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Effect of Ovaprim and Pituitary Extract on Serum Biochemical Parameters and Male Milt of Grass Carp *(Ctenopharyngodon idella*) During Artificial Spawning

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ABSTRACT

The current study aimed to evaluate the effects of carp pituitary extract (CPE) and ovaprim (OVA), administered separately or in combination, on sperm quality parameters, fry mortality after hatching, blood sex hormones, and blood biochemical parameters of grass carp (*Ctenopharyngodon idella*). A total of 81 fish were divided into three treatment groups, each comprising 27 fish (18 females and 9 males; average weight 7200 ± 30g). The treatments were as follows: intramuscular injection of OVA at 0.5ml/ kg body weight (T1), CPE at 4mg/ kg body weight (T2), and a mixture of OVA and CPE (T3). The results demonstrated a highly significant increase in male testosterone and in the levels of blood hormones (FSH and LH) across treatments after four weeks of injection in both sexes. Furthermore, significant elevations in total bilirubin, direct bilirubin, and indirect bilirubin were observed in both female and male fish in all treated groups compared with their corresponding controls. Semen characteristics—including total density, relative density (%), motility duration, motility percentage, milt volume, and pH—were also evaluated. Results indicated improved sperm quality overall. The T1 group (OVA) exhibited significantly higher values in all reproductive performance parameters compared to the other groups, while the T3 group (OVA + CPE) showed the lowest values ($P< 0.05$). However, no significant differences were detected in milt pH among the three treatments ($P> 0.05$). In conclusion, OVA treatment enhanced sperm viability and reproductive performance in male grass carp (*Ctenopharyngodon idella*).

INTRODUCTION

The grass carp (*Ctenopharyngodon idella*) is a commercially significant fish species broadly cultivated in China, Europe, North America, and other regions to meet dietary demands **(Weidong *et al.*, 2012)**. Valued for its high protein content, it plays a crucial role in food security, particularly in developing nations, and serves as an effective

biological agent for managing aquatic vegetation. Additionally, grass carp is a nutritious food source, providing essential proteins, vitamins, and minerals such as calcium, phosphorus, zinc, and omega-3 fatty acids, which support cognitive health and cardiovascular function **(Panda & Sasmita, 2016)**. Grass carp introduced to Egypt for aquaculture in the Nile River and freshwater ponds contributes to both economic growth and aquatic weed control. However, natural reproduction of this species is often hindered by physiological constraints, including insufficient sex steroid hormone synthesis or gonadotropin deficiencies in the pituitary gland (PG), preventing complete maturation **(Abd-Elhakim *et al.*, 2019)**. Captive breeding challenges further arise due to unsuitable environmental or stress-induced conditions that disrupt reproductive cycles.

Progress in genetic engineering, predominantly CRISPR/Cas9 technology, provides significant potential for developing enhanced grass carp varieties, such as the golden grass carp strain, which could revolutionize future aquaculture practices **(Pengfei Zhao, 2025)**. The aquaculture sector has traditionally prioritized the study of fish eggs over sperm, even though both gametes play crucial roles in successful fertilization. Nevertheless, sperm quality remains a key factor in broodstock management, significantly impacting egg fertilization rates. Critical sperm quality indicators encompass spermatozoa concentration, volume, motility, and motility duration **(Billard *et al.*, 1995)**. Among these, motility is particularly vital because spermatozoa require movement to penetrate eggs effectively **(Chauvaud *et al.*, 1995)**. Variations in motility and its duration depend on fish species and environmental conditions, such as temperature during sperm release **(Billard, 1986)**.

For decades, fish farmers and researchers have relied on hormonal treatments to induce artificial breeding in carp and other fish species (such as silver carp, grass carp, and common carp) for both commercial and scientific applications. Over the past eighty years, hatcheries have employed hormonal techniques to stimulate spawning, enhancing fry and fingerling production and significantly boosting aquaculture output **(Rahman *et al.*, 2011)**. The process, commonly known as “hypophysation,” involves injecting fish with crude PG extracts to trigger ovulation. The most widely used agent is the acetone-dried PG from the common carp (*Cyprinus carpio*), which contains gonadotropin, the key reproductive hormone. These glands are typically sourced from mature carp in temperate regions **(Horváth *et al.*, 2015)**.

