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Induced Spawning of Grass Carp *Ctenopharyngodon idella*, Using Common Carp Pituitary Extract with Domperidone

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by authors RS, AS and MB. The first draft of the manuscript was written by author RS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

To test the efficacy of the technique of using dopamine antagonists with pituitary extracts, experiments were conducted in July, 2022, at Masab Al Sen (Al Sen estuary) Fish Farm and Hatchery, General Commission for Fisheries Resources, Syria. In all, 18 sets (2 female x 4 male in each set) were tried individually. 9 sets were treated with domperidone (DOM) and carp pituitary extract (CPE) and they constituted the experimental sets, while the rest 9 were treated with CPE and formed the control sets. The ovulation ratio, absolute fecundity, relative fecundity (number of eggs/kg), fertilization rate and hatching rate was 92.85%, 384645 ± 70990 eggs, 56853.22 ± 5385.68 eggs/kg, 80.02% and 61.54%, respectively in the experimental sets whereas it was

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92.85%, 355172 ± 53008 eggs, 52059.73 ± 3853.71 eggs/kg, 72.50% and 54.46% respectively in case of control sets. Hatching occurred within 20 h and 45 min to 21 h after fertilization in the experimental sets whereas it was 20 h and 45min to 21 h and 20 min in case of control sets at 26-26.5°C. It was observed that body weight has positive influence on absolute fecundity (r = 0.98, 0.99) and relative fecundity (0.97,0.97) in case of experimental sets and control sets respectively.

Keywords: Induced spawning; fecundity; hypophisation; grass carp; Ctenopharyngodon idella.

1. INTRODUCTION

Grass carp is a freshwater cyprinid, represents the only species of the genus Ctenopharyngodon. Cudmore et al. [1], with a maximum recorded length of 150 cm and maximum published weight of 45 kg, and generally lives 5–11 years [2]. It is native to the lakes, ponds and large Asian rivers that generally flow into the Pacific Ocean [3]. “It is extensively used as a means of biocontrol for aquatic vegetation” [4]. It eats a relatively large quantity of macrophytes [5].

It was introduced in Syria from China for the first time in 1972 [6]. “Age at which grass carp attains maturity varies greatly with climate and environmental factors, especially temperature” [7]. “Female grass carp reach full sexual maturity at 4 to 6 years of age while males reach full sexual maturity at 3 to 4 years of age” [8]. “The fish breeds during monsoon months in the flowing waters of its natural habitat, the rivers, but does not spawn naturally in the static waters of ponds and tanks” [7]. “Spawning periods typically range from late April or early May, end in late June or early July in the native range (Yangtze River) and are triggered by water temperature and hydrograph, with the latter believed to be the primary cue” [9,10]. “Induced breeding is a method in which exogenous hormones are injected into the body of mature parent fish for induction of breeding” [11]. Many years ago, fish farmers and scientists have been using hormone preparations for the artificial propagation of carps and other fish species for commercial and scientific purposes. Usually referred to fish injection with crude fish pituitary glands to induction of ovulation in term "hypophysation" [12].

“In practice, the acetone-dried common carp (Cyprinus carpio) pituitary gland is the most commonly used agent to induce spawning, which contains the active hormone (gonadotropin), collected from mixed populations of marketable carp in temperate climates” [13-15].

Artificial breeding was a limitation because wild sulfides cannot be reared under spawning conditions without hormonal stimulation [16]. “Not only was injection of the carp pituitary gland an important first method for inducing spawning and spermatogenesis in fish, it also stood the test of incubation time and remained the method of choice for many fish keepers. In some situations, it has been found to be the most efficient and reliable method of inducing final gamete maturation or spawning” [12]. “The success many commercial aquaculture production programs are dependent upon its continued availability for use as an aid in spawning fish” [17].

“On the other hand, the increasing of the cyprinid culture in the world caused the problem in the presenting of calibrated CPE to aquaculturists. This obliged experts to test alternative hormones such as HCG (human chorionic gonadotropin), LHRH (luteinizing hormone releasing hormone) and led to the development of a new approach in the inducing of spawning for cyprinid fish. In this approach, different ovaprim forms and their analogues, stimulating of endogenous GTH release, are used with a dopamine receptor antagonist (DA), which potentates response to the peptide” [18].

“Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary’s positive response to injected (LHRHa) luteinizing hormone releasing hormone analogues . There is a family of drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for induce spawning” [19].

“The most successful technique used for ovulation and spawning of fishes is based on the use of dopamine antagonists (DA) with analogues of gonadotropin releasing hormones such as Leutinizing Hormone Releasing Hormone (LHRH-A), and is known as the Linpe

Method. A widely used commercial product for spawning of Chinese carps, has been developed on this principle by mixing an analogue of Salmon Gonadotropin Releasing Hormone (sGnRH-A) and DA" [20].

The present study is the modification of the above said technique, adopted in view of non-availability of synthetic analogues of gonadotropin releasing hormones in Syria, and to test the efficacy of the technique for induce breeding of grass carp under Syrian agro-climatic conditions and also to compare it with the traditional hypophysation method on practicality and effectiveness, as far as grass carp are concerned.

2. MATERIALS AND METHODS

The present study was conducted at Masab Al Sen Fish Farm and Hatchery, (General Commission For Fisheries Resources) on the Syrian coast north of Banyas city (Fig. 1).

A total of 84 healthy, sexually mature, 5-year-old brood stock of grass carps (56 males with an average weight of 5.6 kg and 28 females with an average weight of 4.24 kg, with a ratio of 2:1) were used for this study conducted from 3th July up to 27 July 2022. The total weight of female and male grass carps were calculated as 175.47, 272.175 kg respectively.

