Artificial propagation of female Hungarian strain 7 carp (*Cyprinus carpio*) after treatment with carp pituitary homogenate, Ovopel or Dagin

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ABSTRACT: The effects of reproduction were investigated in carp females of the Hungarian strain 7 whose ovulation was stimulated with carp pituitary homogenate (0.3 + 2.7 mg/kg; group I), Ovopel (1/5 + 1 pellet/kg; group II) or Dagin (1 dose/kg; group III). The least-squares means calculated for the weight of eggs expressed in grams show that eggs of the highest weight were given by females treated with Ovopel and those of the lowest weight by females treated with carp pituitary homogenate (1 047.65 g and 769.28 g, respectively). For this parameter a statistically significant ($P \leq 0.05$) difference was found between the means of group I and II and between the means of group II and III. In the percentage of egg fertilization a statistically significant ($P \leq 0.05$) difference was also determined between the means of group I and II and between the means of group II and III. The applied spawning inducing agent did not affect the percentage of living embryos after 48 h incubation. Within group I and II the latency time did not affect any of the investigated parameters significantly, however, after Ovopel stimulation eggs obtained 7 h after the second injection showed higher weight and better quality in comparison with eggs yielded two hours later. Within the latency time of 7 h and 9 h statistically significantly ($P \leq 0.05$) higher weight of eggs and statistically significantly ($P \leq 0.05$) better quality after 12-h incubation were found in the ovulation stimulation with Ovopel. In the group of fish treated with Dagin the latency time affected the weight of eggs. In this group statistically significantly ($P \leq 0.05$) higher weight of eggs was noted for females whose ovulation occurred after 17 h from the application of Dagin while in the latency time of 15 h and 17 h the quality of eggs was similar after the incubation of 12 h and also after 48 h.

Keywords: *Cyprinus carpio;* induced ovulation; carp pituitary homogenate; Ovopel; Dagin; artificial propagation

The hormonal intervention in the processes controlling the final oocyte maturation in fish is possible at two sites (hypophyseal and hypothalamic approach; Yaron, 1995; Yaron et al., 2002). The application of carp pituitary homogenate (hypophysation) as a technique for spawning induction in fish has been used in aquaculture since the 1930ies (Von Ihering, 1937). A method based on the induction of endogenous GtH release by a superactive analogue of gonadotropin-releasing hormone (GnRH-a) was developed in the 1980ies. The cGtH release in Cyprinids is controlled by a strong dopaminergic inhibition (Peter et al., 1986) and in this connection the GnRH-a preparation has to be applied to Cyprinid fish with a dopamine receptor antagonist (the "Linpe method"; Peter et al., 1988). In the reference literature numerous papers discuss the results of spawning induction after the treatment with GnRH-a in carp and in other Cyprinid fish species (Peter et al., 1988; Drori et al., 1994; Alok et al., 1997; Barth et al., 1997; Szabó et al., 2002; Dorafshan et al., 2003; Mikołajczyk et al., 2003). However only scarce data can be found in the world literature with respect to the results of ovulation stimulation in carp (and other species of Cyprinids) using Ovopel or Dagin – the spawning inducing agents which contain both GnRH-a and a dopamine antagonist in their composition.

The investigation described in the present paper was carried out in the Institute of Ichthyobiology

and Aquaculture in Gołysz (Polish Academy of Sciences) as the continuation of many-year studies on the reproduction of females from different breeding strains of carp. In the stimulation of ovulation both natural (Brzuska, 1987, 1990, 1997; Brzuska and Ryszka, 1990) and synthetic spawning inducing agents (Brzuska and Adamek, 1997; Brzuska and Grzywaczewski, 1999; Brzuska, 2000, 2001a, 2003a,b, 2004; Brzuska and Białowąs, 2002) were used. The results of experiments conducted in the hatchery of the Institute with Dagin as the ovulation stimulator applied to carp females from the Polish strain 6 and Hungarian strain W (Brzuska, 2005) encouraged further studies with this preparation in a different breeding strain of this fish species.