The PG performs a central role in fish reproduction, producing and storing gonadotropin hormones (GTH) that regulate both ovulation and sperm release **(Salami *et al.*, 2006)**. Unlike natural hormonal pathways, direct PG injection targets the ovaries and testes, bypassing the brain-pituitary axis and causing a rapid rise in GTH levels—a critical trigger for spawning **(Rottmann *et al.*, 1991)**. Studies indicate that PG-induced spawning

not only improves fertilization and hatching rates but also enhances larval development and survival **(Oyeleye *et al.*, 2016)**.

The activity of PG is triggered by gonadotropin-releasing hormone (GnRH), which initiates the production and secretion of two key protein hormones: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) **(Josep *et al.*, 2000)**. In the initial phases of reproductive development, including spermatogenesis and vitellogenesis, FSH dominates both the PG and bloodstream. This hormone is essential for stimulating the generation of estradiol and testosterone while overseeing gamete formation **(Ojoghoro *et al.*, 2021)**. As reproductive organs mature and approach spawning readiness, LH levels surge dramatically compared to FSH. This hormonal shift triggers the production of 17α-hydroxyl-progesterone (17α-OHP) **(Amenyogbe *et al.*, 2020)**. This compound serves as the foundation for 17α-20β-dihydroxy-4-pregnen-3-one (17-20β-P), recognized as the primary maturation-inducing hormone (MIH) in numerous fish species. MIH is essential for completing the development of both sperm and eggs, while also facilitating spermatogenesis and ovulation **(Nagahama & Yamashita, 2008)**.

The global expansion of cyprinid aquaculture has created challenges in maintaining consistent supplies of standardized CPE for fish breeders. This supply limitation has prompted researchers to develop alternative spawning induction techniques for cyprinid species. Modern methods now combine various OVA peptide formulations and their synthetic analogs with dopamine receptor antagonists (DAs) to enhance endogenous GTH secretion **(Peter *et al.*, 1988)**. Dopamine plays an inhibitory role in piscine reproductive physiology by suppressing pituitary hormone release, particularly counteracting the impacts of administered luteinizing hormone-releasing hormone analogs (LHRHa). Pharmaceutical agents that function as DAs operate through two primary mechanisms: either by preventing dopamine secretion or by blocking its receptor binding sites. Research findings demonstrate that DA administration effectively neutralizes this inhibitory effect, thereby significantly improving LHRHa-induced spawning success rates **(Arabaci *et al.*, 2004)**. A deeper investigation into the hormonal regulation of gonad maturation requires examining both hypophyseal secretions and follicular hormones, including 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), 17β-estradiol, 11-ketotestosterone, and testosterone **(Schulz *et al.*, 2010)**. Interestingly, while testosterone and 11-ketotestosterone are primarily recognized as male androgens, they are also detectable in female fish **(Rinchard *et al.*, 1993)**. In females, testosterone plays a crucial role in reproductive physiology by serving as a precursor for 17β-estradiol synthesis, which subsequently drives vitellogenic development **(Kagawa *et al.*, 1982)**.

In recent years, blood biochemical parameters have gained significant attention as valuable indicators of physiological status in aquatic organisms **(Ejraei *et al.*, 2015)**. These

hematological and biochemical markers serve as effective tools for evaluating the health and physiological condition of fish with high precision **(Svetina *et al.*, 2002)**. It is important to note that variations in these blood parameters are influenced by multiple intrinsic factors, including age **(Melillo-Filho *et al.*, 2021)** and sex **(Bojarski *et al.*, 2023)**. Studies conducted on the common carp (*Cyprinus carpio L.*), the rainbow trout (*Oncorhynchus mykiss* Walbaum), and the Nile tilapia (*Oreochromis niloticus L.*) have demonstrated significant sex-based differences in hematological profiles **(Ezzat *et al.*, 1973)**.

The present investigation aimed to examine the individual and combined impacts of OVA and CPE on various physiological parameters in grass carp (*Ctenopharyngodon idella*) during induced spawning. Specifically, we evaluated serum sex hormone levels, male milt characteristics, and serum biochemical profiles. The study included both male and female specimens subjected to hormonal treatments. The findings from this research could contribute to the development of optimized artificial breeding protocols for intensive aquaculture operations.

MATERIALS AND METHODS

Fish distribution

The fish used in the experiment were maintained under controlled conditions for 14 days to allow acclimatization and to ensure uniformity in their environmental background. Half of the water in each experimental tank was replaced daily. Throughout the experiment, the water temperature ranged between 22 and 23°C, while dissolved oxygen levels were maintained between 6 and 7.5mg/ L.

