopamine antagonists appeared to enhance sex steroid levels and promote ovarian development in African catfish (Shourbela et al., 2020) as well as in European seabass (Prat et al., 2001). The superior performance of $DPG_{catfish}$ extracts suggests a stronger endocrine response to dried pituitary preparations, particularly those of homologous origin. It could be attributed to increased stimulation of gonadal steroid levels, which are strategic hormones involved in the reproductive performance of African catfish. According to Mehrim, Abdelhamid (Mehrim et al., 2014), the observed increase

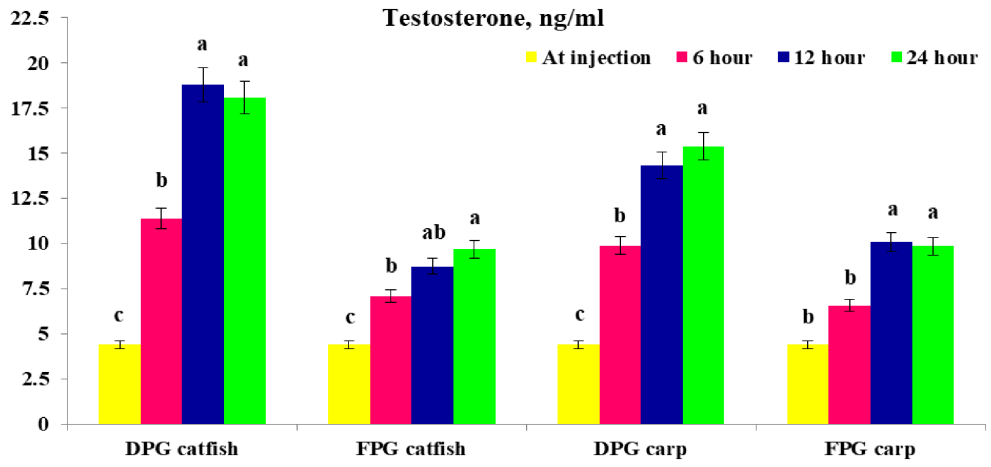

FIGURE 4
Changes in male testosterone concentrations over time after injection. Dissimilar superscript letters in columns are substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

TABLE 5 Stripping response of African catfish.

Items	Source and form of pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
Latency period, hrs	12.00± 0.00^{c}	17.00± 1.00^{b}	12.50± 0.50^{c}	21.00± 1.00^{a}	0.003
Ovulated females, %	100.00± 0.00^{a}	80.00± 0.00^{ab}	90.00± 10.00^{ab}	70.00± 10.00^{b}	0.054
Ovulation index, %	67.60± 1.75^{a}	55.34± 0.17^{bc}	58.49± 1.28^{b}	49.83± 2.31^{c}	0.006
Weight of ovulated eggs, g/ female	94.66± 6.08^{a}	70.78± 1.63^{b}	79.13± 3.29^{b}	66.68± 1.33^{b}	0.019

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

in fecundity of African catfish in their study may be attributed to the administration of native or commercial hormonal agents, which likely motivate the production of key reproductive hormones (FSH, LH, E2, and P4), responsible for egg development and maturation within the ovary. Furthermore, Wu, Zhang (Wu et al., 2009) noted that the secretion of gonadotropins from both the pituitary and gonads may perform a crucial function in the synthesis of sex steroids, the advancement of undifferentiated gonads, and ultimately in sexual differentiation via the brain pituitary gonad axis. Furthermore, Huggard-Nelson, Nathwani (Huggard-Nelson et al., 2002) demonstrated that sexual maturation and gonadal development in fish are linked to elevated levels of circulating gonadotropins and gonadal steroids. Additionally, Sabet, Mohammad (Sabet et al., 2009) and Coccia, Lisa (Coccia et al., 2010) reported that the concentration of 17β-estradiol is directly associated with the gonadosomatic index.

At the time of injection, testosterone levels were similar across all treatment groups. However, after 6 hours, a slight increase was observed in all groups. At 12 h post-injection, substantial alterations were observed between trial groups (P≤ 0.05). Maximum testosterone concentrations were detected at 24 h in the trial groups. Our results align with AdeBIyI, SIRaj (Adebiyi et al., 2013), who confirmed the same testosterone level sequence after 0, 6, 12, and 24 h, respectively. Semenkova, Barannikova (Semenkova et al., 2002) similarly observed a rise in testosterone levels a few hours after administering GnRHa in male stellate sturgeon (*Acipenser stellatus*). In addition, Harmin and Crim (1993) reported that testosterone reached its peak at 24 hours in winter flounder (*Pseudopleuronectes americanus*). The highest testosterone level was confirmed in the dried catfish group, followed by dried carp. The fresh catfish and carp treatments resulted in considerably lower hormone levels with substantial alterations between trial groups (P≤ 0.05). This might be owing to the dried catfish pituitary extract's ability to stimulate plasma sex steroid hormone levels in males, which could boost spermatogenesis and optimize reproductive traits in African catfish.

