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COMPARING THE EFFECTIVENESS OF OVOPEL, OVAPRIM, AND LH-RH ANALOGUE USED IN THE CONTROLLED REPRODUCTION OF IDE, *LEUCISCUS IDUS* (L.)

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ABSTRACT. The aim of the current work was to compare the effectiveness of Ovopel and Ovaprim separately and combined and LH-RH-a in the controlled reproduction of ide, *Leuciscus idus* (L.). The impact of the chosen hormonal preparations on ovulation, spermiation, and the quality of the gametes obtained was determined. The results confirmed the necessity of stimulating ovulation with hormone complexes and dopamine antagonists. The high effectiveness of both Ovopel and Ovaprim for ovulation stimulation in female ide was also confirmed. The greatest degree of ovulation synchronization was obtained after the application of Ovopel (eggs obtained after 36 h); however, the best results of controlled reproduction were obtained after using Ovaprim and a combination of Ovopel and Ovaprim (82 and 85% embryo survival, respectively). The highest percentage of motile sperm was noted in semen taken from males stimulated with Ovaprim (80%) and LH-RH-a (81%).

Key words: CONTROLLED REPRODUCTION, HORMONAL PREPARATIONS, DOPAMINE INHIBITOR, GNRH, RHEOPHILIC CYPRINIDS

INTRODUCTION

Fish propagation is increasingly dependent on artificial reproduction. New hormonal preparations permit improving techniques for the controlled reproduction of species that have been propagated successfully for many years. It is also possible to apply these hormonal preparations in the reproduction of species that, until recently, were not frequently studied, for example rheophilic cyprinid fishes (Kucharczyk 2002, Szabó et al. 2002, Krejszeff et al. 2008) or species that are under protection (Philippart

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1995, Kamiński et al. 2004). The study of the effectiveness of a given preparation for wider application in fish reproduction takes into consideration its impact on quantitative (number of individuals ready to spawn, working and relative fecundity, ejaculate volume) and qualitative parameters of spawners (post spawning mortality) and gametes obtained (sperm motility, embryo survival). The facility of administering the hormonal stimulation is also a consideration.

Ide, *Leuciscus idus* (L.), primarily inhabits the rivers of Central and Eastern Europe and Asia as far east as the Lena River (Kottelat 1997). Ide is of little economic significance, and is fished in larger quantities only locally. This species plays a significant role in recreational fisheries and in the propagation of ornamental fish. River regulation and pollution have caused declines in the abundance of many ide populations (Kruk 2007). One method for combating declining abundance is to stock this species using materials obtained from reproduction under controlled conditions. Interest in this species in Poland has grown in recent years, and, in consequence, effective controlled reproduction techniques have been developed using various hormonal stimulants during and outside of the natural spawning period (Kucharczyk et al. 1999, Targońska-Dietrich et al. 2004). The aim of the current work was to compare the effectiveness of selected hormonal preparations in controlled ide reproduction during the spawning season.

MATERIALS AND METHODS

Ide spawners weighing from 123 to 1040 g were obtained from the Czarci Jar Hatchery near Olsztynek (northeastern Poland). The fish were transported to the Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn. Prior to stocking the fish into tanks, they were segregated by sex. The tanks the spawners were stocked into had a volume of 1000 dm^3, and were fitted with aeration, temperature control, and photoperiod devices (Kujawa et al. 1999). On the day the spawners were stocked into the tanks the water temperature was 10°C. Hormonal injections were performed after an few days to allow the fish to adapt to hatchery conditions. The photoperiod was constant at 12L:12D throughout the period the fish were held in the tanks. The experiment was conducted during one spawning season.

Prior to the first injection, the fish were tagged and then divided into groups according to the hormonal preparation applied. Stimulation was performed with three prepa-

rations: Ovopel (D-Ala^6, Pro^9-Net-mGnRH) (Unic-trade, Hungary) that was homogenized in a 0.9% NaCl solution (Horváth et al. 1997); Ovaprim (D-Arg^6, Pro^9-Net-sGnRH) (Syndel, Canada); LH-RH-a (Argent, USA). Injections of a saline solution were applied as the control (0.9% NaCl). Females were injected peritoneally beneath the ventral fin at dosages presented in Table 1. After injection, the water temperature in the tanks holding the spawners was raised over the subsequent 12 h to 12°C, following which the fish received the second hormonal injection. The males were only injected when the females received the second injection. Following the second injection the water temperature was raised to 14°C. After a subsequent 30 hours had elapsed from the second injection, monitoring for ovulation began. The females from each group were checked over the subsequent 16 hours at 3 to 4 hour intervals.