A total of 81 grass carp (*Ctenopharyngodon idella*) with an average weight of 7200 ± 30g were divided into three groups (27 fish per group: 18 females and 9 males). The fish were stocked in nine fiberglass tanks at a sex ratio of 2 females: 1 male per tank, with three replicate tanks assigned to each treatment. The treatments were as follows:

- **T1:** intramuscular injection with OVA,
- **T2:** intramuscular injection with CPE,
- **T3:** intramuscular injection with a mixture of OVA and CPE.

Preparation of injection

To prepare the pituitary extract (CPE), a single dried gland was pulverized into a fine powder using a mortar. The powder was thoroughly blended with 1 mL of 0.65% saline solution at a ratio of 10 mL saline per gram of pituitary powder. Grinding was performed

with a dry mortar to increase efficiency. A small amount of saline was added to form a paste, after which the remaining saline was incorporated. The mixture was centrifuged at 3000 rpm (1006.2 × g), and the supernatant was collected into a hypodermic syringe for injection.

OVA (containing salmon gonadotropin-releasing hormone analog, domperidone, and dopamine antagonist) was obtained from Syndel Laboratories, Ltd. (Vancouver, British Columbia). Blood samples were collected from both male and female fish prior to injection (controls) to assess baseline biochemical parameters. OVA was administered intramuscularly at a dose of 0.5mL/ kg body weight, while CPE was administered at a dose of 4mg/ kg body weight. Fish were then placed in treatment-specific tanks.

Hormonal injections were administered in a single dose between 7:00 and 9:00 AM. Fish were injected deeply intramuscularly at a 45° angle above the lateral line toward the tail using a 3mL syringe. To minimize loss of the administered solution, the needle was kept within the muscle momentarily before withdrawal, while the injection site was gently massaged with a finger and covered during needle removal **(Laszlo *et al.*, 1985)**.

Biochemical determinations

- **Liver function:** Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined using the kinetic method recommended by the International Federation of Clinical Chemistry, with a Spectrum kit **(Breuer, 1996)**. Albumin, globulin, and alkaline phosphatase (ALP) were measured as additional markers of liver function and physiological status **(Velisek *et al.*, 2009)**. Total bilirubin was determined using a Diamond kit **(Young, 2001)**. Additionally, total protein was measured using the Biuret method **(Cannon *et al.*, 1974)**.

Hormonal immunoassay

- Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were quantified using a sandwich chemiluminescence immunoassay **(McCann *et al.*, 1998)**.

- Testosterone was determined with the LIAISON® testosterone assay, a direct, competitive chemiluminescence immunoassay (CLIA) **(Klee & Heser, 2000)**.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA, 2020). Data were analyzed by one-way ANOVA, and group means were compared at a significance level of 0.05. Results are presented as mean

± standard deviation (SD). Additional analyses were conducted using the Statistical Analysis System **(SAS, 2009)**.

RESULTS

Table 1. Sperm quality parameters (means ± SD) in male grass carp in different treatments during spawning season

Treatments / Item	T1	T2	T3
Milt volume (ml)	18.64±2.13[a]	15.2±2.11 [a]	12.15±1.65 [b]
Motility (%)	83.6 ±6.4[a]	76.5±2.5[b]	70.3±1.8[c]
Movement duration (S)	58.7±3.2 [a]	50.8±2.1 [b]	47.8±3.5 [b]
Density ×10 ^{9}/ml	15.6±1.3 [a]	14.1±1.1 [a]	13.2±0.9[c]
Total density ×10 ^{9}	290.2±15.2 [a]	214.3±17.3 [a]	159.7±14.2[c]
pH	7.9±0.2 [a]	7.9±0.1 [a]	8.0± 0.2 [a]

Values with the different letters are significantly different (P<0.05).

Sperm quality parameters—including milt volume (mL), motility (%), movement duration (s), total density (×10⁹), density (×10⁹/mL), and pH—were recorded in male grass carp across the three treatments.

Table (1) shows that milt volume ranged from 12.15 ± 1.65 mL to 18.64 ± 2.13 mL, with significant differences among treatments. Motility (%) ranged from 70.3 ± 1.8% to 83.6 ± 6.4%, with the highest and most significant values observed in T1. Movement duration ranged from 47.8 ± 3.5 s to 58.7 ± 3.2 s, with T1 again showing a highly significant increase.

Sperm density (×10⁹/mL) ranged from 13.2 ± 0.9 × 10⁹/mL to 15.6 ± 1.3 × 10⁹/mL, with very highly significant results in T1. Similarly, total density (×10⁹) ranged from 159.7 ± 14.2 × 10⁹ to 290.2 ± 15.2 × 10⁹, with T1 showing the greatest and most significant values.

