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Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue

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Abstract The aim of the present study was to study spawning stimulation in artificial reproduction of females pikeperch (*Sander lucioperca* L.) using "Chorulon" containing the human chorionic gonadotropin (hCG) and compare with "Supergestran" containing a mammalian GnRH ([D-Ala^6]GnRHProNhet) analogue. The females were divided into eleven experimental groups and injected with hCG at 250, 500, 750, and 1 000 IU kg^{-1} body weight (BW) and mGnRHa at 1, 2.5, 5, 10, 25, and 50 μg kg^{-1} BW. In all treatments, a single intramuscular injection of hormone was performed. Control group was injected with 0.9 % NaCl, 0.9 cm^3 kg^{-1} BW. The average percentages of ovulating females were 88.5 ± 12.3 and 80.8 ± 10.9 % in hCG- and mGnRHa-treated groups, respectively. The average diameter of eggs was 0.95 ± 0.06 and 0.98 ± 0.06 mm in hCG- and mGnRHa-treated groups, respectively. Neither ovulation rate nor diameter of egg was statistically differed among hormonally treated groups. Statistical difference was observed only in hatching rate, where the average were 73.6 ± 14.4 and 50.6 ± 17.7 % in hCG and mGnRHa-treated groups, respectively. Among hormonally treated groups, the best results were observed in groups treated with hCG at 500 and 750 IU kg^{-1} and in groups treated with mGnRHa at 25 μg kg^{-1}. No ovulation was observed in the control group. This study indicated successful ovulation in pikeperch using a single intramuscular injection of hCG or mGnRHa analogue.

Keywords Chorulon · Egg size · Fecundity · Hatching rate · *Sander lucioperca* · Supergestran

Introduction

Pikeperch (*Sander lucioperca* L.) is a highly valuable commercial fish for inland European aquaculture (Hilge and Steffens 1996), which has an acceptable growth rate to market size

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under intensive culture (Fontaine 2009). Up to now, most market size pikeperch come from open waters (lakes, rivers, ponds, or lagoons) and relatively few are produced in fish farms under intensive and indoor conditions (FAO 2007). The stable mass production of fry for open waters or fish farms is not available yet, because the artificial reproduction of pikeperch, particularly reproductive physiology of broodfish, is not still well developed (Philipsen 2008). Therefore, the studies of optimum methods for artificial reproduction with emphasizes on broodfish reproductive physiology to achieve stable mass production of fry and grow up them to market size are still needed.

Spawning induction using hormonal injections has been frequently used to synchronize maturation of gametes (sperm or eggs) in different fish species (Bromage et al. 1992; Zohar and Mylonas 2001). There are a few studies reporting spawning induction in pikeperch using injection of carp pituitary containing gonadotropins, synthetic analogues of GnRH, human chorionic gonadotropin (hCG), or LHRH-a (Antalfi 1979; Schlumbenger and Proteau 1996; Craig 2000; Kouril and Hamackova 2005; Zakes and Demska-Zakes 2005; Rónyai 2007). Recently, a commercial Czech veterinary product approved for the use in ruminants called "Supergestran" has been successfully used in artificial propagation of perch (Kouril and Hamackova 1999, Policar et al. 2008), but the efficiency of Supergestran has not been studied in other percid fishes. The Supergestran contains mammalian GnRH ([D-Ala6]GnRHProNhet) analogue. The Chorulon has been used for artificial reproduction of African catfish (*Clarias gariepinus*) (Richter et al. 1987; Mollah and Tan 1983), rainbow sharks (*Labeo erythrurus*) (Shireman and Gildea 1989), redtail black sharks (*Labeo bicolor*) (Shireman and Gildea 1989), and of pufferfish (*Tetraodon nigroviridis*) (Watson et al. 2009).

Therefore, the present study was conducted to compare efficiency of the "Chorulon" containing hCG and the Supergestran containing mGnRHa in induction of ovulation in pikeperch under controlled conditions.