TABLE 1

Doses and hormone preparation applied in the reproduction of ide, *Leuciscus idus*

	Control ($cm^3\ kg^{-1}$)	Ovopel (pellets kg^{-1})	Ovaprim ($cm^3\ kg^{-1}$)	Ovopel/Ovaprim (pellets kg^{-1}/$cm^3\ kg^{-1}$)	LH-RH ($\mu g\ kg^{-1}$)
I injection	0.5	0.2	0.5	0.2 Ovopel	0
II injection	0.5	1.0	0.5	0.5 Ovaprim	20

The sex products were stripped from the spawners by gentle massage and pressure on the abdomen. The eggs obtained from each experimental treatment were held in separate plastic bowls. The influence of the stimulants on the biological quality of the gametes from each experimental treatment was determined by taking three egg samples (100-200 grains) and then incubating them in Petri dishes in water at a temperature of 16-18°C. The survival percentage of the embryos was determined on the day the eggs reached the eyed stage. The biological quality of the semen was determined based on sperm motility. Samples were collected from the each of the specimens with separate syringes 30 hours following injection. The samples were held on ice and transported to the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, where sperm motility was analyzed according to methods described by Glogowski et al. (1999) for bream, *Abramis brama* (L.).

The remaining eggs were fertilized with the dry method with milt collected from several males and then covered with water. The adhesiveness was removed from the eggs with Woynarovich fluid (40 g urea + 35 g NaCl in 10 dm^3 water) and a short bath in tannic acid solution (6 g dm^{-3}). The treated eggs were placed in Weiss jars and incu-

bated at a temperature of 16-18°C. The water flow rate in the jars was set at 1.5-2 dm^3 min^{-1}, which allowed the incubating eggs to circulate freely. All manipulations of the fish were performed after the fish had been anesthetized in a bath of 0.5 cm^3 dm^{-3} solution of 2-phenoxethanol (Sigma-Aldrich, Germany).

Sperm motility and embryo survival to the eyed stage were analyzed with analysis of variance (ANOVA), and the post hoc test applied to significant values was Duncan's test ($P < 0.05$).

RESULTS

Ovulation was confirmed in 95% of the females stimulated with Ovopel and in 100% of the fish stimulated with Ovaprim or a combination of these two preparations (Table 2). The percentage of females that ovulated in the group stimulated with LH-RH-a was barely 20%. The time between the first injection and ovulation in the various treatments ranged from 36 and 44 hours. The most synchronized ovulation was noted in the group stimulated with Ovopel. The females injected with Ovaprim exhibited the greatest ovulation distribution. No gametes were obtained from the control group of females. Significant differences were confirmed in the mean survival of the embryos to the eyed stage ($P < 0.05$). The lowest survival was noted in the group stimulated with Ovopel.

TABLE 2

Effects of artificial reproduction of ide, *Leuciscus idus*, after the application of selected hormonal preparations (mean ± SD)

	Control	Ovopel	Ovaprim	Ovopel/Ovaprim	LH-RH
Number of females	20	20	20	20	20
Ovulation (%)	0	95	100	100	20
Latency time (h)	-	36	36-44	38- 42	38-42
Survival to eyed-egg stage (%)*	-	66 ± 6^b	82 ± 6^a	85 ± 4^a	83 ± 5^a

** The data with the same letter index in the same rows did not differ statistically ($P > 0.05$)*

Semen was obtained from all the experimental treatment groups of males (Table 3). Stimulation with Ovopel did not have a substantial impact on the motility of the sperm in comparison with the control group. However, the semen obtained from males stimulated with Ovaprim and LH-RH-a exhibited a statistically significantly higher percentage of motile sperm ($P < 0.05$).

TABLE 3

Results obtained after stimulating males ide, *Leuciscus idus* with selected hormonal preparations (mean ± SD)

	Control	Ovopel	Ovaprim	LH-RH
Number of males	15	15	15	15
Spermiation (%)	100	100	100	100
Sperm motility (%)*	66 ± 6^{a}	67 ± 6^{a}	80 ± 5^{b}	81 ± 6^{b}

** Data in rows with the same letter index do not differ significantly statistically (P > 0.05)*

DISCUSSION

The growing demand for the stocking material requires improvement in the stimulation and control of spawning of many species, while for other species these techniques must be developed from scratch. Hormonal stimulation in fish can happen in the hypothalamus, pituitary gland, or the gonads. The method of using a pituitary gland homogenate from carp, *Cyprinus carpio* (L.), spawners that dates to the early 1970s and then later from bream is based on introducing exogenous gonadotropin to the target organism (Yaron 1995). This method is not problem free since the success of the spawning is dependent on the quality of the hyophysate and its hormone concentration, which makes determining the appropriate dosage difficult. The necessity of increasing the effectiveness of controlled reproduction for both economic and practical reasons provided the impetus for the search for more optimal methods. Human chorionic gonadotropin (hCG) has been used to stimulate fish reproduction as has the synthetic GnRH analogue (Kucharczyk 2002). Since the excretion of gonadotropin in many fish species from the pituitary can be inhibited by dopamine (Peter and Yu 1997, Mylonas and Zohar 2000), it became necessary to administer dopamine antagonists (metoclopramide, domperidone) along with the hormones (Brzuska 1999, Szabó et al 2002). But this complicated the administration of the injections. The breakthrough in controlled reproduction came with the appearance on the market of mixed preparations of GnRH analogues (mammalian or fish) and dopamine antagonists that affect endogenic gonadotropin. In addition to their complex effects, these new preparations are in granulated or fluid forms that are easier to administer.

