

Effect of carp pituitary extract (CPE) and human chorionic gonadotropin (hCG) on reproductive performance and larval production of the Egyptian Sole *Solea aegyptiaca*

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ABSTRACT: A 60-day spawning attempt was designed to compare the hormonal stimulation of human chorionic gonadotropin (hCG) with carp pituitary extract (CPE) on reproduction performance and larvae production of *Solea aegyptiaca*. In two groups, the first (Group I) was injected with 5000IU of hCG, and the second (Group II) was treated with 5 mg/kg brood-stock of CPE. Six circular fiberglass tanks (1.5 m³) were used in this trial. Brood-stock of *Solea aegyptiaca* were within an average weight of 36.75± 0.85 g and 40.25±2.17 of female and male, respectively. Fish were randomly stocked in tanks at a rate of 10 brood stock (5 male: 5 female)/ tank. Brood-stock was fed with minced shrimp at a feeding level of 1.5% of the biomass. Measurements of water physiochemical were within acceptable limits for reproducing *Solea aegyptiaca*. Every group was given four batches of fertilized eggs during the experimental period. Statistical analysis of the independent T-test showed that the hCG group was significantly higher in all parameters of reproduction rate than the CPE group.

Key word: Human chorionic gonadotropin, Carp pituitary extract, *Solea aegyptiaca*, reproduction rate

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1. INTRODUCTION

Notwithstanding the remarkable progress Egypt's fish farming industry has made in the last ten years (FAO, 2020), this progress has mostly been focused on freshwater fish, particularly tilapia, which has reached approximately 971,263 tons, or roughly 61% of the total production of fish farming (MOA, 2021). As a result, aquaculture faces competition from horticulture, which typically uses the most freshwater in Egypt (GFARD,

2020). As a consequence, more maricultural should be practiced lessening reliance on fresh water. Whereas major problems in this sector are related to resource use conflicts (water and land) with the scarcity of fresh water in Egypt. As Egyptian mariculture is limited to sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and meagre (*Argyrosomus regius*), which recorded a weak productivity (42,924, 33,094, 46,064 ton, respectively).

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A serious effort made to expand Egyptian maricultural and increase the species farmed (MOA, 2021). There are other marine species that are very suitable to culture under Egyptian conditions but did not record any production, such as the spinefoot-rabbit fish *Siganus* sp and sole fish (MOA, 2021).

Interestingly sole fish is a species appreciated by the market and is a candidate for rearing in a commercial scale, the sole usually lives on sandy and muddy sea beds in depths between 10 to 60 m. They mainly hunt for food at night and feed on worms, mollusks and small fishes and crustaceans. During the day they lie on the seabed buried in sand with only the eyes showing (Picton and Morrow, 2010). In the wild, spawning is associated with temperature (December/ January, Deniel, 1990) and viable eggs are found at a temperature between of 8-12 °C. In the case of these species, captivity is essential to the development of successful industrial farming (Canavate *et al.*, 2006; and Avendaño-Herrera *et al.*, 2008). They have demonstrated successful flatfish breeding and the necessity of aquaculture diversification. Consequently, they supply both quantity and quality of fry. In captivity with ambient conditions, the culture technology for the sole is mainly impaired by the lack of methods to control reproduction under captivity (Cabral, 2000). Most research and development efforts on the use of hormones to control finfish reproductive cycles in aquaculture have focused on the induction of final oocyte maturation (FOM), ovulation, spermiation, and spawning in fish that do not complete these processes in captivity. Hormonal manipulations have important applications in commercial aquaculture, which mostly depend on administration of gonadotropin-releasing hormone (GnRH_a) agonists, which leads to the releasing of the gonadotropin hormone GtH of the pituitary gland to directly stimulating the gonads, or through using pituitary gland extract (PE) or human chorionic gonadotropin (hCG).