2.1 Selection of Brooders

Sexual state of male and females of grass carp under the present investigation showed ripping signs during experiment, since in the female, the pectoral fin was soft and the abdomen was rounded bulged with reddish fleshy vent, and Ova oozed out on pressing abdomen, while the males characterized by having a rough and large pectoral fin, and milt oozed out when pressed on abdomen.

2.2 Care of Brooders

Brooders of grass carp were reared in a 3000 m^2 earthen pond with 1.5-2 meter depth prior to inducing breeding during the period from January to May 2022. Males and females were placed in two separate earthen ponds, each with an area of 3000 m^2, starting from May to July 2022. Brooders were extensively fed with green fodder in the form of aquatic weeds like *Vallisneria*, *Ceratophyllum* and *Hydrilla*, at the rate of 15-20 % of their body weight and artificial pellet feed with 36% crude protein at the rate of 1-3% of their body weight. So as to make them fully mature.

Fig. 1. Al Sen Estuary Fish Farm, in which the research was carried out. Yellow dot: indicates the experimental site in the Fish Farm on the Syrian coast (35º14´ 36 N, 35 º56 ´32 E .)The arrow indicates an aerial photograph of the fish farm ponds

2.3 Preparing the Females for Spawning

When the water temperature in the ponds stabilized at 27º, hormonal injections were started for the fish, from the 3rd to the 27th of July. In each experiment, 6 females and 12 males distributed in three concrete spawning tank designated for this purpose were used, at a rate of 2 females and 4 males per tank.

2.4 Carp Pituitary Extracts (CPE)

The carp pituitary extracts (CPE) was prepared from the pituitary glands of a live adult common carps (2.3-2.8kg) collected in the pre-spawning season (on 15th March) before the beginning of the experiment. Pituitaries were cleaned and conserved in acetone, and stored at room temperature in the dark bottle. The glands were cleaned in the dark with absolute acetone to remove fat remains, dead cells and any other impurities and then immersed in acetone for 2 hours, then with new acetone for another 12 hours, after that with new acetone for another 24 hours. Cleaned glands were conserved in acetone and stored in a dark bottle at room temperature until use. The average weight of the pituitaries (acetone dried) was about 2.5 mg.

2.5 Preparation of Carp Pituitary Extracts (CPE)

Carp pituitary extracts (CPE) was prepared just before the injection .To prepare the extract, glands were dried using filter paper completely then dry pituitary glands were grinded in a mortar into a fine powder. The required amount of this powder was weighted for each fish separately and placed in a test tube after numbering it. saline solution (0.7% NaCl) was added, and the suspension was centrifuged at 3000 rpm for 5 minutes according to [12,6], then the supernatant was used for fish injection.

2.6 Domperidone

It is a drug that antagonizes dopamine receptors, its chemical formula is (C22H24CLN5O2). It is available in the form of coated tablets in pharmacies, each tablet contains 10 mg of domperidone. Among the trade names for this drug in Syria are: Motalon: Produced by Mediotic Laboratories. Motin: Produced by Oubari Pharma. Motiliosyr: Produced by Pharmasyr. Pure domperidone was secured from one of these companies.

2.7 Method of Injection

The fishes were held firmly and weighed cautiously, a calculated amount of doses of CPE and DOM injection to both sexes of grass carps were given intramuscularly in the region of the caudal peduncle above the lateral line. The needle was inserted under the scale with hypodermic 3 ml syringe through to a depth of about 1.5 cm and injected the fluid slowly.

2.7.1 Doses of CPE and DOM

Males were injected with CPE alone at a rate of 2.5mg/kg body weight dissolved in 2 ml saline solution (0.7% NaCl) in both the experimental and control groups, whereas females were injected with CPE at a rate of 5mg/kg body weight and DOM at a rate of 10mg/kg body weight of fish in the experimental sets. And with CPE alone at a rate of 5mg/kg body weight in the control sets. The hypophysation and domperidone injection for the females was applied in two fractional doses, the first or preparatory injection was 10% of the total dose of CPE, and 50% of the total dose of DOM dissolved in 1 ml saline solution (0.7% NaCl), and the second or decisive injection was the remaining 90% of the total dose of CPE, and 50% of the total dose of DOM dissolved in 2 ml saline solution (0.7% NaCl). Two injections were given at an interval of 12 hours. The males were not given a preparatory injection, only the decisive one, in case they released the milt before the female were ready to spawn. The decisive injection (100% pituitary dosage) was administered into the body of males at the time of the second or decisive injection of the females.

2.7.2 Time of injection

The time of injection depends upon the water temperature and the condition of the spawners. First dose was given in the afternoon at 12;00pm and the second dose was given after 12 hours of the first dose.

2.8 Handling and Transfer of Brooders

After the first injection each two female breeders were transferred at once to a concrete spawning tank (9x4x1m) together with four uninjected males. As sex ratio of one female to two males was used in induced spawning for achievement of best results [21]. No anesthesia was given during transfer of brood fishes. The female were sutured to close the genital opening by using a

waxed cotton thread and sewing needle and by making cross stitches over the genital opening. Immediately after suturing the genital opening, the females were returned to the concrete spawning tank and a water flow was ensured at a current speed of 0.3-0.8 m/s in order to secure an appropriate amount of dissolved oxygen in the range of 5-8 mg/L [8].