In contrast, milt pH showed no significant differences (P> 0.05) among treatments, ranging from 7.9 ± 0.1 to 8.0 ± 0.2.

Table 2. Average mortality rate (%) (means ± SD) of fry after hatching up to eight weeks at the three treatments, OVA (T1), PE (T2) and mixture of OVA and PE (T3) of grass carp

Treatments / Item	T1	T2	T3
Second week (%)	11.8±0.4[c]	14.7±0.3 [a]	13.7±0.2 [b]
Third week (%)	8.3±0.2 [c]	10.5±0.3 [a]	9.8±0.2 [b]
Fourth week (%)	6.2±0.3[b]	9.4±0.2[a]	8.7±0.4 [a]
Fifth week (%)	4.8±0.3 [c]	5.4±0.2 [b]	6.4±0.3 [a]
Sixth week (%)	5.3±0.3[a]	4.8±0.2 [a]	5.4±0.4 [a]
Seventh week (%)	3.8±0.2 [c]	5.4±0.2 [a]	4.5±0.3 [b]
Eighth week (%)	5.2±0.4 [a]	5.5±0.4 [a]	5.5±0.3 [a]

The means have the same latter in the same raw are non-significant (P>0.05).

Table (2) presents the average mortality rate (%) of fry after hatching, measured from the second to the eighth week. Significant differences ($P\leq 0.05$) were observed in all weeks except the sixth and eighth.

During the second week, mortality was at its highest in T2 (14.7 ± 0.3%) and lowest in T1 (11.8 ± 0.4%). By the eighth week, however, no significant differences were observed among treatments, with mortality rates of 5.2 ± 0.4%, 5.5 ± 0.4%, and 5.5 ± 0.3% in T1, T2, and T3, respectively.

Table 3. Average number of fish, male number, female number, and sex ratio of fries after hatching and feeding on treated diet for eight weeks in different treatments

Treatments / Item	T1	T2	T3
Fish No.	1000±17 [a]	1000±15 [a]	1000±19 [a]
Male No.	965±14 [a]	906±9 [b]	874±13 [c]
Female No.	35±0.2[c]	94±0,4 [b]	126±0.5 [a]
Sex ratio %	96.5± 1.7 [a]	90.6±1.2[b]	87.4±1.2 [c]

The means have the same latter in the same raw are non-significant (P>0.05).

Table (3) shows that the initial number of selected fish did not differ significantly among treatments, averaging approximately 1000 fish per group at the start of the experiment.

By the end of the experiment, the number of males differed significantly among treatments, with the highest value recorded in T1 (965 ± 14; very highly significant), followed by T2 (906 ± 9; highly significant) and T3 (874 ± 13; significant).

In contrast, female numbers at the end of the experiment were greatest in T3 (126 ± 0.5; very highly significant), followed by T2 (94 ± 0.4; highly significant) and T1 (35 ± 0.2; significant).

Sex ratio (%) also varied significantly across treatments, being the highest in T1 (96.5 ± 1.7%; very highly significant), followed by T2 (90.6 ± 1.2%; highly significant) and T3 (87.4 ± 1.2%; significant).

Table 4. Blood sex hormones and some serum biochemical changes in male and female grass carp following 30-days of OVA injection

The means have the same latter in the same raw are non-significant (P>0.05).