Dagin, an Israeli agent for spawning induction in fish (Drori et al., 1994; Kulikowsky et al., 1996) combines a superactive GnRH analogue [(D-Arg^6, Pro^{9}NEt)-sGnRH] and a dopamine receptor antagonist, metoclopramide. A dose of this preparation calculated per 1 kg female body weight contains 10 μg s-GnRH and 20 mg metoclopramide (Kulikowsky et al., 1996). Numerous positive characteristics of Dagin (besides the induction of ovulation with one injection, thus reducing the stress to fish in comparison with repeated treatments with carp pituitary homogenate, and an easy method of preparing it for injection, which is a very important factor in conditions of hatcheries, Dagin is free of pathogens since its components are fully synthetic) and the broadening scope of studies on its use in different valuable fish species (Kouřil et al., 2003a,b) justify further experiments with carp as the most important species in the temperate zone.

The Hungarian preparation Ovopel, a complex of GnRH analogue (D-Ala^6, Pro^{9}NEt-m-GnRH) and metoclopramide, a water soluble dopamine receptor antagonist (Horváth et al., 1997), was already tested in the Gołysz hatchery both in pure carp strains and in their crosses (Brzuska and Grzywaczewski, 1999; Brzuska and Białowąs, 2002; Brzuska, 2003a,b, 2005). However in the case of carp this preparation necessitates the application of two doses with a 12 h interval between them (Horváth et al., 1997; Mikołajczyk et al., 2003). Since Ovopel had not been tested in the Hungarian strain 7 yet, this preparation was also included in the presented experiment.

Strain 7 is classified among the best native lines in Hungary. The investigation carried out in Fish Culture Research Institute in Szarvas showed its good growth rate. The strain characterized by a high dorsal index, minor carp scaling and a short caudal trunk was imported in Gołysz in 1973 and for many years tested in selection studies to preserve the traits obtained by Hungarian breeders. Females of Hungarian strain 7 are used in creating inter-strain crosses, particularly good results being obtained in crossing males of this line with females of Polish and French lines (Białowąs, 1999). The genetic characteristics of this strain were described by Irnazarow and Białowąs (1994).

The aim of the present study was to investigate the effectiveness of propagation results in females of Hungarian strain 7, whose ovulation was stimulated with carp pituitary homogenate and two synthetic preparations: Ovopel containing mammalian GnRH-a or Dagin containing salmon GnRH-a. We also attempted to ascertain the dependence between the latency time and the reproduction effects after treatments.

MATERIAL AND METHODS

The investigation was carried out on twenty-seven 8-years-old carp females of Hungarian breeding line 7 with body weights ranging between 4.40 and 7.50 kg. The fish were selected out of a larger population of spawners on the basis of external maturity signs in May, i.e. during the natural spawning period of this species. The females transferred to the hatchery were divided into three groups of nine individuals. Each group was placed in a separate tank 2.5 m^3 in volume in water at 21–23°C. After a two-day adaptation period the ovulation stimulation began with the application of carp pituitary homogenate to females of group I, Ovopel to group II and Dagin to group III. Doses of the applied preparations are given in Table 1. As recommended by Horváth et al. (1997), Ovopel pellets were pulverized in a mortar and dissolved in a vehicle of 0.7% NaCl. Dagin was dissolved in 0.7% saline before injection (Yaron et al., 2002).

In group I and II the checks of ovulation were carried out every hour since the 8th hour after the application of the second injection of pituitary homogenate or Ovopel. In group III the first check of ovulation was done 13 h after the Dagin treatment. Also in this group ovulation was checked every hour during the following 6 hours.