The results revealed significant differences among treatments in terms of latency period, ovulation index and weight of ovulated eggs, while ovulated female percentage approached significance. The latency period was shortest (12 h) in females induced with dried catfish pituitary glands followed by females induced with dried carp pituitary glands (12.50 h), and both were significantly lower than in those treated with fresh catfish pituitary glands (17 h) or fresh carp pituitary glands (21 h) (P ≤ 0.05). This can be due to the fact that homologous hormones, particularly in their dried form, may contain higher gonadotropin levels and therefore tend to induce more synchronized and faster ovulation responses in *Clarias gariepinus*, as reflected by the 12-hour peak in estrogen and progesterone levels. In contrast, the longest latency (21 hours) was observed in the fresh carp pituitary group, possibly due to lower gonadotropin content or slower hormonal activation. Natea, Wakjira (Natea et al., 2017) indicated that prolonged latency time has indirect effects on both the quantity and quality of eggs yielded, as well as on the quality of the larvae. This suggests that a shorter latency period is associated with enhanced reproductive performance.

The enhanced stripping performance of dried pituitary glands (PG) compared to fresh PG in our study may be due to the dehydration of the gland, potentially leading to an elevated concentration of the injected pituitary extract. This is supported by

TABLE 6 Spawning performance of African catfish.

Items	Source and form of pituitary gland				*P*-value
	$DPG_{catfish}$	$FPG_{catfish}$	DPG_{carp}	FPG_{carp}	
No. of ovulated eggs / female	108373± 7282^{a}	75842± 569^{bc}	84827± 3287^{b}	67252± 2102^{c}	0.008
No. of fertilized eggs / female	100924± 6404^{a}	67696± 1456^{bc}	76597± 4027^{b}	58704± 2675^{c}	0.007
No. of larvae / female	78644± 7754^{a}	47589± 2546^{b}	56561± 4311^{b}	40252± 2713^{b}	0.018
Fertilization rate, %	93.15± 0.35^{a}	89.25± 1.25^{ab}	90.25± 1.25^{ab}	87.25± 1.25^{b}	0.045
Hatching rate, %	77.75± 2.75^{a}	70.25± 2.25^{ab}	73.75± 1.75^{ab}	68.50± 1.50^{b}	0.054
Larvae survival after 4 d /%	92.88± 0.38^{a}	89.86± 0.60^{b}	92.26± 0.59^{a}	89.82± 0.41^{b}	0.024

Means subsequently with dissimilar letters ± SE in the identical row is substantially changed (P ≤ 0.05), n=2 (2 groups/treatment). DPG, dried pituitary gland extract; FPG, fresh pituitary gland extract.

Okomoda, Tiamiyu (Okomoda et al., 2017), who noted a substantial ($P< 0.05$) weight loss of 8.10% in the preserved pituitary glands compared to only 0.51% in the fresh samples. Fagbenro, Salami (Fagbenro et al., 1993) reported that PG preserved in ethanol is occasionally mentioned as acetone-dried pituitary extracts. However, these results should be interpreted with caution due to limitations, including the short-term nature of the study, lack of dose-response evaluation, use of a single gland source and the absence of hormonal dosage standardization based on dry mass, protein content, or gonadotropin activity. Therefore, the superior performance of dried pituitary extracts may partly reflect differences in effective dosage due to dehydration rather than preservation effects alone.

The spawning performance of *Clarias gariepinus* was influenced by both the source and form (dried or fresh) of the pituitary gland used for hormonal induction with some significant variation among treatments ($P \leq 0.05$). Females injected with $DPG_{catfish}$ exhibited the highest values for ovulated eggs per female, fertilization rate, hatching rate, and larval survival after four days, followed by those injected with DPG_{carp}. In contrast, decreased levels were seen in those administered fresh pituitary preparations. Gadissa and Devi (2013) observed that *Clarias gariepinus* spawners (1-5 kg) administered with catfish pituitary homogenate produced a greater number of spawned eggs (69,939) than those provided with carp pituitary extract (54,633); the difference was not statistically significant ($P > 0.05$).