2.9 Breeding and Spawning

Ovulation usually occurs about 6 h after the second injection when the temperature is at the optimum range (27 -29 °C) [8]. It is important to know the exact time of ovulation, since any delay in stripping may result in over ripening of the eggs in the ovary [22]. About 6 hours after the second dose of injection to the female, showering and water jets were started so as to create circular water motion, soon males and females got excited and showed sexual play. Males started to chase females to forced them to lay eggs. 10 minutes after the appearance of signs of sexual irritation on the injected fish in the concrete spawning tank, the females were caught and the thread that closed the genital opening was removed and sex product was stripped from the spawners by gentle massage and pressure on the abdomen [23] into a dry plastic bowl. Following the semi dry fertilization method by Chaudhary et al. [24], milt was mixed with the eggs using a bird feather for two minutes, this will decrease the distance from the sperm to the micro Pyle of the egg. Eggs were washed with water for 10 min; they absorb water and attain the size of 1 to 1.4 mm in diameter. Then the eggs stripped from each female were placed in cylindrical hatching incubators with a capacity of 182 liters. Water quality parameters during experiments are given in Table 1.

Table 1. Physicochemical parameters of hatching incubators water

DO(mg/L)	6.5
pH	7.9
Water Temperature(0c)	26-26.5
Co2(mg/L)	9.8
Calcium Ca^{+2} (mg/L)	30
Magnesium Mg^{+2} (mg/L)	5
Bicarbonate HCO_3^- (mg/L)	153
Carbonate CO_3^{-2} (mg/L)	26.5
Chlorine Cl^- (mg/L)	6
Nitrate NO_3^- (mg/L)	5
Nitrite NO_2^- (mg/L)	0.05

2.10 Spawning Performance Parameters

Ovulation rate **= (**Number of ovulated females/ Number of injected females) x 100. [25].

Latency period (hrs): The period from injection till the onset of ovulation (hrs). [26].

2.10.1 Counting of eggs

In fish subjected to hormonally induced spawning, fecundity is determined by the number of oocytes released after stripping [27,28]. The number of eggs released was calculated following the gravimetric method [29] in which 1 g of egg sample was weighed three times and mean value was multiplied with the total weight of eggs.

2.10.2 Hatching

Hatching mainly depends on temperature, hatching period was recorded as 21 hours after fertilization at temperature 26-26.5°C. Immediately after hatching, newly hatched larvae were transferred to the circular incubation pool, 13 samples (with a volume of 100 ml) from each cylindrical hatching incubator were counted and the number of hatched larvae counted.

2.10.3 Calculation of fertilization rate and hatching rate

When fish eggs have developed to the middle gastrula stage, 10 samples were taken from each hatching incubator and in each sample, about 100 eggs were collected with small net at random, they were put into a white dish and the eggs such as turbid eggs, white eggs, empty eggs and rotten eggs with naked eyes were given up. Calculation of the fertilized eggs by percentage:

fertilization rate = (Number of fertilized eggs/Total number of eggs) x 100 [25]

Calculation of the hatched larvae by percentage:

hatching rate = (Number of hatched larvae /Number of fertilized eggs) x 100 [25]

2.11 Statistical Analysis

Data were analyzed statistically with Student's t-test. The software Co-Stat program version 6.311Win (Co-Stat, Co-Hort Software, USA) was performed for Statistical analyses. A probability

at level of 0.05 or less was considered significant. All data are presented as means with standard deviations (SD). Correlation relationships were studied using the Correl statistical function in the Excel program, according to the Pearson correlation coefficient for quantitative data.

3. RESULTS

3.1 Latency Period

The latency period ranged from 5 hours and 50 minutes to 6 hours and 10 minutes (Table,2) with an average of 359.92 ± 5.27 minutes, or about 6 hours for the experimental sets of grass carp, while it ranged from 5 hours and 55 minutes to 6 hours and 30 minutes (Table,3) with an average of 371.15 ± 11.93 minutes, about 6 hours and 11 minutes for the control sets of grass carp.

3.2 Ovulation Rate

The ovulation rate was 92.85% (13 out of 14) in both experiments

3.3 Absolute Fecundity

The number of stripped eggs from each female of the experimental groups ranged between 237148 and 517360 eggs (Table 2) with an average of 384645.3 ± 70990.5, while the number of stripped eggs from each female of the control groups ranged between 280042 and 441777 eggs (Table, 3) with an average of 355171.76 ± 53007.93. Body weight had a positive influence on absolute fecundity (Fig. 2).

3.4 Relative Fecundity

The relative fecundity values ranged between 45649.28 and 65947.7 eggs/kg of female body weight (Table, 2) with an average of 56853.22 ± 5385.678 of female weight in the brooders treated with DOM and CPG (experimental groups), while its values ranged between 46829.77 and 58128.6 eggs/kg(Table, 3) with an average of 52059.73 ± 3853.709 of female weight in the brooders treated with CPG alone (control groups). Body weight had a positive influence on relative fecundity (Fig. 3).

3.5 Fertilization Rate

The fertilization rate was 80.02% in the brooders treated with DOM and CPG (experimental groups), while it reached 72.50% in brooders treated with CPG alone (control groups).

3.6 Hatching Time

The hatching time of fertilized eggs was 20 hours and 45 minutes to 21 hours (Table 2) with an average of 1250.76 ± 4.49 minutes for the females treated with DOM and CPG (experimental groups), while it was 20 hours and 45 minutes to 21 hours and 20 minutes (Table 3) with an average of 1260.61 ± 9.95 minutes for the females treated with CPG alone (control groups), where the water temperature was 26 - 26.5c^{o}, PH was 7.9 and dissolved oxygen was 6.5 mg/l. in cylandrical hatching incubators.