As is well known, the practice known as "hypophysation"-which began in Brazil in the late 1930s- involves using pituitaries on the ground and extracts from them to stimulate fish reproduction. PE is a tool to hormonal induce that has a better rate of fertilization and hatching, as well as improved in larval growth and survival rate and more economically (Oyeleye *et al.*, 2016 and Natea *et al.*, 2017). Also, hCG is a purified gonadotropin hormone the most commonly used to induced spawning in fish. The injected hCG mimics the natural GtH produced. hCG may be more appropriate because it acts much faster, via direct stimulation of the gonad, in inducing FOM, spermiation and spawning. hCG is the popular protocol to induce spawning in many fish species such as African cat fish *Clarias gariepinus* (Attia *et al.*, 2023) sea bream, *Sparus aurata* (Badran *et al.*, 2019) and pigfish, *Orthopristis chrysoptera* (Di Maggio *et al.*, 2014). Accordingly, our study assessed the hormonal inducing of hCG and CPE on the reproduction performance and larval production of the Egyptian sole, *Solea aegyptiaca* brood-stocks.

2. MATERIALS AND METHODS

2.1. Ethics of Animal welfare

All experimental procedures were carried out by institutional guidelines for the National Institute of Oceanography and Fisheries (NIOF, Egypt) Committee for Ethical Care and Use of Animals/aquatic animals with approved code number (NIOF-IACUC, Code: NIOF-AQ5-F-24-R-006).

2.2. Study locations

The wet laboratory at Aquatic Research Station, National Institute of Oceanography and Fisheries (NIOF) in Fayoum, Egypt, provided the facilities for accomplishing this work.

2.3 *Animal source and husbandry producers*

Brood-stocks were obtained from the wild (Wadi-El-Rayan Lake, Fayoum, Egypt) they were caught a month before the breeding season by setting the trammel nets late at night and then withdrawing them early in the morning. After that, the fish were transferred over a 30-minute trip to the experiment location in a plastic tank 1m³ filled with oxygen-saturated water. Once the brood-stock arrived at the laboratory, they were placed in a quarantine tank and treated with Furans (50 ppm for 30 minutes) and then left to adapt for 15 days. Following the adaptation phase, the mothers were chosen based on their maturity level, sex, and overall health. After that, they were put into six 1.5 m³ circular fiberglass tanks. The bottom of these tanks had a layer of sand that was 5 cm thick and was continuously ventilated through a blower (2 HP).

Brood-stock with an average initial weight of 36.75± 0.85 g and 40.25±2.17 for female and male, respectively were distributed at rate of 10 fish/ tank with sex ratio (1:1). During the reproductive season, male and female are distinguished by the appearance of the ovary as a yellow vein on the lighter side of the female's body. Fish fed with minced shrimp at feeding level of 1.5% of biomass. Water temperature ranged from 15 to 17 °C and water salinity ranged from 33 to 35‰ during the experimental period, while oxygen concentration ranged from 6.3 to 7.5 mg/L and pH ranged from 8.00 to 8.21. The duration of this trial was 60 days.

2.4 *Preparing hormones for injecting*

After crushing each pituitary gland in a pestle, 1 ml of saline solution (sodium chloride 0.9%), was used to extract the glands. The suspension was then put into a centrifuge to extract the remaining fluid, which was then put into an injection syringe. Human chorionic gonadotropin (hCG) is obtained from Egyptian International Pharmaceutical Industries Co. in 10th of Ramadan City, industrial area B1 box 149, Egypt. It is also available as a 5000 IU formulation.

2.5 *Treatments and injecting*

Two treatments were performed to compare the effectiveness of injecting hCG at doses of 5000IU/kg of each male and female (Group I) with inducing CPE at a dose of 5 mg/kg of each male and female (Group II) on reproductive performance and larvae production. When water temperature reached 15-17°C, males and females were injected at the same time with a single dose in the morning (7-9 AM) and fishes were injected intramuscularly at an angle 45 degrees toward the tail by using insulin syringe for hCG and 3 ml syringe for CPE injection. Hatching larvae were collected and counted as soon as they appeared in tanks by plankton net 150 µ mesh size .

2.6 *Statistical analysis*

Independent sample T-test was used to determine if there was significant difference in experiments, which involved only two treatments. Using SPSS Statistical Package Program (SPSS, 2008) 17, released version.

3. RESULTS

As shown in table (1) there were significant dissimilarities between measurements of reproduction performance of the induced brood-stock by hCG and CPE. All parameters except No. of batches were higher with stimulating using hCG at dose of 5000IU/kg fish.

Data in table (2) showed significant differences at (P<0.05) between the hCG-induced group and those induced by CPE in each batch in terms of No. of eggs or larvae and hatching rate, whereas, hCG group was significantly higher in these indicators than CPE group.