OVA group	**Before injection (control)**		**After 2 weeks of injection**		**After 4 weeks of injection**	
	♂	♀	♂	♀	♂	♀
Sexual hormones:						
Testosterone	0.46±0.09 ^{c}	-	0.85±0.033 ^{b}	-	0.97±0.007^{a}	-
FSH	2.12±0.13 ^{c}	4.38±0.16 ^{c}	3.08±0.15 ^{b}	5.66±0.14 ^{b}	4.1±0.12^{a}	9.56±0.2^{a}
LH	1.8±0.02^{c}	3.6±0.4 ^{c}	5.8±.0.34^{b}	7.38±0.25 ^{b}	9.21±0.22^{a}	17.33±0.57^{a}
Liver function:						
Total bilirubin	0.36±0.04 ^{b}	0.34±0.03^{c}	0.469±0.02 ^{b}	0.53±0.02 ^{b}	0.588±0.02 ^{a}	0.584±0.017 ^{a}
Direct bilirubin	0.104±0.02^{a}	0.10±0.02^{c}	0.15±0.01 ^{b}	0.226±0.01 ^{b}	0.194±0.009^{c}	0.25±0.007 ^{a}
Indirect bilirubin	0.33±0.03 ^{a}	0.34±0.04^{c}	0.446±0.014^{b}	0.49±0.02 ^{b}	0.542±0.015^{c}	0.55±0.007 ^{a}
Total protein	5.6±0.13 ^{a}	5.3±0.2 ^{a}	5.54±0.108 ^{a}	5.62±0.19 ^{a}	5.24±0.21 ^{a}	5.36±0.22 ^{a}
Albumin	2.92±0.23 ^{b}	2.8±0.21 ^{b}	3.28±0.128 ^{a}	3.6±0.07^{a}	3.46±0.136 ^{a}	3.44±0.11 ^{a}
Globulins	2.92±0.31 ^{b}	3.2±0.16 ^{b}	3.5±0.13 ^{a}	4.78±0.26 ^{a}	3.6±0.13 ^{a}	4.64±0.18 ^{a}
ALT	43.4±2.8 ^{b}	43.4±2.4 ^{b}	50.4±1.9 ^{a}	50.8±1.56 ^{a}	51.8±1.66 ^{a}	50.2±1.53 ^{a}
AST	68.2±2.8 ^{a}	67.6±1.5 ^{b}	71±1.3 ^{a}	71.2±1.28 ^{a}	74±3.09 ^{a}	69.2±1.99 ^{a}
ALP	143.6±2.29^{c}	141.2±3.04 ^{b}	152.8±1.28 ^{b}	155.8±3.9 ^{a}	165.8±1.655 ^{a}	142.3±2.1 ^{b}

Table (4) summarizes the blood sex hormone and liver function results in male and female grass carp over a 30-day period of OVA treatment, assessed at three stages: before injection (control), and after 2 and 4 weeks of injection.

Male testosterone levels differed highly significantly ($P \leq 0.05$) among treatments, reaching the highest value after 4 weeks (0.97 ± 0.007). Similarly, all measured hormones (FSH and LH) showed highly significant variations in both sexes after 2 and 4 weeks compared with controls. FSH reached its maximum level after 4 weeks in females (9.56 ± 0.2) and males (4.1 ± 0.12). LH also peaked after 4 weeks, recording 17.33 ± 0.57 in females and 9.21 ± 0.22 in males.

Serum biochemical parameters revealed significant changes after OVA injection. In females, after 4 weeks, total bilirubin, direct bilirubin, and indirect bilirubin were 0.584 ± 0.017, 0.25 ± 0.007, and 0.55 ± 0.007, respectively. In males, corresponding values were 0.588 ± 0.02, 0.194 ± 0.009, and 0.542 ± 0.015.

Albumin, globulin, ALT, AST, and ALP levels were also significantly affected by OVA treatment. In females, the highest levels were observed after 2 weeks (3.6 ± 0.07, 4.78 ± 0.26, 50.8 ± 1.56, 71.2 ± 1.28, and 155.8 ± 3.9, respectively). In males, peak values were recorded after 4 weeks (3.46 ± 0.136, 3.6 ± 0.13, 51.8 ± 1.66, 74 ± 3.09, and 165.8 ± 1.655, respectively).

In contrast, total protein levels did not differ significantly across the three stages in either sex.

Table 5. Blood sex hormones and some serum biochemical changes in male and female grass carp following 30-days of CPE injection

CPE group	Before injection (control)		After 2 weeks of injection		After 4 weeks of injection	
	♂	♀	♂	♀	♂	♀
Sexual hormones:						
Testosterone	0.43±0.13^{c}	-	1.3±0.12^{a}	-	1.16±0.047^{a}	-
FSH	2.08±0.11^{c}	4.54±0.09^{c}	3.18±0.16^{b}	6.18±0.16^{b}	4.92±0.15^{a}	10.24±0.17^{a}
LH	2.1±0.13^{c}	3.17±0.29^{c}	5.73±0.42^{b}	7.592±0.24^{b}	10.216±0.21^{a}	19.06±0.56^{a}
Liver function:						
Total bilirubin	0.304±0.022^{c}	0.378±0.023^{c}	0.476±0.012^{b}	0.518±0.024^{b}	0.602±0.017^{a}	0.6±0.014^{a}
Direct bilirubin	0.108±0.013^{c}	0.096±0.017^{c}	0.144±0.01^{b}	0.262±0.017^{b}	0.22±0.01^{a}	0.296±0.011^{a}
Indirect bilirubin	0.29±0.069^{c}	0.358±0.022^{c}	0.458±0.012^{b}	0.5±0.016^{b}	0.549±0.017^{a}	0.6±0.04^{a}
Total protein	5.12±0.13^{a}	5.4±0.18^{a}	5.52±0.12^{a}	5.38±0.21^{a}	5.00±0.4^{a}	5.1±0.15^{a}
Albumin	2.46±0.14^{b}	2.7±0.18^{b}	3.46±0.12^{a}	3.7±0.17^{a}	3.5±0.123^{a}	3.52±0.128^{a}
Globulins	3.12±0.13^{b}	3.32±0.15^{b}	3.48±0.15^{a}	4.76±0.21^{a}	3.46±0.26^{a}	5.08±0.156^{a}

