Original Research Article

Stimulation spawning of Grass carp *Ctenopharyngodonidella* , by carp pituitary extract with Domperidone.

Abstract

To test the efficacy of the technique of using dopamine antagonists with pituitary extracts, experiments were conducted in July, 2022,at MasabAl sen (Al Sen estuary) Fish Farm and Hatchery, General Commission For Fisheries Resources, Syria. In all, 18 sets (2 female *4 male in each set) were tried individually. 9 sets were treated with Domperidone (DOM) and Carp Pituitary Extract (CPE) and theyconstituted the experimental sets, while the rest 9 were treated withCPE and formed the control sets. The ovulation ratio, absolute fecundity, relative fecundity (number of eggs/kg), fertilization rate and hatching ratewas 92.85%, 384645 ± 70990 eggs, 56853.22 ± 5385.68 eggs/kg, 80.02% and 61.54%, respectively in the experimental sets whereas it was 92.85%, 355172 ± 53008 eggs, 52059.73 ± 3853.71 eggs/kg, 72.50% and 54.46% respectively in case of controlsets. 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 absolutefecundity (r = 0.98, 0.99) and relative fecundity (0.97,0.97) in case of experimental sets and control sets respectively.

Key words: Induced spawning, fecundity, hypophisation, grass carp, *Ctenopharyngodonidella*.

INTRODUCTION:

Grass carp *Ctenopharyngodonidella* was introduced in Syria from China for the first timein 1972. The fish is known as one of the most efficient biological agents for controlling aquatic weeds(Hussain1982).
Age at which grass carp attains maturity varies greatly with climate and environmental factors, especially temperature (Jhingran and Pullin, 1985. 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 (Jehan and Egg, 1977).The fish breeds during monsoon months in the flowing waters of its natural habitat, the rivers, but does not spawn naturally in the static

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waters of ponds and tanks(Jhingran and Pullin, 1985).Spawning is the release of sexual products (ova in the case of female and milt in the case of male) to the exterior of the body (Basaran*et al*., 2008). Induced breeding is a method in which exogenous hormones are injected into the body of mature parent fish for induction of breeding (Heggberget, 1996).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".

Kuronuma (1968) and Vinogradov (1968) reported that the success of induced spawning of grass carp by pituitary hormone injection was achieved for the first time in China (1960) and in the USSR (1961), respectively. Successful spawning of grass carp by injection of pituitary homogenate were also reported in other regions(Lin1965; Chaudhury*et al*. 1966; chen*et al*. 1969; Anon. 1970; Boyd and Baily 1972; Shrestha 1973; Prabhavathy and Sreenivasan 1977; Bohl 1979; Stanley 197; Shireman and Smith 1983).In Syria, successful artificial propagation of grass carp was carried out for the first time by Jehan and Egg (1977). After that, Hussein *et al*. (1981, 1985) succeeded in investigating the induced spawning of fish in Syria as well.

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In practice, acetone-dried common carp *Cyprinuscarpio* pituitary gland is the most commonly used agent to induce ovulation, which contains the active hormone (gonadotropin), is collected from mixed populations of marketable carp in temperate climate (Saad and Billard, 1987; Brzuska, 2004; Szabó*et al*., 2014 and Horváth*et al*., 2015). Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances.(Billard The pituitary gland produces and stores gonadotropin hormones (GTH), which play a decisive role in ovulation and spermiation. Injected pituitary material bypasses the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood (GTH) levels that normally precedes spawning (Rottmann*et al*., 1992; Brzuska and Bialowas, 2002). Artificial reproduction has been one of the bottlenecks because it has not been possible to reproduce wild cyprinids in hatchery conditions without hormonal stimulation (Billard*et al*.,1989; Żarski*et al.*, 2009). Not only was carp pituitary injection being one of the first important methods of inducing ovulation and spermiation in fish, but also it has stood the test of incubation time and is still the preferred

methodology for many fish culturists. In some situations, it has been found to be the most efficient and reliable method of inducing final gamete maturation or spawning. The success many commercial aquaculture production programs are dependent upon its continued availability for use as an aid in spawning fish (Erdahl, 1996).

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 (Peter *et al*., 1988). 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 (Arabaci*et al*., 2004).

The most recent and 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. 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.