In harmony with our results, Ruaza Jr (Ruaza, 2018) reported that the administration with *C. gariepinus* pituitary extract yielded a higher fertilization rate (88.4%) than *C. carpio* one (76.6%). Findings from other studies indicated that induced spawning using catfish pituitary extracts resulted in a fertility rate of 80% for *C. gariepinus* (Hecht et al., 1982; Britz and Hecht, 1988). Furthermore, Okomoda, Tiamiyu (Okomoda et al., 2017) recorded 97.5% and 95.5% fertilization rate values for preserved and fresh *C. gariepinus* pituitary trial groups, respectively, with substantial alterations among both trial groups ($P< 0.05$). The peak fertilization and hatching rates in ♂ *Heterobranchus bidorsalis* x ♀ *Clarias gariepinus* were recorded in T1 (dried) as compared to T2 (fresh) *Clarias gariepinus* pituitary extracts (Ibrahim et al., 2019). Moreover, our values regarding the fertilization rate contrasted with the study of Gadissa and Devi (2013), who reported 76.93% and 80.53% for catfish and carp hormones-induced fish, respectively.

In line with the current investigation, Okomoda, Tiamiyu (Okomoda et al., 2017) indicated that the use of preserved pituitary gland (PG) extract showed significantly higher hatchability (59.74%) compared to fresh PG extract (51.39%), which may be attributed to its ability to induce the ovulation of larger eggs. Literature has shown that egg size is significantly and positively linked to several reproductive traits, including hatchability (Arome Ataguba et al., 2013), larval length, and survival rates (Buckley et al., 1991; Rideout et al., 2005). Hempel (1979) also reported that larger eggs offer greater energy reserves for larval development, which indicated by the presence of a larger yolk sac. Moreover, our values exceeded those reported by Adebayo and Popoola (2008), who observed hatching rates ranging from 51.1% to 73% across various hormone treatments. Our findings contrasted with the study of Ruaza Jr (Ruaza, 2018) who stated that the extract derived from the pituitary gland of *C. carpio* revealed superior hatchability rate, followed by that of *C. gariepinus*. Moreover, Okomoda, Tiamiyu (Okomoda et al., 2017) indicated that the growth of fry after 15 days since first feeding, which was obtained from preserved pituitary gland extract, was comparable to the control group (Ovaprim synthetic hormone), while the lowest growth performance was detected in fry spawned with fresh pituitary gland extract. Future studies should also assess egg and larval quality parameters, such as deformities, and survival to better evaluate reproductive outcomes.

The enhanced performance of dried pituitary glands (DPG) compared to fresh (FPG) in the present work may be attributed to the elimination of moisture content during the drying process, which could result in a higher hormone concentration and, consequently, greater hormonal effectiveness. Okere, Erondu (Okere et al., 2015) previously suggested that hatching success depends on the hormonal dosage administered. According to Mosha and Mlingi (2018), increasing the dosage of pituitary gland extract led to a corresponding rise in the number of eggs and hatchlings, suggesting the high effectiveness of this ovulation-inducing agent. This enhanced response is likely because of the efficient activation of gonadotropin hormones, which attach to specific receptors in the ovarian granulosa cells, stimulating the synthesis of steroid hormones that facilitate ovulation (Mosha et al., 2016). However, as hormone concentrations were not directly quantified, this explanation should be considered a hypothesis rather than a confirmed mechanism. Another possible reason for the improved results at higher pituitary extract doses is the stronger stimulation effect, which may induce contractions in the smooth muscles of the female gonoducts prior to ovulation, thereby enhancing the spawning process (Yeung et al., 2006).

Hormonal analyses revealed that $DPG_{catfish}$ produced the highest elevations in estrogen, progesterone, and testosterone levels, suggesting stronger endocrine stimulationin response to the dried homologous pituitary gland. This effect was consistently reflected across all measured reproductive parameters, with some significant variation among treatments. The $DPG_{catfish}$ treatment achieved superior fecundity, ovulation rate, fertilization rate, and hatching percentages compared with other treatments. These hormonal elevations, likely reflecting more effective endocrine regulation, may have promoted more efficient reproductive processes, thereby explaining the improved spawning outcomes. Accordingly, preserving natural hormonal sources may represent a superior strategy for fish producers (Okomoda et al., 2017).

5 Conclusion

The study demonstrated that both the source and preparation method of pituitary extracts significantly influence the reproductive performance of *Clarias gariepinus*. The data indicated that dried catfish pituitary extract consistently showed superior results compared to other treatments across all evaluated spawning parameters. Dried catfish pituitary extract appeared as the most effective

treatment, suggesting its suitability for enhancing seed production in catfish aquaculture. We recommend incorporating histological and gene expression analyses that would significantly strengthen the validation and provide deeper mechanistic insights into the observed outcomes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by This study and the experimental protocol were performed in accordance with the stringent guidelines, authorization and approved by the National Institute of Oceanography and Fisheries (NIOF-IACUC, Egypt) committee for the ethical treatment of animals/aquatic animals. All methods were performed in accordance with the relevant guidelines and regulations (Approval no.: NIOF-AQ5-F-25-R-018). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

