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Comparing the Effectiveness of Ovopel and Carp Pituitary Extract (CPE) on Artificial Spawning of Scaly Carp (*Cyprinus carpio*)

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Abstract: The effect of ovulation stimulators (Ovopel and carp pituitary extract) on spawning of scaly carp females were investigated. Following application of Ovopel, eggs were obtained from higher percentage of females than CPE treatment (92.5 and 75.0%, respectively. In the case of Ovopel stimulator, female weights were significantly correlated with living embryo ($r = 0.576$, $p<0.01$) and hatching rates ($r = 0.684$, $p<0.01$). Also interaction between egg weights and fertilization rates ($r = 0.603$, $p<0.01$) and between hatching and living embryo rates ($r = 0.679$, $p<0.01$) were determined significant. On the other hand, egg weights were negatively correlated ($r = -0.368$, $p<0.05$) with living embryo rates. For the CPE stimulator, egg weights were significantly correlated with fertilization ($r = 0.662$, $p<0.01$), living embryo ($r = 0.390$, $p<0.05$) and hatching rates ($r = 0.443$, $p<0.01$). Also hatching were significantly correlated with fertilization ($r = 0.665$, $p<0.01$) and living embryo rates ($r = 0.903$, $p<0.01$). In addition, fertilization and living embryo rates significantly correlated ($r = 0.615$, $p<0.01$) with each other.

Key words: *Cyprinus carpio*, stimulation of ovulation, ovopel, carp pituitary extract, fertilization, embryo

INTRODUCTION

In fish reproduction to produce the highest possible number of good quality hatch, several attempts have been made to obtain highest weight and best quality of eggs under controlled conditions. For this purpose various stimulating ovulation preparations were experimentally tested to determine stimulators that would ensure such effects.

The application of Carp Pituitary Extract (CPE) is based on the induction of endogenous GtH released by gonadotropin-releasing hormone (GnRH-a) (Horvath and Szabo, 1996). In fishery practice, the application of GnRH-a is associated with numerous difficulties such as preparation of weighed portions, storage of prepared solution of the analogue and precise determination of the ovulation time (Kozlowski, 1994). On account of the above difficulties, researchers are reluctant to apply the CPE to the spawners in hatchery conditions.

The simple and new method of treating fish with complex substances such as Ovopel that containing GnRH-a encourages their use in hatchery practice. Ovopel is available in pellet form and each pellet contains superactive gonadotropin releasing hypothalamic hormone. Giving the possibility of precise dosing without the necessity of weighing the preparation, simple method of preparing and storing of injections seem to justify further investigations using Ovopel (Klodzinska and Okoniewski, 1998; Kucharczyk and Szabo, 1998).

In the present research, an attempt was made to stimulate ovulation in scaly carp with most popular ovulation stimulators applied to numerous fish species. The aim of this research was to compare the effects on the reproduction in females treated with Ovopel and CPE.

MATERIALS AND METHODS

The experiment was carried out on 60 scaly carp females that body weights ranged from 2.7-6.8 kg. The broodstock were held in sand ponds at 24±1°C water temperature under natural photoperiod regime and fasted 48 h before gamete collection in State Hydrolic Works (SHW) Fish Reproduction Station during spawning season of scaly carp. For gamete collection, broodfish were anesthetized in 100 ppm quinaldine sulphate.

The females were randomly selected for reproduction and divided to 6 groups. In group I, II and III ovulation was stimulated with Ovopel and in group IV, V and VI with CPE. Carp pituitaries were prepared for injections according to Matlak (1970). Ovopel was purchased from

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Table 1: Number of females, substances used in stimulating ovulation, doses and method of application

Groups	No. of females	Substance	Doses*
I	10	Ovopel	0.5 pellet (i.p.)
II	10	Ovopel	1 pellet (i.p.)
III	10	Ovopel	0.5 pellet+1 pellet after 12 h (i.p.)
IV	10	CPE	0.1+0.9 mg after 12 h (i.p.)
V	10	CPE	0.2+1.8 mg after 12 h (i.p.)
VI	10	CPE	0.3+2.7 mg after 12 h (i.p.)
Total	60		