Materials and Methods

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

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A total of 84 healthy, sexually mature, 5-year-oldbroodstock 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 July2022. The total weight of female and malegrass carps were calculated as 175.47, 272.175 kgrespectively.

Figure 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.

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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, andOva 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.

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 breedingduring 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 Mayto 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 weightand artificial pellet feed with 36% crude protein at the rate of 1-3% of their body weight(Saad, 2000). so as to make them fully mature.

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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 purposewere used, at a rate of 2 females and 4 males per tank.

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 15thMarch) 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 temperatureuntil use.The average weight of the pituitaries (acetone dried) was about 2.5 mg .

Preparation of Carp pituitary extracts (CPE):

Carp pituitary extracts (CPE) was preparedjust before the injection .To prepare the extract, glands were dried using filter paper completelythen dry pituitary glands weregrinded in a mortar into a fine powder.Then, 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 (Faraget al., 2017; Hussain, 1982), then the supernatant was used for fish injection.

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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

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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.

Doses of CPE and DOM:

Males were injected with CPE alone at a rate of 2.5mg/kg body weightdissolved 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 weightand 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.

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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.

Handling and transfer of Brooders

After the first injection each two female breeders were transferred at once to a concrete spawning tank (9*4*1m) together with fouruninjected males, Assex ratio of one female to two males was used in induced spawning for achievement of best results (Naeem*et al*., 2011b). 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 (Jehan and Egg, 1977).

Breeding and spawning

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 andsex product was stripped from the spawners by gentle massage and pressure on the abdomen (Jamroz*et al*., 2008) into a dry plastic bowl. Following the semi dry fertilization method by Chaudhary *et al*., (1984), 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
Sodium Na^{+} (mg/L)	6
Potassium K^{+} (mg/L)	0.5
Iron Fe^{+2} (mg/L)	0.02
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
Sulfate SO_4^{-2}(mg/L)	7
Phosphorous PO_4^{-3} (mg/L)	0.09

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Spawning performance parameters

Ovulation rate = **(**Number of ovulated females/ Number of injected females) * 100. (Hossain *et al*.,2012).

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Latency period (hrs): The period from injection till the onset of ovulation (hrs). (El-Hawaeey*et al*.,2016).

Counting of eggs

In fish subjected to hormonally induced spawning, fecundity is determined by the number of oocytes released after stripping (Arantes*et al*., 2013; Sato *et al*., 2003)**.** The number of eggs released was calculated following the gravimetric method (Haniffa and Sridhar, 2002) in which 1 g of egg sample was weighed three times and mean value was multiplied with the total weight of eggs.

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 circularincubation pool, 13 samples (with a volume of 100 ml) from each cylindrical hatching incubator were counted and the number of hatched larvae counted.

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 thefertilized eggs by percentage:

fertilization rate = (Number of fertilized eggs/Total number of eggs) * 100. (Hossain *et al*.,2012).

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Calculation of the hatched larvae by percentage:

hatching rate = (Number of hatched larvae /Number of fertilized eggs) * 100. (Hossain *et al*.,2012).

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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 (Bailey, 1981).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.

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Results

Latency period

The latency period ranged from 5 hours and 50 minutes to 6 hours and 10 minutes (Table2) 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 (Table3) with an average of 371.15 ± 11.93 minutes, about 6 hours and 11 minutes for the control sets of grass carp. With a water temperature of 27-29º, PH of 7.9 and dissolved oxygen DO of 6.5 mg/l.

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Ovulation rate

The ovulation rate was 92.85% (13 out of 14) in both the experimental and control groups.

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Absolute fecundity

The number of stripped eggs from each female of the experimental groups ranged between 237148 and 517360 eggs (Table2) with an average of 384645.3± 70990.5, While the number of stripped eggs from each female of the control groups ranged between 280,042 and 441,777 eggs (Table3) with an average of 355171.76 ± 53007.93. Body weight had a positive influence on absolute fecundity(Figure2).

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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.