HK: Project administration, Data curation, Writing – review & editing, Visualization, Writing – original draft, Formal analysis, Validation, Conceptualization, Software, Resources. HS: Formal analysis, Writing – review & editing, Methodology, Writing – original draft, Conceptualization. EA: Methodology, Conceptualization, Investigation, Writing – review & editing, Formal analysis. AE: Investigation, Data curation, Writing – review & editing, Conceptualization, Writing – original draft. FA: Resources, Writing – review & editing, Investigation. GH: Conceptualization, Investigation, Data curation, Visualization, Writing – review & editing. RA: Investigation, Conceptualization, Writing – review & editing, Resources, Formal analysis.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abdel-Hady, M. M., Zaki, M. A. A., Barrania, A. A., Abdel-Khalek, Z. M., and Haggag, S. M. (2025). Sustainable development of aquaculture in Egypt: a review of key challenges and solutions. *Rev. Fish. Sci. Aquac.* 34 (2), 146–174. doi: 10.1080/23308249.2025.2531206

Adebayo, O., and Fagbenro, O. (2004). Induced ovulation and spawning of pond raised African giant catfish, Heterobranchus bidorsalis by exogenous hormones. *Aquaculture* 242, 229–236. doi: 10.1016/j.aquaculture.2004.07.019

Adebayo, O., and Popoola, O. (2008). *Comparative Evaluation of Efficacy and Cost of Synthetic and Non-Synthetic Hormones for Artificial Breeding of African Catfish (Clarias Gariepinus Burchell, 1822).*

Adebiyi, F. A., Siraj, S. S., Harmin, S. A., and Christianus, A. (2013). Effects of GnRHa on plasma sex steroid hormones of river catfish Hemibagrus nemurus (Valenciennes 1840). *Sains Malaysiana* 42 (5), 635–642.

Alvarado, M., Serrano, E., Carlos Sánchez, J., and Valladares, L. (2015). Changes in plasma steroid hormones and gonadal histology associated with sexual maturation in wild southern hake (Merluccius australis). *Lat. Am. J. Aquat. Res.* 43 (4), 632–640. doi: 10.3856/vol43-issue4-fulltext-2

Arome Ataguba, G., Tosin Okomoda, V., and Chukwuemeka Onwuka, M. (2013). Relationship between broodstock weight combination and spawning success in African catfish (Clarias gariepinus). *Croat. J. Fish.* 71 (4), 176–181. doi: 10.14798/71.4.694

Attia, E.-S. I., Abdel-Aziz, M. F. A., Hassan, H. U., Ahmed, M. Z., Arai, T., and Saleh, H. H. E. (2025). A comparative study to estimate three commercial products of human chorionic gonadotropin (hCG) on blood biochemistry, sexual hormones concentrations, reproductive indices, and larvae production of African catfish Clarias gariepinus brood-stock. *Ital. J. Anim. Sci.* 24 (1), 222–232. doi: 10.2139/ssrn.4833138

Bosworth, B. (2005). Comparison of carp pituitary extract, catfish pituitary extract, or LHRHa for induced spawning of channel catfish females to produce channel catfish × blue catfish hybrid fry. In: *Aquaculture America Conference*, 17–20.

Britz, P. (1991). The utility of homoplastic pituitary glands for spawning induction of the African catfish (Clarias gariepinus) in commercial aquaculture in Africa. *Water SA.* 17, 237–242.

Britz, P., and Hecht, T. (1988). "Artificial propagation and fry production," in *The Culture of Sharptooth Catfish, Clarias gariepinus in Southern Africa. South African National Scientific Programmes, Report*, vol. 153. .

Brzuska, E. (2004). Artificial spawning of carp (Cyprinus carpio L.); differences between the effects of reproduction in females of Hungarian, Polish and French origin treated with carp pituitary homogenate or [D-Tle6, ProNHEt9] GnRH (Lecirelin). *Aquacult. Res.* 35, 1318–1327. doi: 10.1111/j.1365-2109.2004.01153.x

Brzuska, E., Raczkevi, R., Judit, Adamek, J., and Radics, F. (1999). Preliminary investigation on the influence of different hormones treatments on the ovulation, embryonic survival and larval morphology in African catfish (Clarias gariepinus Burchell). *Halaszat Hungary* 922, 88–92.

Buckley, L., Smigielski, A. S., Halavik, T. A., Caldarone, E. M., Burns, B. R., and Laurence, G. C. (1991). Winter flounder Pseudopleuronectes americanus reproductive success. II. Effects of spawning time and female size on size, composition and viability of eggs and larvae. *Mar. Ecol. Prog. Ser.* 74, 125–135. doi: 10.3354/meps074125

Budi, D. S., Hartono, D., Maulana, F., Bodur, T., and Lutfiyah, L. (2020). Some fecundity parameters and ovarian maturity criteria of ornamental red cherry shrimp (Neocaridina davidi). *Turk. J. Vet. Anim. Sci.* 44 (2), 456–462. doi: 10.3906/vet-1910-13

Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., and Mañanos, E. (1995). *Sea Bass (Dicentrarchus Labrax L.). In-Broodstock Management and Egg and Larval Quality*. Eds. N. R. Bromage and R. J. Roberts (Oxford: Blackwell Scientific).