Materials and methods

Broodstock, transport, manipulation, and culture conditions

Broodfish of pikeperch were obtained from Fish farm (Rybářství Třeboň a.s., Czech Republic) during the first half of March 2010. Fish were harvested from a pond at the same time. Fish were placed in 2-m^3 plastic tank (water characteristics were: pH, 7.2 ± 0.2; oxygen saturation, 90.5 ± 4.2 %) and transferred by truck and kept in storage pond at the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB, FFPW) and fed forage fish *Pseudorasbora parva* (Total length = 40–60 mm; weight = 0.3–0.4 g) until the spawning season (April). Before the spawning season, broodfish were harvested from storage pond and sexually separated based on fish shape and genital papilla characteristics. The males (50 individuals) and females (77 Individuals) were separately kept in tank (6 m^3) of a recirculating aquaculture system (RAS) at USB, FFPW. At the time of spawning, females were divided into eleven groups and kept in 1-m^3 tanks ; each group contains seven individuals. Water characteristics were as follows: pH, 7.2 ± 0.2; oxygen saturation, 80.4 ± 3.1 %; flow rate, 12.5 L min^{-1}; temperature, 15 ± 0.5 °C; ammonia concentration, <0.02 mg L^{-1}; nitrite, <0.02 mg L^{-1}; nitrate, <0.05 mg L^{-1}; and photoperiod, 14L:10D. Water temperature was measured four times daily with an auto-recording thermometer (model RT-F5x, QiAnatytical Ltd., Czech Republic). Oxygen saturation (%) and pH were measured twice daily (06:30 and 14:00 h) with a combined pH and oxygen

meter (MultiLine P4, WTW). Other water quality parameters (nitrite, nitrate, and ammonia levels) were weekly evaluated at the chemical laboratory of USB, FFPW.

Hormonal stimulation

Before hormonal stimulation, females were tagged with PIT tags (AEG Identifikationssysteme GmbH (AEG ID), Ulm, Germany). To have no significant differences in fish size among treated groups, body weight (BW) was recorded. Maturity stage of the oocytes from each female was determined according to the method described by Kucharczyk et al. (2007). Then, females were intramuscularly under dorsal fin injected with the Chorulon containing hCG (Intervet, the Netherlands) or the Supergestran containing mGnRHa (Lecirelin (D-Tle6) GnRH*m* ProNHEt) (FERRING LÉČIVA, Czech Republic) as described by (Policar et al. 2008; Kouril et al. 2007). The biological characteristics of females in different hormonally treated groups as well as doses of injection are presented in Table 1. All individuals were anesthetized in clove oil water bath 0.03 ml L^{-1} (Dr. Kulich Ltd.) for 10 min before manipulation (Hamackova et al. 2001).

Stripping of broodfish: absolute and relative fecundity and size of eggs

Two days after hormonal treatment, females were controlled each hour by a gentle massage of the abdominal cavity. Females showing ovulation were immediately transferred to clove oil water bath, and eggs were stripped by gentle massage along abdominal cavity. Obtained eggs were weighted using a balance (PCB 1000-2, Kern, Germany with accuracy of 0.01 g) from each individual. Then, three small samples (approximately 1 g) were randomly selected and weighed using balance (ALJ 220-4, Kern, Germany with accuracy of 0.0001 g) for determination of absolute fecundity (the total number of eggs) and relative fecundity (total number of eggs per 1 kg). To measure size of eggs, pictures were taken from egg samples (100 eggs in each group) using Olympus E-510 digital camera mounted on binocular microscope Olympus BX51. Data were then collected using Quick PHOTO CAMERA 2.2 software (Olympus, Hamburg, Germany).

Table 1 Details of hormonally treated groups of pikeperch (*Sander lucioperca* L.) used in the present study

Group	Number of females	Mean ± SD body weight (g)	Treatment	Dose
Group 1	7	1,202 ± 259	hCG (IU kg^{-1} BW)	250
Group 2	7	1,188 ± 199	hCG (IU kg^{-1} BW)	500
Group 3	7	1,204 ± 295	hCG (IU kg^{-1} BW)	750
Group 4	7	1,309 ± 359	hCG (IU kg^{-1} BW)	1 000
Group 5	7	1,265 ± 565	mGnRHa (μg kg^{-1})	1
Group 6	7	1,104 ± 384	mGnRHa (μg kg^{-1})	2.5
Group 7	7	1,212 ± 392	mGnRHa (μg kg^{-1})	5
Group 8	7	1,268 ± 396	mGnRHa (μg kg^{-1})	10
Group 9	7	1,238 ± 412	mGnRHa (μg kg^{-1})	25
Group 10	7	1,283 ± 197	mGnRHa (μg kg^{-1})	50
Group 11	7	1,247 ± 282	0.7 % NaCl	