The application and usefulness of Ovopel in controlled fish reproduction has been confirmed in numerous scientific studies (Brzuska and Grzywaczewski 1999, Szabó et al. 2002, Kucharczyk et al. 2005). Ovaprim is a new preparation in Polish aquaculture

and reports on its effectiveness in fish reproduction are still few (Kucharczyk et al. 2007, Kujawa et al. 2007). This preparations is, however, applied successfully in Asian countries (Das 2004) and in the USA (Viveiros et al. 2002).

The results of the hormonal stimulation of ide females in the current work confirm the effectiveness and usefulness of both Ovopel and Ovaprim for the controlled reproduction of ide, and were similar to the results reported by Kucharczyk et al. (1999) for a wild population (79.3% embryonic survival to the eyed stage), in which stimulation with Ovopel produced better reproduction results in comparison with stimulation with carp pituitary gland homogenate and hCG. Additionally, these were similar to the results obtained by Targońska-Dietrich et al. (2004) for cultured stocks from ponds and those reared in recirculating systems (84.1 and 68.1% embryonic survival, respectively). It is noteworthy that the results obtained in the current work following stimulation with Ovaprim, including embryonic survival and semen parameters, are higher than with the application of Ovopel. This is also confirmed by Kucharczyk et al. (2007). While the time until ovulation was longer in the groups injected with Ovaprim and asynchronization in female spawning readiness was the highest, embryonic survival was 20% higher and sperm motility was over 12% higher in these groups. It is noteworthy that the application of a combination of Ovopel and Ovaprim produced gametes of the highest biological quality and greater ovulation synchronization in comparison with the group injected only with Ovaprim. Similar dependencies were noted by Żarski et al. (unpublished data) during the controlled reproduction of ide and dace, *Leuciscus leuciscus* (L.). The results obtained regarding the analyzed quality parameters of the gametes confirm the greater effectiveness of the Ovopel and Ovaprim combination in comparison to results reported in earlier studies (Kucharczyk et al. 1999, Targońska-Dietrich et al. 2004). Szabó et al. (2002) noted that the stimulation of nase, *Chondrostoma nasus* (L.), with pure GnRH analogue did not produce satisfactory reproduction results. In the current work, the administration of LH-RH-a was similarly unsatisfactory. It was observed, however, that administering LH-RH-a to induce spermiation in males resulted in the highest percentage of motile sperm, which was comparable to the results obtained following the application of Ovaprim.

The results obtained confirm the possibility of obtaining much better results from the controlled reproduction of ide after administering the Ovaprim preparation. This is indicated by the high biological quality of the gametes obtained. Greater ovulation syn-

chronization after applying this preparation might be possible if the first injection is of Ovopel. However, to achieve fully satisfactory results from controlled reproduction with the stimulant Ovaprim further studies are required that take into account other factors that influence the success of controlled fish reproduction.

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STRESZCZENIE

PORÓWNANIE SKUTECZNOŚCI ZASTOSOWANIA OVOPELU, OVAPRIMU ORAZ ANALOGU LH-RH W KONTROLOWANYM ROZRODZIE JAZIA, *LEUCISCUS IDUS* (L.)

Celem niniejszej pracy było porównanie skuteczności stymulacji owulacji u samic jazia, *Leuciscus idus* (L.) przy zastosowaniu Ovopelu, Ovaprimu i ich kombinacji oraz LH-RH-a. Ponadto określono przydatność tych preparatów do stymulacji spermacji po zastosowaniu pojedynczej iniekcji. Wyniki potwierdziły konieczność stymulacji owulacji za pomocą kompleksów „hormon + antagonista dopaminy". Potwierdzono tym samym wysoką przydatność zarówno Ovopelu, jak i Ovaprimu do stymulacji owulacji samic jazia. Największą synchronizację owulacji uzyskano po zastosowaniu Ovopelu, gdzie ikrę od wszystkich samic pozyskano po 36 h. Jednakże najlepsze rezultaty kontrolowanego rozrodu odnotowano po zastosowaniu Ovaprimu oraz kombinacji Ovopelu z Ovaprimem (odpowiednio 82 i 85% przeżywalności embrionów). Największym odsetkiem ruchliwych plemników charakteryzowało się nasienie pobrane od samców stymulowanych zarówno Ovaprimem (80%) jak i LH-RH-a (81%).