Eggs yielded by stripping females were weighed (from each fish separately) and fertilized with mixed

Table l. Substances used to stimulate ovulation in carp females of Hungarian line 7, doses applied, method of application and number of fish in groups

Group	No. of females	Substance	Doses*
I	9	Carp pituitary	0.3 mg and 2.7 mg after 12 h (i.p.)
II	9	Ovopel	1/5 pellet and 1 pellet after 12 h (i.p.)
III	9	Dagin	1 dose** (i.p.)
Σ	27		

Dagin was kindly provided by Mr. Z. Kulikovsky, Gan-Shmuel Fish Breeding Centre, Hefer, Israel

*doses per 1 kg of female body weight; i.p. = intraperitoneally

**1 dose of Dagin contains 10 μg of s-GnRH-a and 20 mg of metoclopramide

milt from three males at a ratio of 1 ml milt to 100 g eggs. Sperm production was induced using a carp pituitary homogenate (2 mg pituitary per 1 kg body weight). The incubation of fertilized eggs from each female separately was conducted in a Weiss glass at 21–22°C. During the incubation the water flow was ~3 l/min. The percentage of fertilization was calculated after 12-h incubation and the percentage of living embryos after 48 h, each parameter being calculated from three replications. The statistical characteristics of the obtained data are presented in Table 2.

The data were statistically verified using analysis of variance according to the least-squares method to find out if the applied ovulation stimulator (group) affected the parameters describing the effects of reproduction. The investigated parameters were: weight of eggs in grams, percentage of fertilized eggs and percentage of living embryos after 48-h incubation of eggs.

Analysis of variance was conducted according to this model:

$$Y_{ij} = \alpha + p_i + bW_{ij} + e_{ij} \quad (1)$$

where:

α = the theoretical general mean with the assumption that $W_{ij} = 0$

p_i = the effect of treatment (group) (i = 1,....3)

b = the regression on female body weight

W_{ij} = the body weight of female j

e_{ij} = the random error connected with observation j

The estimated least-squares means for the investigated parameters of each treatment are given in Table 3. The significance of the treatment for the investigated parameters was verified using the F-test (Table 3). Duncan's multiple range test was used to check the significance of differences between the means of the investigated parameters within the groups (Table 2). Phenotypic correlations between the investigated parameters were calculated separately for each group (Table 4).

Since after the treatments with pituitary homogenate, Ovopel and Dagin the investigated females

Table 2. Arithmetical means (±SD) for body weight of females, weight of eggs, percentage of egg fertilization and percentage of living embryos after 48-h incubation and the results of Duncan's multiple range test

Group	Parameters			
	weight of females (kg)	weight of eggs (g)	fertilized eggs after 12-h incubation	percentage of living embryos after 48 h-incubation
	1	2	3	4
I	6.28 ± 0.93[a]	823.70 ± 262.20[a]	86.17 ± 5.20[a]	74.44 ± 12.82[a]
II	6.33 ± 0.77[a]	1181.06 ± 353.41[b]	94.87 ± 4.81[b]	75.54 ± 16.07[a]
III	5.54 ± 0.91[a]	817.52 ± 317.77[a]	80.11 ± 23.69[a]	71.83 ± 26.37[a]

Group means designated by the same letter do not differ significantly from each other. Mean values marked with different letters are significantly different at $P \leq 0.05$

Parameter 1 for all groups n = 9, parameters 2–4 for group I and III n = 6 and for group II n = 8

SD = standard deviation

yielded eggs at two time limits, we attempted to find out if the effects on reproduction within a group depended on latency time. For the solution of this problem three separate analyses of variance were carried out using the least-squares method according to the following linear model:

$$Y_{ij} = \alpha + c_i + bW_{ij} + e_{ij} \quad (2)$$

where:

α = the theoretical general mean with the assumption that $W_{ij} = 0$

c_i = the effect of time i on the ovulation (i = 1,....2)

b = the regression on female body weight

W_{ij} = the body weight of female j

e_{ij} = the random error associated with observation j

The present paper also tried to answer a question if within the same latency time different reproduction effects were obtained after the ovulation stimulation with pituitary homogenate or Ovopel. In this connection two analyses of variance were conducted using the least-squares method (for latency time of 7 h and 9 h) according the following linear model:

$$Y_{ij} = \alpha + s_i + bW_{ij} + e_{ij} \quad (3)$$

where:

α = the theoretical general mean with the assumption that $W_{ij} = 0$

s_i = the effect of the ovulation stimulator (i = 1,....2)

b = the regression on female body weight

W_{ij} = the body weight of female j

e_{ij} = the random error associated with observation j

Analyses of variance carried out according to models 2 and 3 permitted to estimate the least-squares means characterizing the effects of reproduction connected with the ovulation time. The least-squares means are given in Table 5.