There was no significant difference in body weight

In vitro fertilization

To study whether different hormone affect fertilization rate of obtained eggs, in vitro fertilization test was performed on collected eggs. Sperm of 4 males was collected with a syringe after bathing in clove oil (Hamackova et al. 2001). Genital papilla of males was firstly dried to avoid sperm contamination by water, urine, and blood. Sperm quality was then tested after activation in hatchery water under microscope (Alavi et al. 2009). Samples with 90 % of motile spermatozoa were chosen for fertilization test. The sperm from three males (without hormonal injection) was directly added into the batches of eggs (50 μL of sperm to 100 eggs), and then hatchery water was added and mixed for 2 min. After fertilization, the eggs were washed to remove adhesiveness of the fertilized eggs with milk and talc (Gela et al. 2003). Then, three samples from each female, each contains 100 eggs, were separately incubated in experimental small cages (water temperature: 14 ± 0.7 °C and oxygen saturation: 88.5 ± 2.9 %). The incubation cages were connected to the re-circulating system. The hatching rate was determined as follows:

$$\text{Hatching rate} = (\text{Hl}/\text{Te}) \times 100$$

where "Hl" is the number of hatched larvae and "Te" is number of eggs at the beginning of fertilization.

Statistical analysis

The reproductive performances of the fish were evaluated with program Statistica 9.0 (StatSoft, Inc., Czech Republic). Reproductive parameters (latency time, relative and absolute fecundity, size of eggs, and hatching rate) were statistically analyzed by one-way analyses of variance, ANOVA ($P < 0.05$) followed by the post hoc Tukey's multiple-comparison tests. Percentage data were transformed prior to analyses with arcsin function. Differences between hormonal preparations were statistically analyzed by one-way analyses of variance ANOVA ($P < 0.05$) by the post hoc mean comparisons (Unequal N HSD comparison test).

Results

Ovulation rate, latency time, and hatching rate

The all females had oocytes exclusively in maturity stage II or in stages III and IV. The highest ovulation rate (100 %) was observed in females treated with mGnRHa (25 μg kg^{-1}) or with hCG (500 and 750 IU) (Table 2). The average percentages of ovulated females were 88.5 ± 12.3 % in the hCG-treated groups and 80.8 ± 10.9 % in mGnRHa-treated groups. Ovulation rate did not differ among hormonally treated groups, statistically. The latency time ranged from 78.05 to 89.29 h after injection (Table 2), but no significant difference was observed among hormonally treated groups. Hatching rate showed significant differences among treated groups (Table 2). The highest hatching rate was observed in groups stimulated with hCG at 500 and 750 IU. The average hatching rate was 73.6 ± 14.4 and 50.6 ± 17.7 % in hCG- and mGnRHa-treated groups, respectively.

Table 2 Ovulation rate, latency time, and hatching rate in females of pikeperch (*Sander lucioperca* L.) treated with single intramuscular injections of Chorulon containing human chorionic gonadotropin (hCG) and Supergestran containing mammalian GnRH without dopamine inhibitor

Group ($n = 7$)	Ovulation (%)	Latency time (h)	Hatching rate (%)
hCG 250 IU	71	84.98 ± 9.89	70.9^{bc} ± 4.1
hCG 500 IU	100	78.05 ± 6.93	84.2^{c} ± 6.2
hCG 750 IU	100	78.59 ± 7.48	86.8^{c} ± 3.8
hCG 1 000 IU	83	88.0 ± 12.05	52.5^{b} ± 4.5
mGnRHa 1 μg kg^{-1}	86	89.29 ± 11.73	52.3^{b} ± 5.5
mGnRHa 2.5 μg kg^{-1}	71	84.42 ± 11.46	65.5^{b} ± 2.5
mGnRHa 5 μg kg^{-1}	71	83.90 ± 21.64	51.1^{b} ± 3.6
mGnRHa 10 μg kg^{-1}	71	79.4 ± 9.24	52.2^{b} ± 4.5
mGnRHa 25 μg kg^{-1}	100	83.0 ± 9.13	60.5^{b} ± 6.4
mGnRHa 50 μg kg^{-1}	86	86.46 ± 27.67	22.10^{a} ± 7.2
Control	n.d.	n.d.	n.d.