*Dose per 1 kg of body weight; i.p., intraperitoneally

Interfish (Budapest, Hungary). The Ovopel pellets were pulverized in a mortar and dissolved in 0.7% NaCl (Horvath *et al*., 1997). In experimental groups I, II and III Ovopel was injected in single dose of 0.5 and 1.0 pellet kg^{-1} but 1.5 pellets kg^{-1} was injected in two doses as 1/2 pellet and 1 pellet following 12 h. In group IV, V and VI CPE was injected in two doses of 1, 2 and 3 mg kg^{-1}. The first injection (10%) was given 12 h before second (90%). (Table 1). About 10 males that body weights ranging between 2.3 and 5.7 kg were stimulated with 1 mg kg^{-1} of Carp Pituitary Extract (CPE) at single dose.

The ovulation were checked every hours. The same person carried out all the strippings. Only females releasing >500 g of eggs were considered as ovulated. Eggs stripped from each female were fertilized with the pooled sperm that stripped from three males. The incubation of fertilized eggs stripped from each female were conducted in Zuger glasses at 23-24°C water temperature separately. During incubation, the water flow was ~3 L min^{-1} in Zuger glasses. The percentages of fertilization and living embryo were calculated following 12 and 24 h incubation, respectively. For this aim, a small sample of eggs was taken from the central part of the Zuger glass and 100 of these were placed on the petri dish. Three such measurements were carried out from one Zuger glass and the mean percentage was calculated from each stripped female in this way. Also hatching rates of larvae were calculated with similar method.

The data were statistically verified using analysis of variance. Duncan's multiple range test was used to check the significance of differences between the means of the investigated parameters within the groups at a minimum significance of $p<0.05$. Correlations between were estimated using Pearson's correlation test. Results are presented as mean±SD. Statistical analyses were performed with SPSS 10 for Windows statistical software package.

RESULTS AND DISCUSSION

Following CPE stimulation eggs were obtained from 70.0% of females while in the group of fish treated with Ovopel this value was 92.5%. Ovulation occured in all groups at the end of the 14 h following hormonal induction. The results of variance analysis showed a signifcant ($p<0.05$) effect of the ovulation stimulators on the eggs weights. More eggs were obtained by females stimulated with Ovopel compared to CPE. The fertilization and living embryo rates were higher in the case of Ovopel stimulation (Table 2). Correlations regarding to the stimulation with Ovopel and CPE was shown in Table 3 and 4, respectively.

Table 2: Arithmetical means (±SD) for body weight of females, weight of eggs, percentage of egg fertilization and percentage of living embryos after 24 h incubation and the results of Duncan's multiple range test

Descriptive statistics				
Groups	n	Means±SD	Minimum	Maximum
Weight of females (kg)				
Group I	10	4.79±0.78^{b}	3.80	6.40
Group II	10	4.47±0.19^{ab}	3.60	6.30
Group III	10	4.26±0.47^{ab}	4.20	6.80
Group IV	10	4.48±0.26^{ab}	3.20	6.10
Group V	10	3.75±0.23^{a}	2.70	5.60
Group V	I0	4.78±0.85^{b}	3.60	6.20
Weight of eggs (g)				
Group I	10	930.59±28.37^{a}	725.43	1096.24
Group II	10	1100.47±57.84^{b}	870.27	1420.37
Group III	10	1850.32±43.72^{c}	1690.37	2045.20
Group IV	10	860.37±19.46^{a}	693.28	1025.45
Group V	10	900.27±26.48^{a}	684.48	1125.46
Group VI	10	950.25±32.69^{a}	728.84	1025.28
Percentage of fertilized eggs after 12 h incubation				
Group I	10	91±4.26^{c}	86.00	95.00
Group II	10	95±2.89^{d}	83.00	97.00
Group III	10	99±2.47^{e}	87.00	100.00
Group IV	10	88±2.56^{b}	84.00	93.00
Group V	10	89±3.27^{b}	82.00	92.00
Group VI	10	86±2.86^{a}	79.00	92.00
Percentage of living embryos after 24 h incubation				
Group 1	10	72±3.26^{c}	64.00	86.00
Group II	10	77±2.19^{d}	69.00	85.00
Group III	10	79±3.47^{d}	67.00	83.00
Group IV	10	70±6.54^{c}	56.00	82.00
Group V	10	64±2.46^{a}	50.00	69.00
Group VI	10	67±4.59^{b}	59.00	76.00
Hatching rates (%)				
Group I	10	72±2.46^{cd}	67.00	79.00
Group II	10	72±3.62^{cd}	64.00	82.00
Group III	10	77±2.59^{d}	69.00	86.00
Group IV	10	70±2.45^{c}	64.00	82.00
Group V	10	63±3.27^{a}	50.00	69.00
Group VI	10	67±4.15^{b}	60.00	74.00