Coccia, E., Lisa, E. D., Cristo, C. D., Cosmo, A. D., and Paolucci, M. (2010). Effects of estradiol and progesterone on the reproduction of the freshwater crayfish Cherax albidus. *Biol. Bull.* 218 (1), 36–47. doi: 10.1086/bblv218n1p36

De Graaf, G., Galemoni, F., and Banzoussi, B. (1995). Artificial reproduction and fingerling production of the African catfish, Clarias gariepinus (Burchell 1822), in protected and unprotected ponds. *Aquac. Res.* 26 (4), 233–242. doi: 10.1111/j.1365-2109.1995.tb00908.x

Dhara, K., and Saha, N. C. (2013). Controlled breeding of Asian catfish Clarias batrachus using pituitary gland extracts and ovaprim at different temperatures, latency periods and their early development. *J. Aquacult. Res. Dev.* 4, 186. doi: 10.4172/2155-9546.1000186

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics* 11, 1–42. doi: 10.2307/3001478

El-Hawarry, W. N., Abd El-Rahman, S. H., and Shourbela, R. M. (2016). Breeding response and larval quality of African catfish (Clarias gariepinus, Burchell 1822) using different hormones/hormonal analogues with dopamine antagonist. *Egypt J. Aquat. Res.* 42, 231–239. doi: 10.1016/j.ejar.2016.06.003

Eyo, J., and Mgbenka, B. (1992). Aspects of the biology of Clarias gariepinus in Anambra River Basin. 1. Oocyte diameter, fecundity and sex ratio. *J. Agric. Sci. Technol.* 2 (1), 47–51.

Fagbenro, O., Salami, A., and Sydenham, D. (1993). Induced ovulation and spawning in the catfish, Clarias isheriensis, using pituitary extracts from non-piscine sources. *J. Appl. Aquac.* 1 (4), 15–20. doi: 10.1300/j028v01n04_02

Foster, L. B., and Dunn, R. T. (1974). Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clin. Chem.* 20, 365–368. doi: 10.1093/clinchem/20.3.365

Gadissa, S., and Devi, L. P. (2013). Evaluation of spawning induction of African catfish (Clarias gariepinus) by heteroplastic hypophysation. *Int. J. Fish. Aquat. Stud.* 1, 22–25.

Haffray, P., Enright, W. J., Driancourt, M. A., Mikolajczyk, T., Rault, P., and Breton, B. (2005). Optimization of breeding of salmonids: Gonazon™, the first officially approved inducer of ovulation in the EU. *Cybium* 36 (1), 52–56. Available online at: https://hal.inrae.fr/hal-02675922.

Harmin, S., and Crim, L. (1993). Influence of gonadotropic hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder, Pseudopleuronectes americanus (Walbaum). *Fish. Physiol. Biochem.* 10, 399–407. doi: 10.1007/bf00004506

Hasimuna, O. J., Mphande, J., Maulu, S., Chibesa, M., Siankwilimba, E., Mumbula, I., et al. (2025). The status, challenges, and potential opportunities of fish seed production in Zambia. *Aquac. Int.* 33 (6), 399. doi: 10.1007/s10499-025-02073-x

Hecht, T. (1985). Recent developments in aquaculture in South Africa: the sharptooth catfish, Clarias gariepinus. *Aquac. South Afr.* 47 (2-3), 173–183. doi: 10.1016/0044-8486(85)90063-8

Hecht, T., Saayman, J. E., and Polling, L. (1982). Further observations on the induced spawning of the sharptooth catfish, Clarias gariepinus (Clariidae: Pisces). *Water SA* 8 (2), 101–107.

Hempel, G. (1979). *Early Life History of Marine Fish: the Egg Stage*.

Hossain, M. B., Rahman, M. M., Sarwer, M. G., Ali, M. Y., Ahamed, F., Rahman, S., et al. (2012). Comparative study of carp pituitary gland (PG) extract and synthetic hormone ovaprim used in the induced breeding of stinging catfish, Heteropneustes fossilis (Siluriformes: Heteropneustidae). *Our Nature* 10 (1), 89–95. doi: 10.3126/on.v10i1.7755

Houssay, B. (1931). Action sexuelle de l'hypophyse sur les poissons et les reptiles. *CR. Seances. Soc Biol. Ses. Fil.* 106, 377–378.