ALT	43.2±2.42 [b]	50.6±1.5 [a]	49.6±2.25 [a]	49.6±2.29 [a]	48.0±1.52 [a]	49.6±2.36 [a]
AST	68.8±2.02 [a]	70.4±1.03 [a]	69.4±2.8 [a]	69.0±2.67 [a]	69.8±2.52 [a]	68.6±2.58 [a]
ALP	148.6±1.63 [b]	143.2±2.08 [c]	152±1.73 [a]	160±1.79 [a]	167.2±1.88 [a]	155±2.3 [b]

The means have the same latter in the same raw are non-significant (P>0.05).

Table (5) presents the blood sex hormone and liver function results in male and female grass carp over a 30-day period of CPE treatment, assessed at three stages: control (before injection), and after 2 and 4 weeks of injection.

Male testosterone levels showed highly significant variation, reaching the highest value after 2 weeks of injection (1.3 ± 0.12). Similarly, all measured hormones (FSH and LH) exhibited highly significant differences in both sexes after 2 and 4 weeks compared with controls. FSH reached its peak after 4 weeks in females (10.24 ± 0.17) and males (4.92 ± 0.15). LH also reached maximum values after 4 weeks, with 19.06 ± 0.56 in females and 10.216 ± 0.21 in males.

Serum biochemical parameters also showed notable changes. After 4 weeks, total bilirubin, direct bilirubin, and indirect bilirubin levels were significantly higher in females (0.6 ± 0.014, 0.296 ± 0.011, and 0.6 ± 0.04, respectively) and in males (0.602 ± 0.017, 0.22 ± 0.01, and 0.549 ± 0.017, respectively) compared with the controls.

Albumin, globulin, and ALP were also significantly affected by CPE injection. The highest values were observed after 2 and 4 weeks, reaching 3.7 ± 0.17, 5.08 ± 0.156, and 160 ± 1.79 in females, and 3.5 ± 0.123, 3.48 ± 0.15, and 167.2 ± 1.88 in males, respectively.

In contrast, total protein, ALT, and AST did not differ significantly across the three stages in either sex.

Table 6. Blood sex hormones and some serum biochemical changes in male and female grass carp following 30- days of injection with a mixture of OVA and CPE

OVA and CPE group	**Before injection (control)**		**After 2 weeks of injection**		**After 4 weeks of injection**	
	♂	♀	♂	♀	♂	♀
Sexual hormones:						
Testosterone	0.318±0.128[c]	-	1.9±0.158 [a]	-	1.55±0.095 [b]	-
FSH	2.02±0.124 [c]	4.32±0.136 [c]	3.38±0.09 [b]	7.52±0.258 [b]	5.76±0.21 [a]	11.44±0.275 [a]
LH	2.1±0.09 [c]	2.91±0.308 [c]	7.04±0.17 [b]	9.06±0.275 [b]	11.204±0.232 [a]	22.64±0.49 [a]