Data are shown as mean ± SD

n.d. values are not determined due to no ovulation of females

There was no significant difference in latency period among treated groups

Values within the column with different superscripts are significantly different ($P < 0.05$)

Weight of eggs, relative fecundity, absolute fecundity, and size of eggs

The percentage of weight of eggs was similar among treated groups and ranged from 5.22 % observed in group treated with mGnRHa at 50 μg kg^{-1} to 10.89 % observed in group treated with mGnRHa 2.5 μg kg^{-1} (Table 3). No significant differences were found between groups in terms of relative fecundity (ranged from 88.0 to 144.6 × 10^3 eggs kg^{-1}) and absolute fecundity (ranged from 164.6 to 208.3 × 10^3 eggs) (Table 3). Size of non-fertilized eggs did not also differ among treated groups and ranged from 0.917 to 1.012 mm. Average diameter of eggs was 0.949 ± 0.06 mm in the hCG-treated groups and 0.976 ± 0.057 mm in the mGnRHa-treated groups.

Discussion

The present study showed successful ovulation induction in pikeperch hormonally treated with a single intramuscular injection of Supergestran and Chorulon. All females were determined in stages II, III, IV before injection, and during the ovulation were also observed stages V and VI (Zarski et al. 2012). The ovulation rate observed in the present study (88.5 and 80.8 % in the hCG- and mGnRHa-treated groups) is similar to those of Zakes and Demska-Zakes (2005) and Rónyai (2007), where two injections were performed for stimulation of ovulation. Therefore, a single injection is suggested to reduce stress in females due to hormonal treatment, which may affect the quality of ovulated eggs (Schreck et al. 2001).

It is shown that Supergestran, containing mGnRHa, could be introduced for artificial reproduction of fish in hatcheries as has been used for induction of ovulation in perch (Policar et al. 2008). In the present study, fully ovulated females of pikeperch were observed when hCG at 500–750 IU kg^{-1} was injected. Very similar ovulation rate (93 %)

Table 3 Weight of eggs, relative fecundity, absolute fecundity, size of eggs in females of pikeperch (*Sander lucioperca* L.) treated with single intramuscular injections of Chorulon containing human chorionic gonadotropin (hCG) and Supergestran containing mammalian GnRH without dopamine inhibitor

Group	Percentage eggs (% of female BW)	Relative fecundity (number of eggs $\times 10^3$ per 1 kg)	Absolute fecundity (number of eggs $\times 10^3$)	Size of egg (mm)
hCG 250 IU	$8.08^a \pm 1.3$	$88.7^a \pm 18$	$180.7^a \pm 99$	$1.011^a \pm 0.6$
hCG 500 IU	$7.00^a \pm 3.0$	$101.3^a \pm 23$	$189.2^a \pm 64$	$0.971^a \pm 0.5$
hCG 750 IU	$9.21^a \pm 5.3$	$116.7^a \pm 58$	$181.6^a \pm 89$	$0.982^a \pm 0.5$
hCG 1 000 IU	$6.51^a \pm 2.6$	$106.1^a \pm 45$	$168.8^a \pm 50$	$0.946^a \pm 0.5$
mGnRHa 1 μg kg^{-1}	$9.26^a \pm 2.8$	$120.1^a \pm 64$	$208.3^a \pm 86$	$0.928^a \pm 0.5$
mGnRHa 2.5 μg kg^{-1}	$10.89^a \pm 1.8$	$88.0^a \pm 82$	$124.5^a \pm 73$	$0.949^a \pm 0.4$
mGnRHa 5 μg kg^{-1}	$9.52^a \pm 6.2$	$133.9^a \pm 60$	$175.0^a \pm 78$	$1.012^a \pm 0.6$
mGnRHa 10 μg kg^{-1}	$7.93^a \pm 2.5$	$142.3^a \pm 79$	$168.7^a \pm 86$	$0.978^a \pm 0.2$
mGnRHa 25 μg kg^{-1}	$7.58^a \pm 3.9$	$144.6^a \pm 82$	$196.0^a \pm 80$	$0.920^a \pm 0.5$
mGnRHa 50 μg kg^{-1}	$5.22^a \pm 3.5$	$127.6^a \pm 67$	$164.6^a \pm 82.6$	$0.917^a \pm 0.1$
Control	n.d.	n.d.	n.d.	n.d.