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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

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

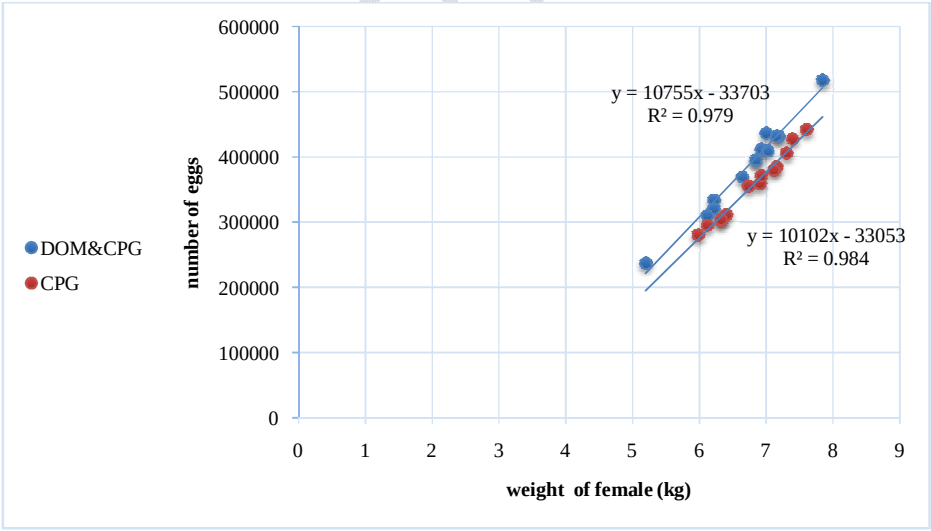

Figure2: The correlation between the weight of females of grass carp and the absolute fecundity (number of eggs / female) when treated with DOM and CPG and when treated with CPG alone.

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Relative fecundity

The relative fecundity values ranged between 45649.28 and 65947.7 eggs/kg of female body Wight(Table2)with an average of 56853.22 ± 5385.678 of female weight in the broodstock treated with DOM and CPG (experimental groups), while its values ranged between 46829.77 and 58128.6 eggs/kg(Table3)with an average of 52059.73 ± 3853.709of female weight in the broodstock treated with CPG alone (control groups). Body weight had a positive influence on relative fecundity (Figure3).

Fertilization Rate

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

Hatching time

The hatching time of fertilized eggs was 20 hours and 45 minutes to 21 hours (Table2) with an average of 1250.76 ± 4.49 minutesfor the females treated with DOM and CPG (experimental groups), while it was 20 hours and 45 minutes to 21 hours and 20 minutes (Table3) with an average of 1260.61 ± 9.95 minutes for the females treated with CPG alone (control groups), where the water temperature was 26 - 26.5º, PH was 7.9 and dissolved oxygen DO was 6.5 mg/l. in cylindrical hatching incubators.

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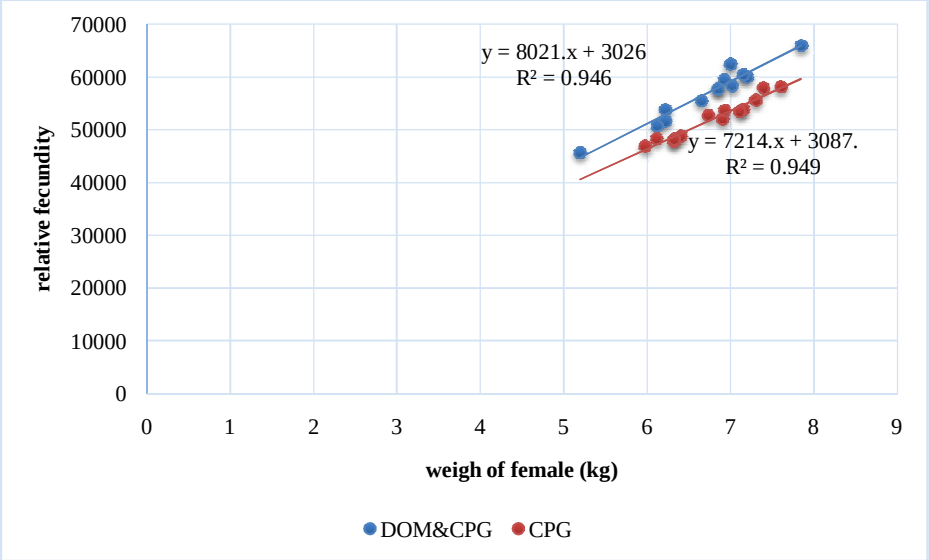

Figure 3: The correlation between the weight of females of grass carp and the relative fecundity (number of eggs/kg of body Wight) when treated with DOM and CPG and when treated with CPG alone.