Huggard-Nelson, D. L., Nathwani, P. S., Kermouni, A., and Habibi, H. R. (2002). Molecular characterization of LH-β and FSH-β subunits and their regulation by estrogen in the goldfish pituitary. *Mol. Cell. Endocrinol.* 188 (1-2), 171–193. doi: 10.1016/S0303-7207(01)00716-X

Hussein, G. H. G., Chen, M., Qi, P. P., Cui, Q. K., Yu, Y., Hu, W. H., et al (2020). Aquaculture industry development, annual price analysis and out-of-season spawning in largemouth bass Micropterus salmoides. *Aquaculture* 519, 734901. doi: 10.1016/j.aquaculture.2019.734901

Ibrahim, J., Ovie, S. O., Maradun, H. F., Asuwaju, F. P., Mohammed, Y. S., Sahabi, A. M., et al. (2019). Breeding response of Clarias gariepinus induced with pituitary gland and synthetic hormone (Ovulin) and the effect on growth performance of its hybrid in New Bussa, Nigeria. *Asian J. Res. Anim. Vet. Sci.* 4 (3), 1–7. doi: 10.9734/ajravs/2019/v2i461

Khalil, H. S., Momoh, T., Al-Kenawy, D., Yossa, R., Badreldin, A. M., Roem, A., et al. (2021). Nitrogen retention, nutrient digestibility and growth efficiency of Nile tilapia (Oreochromis niloticus) fed dietary lysine and reared in fertilized ponds. *Aquac. Nutr.* 27 (6), 2320–2332. doi: 10.1111/anu.13365

Khalil, H. S., and Nasr-Allah, A. (2025). Comparative study on the effect of raceway and in-pond raceway systems on different Nile tilapia, Oreochromis niloticus strains fed diets replacing soybean meal by poultry byproduct meal on: Water quality, growth performance and production efficiency. *Aquacult. Int.* 33, 258. doi: 10.1007/s10499-025-01920-1

Kucharczyk, D., Nowosad, J., Wyszomirska, E., Cejko, B. I., Arciuch-Rutkowska, M., Juchno, D., et al. (2020). Comparison of artificial spawning effectiveness of hCG, CPH and GnRHa in combination with dopamine inhibitors in a wild strain of ide Leuciscus idus (L.) in hatchery conditions. *Anim. Reprod. Sci.* 221, 106543. doi: 10.1016/j.anireprosci.2020.106543

Kucska, B., Quyen, N. N., Szabo, T., Gebremichaela, A., Alebachewb, G. W., Bogo, B., et al. (2022). The effects of different hormone administration methods on propagation successes in African catfish (Clarias gariepinus). *Aquac. Rep.* 26, 101311. doi: 10.1016/j.aqrep.2022.101311

Lambert, D. M., Argue, B. J., Liu, Z., and Dunham, R. A. (1999). Effects of seasonal variations, thyroid and steroid hormones, and carp pituitary extract on the artificial production of channel catfish Ictalurus punctatus × blue catfish I. furcatus hybrids. *J. World Aquac. Soc.* 30 (1), 80–89. doi: 10.1111/j.1749-7345.1999.tb00320.x

Liu, Z., Li, P., Argue, B. J., and Dunham, R. A. (1997). Gonadotropin a-subunit glycoprotein from channel catfish (Ictalurus punctatus) and its expression during hormone-induced ovulation. *Mol. Mar. Biol. Biotech.* 6, 217–227.

Marimuthu, K., and Haniffa, M. (2010). Induced spawning of native threatened spotted snakehead fish Channa punctatus with ovaprim. *Asian Fish. Sci.* 23, 60–70. doi: 10.17485/ijst/2011/v4is.143

Marimuthu, K. H., M.A.,, and Aminur, R. (2009). Induced breeding of the Indian catfish Mystus montanus using HCG and ovaprim. *Acta. Ichthyol. Piscatoria.* 39, 1–5.

Mehrim, A. I., Abdelhamid, A. M., Radwan, I. A., and Abdelhamid, A. F. (2014). Comparative study for different sources of reproductive stimulating materials and their effects on the reproductive performance of African catfish Clarias gariepinus (Burchell, 1822). *Asian J. Anim. Vet. Adv.* 9 (7), 414–427. doi: 10.3923/ajava.2014.414.427

Mikolajczyk, T., Chyb, J., Szczerbik, P., Sokolowska-Mikolajczyk, M., Epler, P., Enright, W. J., et al. (2004). Evaluation of the potency of azagly-nafarelin (GnRH analogue), administered in combination with different formulations of pimozide, on LH secretion, ovulation and egg quality in common carp (Cyprinus carpio L.) under laboratory, commercial hatchery and natural conditions. *Aquaculture* 234 (1-4), 447–460. doi: 10.1016/j.aquaculture.2004.01.012

Mosha, S. S., Kang'ombe, J., Jere, W., and Madalla, N. (2016). Effect of organic and inorganic fertilizers on natural food composition and performance of African catfish (Clarias gariepinus) fry produced under artificial propagation. *J. Aquac. Res. Dev.* 7 (8).