Data are shown as mean ± SD

n.d. values are not determined due to no ovulation of females

There was no significant difference in any measured parameters among treated groups

has been reported at 700 IU kg^{-1} (Kucharczyk et al. 2008). It is well known that hCG directly acts at the level of gonads and does not require the existence of LH stores or activation of the pituitary gonadotropins (Zohar and Mylonas 2001). The higher cost of Chorulon preparation is a disadvantage compared with that of Supergestran. Better ovulation rate, spawning synchronization, and hatching rate were observed in treated group with Chorulon (hCG) than with Supergestran, but it is possible that the treated females do not response to hormonal treatment in the next spawning season (Van der Kraak et al. 1989; Watanabe et al. 1998). This is probably due to the large size of the GtH molecule and its heterogenous nature, which may consequently result in inducing the immune response of some fish species. (Mylonas and Zohar 1997). It is also shown that that low dose of mGnRHa (25 μg kg^{-1}) could induce ovulation in a substantial number of pikeperch. This is lower than the dose that has been used for ovulation in perch (50–125 μg kg^{-1}) (Policar et al. 2008; Kouril and Hamackova 1999). Schlumbenger and Proteau (1996) also showed ovulation induction in pikeperch at 100 μg kg^{-1} mGnRHa. In the control group, no ovulated female was observed, suggesting no possible effects pheromones in ovulation of pikeperch (Rónyai 2007).

Several studies show shorter latency period in females treated with hormones acting on gonads (such as hCG, carp pituitary) compared with hormones acting on brain (GnRHa) (Zohar and Mylonas 2001). On the other hand, no differences were found between hCG- and mGnRH-treated groups in this study. Latency time in the hCG-treated groups was similar to the results reported by Rónyai (2007), who performed two injections. Nevertheless, latency time in the mGnRH was also similar to the results described by Policar et al. (2008), who used only one injection.

The relative fecundity (from 88.0 to 144.6 $\times 10^3$ eggs kg^{-1}) of pikeperch obtained in this study is lower compared with Lappalainen et al. (2003) (250 $\times 10^3$ eggs kg^{-1}) and to Schlumbenger and Proteau (1996) (200 $\times 10^3$ eggs kg^{-1}). The absolute fecundity (from

164.6 to 208.3 $\times$ 10^3 eggs) of pikeperch recorded in this study is similar to the results reported by Demska-Zakes and Zakes (2002) and Lappalainen et al. (2003). Also, size of eggs showed no difference with the previous studies (Horvath et al. 1984; Schlumbenger and Proteau 1996; Lappalainen et al. 2003). The size of unfertilized eggs is very constant and not responded results corresponded by Demirkalp (1992) and Barus and Oliva (1995), which reported ranged size of unfertilized eggs (0.5–1.4 mm).

In conclusion, our study on pikeperch demonstrated that a single hormonal injection of Chorulon containing hCG or Supergestran containing mGnRHa could be used for induction of ovulation. Although we observed better efficiency of hCG compared with that of mGnRHa, further studies are needed to look at reproductive status of broodfish in the next spawning season. This study showed the highest quality of eggs at 500 and 750 IU kg^{-1} hCG and in result at 25 μg kg^{-1} mGnRHa. The Supergestran is suggested to be used in fish farm due to lower price and its availability in the Czech Republic. Further studies are required to optimize protocols for using Supergestran in artificial reproduction of pikeperch via looking at physiological functions of hypothalamus–pituitary–gonad axis.

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