RESULTS

Percentage of spawning females after hormonal stimulation

After a double injection of pituitary homogenate (group I) eggs were obtained from eight out of the nine treated females, however, already after 12-h incubation the quality of eggs from two fishes was very poor (the fertilization percentage < 15). After the treatment with Ovopel (group II) ovulation also occurred in eight out of the nine females. In the group treated with Dagin (group III) eggs were yielded by seven females but the fertilization percentage of eggs from one female was very low (<10) (Figure 1). Data concerning the percentage fertilization of eggs of very poor quality were omitted in further calculations.

Ovulation time

In a part of females of both group I (pituitary homogenate) and group II (Ovopel), the latency time was 7 h, however, the remaining fish of these two groups yielded eggs after further 2 h. Two periods

Table 3. The least-square means (LSM) estimated for investigated reproduction parameters and significance of the *F*-test for treatments and for regression on the female body weight (*$P \leq 0.05$)

Treatments	Parameters								
	weight of eggs (g) α = 886.37			percentage of fertilized eggs after 12-h incubation α = 79.86			percentage of living embryos after 48-h incubation α = 65.65		
	LSM	SE	*F*	LSM	SE	*F*	LSM	SE	*F*
Ovulation stimulator			*			*			–
Carp pituitary (group I)	769.28	88.18		80.21	5.80		67.16	8.25	
Ovopel (group II)	1047.65	78.96		88.96	5.17		67.59	7.37	
Dagin (group III)	842.19	108.87		70.40	7.16		60.99	10.19	
Regression/body weight	86.31	69.10	–	9.79	4.54	*	11.95	6.47	*

SE = standard error of the least-squares means; α = theoretical general mean

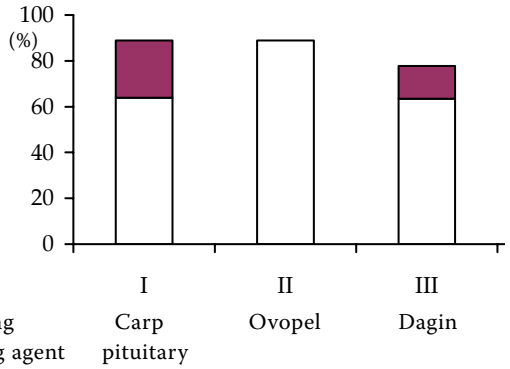

Figure 1. Percentage of spawning females after hormonal stimulation

Percentage of females which gave eggs ■ of very poor quality (fertilization percentage below 15) and □ of satisfactory quality

of spawning were also recorded in group III; for a part of fish the latency time was 15 h while the other part of females yielded eggs 17 h after the Dagin treatment.

Effect of treatments on weight and quality of eggs

Statistically significant ($P \leq 0.05$) effects of treatments on the weight of eggs expressed in grams was noted. The least-squares means estimated for the investigated groups show that the highest weight of eggs was obtained from fish after the application of Ovopel (group II) and the lowest after pituitary homogenate (group I) (1 047.65 g and 769.28 g, respectively; Table 3). The results of Duncan's test show that the mean weight of eggs in fish of group I significantly ($P \leq 0.05$) differed only from the mean weight of eggs yielded by fish of group II (Table 2). A statistically significant ($P \leq 0.05$) difference was also recorded between the means calculated for this parameter in group II and III (Table 2).

The statistically significant ($P \leq 0.05$) effect of the spawning inducing agent was demonstrated only for one parameter describing the quality of eggs, i.e. the fertilization percentage. The highest value of the least-squares mean for this parameter was noted in group II and the lowest in group III (88.96% and 70.40%, respectively; Table 3). Statistically significant ($P \leq 0.05$) differences were found only between the means for group I and II and also for group II and III (Table 2).