Mosha, S., and Mlingi, F. (2018). Effects of different catfish pituitary gland extract dosages on eggs and hatchlings quantity of African catfish, Clarias gariepinus at a constant latency period. *J. Aquacult. Res. Dev.* 9, 1–3.

Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P. R., Thorsen, A., and Junquera, S. (2003). Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. *J. Northwest Atl. Fish. Sci.* 33, 33–54. doi: 10.2960/j.v33.a3

Mylonas, C. C., Duncan, N. J., and Asturiano, J. F. (2017). Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. *Aquaculture* 472, 21–44. doi: 10.1016/j.aquaculture.2016.04.021

Mylonas, C. C., Fostier, A., and Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocrinol.* 165, 516–534. doi: 10.1016/j.ygcen.2009.03.007

Natea, G., Wakjira, M., Negisho, T., and Endebu, M. (2017). Spawning response of African catfish (Clarias gariepinus (Burchell 1822), Claridae: Teleost) exposed to different piscine pituitary and synthetic hormone. *Int. J. Fish. Aquat. Stud.* 5 (2), 264–269.

Nguenga, D., Teugels, G. G., Legendre, M., and Ollevier, F. (2004). Influence of tropical seasonal changes on oocyte diameter, responses to hormonal induction and hatching quality in two strains of the catfish, Heterobranchus longifilis Val. (Clariidae). *Aquac. Res.* 35 (14), 1349–1357. doi: 10.1111/j.1365-2109.2004.01160.x

Nwokoye, C. O., Nwuba, L. A., and Eyo, J. E. (2007). Induced propagation of African clariid catfish, Heterobranchus bidorsalis (Geoffrey Saint Hillarie, 1809) using synthetic and homoplastic hormones. *Afr. J. Biotechnol.* 6 (23), 2687–2693. doi: 10.5897/ajb2007.000-2430

Okere, E., Erondu, E. S., and Zabbey, N. (2015). Evaluating the efficacy of pituitary gland extracts and ovaprim in induced breeding and fry quality of Clarias gariepinus,

Burchell (Pisces: Claridae). *Agricult. Forestr. Fish.* 4, 71–76. doi: 10.11648/j.aff.20150402.18

Okomoda, V., Tiamiyu, L., and Kwaghger, D. (2017). Spawning performance of Clarias gariepinus (Burchell, 1822) induced with ethanol preserved and fresh catfish pituitary extract. *Zygote* 25 (3), 376–382. doi: 10.1017/s0967199417000247

Otoh, A., Ekanem, I., Okoko, A., Asangusung, P., and George, U. (2024). Comparative study of inducing broodstock with natural and artificial hormones on reproductive performances of Clarias gariepinus. *Asian J. Fish. Aquat. Res.* 26 (3), 31–38. doi: 10.9734/ajfar/2024/v26i3744

Prat, F., Zanuy, S., and Carrillo, M. (2001). Effect of gonadotropin-releasing hormone analogue (GnRHa) and pimozide on plasma levels of sex steroids and ovarian development in sea bass (Dicentrarchus labrax L.). *Aquaculture* 198, 325–338. doi: 10.1016/s0044-8486(00)00600-1

Qadri, S., Shah, T. H., Balkhi, M. H., Bhat, B. A., Bhat, F. A., Najar, A. M., et al. (2015). Absolute and relative fecundity of snow trout, Schizothorax curvifrons Heckel, 1838 in River Jhelum (Jammu & Kashmir). *SKUAST J. Res.* 17 (1), 54–57.

Rideout, R. M., Trippel, E., and Litvak, M. (2005). Effects of egg size, food supply and spawning time on early life history success of haddock Melanogrammus aeglefinus. *Mar. Ecol. Prog. Ser.* 285, 169–180. doi: 10.3354/meps285169

Ruaza, F. C. (2018). Induced spawning activity of African catfish (Clarias gariepinus, BURCHELL 1822) using different fish pituitary gland. *SDSSU. Multidiscip. Res. J.* 6, 14–17. doi: 10.69546/94gbkl03

Saadony, S., Eldanasoury, M., Ali, B., and Sharf, S. (2014). Seasonal reproductive biology and artificial propagation of female African catfish (Clarias gariepinus) after hormonal stimulation. *J. Anim. Poult. Fish. Prod.* 2 (1), 21–31. doi: 10.21608/japfp.2014.7438

Sabet, S. S., Mohammad, I., Bagher, A. F., and Saeed, G. (2009). Study on sexual maturity and levels of gonad steroid hormones in female kutum Rutilus frisii kutum (Kamenskii, 1901) during spawning season from river Sefid-Rood of the Southern Caspian Sea. *J. Cell Anim. Biol.* 3 (11), 208–215.