Table (1). Numbers of fertilized eggs and larvae of each tank and /gram of female *Solea aegyptiaca* induced by hCG or CPE.

Table 1. Numbers of fertilized eggs and larvae of each tank and /gram of female *Solea aegyptiaca* induced by hCG or CPE.

Items	Groups		Sig.
	Group I: hCG	Group II: CPE	
No. fertilized eggs/ tank	16325±475 ^a	9450±300 ^b	0.007
No. Larvae/ tank	1443±487.5 ^a	7157±267.5 ^b	0.006
No. fertilized eggs/ gram female	85.91±0.24 ^a	53.23±0.94 ^b	0.001
No. Larvae/ gram female	75.94±0.56 ^a	40.31±0.91 ^b	0.001
No. batches for 60 days	4±0.00	4±0.00	-

Values in the same row having different superscripts are differ significantly ($P < 0.05$).

Table 2. Number of fertilized eggs/female, number of larvae/ female and hatching rate of each spawning batch of *Solea aegyptiaca* brood-stock that induced using hCG or CPE.

Items	Groups	Batches (1)	Batches (2)	Batches (3)	Batches (4)	Total
NO. fertilized eggs / female	Group I: hCG	1110±20.0 ^a	750±40.0 ^a	965±15.0 ^a	425±45.0 ^a	3360±240 ^a
	Group II: CPE	732±22.50 ^b	524±16.0 ^b	440±20.0 ^b	225±5.00 ^b	1921±31.5 ^b
	Sig.	0.007	0.034	0.002	0.042	0.027
NO. larvae/ female	Group I: hCG	996±25.0 ^a	691±13.0 ^a	849±17.00 ^a	378±42.5 ^a	2887±97.0 ^a
	Group II: CPE	554.5±18.5 ^b	372.5±12.5 ^b	335±17.5 ^b	170±5.0 ^b	1431±53.5 ^b
	Sig.	0.006	0.003	0.002	0.042	0.006
Hatching rate (%)	Group I: hCG	88.08 ^a	89.1 ^a	87.97 ^a	88.76 ^a	88.49 ^a
	Group II: CPE	75.69 ^b	75.62 ^b	76.0 ^b	75.5 ^b	75.71 ^b
	Sig.	0.003	0.002	0.003	0.004	0.002

Values in the same column/ indicator having different superscripts are differ significantly ($P < 0.05$).

4. DISCUSSION

Spawning inducing hormones are known to offer a viable approach for breeding fish in captivity, improving genetic loss, guaranteeing fry supply, and lowering reliance on fingerlings obtained from the wild. The two different hormonal stimulants used to spawn fish were thoroughly discussed and compared in this study. Pituitary extraction of carp fish was used as the induced spawning of *Solea aegyptiaca* which was injected at rate one gland (5 mg/kg of brood-stock), the responses of this treatment were compared with responses of another group stimulated by 5000IU of hCG. Our results showed that, the reproductive performance and hatching rate significantly enhanced with hCG-group compared to CPE-group.

As it is widely known carp pituitary extraction (CPE), the first hormone used in fish reproduction is efficient for artificial propagation of many fish species (Donaldson and Hunter, 1983). It is the most frequently utilized hormone for inducing spawning in fish worldwide and is used by culturists, especially the common carp, major Indian carp, and Chinese carp (Kahkesh *et al.*, 2010). CPE injection acting directly on the ovaries and testicles instead of being through the brain-pituitary axis provides the spike in blood GtH levels that often occurs before spawning (Rottmann *et al.*, 1991). CPE is more affordable and readily available (Kahkesh *et al.*, 2010). Furthermore, pituitary gland extract for carp fish (total dose 4 mg/kg of fish body weight) was successfully used to spawn *Solea vulgaris*