Group means designated by the same letter do not differ signifcantly from each other. Mean values marked with different letters are signifcantly different at $p\leq0.05$. SD = standard deviation

The results of the present research showed that Ovopel stimulation much better effecive on spawning than CPE. According to the results, more females were spawned and many more eggs were obtained in the case of Ovopel stimulation. As a result of Ovopel application, double doses administration yielded more eggs, higher fertilization and living embryo rates compared to one dose of this preparation.

Table 3: Correlation between the traits of females treated with Ovopel

Variables	Weight of females (kg)	Weight of eggs (g)	Percentage of fertilized eggs after 12 h incubation	Percentage of living embryos after 24 h incubation
Weight of eggs (g)	0.087			
Percentage of fertilized eggs after 12 h incubation	0.201	0.603**		
Percentage of living embryos after 24 h incubation	0.576**	-0.368*	0.190	
Hatching rates	0.684**	0.073	0.177	0.679**

*Correlation significant at $p \leq 0.05$; **Correlation significant at $p \leq 0.01$

Table 4: Correlation between the traits of females treated with Carp Pituitary Extract (CPE)

Variables	Weight of females (kg)	Weight of eggs (g)	Percentage of fertilized eggs after 12 h incubation	Percentage of living embryos after 24 h incubation
Weight of eggs (g)	0.226			
Percentage of fertilized eggs after 12 h incubation	0.168	0.662**		
Percentage of living embryos after 24 h incubation	0.328	0.390*	0.615**	
Hatching rates	0.209	0.433*	0.665**	0.903**

*Correlation significant at $p \leq 0.05$; **Correlation significant at $p \leq 0.01$

Application of Ovopel at double doses in Cyprinidae family was also supported by Horvath *et al.* (1997) and Brzuska and Grzywaczewski (1999).

On the other hand, more dead embryo was determined with CPE stimulation compared to Ovopel treatment. Hormonal stimulation can induce ovulation only when the ovary is at appropriate stage of maturation (Bieniarz *et al.*, 1991; Secer *et al.*, 2009). Therefore, differences in ovary maturation might be the reason why some scaly carp females did not ovulate. Evaluation of female ripeness based not only on fish condition and body shape but also on the gonad maturation assessment (Bozkurt, 2006).

The results of the experiment showed that spawning was better in larger fish that ranging between 4 and 6 kg in weight. As a result of this situation, higher percentage of larger fish spawned within the groups treated with Ovopel and CPE. As a result of this situation, the eggs obtained from them were much better quality than those from females of smaller body weight. The practical aspect of this research is that controlled spawning shoud be conducted in females of 4-6 kg body weight. As connecting to this situation, numerous hatchery operations might be less labour consuming and ovulation stimulation might be less expensive.

CONCLUSION

In conclusion, the application of Ovopel was found less stressful and effective for brood scaly carp than application of the CPE.

However, to achieve fully satisfactory results from controlled reproduction with the stimulant Ovopel further studies are required that take into account other factors that influence the success of controlled carp reproduction.

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