Table 2. Latency period, absolute fecundity, relative fecundity, number of fertilized eggs, number of hatched larvae and hatching time of experimental sets of grass carp

Experiment date	female weight (kg)	Latency period (min)	Absolute fecundity (egg)	Relative fecundity (egg/kg)	Number of fertilized eggs	Number of hatched larvae	hatching time (min)
03/07/2022	7.195	362	431258	59938	347593	218400	1250
	6.65	362	369117	55506	299723	191100	1245
07/07/2022	6.85	355	395460	57731	325463	207610	1255
	7.15	350	432264	60456	352727	221910	1250
11/07/2022	7	360	436410	62344.29	356547	220350	1245
	5.195	355	237148	45649.28	196595	123890	1250
15/07/2022	6.925	360	411536	59427.58	341986	220350	1260
	6.215	355	334080	53753.82	279959	183690	1255
19/07/2022	7.845	365	517360	65947.7	411818	240110	1250
	6.85	360	395274	57704.2	317009	187720	1255
23/07/2022	6.215	370	320682	51598.07	238908	136630	1250
	7.02	365	409500	58333.33	299344	174850	1245
27/07/2022	6.12	360	310300	50702.6	233655	135980	1250
	7.105	0	0	0	0	0	0

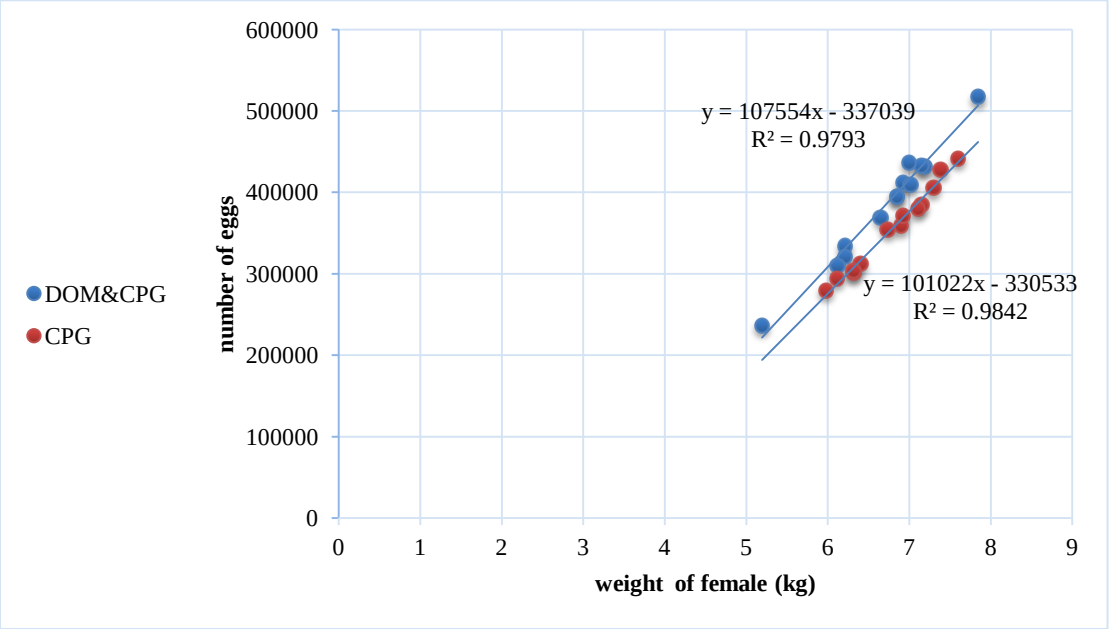

Fig. 2. The correlation between the weight of females and the absolute fecundity (number of eggs / female) when treated with DOM and CPG and when treated with CPG alone