Differences between the mean body weights of females in the investigated groups were statistically insignificant (Table 2), but the regression on female body weight was significant ($P \leq 0.05$) for the percentage of egg fertilization and percentage of living embryos after 48 h of egg incubation (Table 3).

Ovulation time and weight and quality of eggs

In the group of fish whose ovulation was stimulated with carp pituitary homogenate, no statistically significant effect of latency time on the weight of eggs was determined. In fish spawning both 7 h and 9 h after the second pituitary treatment the values of the least-squares means for this parameter were similar: 574.91 g and 581.79 g, respectively (Table 5). In group I the latency time did not affect the quality of eggs significantly. However, it should be noted that with the longer time lapse between the second pituitary injection and the ovulation the least-squares mean for the percentage of living embryos after 48 h incubation was lower by 10% in comparison with the least-squares mean calculated for a shorter latency time (65.75 and 76.43, respectively; Table 5).

In group II the values of the least-squares means ascertained for the weight of eggs slightly differed by their latency time of 7 and 9 h (1 329.56 g and 1 253.87 g, respectively; Table 5). In this group no statistically significant effect of the ovulation time on the investigated parameters of egg quality was determined (Table 5). It should be stressed, however, that the least-squares means calculated for the percentage of living embryos after 48-h and for females whose ovulation occurred earlier were higher in comparison with the respective means calculated for a longer latency time (85.40 and 77.92, respectively, Table 5).

In the latency time of 7 h statistically significantly ($P \leq 0.05$) higher weight of eggs was ascertained after Ovopel treatment in comparison with the weight of eggs from fish treated with carp pituitary (1 286.16 g and 872.37 g, respectively; Table 5). The effect of the applied ovulation stimulator was statistically significant ($P \leq 0.05$) only with respect to the percentage of fertilized eggs, higher quality of eggs being observed after the treatment with Ovopel (Table 5).

With the latency time of 9 h the least-squares means for the weight of eggs calculated for fish

treated with Ovopel as the ovulation stimulator were also significantly ($P \leq 0.05$) higher in comparison with the means ascertained for females treated with carp pituitary homogenate (1 080.6 g and 769.50 g, respectively; Table 5). No statistically significant difference was found between the means

Table 4. Correlation between the parameters of females treated with carp pituitary homogenate (group I), females treated with Ovopel (group II) and females treated with Dagin (group III) (*$P \leq 0.05$)

Parameters	Group	Weight of females (kg)	Weight of eggs (g)	Percentage of fertilized eggs after 12-h incubation	Percentage of living embryos after 48-h incubation
		1	2	3	4
	I		0.61	0.53	0.35
1	II		0.96*	0.44	0.47
	III		0.81	–0.01	0.01
	I			0.16	0.54
2	II			0.60	0.61
	III			0.43	0.42
	I				0.47
3	II				0.99*
	III				1.00*

Table 5. Least-squares means (LSM) characterizing the effect on reproduction associated with the time of ovulation and results of *F*-test (*$P \leq 0.05$)

Classification factor	Weight of eggs (g)			Percentage of fertilized eggs after 12-h incubation			Percentage of living embryos after 48-h incubation		
	α	LSM	*F*	α	LSM	*F*	α	LSM	*F*
Carp pituitary (group I)	578.35			85.08			71.10		
Time of ovulation 7 h		574.91	–		84.40	–		76.43	–
Time of ovulation 9 h		581.79			85.76			65.75	
Ovopel (group II)	1 291.71			97.44			81.66		
Time of ovulation 7 h		1 329.56	–		99.01	–		85.40	–
Time of ovulation 9 h		1 253.87			95.87			77.92	
Time of ovulation 7 h	1 079.26			88.40			79.73		
Carp pituitary (group I)		872.37			81.50			77.66	_
Ovopel (group II)		1 286.16	*		95.30	*		81.79	
Time of ovulation 9h	925.07			91.56			70.13		
Carp pituitary (group I)		769.50			87.73			68.35	_
Ovopel (group II)		1 080.64	*		95.38	*		71.91	
Dagin (group III)	725.97			66.40			57.11		
Time of ovulation 15 h		586.80			64.34	–		55.14	–
Time of ovulation 17 h		865.15	*		68.46			59.09	

α = theoretical general mean

determining the percentage of living embryos after 48-h incubation developed in eggs from females stimulated with the above-mentioned spawning inducing agents (Table 5).