Liver function:						
Total bilirubin	0.286±0.024 [c]	0.392±0.01 [c]	0.584±0.019 [b]	0.496±0.019 [b]	0.65±0.012 [a]	0.63±0.013 [a]
Direct bilirubin	0.098±0.017 [c]	0.102±0.015 [b]	0.18±0.015 [b]	0.288±0.012 [a]	0.228±0.016 [a]	0.31±0.014 [a]
In direct bilirubin	0.262±0.017 [c]	0.304±0.015 [c]	0.372±0.014 [b]	0.456±0.02 [b]	0.536±0.011 [a]	0.53±0.014 [a]
Total protein	5.12±0.128[a]	5.16±0.16 [a]	5.3±0.14 [a]	5.14±0.129 [a]	5.00±0.32 [a]	4.86±0.163 [b]
Albumin	2.38±0.107[b]	2.56±0.15 [b]	3.28±0.143 [a]	3.16±0.15 [a]	3.24±0.11 [a]	3.1±0.123 [a]
Globulins	3.00±0.18 [a]	3.12±0.13 [c]	3.3±0.14 [a]	4.22±0.11[b]	3.2±0.17[a]	4.58±0.21 [a]
ALT	38.6±1.86 [b]	51.6±1.44 [a]	45.6±1.89 [a]	41.1±1.28 [c]	44.8±2.06 [a]	46.6±1.63 [b]
AST	65.4±1.72 [a]	69.4±1.2 [a]	63.0±1.4 [a]	57.6±1.99 [b]	54.8±2.29 [b]	60.2±0.86 [b]
ALP	146.8±2.92 [b]	139.2±2.69 [c]	150.4±2.27 [b]	154.4±1.86 [b]	172±2.88 [a]	196.2±2.06 [a]

The means have the same latter in the same raw are non-significant (P>0.05).

The change in blood sex hormone and liver function in both male & female grass carp through 30-days of OVA and CPE mixture treatments in three stages; control, after 2 and 4weeks of injection are summarized in Table (6). There were highly significant differences (P≤0.05) in the levels of male testosterone recording the highest level after 2 weeks of injection (1.9±0.158). It's obvious that in all measured hormones (FSH, LH) there was a highly significant variant among treatments in both male & female grass carp after 4weeks of injection compared to the control groups. FSH recorded the highest level in female (11.44±0.275), while in male values of 5.76±0.21 were recorded. Furthermore, LH recorded the highest level in female (22.64±0.49), while in male recorded values of 11.204±0.232 were detected.

Serum biochemical parameters of grass carp showed some changes as follows; there were highly significant changes among both male & female grass carp after 4 weeks of injection comparing with the control groups in the levels of total bilirubin, direct bilirubin, and indirect bilirubin.

Moreover, albumin was highly significant affected by using mixture of OVA and CPE injection among groups, the highest level was observed after 2 weeks in both sexes recording the highest values (3.16±0.153) in female and (3.28±0.143) in male. Furthermore, female globulins, and ALP were highly significant after 4 weeks (4.58±0.21) and (196.2±2.06) respectively, but male globulins weren't significant at the three stages. Wherein, ALP had high significant after 4 weeks (172±2.88). While, female ALT & AST values were observed very highly significant before injection. Meanwhile, the male ALT showed a high significant increase after 2 weeks, but AST recorded an extremely significant decrease after 4 weeks of injection. On the contrary, total protein, had no significant differences during the three stages in both sexes.

DISCUSSION

The present study provides the first evidence that hormonal therapy enhances spermiation in grass carp, regardless of the hormone used. However, the highest sperm quality parameters were consistently observed in fish treated with OVA. Sperm quality indices—including movement duration, motility, milt volume, density, and total density—were significantly improved across all treatments, with OVA (T1) producing the greatest improvements.

In teleosts, sperm motility is a primary indicator of sperm quality **(Lahnsteiner *et al.*, 1998)**, while milt volume serves as an important measure of semen yield and spermatozoa density. Previous research has demonstrated that OVA and other GnRHa-based therapies improve these parameters in a variety of fish species. For example, administering OVA with domperidone prolonged sperm motility in European smelt (*Osmerus eperlanus*) **(Krol *et al.*, 2009)**. Similar enhancements in sperm motility have been observed in the European catfish **(Linhart & Billard, 1994)**, yellowtail flounder **(Clearwater & Crim, 1998)**, paddlefish **(Linhart *et al.*, 2000)**, and Atlantic halibut **(Vermeirssen *et al.*, 2004)**. These findings confirm that GnRHa-based treatments stimulate the hypothalamic–pituitary–gonadal (HPG) axis more effectively than gonadotropins alone, promoting spermiation and milt production **(Zohar & Mylonas, 2001)**.

In the present study, both OVA and CPE improved sperm motility in grass carp, consistent with findings in other teleosts. Importantly, the OVA group displayed the highest values for motility rate, movement duration, and sperm density. These parameters are crucial because they directly determine fertilization potential **(Gage *et al.*, 2004)**. Improved sperm motility and movement time have been previously linked with higher fertilization success in salmonids **(Gage *et al.*, 2004)** and northern pike **(Beata *et al.*, 2018)**. Our results suggest that these parameters can serve as species-specific indicators of terminal sperm maturation in grass carp following hormonal stimulation.