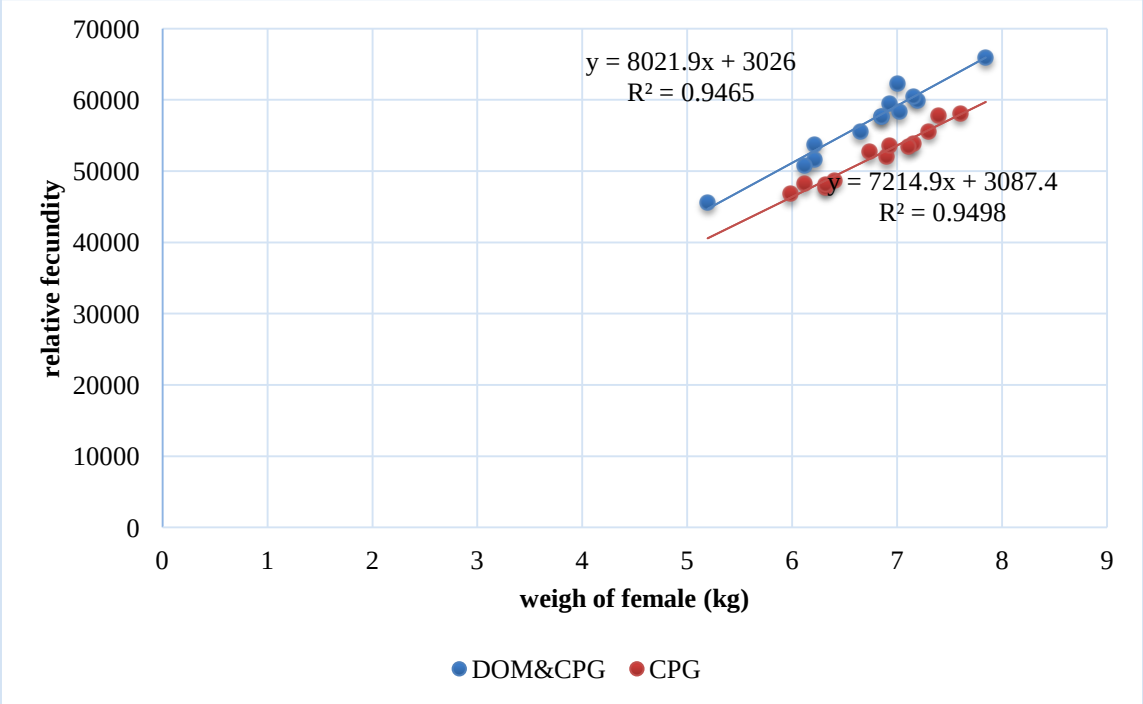

Fig. 3. The correlation between the weight of females and the relative fecundity (number of eggs/kg of body Wight) when treated with DOM and CPG and when treated with CPG alone

3.7 Hatching Rate

The hatching rate of fertilized eggs was 61.54% in experimental groups, while it was 54.46% in control groups.

4. DISCUSSION

“Many difficulties have been encountered with the traditional method of hypophysation. Apart from problems of procurement a:nd preservation

Table 3. Latency period, absolute fecundity, relative fecundity, number of fertilized eggs, number of hatched larvae and hatching time of control sets of grass carp

Experiment date	female weight (kg)	Latency period (min)	Absolute fecundity (egg)	Relative fecundity (egg/kg)	Number of fertilized eggs	Number of hatched larvae	hatching time (min)
03/07/2022	7.15	390	384720	53806	286231	158340	1265
	6.9	390	358976	52025	267437	149240	1265
07/07/2022	7.3	360	405697	55574	303461	169130	1260
	6.4	355	311952	48742	234276	130910	1250
11/07/2022	7.39	370	427720	57878.21	324639	181870	1265
	5.98	365	280042	46829.77	210311	120380	1260
15/07/2022	6.735	365	355020	52712.7	270525	156390	1265
	6.115	360	294920	48228.95	224139	130910	1260
19/07/2022	7.6	375	441777	58128.6	315870	165620	1280
	6.925	370	371259	53611.4	264707	136370	1273
23/07/2022	7.11	385	379746	53410.13	247594	123890	1250
	6.32	380	301600	47721.52	199357	100230	1245
27/07/2022	6.315	360	303804	48108.3	198991	99840	1250
	7.150	0	0	0	0	0	0

there is total lack of information on potency of pituitary glands. HCG has been reported to be immunogenic to some of the species. Both pituitary glands and HCG loss potency when stored so it is not very easy to estimate reliable dosages and variable results have been obtained with different lots of glands or HCG" [20]. The other synthetic alternatives used in the second generation of techniques are very costly and are scarce in developing countries like Syria.

Studies of Stacey et al. [30] and Billard et al. [31] established that "it was the gonadotropin release inhibitory factor (GRIF) which hindered ovulation in teleost fishes and abolition of this factor increased natural surge of GTH (Pituitary Gonadotropin), and the increased level of GTH in the blood serum is the common prerequisite for spontaneous ovulation". Studies of Crim and Evans [32] and Chang et al. [33] suggested that dopamine served as an inhibitor of GTH release. Peter et al. 1986) showed the direct inhibitory action of dopamine on GTH cells and they further demonstrated that administration of dopamine antagonists along with LHRH -analogues caused the elevation of GTH level in the blood serum which induced the fish to breed and it resulted in the development of Linpe Method [34].

Taking into account the results of above studies and the non-availability of LHRH-analogues in Syria, it was proposed to use DA with ovulating agents such as HCG and CPE as the former is believed to act as LH and the latter to contain both LH and FSH.

The statically analysis of the result of current experiment revealed that there were significant differences ($P \leq 0.05$) in the latency period, relative fecundity, number of fertilized eggs, number of hatched larvae, and hatching time between the females treated with DOM and CPG (experimental groups), and the females treated with CPG alone (control groups). The females treated with CPG took longer time for the appearance of the stimulus activity than the females treated with DOM and CPG together, and the difference was significant. In a study conducted by Weerakoon [35] on grass carp and bighead carp *Aristychthys nohilis* in Sri Lanka, it was found that the latency period was 6-7 hours at a water temperature of 28-30°C,whille in the experiment conducted by Farag et al. [12] the latency period of grass carp was 16, 19, 20, 18, and 17 hours in groups G1 treated with CPE, G2 treated with a mixture of HCG and CPE, G3 treated with HCG, G4 treated with Ovaprim, and G5 treated with Receptal, respectively, With a water temperature of 22-25 °C. In the study conducted by Rashid et al. [36] on grass carp and silver carp *Hypophthalmichthys molitrix* using Ovatide in Kashmir, eggs were laid 14-16 hours after injection at a water temperature of 24-27 °C, and in the experiment by Naeem et al. [37], latency period of grass carp was 8 hours and 30 minutes at a water temperature of 20-24.5 °C. The ovulation rate was 92.85% in both the females treated with DOM and CPG together, and the females treated with CPG alone. In the experiment by Naeem et al. [21], the ovulation rate of grass carp was 100%,whille in the study