In the group of fish treated with Dagin the comparison of the least-squares means of the weight of eggs in the case of latency time of 15 h and 17 h showed that eggs of significantly higher weight were obtained from females spawning 17 h after the application of this stimulator (586.80 g and 865.15 g, respectively; Table 5). In the case of Dagin treatment the latency time did not significantly affect the investigated parameters characterizing the quality of eggs (Table 5).

Dependences between investigated parameters

The highest (and statistically significant $P \leq 0.05$) value of the coefficient of correlation between the body weight of females and the weight of eggs was found for group II and the lowest for group I (0.96 and 0.61, respectively; Table 4). The highest values of the coefficient of correlation between the weight of eggs and the fertilization percentage and between the weight of eggs and the percentage of living embryos after 48h incubation were also found for group II (Table 4). The correlation between the percentage fertilization and the percentage of living embryos after 48-h incubation was 0.99 for group II, 1.00 for group III and 0.47 for group I (Table 4).

DISCUSSION

The data obtained in the presented experiment showed that in the investigated strain the best reproduction effects were obtained from females whose ovulation was stimulated with Ovopel. Out of the nine fishes treated with this stimulator only one failed to spawn while the eggs from all the spawning females were characterized by sufficiently good quality. In this group significantly higher weight of eggs was recorded in comparison with the weight of eggs from fish treated with pituitary homogenate or Dagin. The results found in the experiment with fish from two genetically distant strains of carp (i.e. Polish line 6 and Hungarian line W) treated with carp pituitary homogenate, Ovopel or Dagin also showed the highest percentage of spawning females of these lines and the highest weight of eggs after the application of Ovopel (Brzuska, 2005).

In comparison with the pituitary treatment higher percentages of spawning females treated with Ovopel were also observed in earlier studies of carp (Israeli strain Dor-70 and a cross of this strain with Hungarian strain 8 – Brzuska and Grzywaczewski, 1999; French line F and a crossbred 1X – Brzuska and Białowąs, 2002; Polish line 3 – Brzuska, 2003b; Polish line 6 and Hungarian line W – Brzuska, 2003a; and line M 2 – Kouřil et al., 2003a) and also of other fish species from Cyprinidae (e.g. grass carp *Cthenopharyngodon idella* – Kouřil et al., 2003a; tench *Tinca tinca* L. – Kouřil et al., 2003b).

As for the three ovulation stimulators used in the experiment, the lowest percentage of spawning females was recorded in the case of Dagin. Earlier studies showed that after the application of this spawning inducing agent females of Polish line 6 and Hungarian line W yielded eggs in a fairly high percentage in comparison with females stimulated with pituitary homogenate (Brzuska, 2005), however the percentage was much higher in the Hungarian line W. It should be noted that when the ovulation in carp (*Cyprinus carpio* L.) was stimulated with Ovaprim (which combines D-Arg^6, Pro^{9}NEt-sGnRH analogue – the same that is contained in Dagin, but a different dopamine receptor blocker – domperidon) the percentage of ovulating females also distinctly depended on their origin (Brzuska and Adamek, 1997).

Much higher values of the percentage of spawning females after the Dagin treatment in comparison with fish treated with carp pituitary homogenate were reported not only for carp (*Cyprinus carpio* L.) but also for grass carp (*Cthenopharyngodon idella* Val.) and tench (*Tinca tinca* L.) (Kouřil et al., 2003a,b). The results of some experiments conducted on common carp (Koi and Dor-70 varieties) under routine hatchery conditions in Israel showed that the differences between treatments (Dagin or carp pituitary extract) in the spawning ratios (number of ovulating females after injection per total number of injected females) were statistically insignificant in each experiment (Kulikovsky et al., 1996).