(Sharaf *et al.*, 2017). However, CPE contains other non-sex hormones such as prolactin cells and it was found that some of these hormones have opposite effects to sex gland stimulants such as prolactin and Thyroid-stimulating hormone (TSH). Furthermore, CPE is unpredictable in terms of quality, has a low activity level in the HPG axis or the great variability in pituitary LH content. Also, marine fish may be showed a poor response of the hormonal inducing by CPE in comparison with LHRHa or hCG. Assurance of this believing a study of Chebanov and Savelyeva (1999) cleared that LHRHa was more effective than pituitary gland extract for the induction of breeding in *A. stellatus*. Su *et al.* (2013) LHRHa implants produced significantly more ($P < 0.001$) fry/kg of brood-stock than CPE. Williot *et al.* (2002) demonstrated that the GnRH α D-Phe-6 NH $_2$ was as potent as CPE for inducing gamete release in Siberian sturgeon (*Acipenser baerii*). Furthermore, in European catfish, *Silurus glanis* L., the percentage of ovulating females following LHRHa treatments was higher than that following pituitary extract treatment (Brzuska and Adamek, 1999). Our results showed that decreased in No. of fertilized egg/ female and hatching rate of CPE group compared with hCG group. This may be attributed to other maternally derived components than nutrients, such as mRNAs and steroid hormones, present in the egg cytoplasm, might govern the essential processes during early embryogenesis, recently this was confirmed in European eel by (Kottmann *et al.*, 2021). As it's known pituitary gonadotropins stimulate the formation of sex steroids by the follicle cells surrounding developing eggs, hence regulating oogenesis (Nagahama and Yamashita, 2008). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the two forms of gonadotropins that the pituitary gland generates (Brooks *et al.*, 1997). As a result, a variety of factors, including the dosages administered, the preservation techniques used, time to get the gland from the fish (during or outside of the mating season),

and the source of the pituitary gland (freshwater or marine water fish), influencing the response of broodstock for stimulating the spawning. Consequently, it can be assumed that hormonal stimulation of marine spawning fish using pituitary extracts from carp or catfish is less effective than hormonal stimulation using extracts from marine fish, such as extract from salmon. In the same trend, Bebini *et al.* (2022) demonstrated that produced eggs of European eel females that were induced by salmon pituitary extract (SPE) showed a higher percentage of buoyant eggs and fertilization rate than those were induced by carp pituitary extract (CPE). They added larvae from the SPE group were larger at hatch than CPE. Considering the "bigger is better hypothesis" (Bailey and Houde, 1989), having a larger body area at hatch is generally considered an advantage and has been related to higher survival rate.

On the other hand, hCG is the most popular purified gonadotropin hormone utilized to induce spawning in fish. The injected hCG in fish mimics the natural GtH released by the fish's pituitary. Similarly just like the case with pituitary extracts, hCG bypass the brain-pituitary link, acts directly on gonads (ovaries and testes), so hCG may be more appropriate because it acts much faster, via direct induction of the gonad to release sex steroid hormones which in turn act a key role in final oocyte maturation (FOM), spermiation and spawning (Rottmann *et al.*, 1991). Besides, Morretti (1999) hCG is often given in a single dose, which ranges between 100 and 4000 international units (IU) per kg body weight. There is one situation in which hCG is preferred over GnRH α . The advantage of hCG does not require the existence of LH stores or activation of the pituitary gonadotrophs. Additionally, the hCG hormone, which stays in the fish's systems longer, greatly stimulates ovulation and spermatism (Rottmann *et al.*, 1992).

Several studies have reviewed the success of artificial spawning using hCG in *Solea* fish, Ramos (1986) said that, spawning in the common sole was done by injecting hCG intramuscularly in single doses ranging from 250 to 1000 IU/kg fish. He added low doses of hCG (250-500 IU/kg fish), injected into females with oocytes in the final stages of vitellogenesis induced spawning with the highest fertilization rate, number of eggs and number of spawnings. Saleh *et al.* (2016) reported that, artificial spawning of *Solea aegyptiaca* were achieved using hCG (total dose 7000 IU/kg fish body weight).

CONCLUSION

Generally, there is great mystery and unknowns about the hormonal stimulation of marine fish to reproduce artificially, especially sole fish, due to the lack of studies on their spawning. Whereon, there are several points to be studied, such as the optimal determination of the pituitary source and its dosage if used as an available and less expensive method, as well as testing of synergistic combinations of CPE and other synthetic hormones can significantly. Finally, our finding affirmed that the injected hormonal of *Solea aegyptiaca* broodstock using 5000 IU of hCG achieved higher reproductive indicators than using carp pituitary extract at dose of 5mg/kg broodstock.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Data Availability Statement

The data that support this study are available in the article and accompanying online supplementary material.

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