of Szabó et al. [22] the ovulation rate of grass carp was 79.1%. In the experiment of Hussain [38] the ovulation rate of grass carp was 91.6%, and in the study of Rashid et al. [36] the ovulation rate of grass carp was 100%. Absolute fecundity was 384645.3 ± 70990.5 eggs in the females treated with DOM and CPG together, while it was 355171.76 ± 53007.93 eggs in the females treated with CPG alone. In the study conducted by Weerakoon [35] the absolute fecundity was 600000 eggs for mature female grass carp weighing 8-9 kg, in the study carried out by Naeem et al. [21] the number of eggs stripped from 22 females with a total weight of 115.3 kg was 7210000 eggs. In the study conducted by Rashid et al. [36] the number of eggs stripped from 7 females with a total weight of 15.504 kg was 1240320 eggs. Inaba et al. [39] estimated 485000 eggs in a grass carp weighing 7.1 kg, and Alikunhi and Parameswaran (1963) reported that a female weighing 7.036 kg, with her ovaries weighed 553 g, the number of her eggs reached 308,800 eggs, and that a female weighed 5.724 kg, with her ovaries weighed 1129 g, the number of her eggs reached 618100 eggs.

The relative fecundity value was 56853.22 ± 5385.678 eggs/kg in the females treated with DOM and CPG together, while it was 52059.73 ± 3853.709 eggs/kg in the females treated with CPG alone. In the study conducted by Rashid et al. [36] the relative fecundity of grass carp and silver carp was recorded as 70000-80000 and 100000-110000 eggs/kg body weight of fish, respectively and in the study carried out by Naeem, et al. [21] the relative fecundity was 62532 eggs/kg.

It was observed that body weight has positive influence on absolute fecundity (r = 0.98, 0.99) and relative fecundity (r = 0.97,0.97) in case of experimental sets and control sets respectively. This may be due to high nutritional status of the brood fish as brood stock were given artificial feed beside green fodder throughout the rearing period. Earlier studies showed that body weight had positive influence on absolute fecundity (r= 0.926) in grass carp [21] but had no influence on relative fecundity (number of eggs / kg) neither in silver carp [37] nor in grass carp [21].

The fertilization rate was 80.02% in the females treated with DOM and CPG together, while it was 72.50% in the females treated with CPG alone. In the study by Rashid et al. [36] the fertilization rate for grass carp and silver carp was 80.03% and 78.12% respectively and in the study carried out by Naeem et al. (2011) the fertilization rate was 80.36%. The hatching time of fertilized eggs was 20 hours and 45 minutes to 21 hours with an average of 1250.76 ± 4.49 minutes for the females treated with DOM and CPG together, while it was 20 hours and 45 minutes to 21 hours and 20 minutes with an average of 1260.61 ± 9.95 minutes for the females treated with CPG alone. In the study conducted by Weerakoon [35] the hatching time of fertilized eggs was 15 to 17 hours and in the study conducted by Rashid et al. [36] the hatching time of fertilized eggs was 20 to 30 hours. The hatching rate of fertilized eggs was 61.54% in the females treated with DOM and CPG together, while it was 54.46% in the females treated with CPG alone. In the experiment conducted by Farag et al. [26] the hatching rate percentages of G1 treated with CPE showed the highest ratios; 50, 49 and 48% followed by G5 treated with Receptal in common carp, grass carp and silver carp respectively. In the study by Rashid et al. [36] the hatching rate for grass carp and silver carp was 70.10% and 69.71%, respectively and in the study carried out by Naeem et al. [21] the hatching rate was 79.49%. In the experiment conducted by Singh et al. [20] to test the efficacy of the technique of using dopamine antagonists with pituitary extracts on induced spawning of Indian major carps *Cirrhinama mrigala*, *Catla catla*, *Labeo rohita*, the hatching rate of fertilized eggs was 85.7% in the females treated with DOM and CPG together, while it was 72.7% in the females treated with CPG alone.

The results of the present study compare well with those of commercial Linpe-based products such as ovaprim-c reported by Naeem, et al. [21] on grass carp, which indicates an ovulation rate of 100%, a fertilization rate of 80.36%, and a relative fecundity of 62532 eggs/kg. Ovatide reported by Rashid et al. [36] on grass carp and silver carp indicating that the ovulation rate was 100%, relative fecundity of grass carp and silver carp were recorded as 70000- 80000 and 100000-110000 eggs/kg body wt. of fish respectively. The fertilization percentage of grass carp and silver carp were recorded as 80.03% and 78.12% respectively. The hatching percentage of grass carp and silver carp were recorded as 70.10% and 69.71% respectively [36]. The results also compare very favorably with those obtained by using the traditional hypophysation methods on grass carp. The technique may be have problems of using CPE. But it has proved quite efficient and cost

effective. Much more work is required to be done to replace the pituitary extract as far as possible and to develop a ready to use product [40-42].

5. CONCLUSION

The present study demonstrates the advantages of the technique of using dopamine antagonists with pituitary extracts over pituitary extracts alone: e.g. it increases the number of stripped eggs, the fertilization rate, and hatching rate. It also reduces the latency period, and the time for eggs to hatch. This work demonstrated that the use of CPE coupled with domperidone is an effective and reliable procedure for induction of ovulation and spawning in Grass carp in two injections protocol in a total dose of 5mg/kg body weight CPE and 10 mg/kg body weight DOM.