In the present experiment after the ovulation stimulation with Dagin the weight of eggs was much lower in comparison with the weight of eggs yielded by females treated with Ovopel, although it did not differ significantly from the weight of eggs obtained from females after pituitary homogenate applica-

tion. This observation was also confirmed by the results of studies conducted by Brzuska (2005) if the weight of eggs in grams was taken into consideration. Kouřil et al. (2003a) reported that the weight of eggs expressed as the percentage of body weight of carp females was almost equal in groups treated with Dagin and carp pituitary homogenate (3.67% and 3.02%, respectively).

It should be stressed that in the presented experiment the quality of eggs from females of the three investigated groups significantly differed only after 12-h incubation while the highest fertilization percentage was found after the treatment with Ovopel and the lowest after Dagin. However, after 48-h incubation the quality of eggs was similar after pituitary homogenate or Ovopel treatment and only slightly lower after Dagin. In comparison with the quality of eggs after pituitary homogenate or Ovopel much poorer quality of eggs was obtained after the treatment with Dagin in an earlier study, but only in one of the two genetically distant lines of carp (Brzuska, 2005).

In reporting a spawning success in Dagin-treated carp (*Cyprinus carpio* L.) females Kulikovsky et al. (1996) noted that in seven out of the eight experiments carried out in one year there was no differences in embryo viability in the spawns of Dagin- or carp pituitary-treated fish. However, in the above-quoted paper no statistical significance of differences between the treatments was documented in respect of embryo viability 24 h after fertilization in spite of considerable differences appearing in favour of carp pituitary extract in experiments conducted earlier in that spawning season. The results of experiments conducted in the Gołysz Institute suggest that in testing various ovulation stimulators the time of observations of developing embryos should exceed the period of 24 h after fertilization (if it is possible in a given species). It is essential because after the application of synthetic spawning inducing agents the survival of embryos can decrease considerably in spite of incubation conditions maintained at the most favourable level. Among other cases this was observed in European catfish (*Silurus glanis* L.) after the application of desGly^{10}[D-Ala^{6}]LHRH-ethylamide with primozide (Brzuska and Adamek, 1999) and of [D-Tle^{6}, ProNHEt^{9}] GnRH (Lecirelin) in African catfish *Clarias gariepinus* (Brzuska et al., 2004).

Like some previous studies aimed at the effects of carp reproduction after ovulation stimulation with different preparations (Brzuska and Grzywaczewski, 1999; Brzuska and Białowąs, 2002; Brzuska, 2002, 2004), the present paper deals with the problem of dependence between latency time and spawning success. The results presented here distinctly show that in the latency time of 15 h after the application of Dagin the obtained weight of eggs was statistically significantly lower than the weight of eggs from females spawning two hours later. It is worth stressing that the eggs of fish spawning both 15 and 17 h after the Dagin treatment did not differ either by fertilization percentage or the percentage of living embryos after 48 h incubation.

According to the figure "dependence of latency time on temperature", given by Drori et al. (1994), the latency of 17 h is the time when in water at 21–22°C ovulation can be expected. Earlier studies with the use of Dagin carried out on females of two breeding lines of carp showed that the synchronization of ovulation occurred in one strain only, i.e. Polish line 6 and the latency time was 14 h at the same water temperature. Females of the other breeding line investigated, i.e. Hungarian line W, spawned 17, 19 or 21 h after the Dagin treatment (Brzuska, 2005). It should be noted that both in the present experiment and in earlier studies (Brzuska, 2005) the latency time in carp pituitary homogenate-treated fish was shorter than in Dagin-treated females, this being in agreement with data for the carp (Drori et al., 1994; Kouřil et al., 2003a).

It is striking that after the treatment with both carp pituitary homogenate and Ovopel two periods of spawning are reported: 7 or 9 h after the second application with either of the two preparations. However, in the case of these ovulation stimulators the latency time did not affect the weight or quality of the obtained eggs significantly.

In recapitulating, it seems worth stressing that the experiments conducted in Gołysz with Dagin treatment and three breeding lines of carp (*Cyprinus carpio* L.) gave interesting results, justifying further studies on this spawning inducing agent applied to a higher number of females of various breeding lines.

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