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Hatching Rate

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

DISCUSSION

Many difficulties have been encountered with the traditional method of hypophysation. Apart from problems of procurement a:nd preservation 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. The other synthetic alternatives used in the second generation of techniques are very costly and are scarce in developing countries like Syria.

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Studies of Stacey et *al*. (1979 a,b) and Billard*et al*. (1983) 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 (1980) and Chang *et al*. (1984) suggested that Dopamine served as an inhibitor of GTH release. Peter *etal*. (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 (Lin *et al*., 1988).

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 results of the present study showed that there were statistically significant differences 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, as the average of latency period was 359.92 ± 5.27 minutes, or about 6 hours for females treated with DOM and CPG, and 371.15 ± 11.93 minutes, about 6 hours and 11 minutes for females treated with CPG alone, and the difference was significant.In a study conducted by Weerakoon. (1979) on grass carp*C. idella*and bighead carp *Aristychthysnohilis* in Sri Lanka. The latency period was 6-7 hours at a water temperature of 28-30°C,in the experiment conducted by Farag*et al*. (2017) 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*. (2014) on grass carp *C.idella*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*. (2011b), 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*. (2011b), the ovulation rate of grass carp was 100%, in the study bySzabó*et al*. (2019) the ovulation rate of grass carp was 79.1%,in the experiment by Hussain (1988) the ovulation rate of grass carp was 91.6%, andin the study by Rashid *et al*. (2014)the ovulation rate of grass carp was 100%. Absolute fecunditywas 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. (1979) The absolute fecundity was 600,000 eggs for mature female grass carp weighing 8-9 kg, in the study carried out by Naeem,*et al*. (2011b) the number of eggs stripped from 22 femaleswith a total weight of 115.3

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kg was 7210000 eggs,in the study conducted by Rashid *et al*. (2014) the number of eggs stripped from 7 femaleswith a total weight of 15.504 kg was 1240320eggs,Lin. (1935) reported that in China a female grass carp weighing 7 kg had 100,000 ova, Inaba*et al*. (1957) estimated 485,000 ovain a grass carp weighing 7.1 kg, and Alkunhi and Parameswaran (1963) reported that a female weighing 7,036 kg, her ovaries weighed 553 g, and the number of her eggs reached 308,800 eggs, and that a female weighed 5,724 kg, her ovaries weighed 1129 g, and the number of her eggs reached 618,100 eggs.The relative fecundity value was56853.22 ± 5385.678 eggs/kgin the females treated with DOM and CPG together, while it was52059.73 ± 3853.709 eggs/kg in the females treated with CPG alone. In the study conducted by Rashid *et al*. (2014) 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*. (2011b) 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 (Naeem*et al*., 2011b) but had no influence on relative fecundity (number of eggs / kg) neither in silver carp (Naeem*et al*., 2011a) nor in grass carp (Naeem*et al*., 2011b) induced to ovulate by hormonal treatment.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*. (2014)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 minutesfor 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. (1979) the hatching time of fertilized eggs was 15 to 17 hours andin the study conducted by Rashid *et al*. (2014)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*. (2017)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

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carp and silver carp, respectively,In the study by Rashid *et al*. (2014) the hatchingrate for grass carp and silver carp was 70.10% and 69.71%, respectively andin the study carried out by Naeem,*et al*. (2011b) the hatching rate was 79.49%.In the experiment conducted by Singh *et al*. (1996) to test the efficacy of the technique of using dopamine antagonists with pituitary extracts on induced spawning of Indian Major Carps, 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*.2011b) on grass carp, which indicates an ovulation rate of 100%, a fertilization rate of 80.36%, and a relative fecundity of 62532. egg/kg,andOvatide reported by (Rashid *et al*.2014) 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.The results also compare very favorably with those obtained by using the traditional hypophysation methods on grass carp. The technique may be said to 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.

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 fertilizationrate, 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 c. idellain two injections protocol in a total dose of 5mg/kg body weight CPE and 10 mg/kg body weight DOM. To induce spawning of female grass carp.

Recommendations

We recommend 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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