6. RECOMMENDATIONS

It was recommended to use CPE of 0.5 mg/kg body weight with DOM of 5 mg/kg body weight as a preparatory injection and CPE of 4.5 mg /kg with DOM of 5 mg/kg as a decisive injection at an interval of 12 hours. To induce spawning of males, a single CPE injection of 2.5 mg/ kg is recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cudmore B, Mandrak NE. Biological synopsis of grass carp (*Ctenopharyngodon idella*). Burlington, Fisheries and Oceans Canada/Great Lakes Laboratory for Fisheries and Aquatic Sciences. 2004;44.
2. Schofield PJ, Williams JD, Nico LG, Fuller MT. Foreign nonindigenous carps and minnows (Cyprinidae) in the continental United States: a guide to their identification, distribution, and biology. Scientific Investigations Report 2005-5041. U.S. Geological Survey, Denver, CO. 2005;104.
3. Adams BM, Bertrand KN, Brown ML, Auger D. Genetic structure of grass carp populations in the Missouri and Mississippi River basins, USA. Praire Nat. 2011;43(3/4):84–91.
4. Pípalová I. A review of grass carp use for aquatic weed control and its impact on water bodies. J. Aquat. Plant Manage. 2006;44:1–12.
5. Jones LA, Drake DAR, Mandrak NE, Jerde CL, Wittman ME, Lodge DM, van der Lee AS, Johnson TB, Koops MA. Modelling survival and establishment of grass carp, ctenopharyngodon idella, in the great lakes basin. DFO Can. Sci. Advis. Sec. Res. Doc. 2016/101. vi + 52.
6. Hussain MG. A guide to fish farming in Syria, A Training Manual of UNV Multi-Sectorial Assistance Project SYR/78/007, UNDP, Damascus, Syria. 1982;112.
7. Jhingran VG, Pullin RSV. A hatchery manual for the common, Chinese and Indian major carps. Contribution No. 252, ICLARM Studies and Reviews, 11. International Center for Living Aquatic Resources Management: Manila, Philippines, ISBN 971-1022-17-6. 1985;191.
8. Jehan Y, Egg LY. Final report of artificial breeding of grass carp in Syria. Tech, Rep, Korean Mission. 1977;195.
9. Zhang G, Wu L, Li H, Liu M, Cheng F, Murphy BR, Xie S. Preliminary evidence of delayed spawning and suppressed larval growth and condition of the major carps in the Yangtze River below the Three Gorges Dam. Environ. Biol. Fish. 2012;93:439–447.
10. Kocovsky PM, Chapman DC, McKenna JE. Thermal and hydrologic suitability of Lake Erie and its major tributaries for spawning of Asian carps. J. Great Lakes Res. 2012;38:159–166.
11. Heggberget TG. The role of aquaculture in world fisheries. Oxford & IBH Publishing co. Pvt. Ltd. New Delhi; 1996.

12. Farag ME; Zeinhom MM, Ibrahim IH. Stimulation spawning of common carp, grass carp and silver carp by carp pituitary extract, human chorionic gonadotrophin, receptal and ovaprim hormones for commercial purposes. 1st International Conference (Central Laboratory For Aquaculture Research In Cooperation With Worldfish), Cairo, Egypt. 2017;2-346.
13. Saad A, Billard R. Spermatozoa production and volume of semen collected after hormonal stimulation in the carp. *Cyprinus carpio* Aquaculture. 1987;65(1):67-77. DOI;https://doi.org/10.1016/0044-8486(87)90271-7
14. Szabó T, Ditrói B, Szabó K, Bokor Z, Urbányi B. Comparison of the efficiency of common carp and silver carp in the breeding of common carp (*Cyprinus carpio*) and Northern Pike (*Esox lucius*). Turkish Journal of Fisheries and Aquatic Sciences. 2014;14:841-844.
15. Horváth L, Tamás G, Coche AG, Kovács É, Moth-Poulsen T, Woynarovich A. Training manual on the artificial propagation of carps: A handout for on-farm training workshops on artificial propagation of common carp and Chinese major carps in Central and Eastern Europe, the Caucasus and Central Asia. Food and Agriculture Organization of the United Nations, FAO, Budapest. 2015;38.
16. Billard R, Bieniarz K, Popek WM, Popek W, Saad A. Observations on a possible pheromonal stimulation of milt production in carp (*Cyprinus carpio* L.). Aquaculture. 1989;77(4):387-392.
17. Erdahl D. Clinical field trials to determine the efficacy of CCP to induce gamete maturation (ovulation and spermiation) in a variety of fish species. U.S. Fish and Wildlife Service, INAD. 1996;;8391: 1–12.
18. Peter RE, Lin HR, Van Der Kraak G. Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture. 1988;74: 1–10.
19. Arabaci M, Cagirgan H, Sar M. Induction of spawning in ornamental common carp (Koi, *Cyprinus carpio* L.) using LHRHa (DSer (tBu) 6, Pro9- Net) – LHRH) combined with Haloperidol and carp pituitary extract. Aquaculture Research. 2004;35:10.
20. Singh K, Dutt S, Ali M, Biswas RK. Use of dopamine antagonsts on induced spawning of Indian Major Carps. Journal of the Indian Fisheries Association NO. 1996;26:75-84.
21. Naeem M, Zuberi A, Salam A, Ashraf M, Elahi N, Ali M, Ishtiaq A, Malik T, Khan MJ, Ayaz MM, Iqbal MJ, Ahmad B. Induced spawning, fecundity, fertilization rate and hatching rate of grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim–C at a fish hatchery Faisalabad, Pakistan. Afr. J. Biotechnol. 2011b;10:11048–11053. DOI:https://doi.org/10.5897/AJB10.1481.
22. Szabó T, Urbányi B, Müller T, Szabó R, Horváth L. Assessment of induced breeding of major Chinese carps at a large-scale hatchery in Hungary. Aquac. Rep. 2019;14:100193.
23. Jamroz M, Kucharezky D, Hakuc-Blazowska A, Krejszeff, S; Kujawa R, Kupren K, Kwiatkowski M, Targonska K, Zarski D, Cejko BI, Glogowski. Used in the controlled production of IDE, LEUCISCUS IDUS. Arch. Polish Fish. 2008;16:363-370.
24. Chaudhary H, Singh Sp, Sukumaran KK. Induced breeding ofcarp ICAR. New. Delhi, India. 1984;82.
25. Hossain MB, Rahman MM, Sarwer MG, Ali MY, Ahamed F, Rahman S, Fulanda B, Rahman MM, Subba BR, Hossain MY. 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. 2012;10:89-95.
26. El-Hawarry WN, Abdel-Rahman SH, Shourbela RM. Breeding response and larval quality of African catfish (*Clarias gariepinus,* Burchell 1822) using different hormones/hormonal analogues with dopamine antagonist. Egypt. J. Aquat. Res. 2016;42:231-239. DOI:10.1016/j.ejar.2016.06.003.
27. Arantes CC, Castello L, Cetra M, Schilling A. Environmental influences on the distribution of arapaima in Amazon floodplains. Environmental Biology of Fishes. 2013;96:1257– 1267.
28. Sato Y, Verani NF, Nuner APO, Godinho H, Pand Verani JR. Padroes reprodutivos de peixes da bacia do Sao Francisco (Reproductive patterns of fishes of the Sao Francisco basin). In: A ´ guas, peixes e pescadores do Sao Francisco das Minas Gerais (Water, fish and fishermen of the Sa˜o Francisco from Minas Gerais). H. P.