Comparing the two hormones, OVA and CPE act at different levels of the HPG axis. CPE functions at the gonadal level, directly stimulating sex steroid secretion, whereas OVA acts at the pituitary level, stimulating the release of gonadotropins **(Mylonas *et al.*, 2017)**. Although both agents enhanced spermiation, OVA-treated fish showed consistently superior performance, indicating greater efficacy. Furthermore, seminal plasma plays an essential role in preserving sperm motility and protecting against oxidative stress **(Rurangwa *et al.*, 2004)**. Hormonal stimulation likely enhances seminal plasma function by elevating steroid hormone levels, which may explain the improved sperm performance observed.

In Egypt, CPE has historically been the preferred spawning inducer for cyprinids, but its high cost and limited availability have reduced its practicality for hatcheries. OVA provides a more cost-effective and widely available alternative, making it a promising

option for large-scale aquaculture. Our findings demonstrated that fish treated with OVA produced the highest male numbers (965 ± 14) and achieved the greatest fry sex ratio (96.5 ± 1.7%). By contrast, females were more abundant in T3 (126 ± 0.5). Importantly, control groups failed to spawn or spermiate, while both OVA- and CPE-treated groups achieved full reproductive success. These results align with earlier reports showing that hormonal injections elevate sex hormone levels and stimulate spawning in carp and other teleosts **(Shokr, 2020; El-Sayed *et al.*, 2023)**.

The hormonal effects observed here were further reflected in blood sex hormone levels. Testosterone, FSH, and LH were significantly elevated in both sexes after OVA and CPE administration. Peak testosterone was observed at different times depending on the hormone: after 2 weeks with CPE and after 4 weeks with OVA. Similar patterns have been reported in the white silver carp **(Abol-Munafi *et al.*, 2006)** and grass carp **(Metwally & Fouad, 2008)**. These findings confirm that spermatogenesis can be modulated by exogenous hormonal stimulation, consistent with earlier studies on the catfish **(Coccia *et al.*, 2010)**.

Female grass carp treated with CPE displayed the highest FSH and LH levels, consistent with the natural reproductive cycle in teleosts, where FSH dominates early gonadal development and LH surges later to induce final oocyte maturation and ovulation **(Mylonas *et al.*, 2010)**. The hormonal profiles observed in this study suggest that both CPE and OVA effectively trigger endocrine pathways required for final gamete maturation.

Biochemical markers also reflected treatment effects. OVA and CPE elevated liver function enzymes (ALT, AST, ALP) and bilirubin fractions, particularly after 4 weeks. These changes may be attributed to stress-induced modulation of the HPG axis and increased metabolic activity during gametogenesis, as previously reported in the catfish **(Shokr, 2020)**. While albumin and globulin levels rose significantly in treated fish, total protein remained unaffected, consistent with studies in grass carp **(Ejraei *et al.*, 2015)**. Increased ALT, AST, and ALP activities are often associated with hepatic stress or metabolic adaptation, as noted in other teleosts exposed to environmental or hormonal stressors **(Bhattacharya *et al.*, 2005; Velisek *et al.*, 2009; Medhat *et al.*, 2017)**.

Proteins in seminal plasma play a key role in sustaining sperm motility and protecting spermatozoa from oxidative damage **(Lahnsteiner *et al.*, 2004)**. The observed correlations between protein levels and motility in this study support the idea that seminal proteins enhance fertilization potential. As reported in salmonids, protein concentrations decline during later stages of spermiation, possibly due to their utilization in sperm maintenance and protection **(Sanchez-Rodriguez *et al.*, 1978; Lahnsteiner *et al.*, 1995)**. Taken together, the results of this study demonstrate that both OVA and CPE effectively stimulate spermiation, elevate sex hormone levels, and modulate biochemical markers in grass carp. However, OVA consistently produced superior sperm quality, higher male

yields, and improved fry sex ratios, while also representing a more practical and cost-effective option for hatchery operations in Egypt.

CONCLUSION

The present study demonstrates the advantages of ovaprim over commercial pituitary extract, including reduced handling of broodfish due to its single-dose application. This not only decreases post-spawning mortality but also enhances spawning response, minimizes adverse effects on health and growth, and offers ease of use even for unskilled farmers. The findings presented here, together with those of previous studies, emphasize the importance of considering these variables in aquaculture research and practice. Nonetheless, the physiological mechanisms underlying the variations observed in this experiment require further investigation to fully clarify their roles in reproductive performance.

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