Sahoo, S., Giri, S. S., Chandra, S., and Mohapatra, B. C. (2008). Evaluation of breeding performance of Asian catfish Clarias batrachus at different dose of HCG and latency period combinations. *Turk. J. Fish. Aquat. Sci.* 8 (2), 249–251. Available online at: https://dergipark.org.tr/en/download/article-file/141867.

Saleh, H. H., Abdel-Aziz, M. F. A., Abdelaty, B. S., Bazina, W. K., El Basuini, M. F., Darwish, S. I., et al. (2025). Watery yucca (Yucca schidigera) additions and biofloc as closed water systems: an assessment of their effects on reproductive efficiency, biochemistry and blood gases, and fry performance of Nile tilapia (Oreochromis niloticus). *Aquac. Int.* 33 (5), 390. doi: 10.1007/s10499-025-02063-z

Semenkova, T., Barannikova, I., Kime, D. E., McAllister, B. G., Bayunova, L., Dyubin, V., et al. (2002). Sex steroid profiles in female and male stellate sturgeon (Acipenser stellatus Pallas) during final maturation induced by hormonal treatment. *J. Appl. Ichthyol.* 18, 375–381. doi: 10.1046/j.1439-0426.2002.00368.x

Sharaf, S. (2005). Effect of gonadotropin–releasing gormone (GnRHs) and human chronic gonadotropin (HCG) on ovarian development and plasma levels of sex steroids in African catfish Clarias gariepinus. *Egypt. J. Appl. Sci.* 20, 23–37.

Shourbela, R. M., Tohamy, H. G., and El Hawarry, W. N. (2020). Induced spawning of African catfish (Clarias gariepinus Burchell 1822) after pre-spawning prophylactic disinfection; the breeding performance and tissue histopathological alterations are under scope. *Iran. J. Fish. Sci.* 19 (1), 309–324. doi: 10.22092/IJFS.2018.115523

Szabó, T., Medgyasszay, C., and Horváth, L. (2002). Ovulation induction in nase (Chondrostoma nasus, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture* 203 (3-4), 389–395.

Wu, F., Zhang, X., Zhang, W., Huang, B., Liu, Z., Hu, C., et al. (2009). Expression of three gonadotropin subunits in Southern catfish gonad and their possible roles during early gonadal development. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 153 (1), 44–48. doi: 10.1016/j.cbpa.2008.12.013

Xu, L., Zhao, M., Ryu, J. H., Hayman, E. S., Fairgrieve, W. T., Zohar, Y., et al. (2023). Reproductive sterility in aquaculture: A review of induction methods and an emerging approach with application to Pacific Northwest finfish species. *Rev. Aquac.* 15 (1), 220–241. doi: 10.1111/raq.12712

Yaron, Z., Bogomoinaya, A., Drori, S., Biton, I., Aizen, J., Kulikovsky, Z., et al. (2009). Spawning induction in the carp: past experience and future prospects-a review. *Isr. J. Aquac.* 61 (1), 5–26. doi: 10.46989/001c.20537

Yeung, C.-M., Chan, C. B., Leung, P. S., and Cheng, C. H. (2006). Cells of the anterior pituitary. *Int. J. Biochem. Cell. Biol.* 38 (9), 1441–1449. doi: 10.1016/j.biocel.2006.02.012

Yusuf, A., Yunusa, A., Onimisi, M. M., Aaron, B. O., and Emmanuel, V. S. (2023). Comparative effect and economic analysis of pituitary extracts and synthetic hormone (Ovaprim) on the hatchability and survival rate of African catfish (Clarias gariepinus) fry. *Int. J. Global Aff. Res. Dev.* 1 (2), 122–131.

Zaki, E. M., and Abd El-Ghaffar, H. A. (2020). Effects of Ovaprim, pituitary gland extract and human chorionic gonadotropin on testosterone, 11-ketotestosterone, and 17β-estradiol hormones of catfish (Clarias gariepinus). *Egypt J. Aquac.* 10 (4), 1–16. doi: 10.21608/eja.2020.52848.1043

Zidan, S., Saleh, H., Semaida, A., Abou-Zied, R., and Allam, S. (2020). Effect of different doses of human chorionic gonadotropin (HCG) hormone on stripping response and reproductive performance of the African catfish (Clarias gariepinus). *Egypt J. Aquat. Biol. Fish.* 24 (6), 225–242. doi: 10.21608/ejabf.2020.111531