Godinho, A. L. Godinho (Eds). Ed. PUC Minas, Belo Horizonte, Brazil. 2003;229–274:468. ISBN: 8586480142.

29. Haniffa M, Sridhar S. Induced spawning of spotted murrel (*Channa punctatus*) and Catfish (*Heteropneustes fossilis*) using human chorionic hormone and synthetic hormone Ovaprim. Veterinarski Arch. 2002;72(1): 51-56.
30. Stacey NE, Cook AF, Peter RE. Ovulatory surge of gonadotropin in the goldfish, *Carassius auratus*. Gen. Camp. Endocrinol. 1979 a;37:246-249.
31. Stacey NE, Cook AF, Peter RE. Spontaneous and gonadotropin. ovulation in the goldfish, Carassius auratus L : effects of external factors. J. Fish. Biol. 1979 b;15:349-361.
32. Crim LW, Evans DH. LHRH stimulated gonadotropin release from. The rainbow trout pituitary gland on in vitro assay for detection of teleost gonadotropin releasing factors. Gen. Camp. Endorinol. 1980;40:283-290.
33. Chang JP, Peter RE, Nathorniak CS, Sokolowska M. Effects of catecholaminergic agonists and antagonists on serum gonadotropin concentrations and ovulation in Goldfish: evidence for specificity of dopamine inhibition of gonadotropin secretion. Gen. Comp. Endocrinol. 1984;55: 351-360.
34. Lin HR, Van Der Kraak C, Zhou XJ, Liang JY, Peter RE, Rivier JE, Vole WW. Effects of (D-Ala6, Tra7, Leu8, Pro9)LHRH (sGnRH-A), and (D-Ala6 - Pro9 NEt)-LHRH (LHRH-A), in combination with pimozide of domperidone, on gonadotropin release and ovulation in the Chinese loach and common carp. Gen. Comp. Endocrinol; 1988.
35. Weerakoon DEM. Induced spawning of two major species of Chinese carps, *Ctenopharyngodon idella* and *Aristychthys nobilis* in Sri Lanka. .Bull. Fish. Res. Stn., Sri Lanka. 1979;29:55-62.
36. Rashid M, Balkhi MH, Naiko GA, Ahamad T. Induced breeding of grass carp (*Ctenopharyngodon idella*) and Silver Carp (*Hypophthalmichthys molitrix*) using ovatide as synthetic hormone at national fish seed farm (Nfsf) Manasbal, Kashmir, J&K. Fish Aquac. J. 2014;5:110.
37. Naeem M, Salam A, Elahi N, Ali M, Ishtiaq A, Andleeb A. Effect of body weight on absolute and relative fecundity of *Hypophthalmichthys molitrix* with intramuscular injection of Ovaprim–C. Int. J. Agric. Biol. 2011a;13:141–144.
38. Hussain MG. Development of induced spawning procedures for grass carp, *Ctenopharyngodon idella*, in Syria. Asian Fis, Sci. 1988;2:115-119.
39. Inaba D, Nomura M, Nakamura M. Preliminary report on the spawning of grass carp and silver carp in the Tone river Japan and the development of their eggs. J. Tokyo Univ. Fish. 1957; 43(1): 81-96.
40. Billard R, Alagarswami K, Peter RE, Breton B. Potentialisation par is pimozide des effets du LHRH-A sur la secretion gonadotrope hypophysaire !'ovulation et la spermiation chez la carpe commune (*Cyprinus carpio*). C.R. Acad. Sci. Ser. 1983;III. 296:181-184.
41. Brzuska E, Bialowas H. Artificial spawning of carp (*Cyprinus carpio* L). Aquacul. Res. 2002;33: 753- 765.
42. Zarski D, Kucharczyk D, Targonska K, Jamróz M, Krejszeff S, Mamcarz A. Application of Ovopel, Ovaprim and their combination in artificial reproduction of two rheophilic cyprinid fishes. Polish Journal of Natural Science. 2009;24:235-244.

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