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ARTIFICIAL SPAWNING OF PIKEPERCH (*SANDER LUCIOPERCA* (L.)) STIMULATED WITH HUMAN CHORIONIC GONADOTROPIN (HCG) AND MAMMALIAN GNRH ANALOGUE WITH A DOPAMINE INHIBITOR

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ABSTRACT. The aim of the experiment was to identify the possibilities of stimulating pikeperch spawning with human chorionic gonadotropin (hCG) and Ovopel, a mammalian GnRH analogue (D-Ala^6 Pro^{9}NEt-mGnRH) with a dopamine inhibitor (metoclopramide). Pikeperch spawners were caught with trap gear (fyke-nets) during the pre-spawning season from the Tałty and Tałtowisko lakes (Masurian Lakeland, northern Poland). After transport that lasted an hour, the fish were placed in tanks in a recirculating system and then segregated by sex. The females were divided into five experimental groups, each containing six specimens. The fish were injected twice at 24 hour intervals with hCG (group I – 200 and 200 IU kg^{-1} body weight (BW); group II – 200 and 500 IU kg^{-1} BW), Ovopel (group III – 0.25 and 0.50 pellets kg^{-1} BW; group IV – 0.25 and 1.0 pellets kg^{-1} BW) or 0.7% NaCl solution (group V, control – 0.2 and 0.5 cm^3 kg^{-1} BW). The effects of the hormonal stimulation expressed as the percentage of ovulating females, the degree of spawning synchronicity, and survival of the embryos to the eyed-egg stage were highly differentiated. The highest percentages of spent fish were obtained in the group stimulated with hCG – 83.3% (group I) and 100% (group II) of the females ovulated. The development of the oocytes in this group was rapid and synchronous, which was reflected in the shortened and relatively similar latency period (47-57 hours following the first injection; mean ≈ 51 hours) in individual females. No impact was noted with regard to hCG dose (400 vs. 700 IU kg^{-1} BW) on the latency time or on egg quality. Ovopel did not positively affect either oocyte maturation or pikeperch ovulation. None of the fish from group III ovulated, and in group IV, as in the control group, eggs were obtained only from three (50%) females. In contrast to group V, the eggs of females stimulated with Ovopel were of low biological quality and survival to the eyed-egg stage ranged from 0 to 8%. Higher mortality among the females was also noted, especially in group III. The experiment indicated that hormonal stimulation with hCG is effective, while that with Ovopel was surprisingly ineffective.

Key words: PIKEPERCH (*SANDER LUCIOPERCA*), SEMEN, OOCYTES, ARTIFICIAL SPAWNING, HORMONAL STIMULATION, HCG, OVOPEL

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INTRODUCTION

In recent years, significant progress has been made in the development of the artificial spawning of many fish species (Bromage et al. 2001, Lee and Donaldson 2001). Familiarity with the endocrinological processes of fish has permitted developing effective methods for hormonally stimulating gonad maturation as well as spawning itself (Bieniarz and Epler 1991, Peter et al. 1991, Zohar and Mylonas 2001, Epler and Popek 2002). Percid fishes have recently become the subject of intense research into developing artificial spawning methods and other techniques (Kestemont and Mélard 2000). Yellow perch, *Perca flavescens* (Mitch.), and walleye, *Sander vitreus* (Mitch.), have been the subject of recent research on hormonally stimulated gonad maturation and of investigations of endocrinological processes and the impact hormonal stimulation has on them (among other researchers, Dabrowski et al. 1994, Barry et al. 1995, Malison and Held 1996). Research has also been conducted on the hormonally stimulated reproduction of pikeperch, *Sander lucioperca* (L.) (Schlumberger and Proteau 1996, Steffens et al. 1996); however, the pool of knowledge on this topic is decidedly smaller.

Research conducted in recent years has indicated that gonadotropin (GtH), and specifically human chorionic gonadotropin (hCG), might be successfully applied in the artificial reproduction of pikeperch. Although mention was made in earlier publications regarding the positive impact of hCG (Steffens et al. 1996), the data presented was only of a preliminary character. Studies conducted at the Inland Fisheries Institute in Olsztyn were the first to indicate that this hormone can be used successfully to both synchronize the spawning of pikeperch in lake cages (Demska-Zakęś and Zakęś 2002), as well as to induce out-of-season spawning in this species (Zakęś and Szczepkowski 2004). Currently, a commercial product known as Ovopel, which contains mammalian GnRH analogue with the dopamine inhibitor metoclopramide (Horváth et al. 1997), is gaining popularity as a preparation for synchronizing the spawning of many fish species, especially cyprinids. This preparation has proven to be very effective in stimulating the reproduction of carp, *Cyprinus carpio* L., silver carp, *Hypophthalmichthys molitrix* (Val.), grass carp, *Ctenopharyngodon idella* (Val.), tench, *Tinca tinca* (L.) (Horváth et al. 1997, Kłodzińska and Okoniewski 1998, Brzuska and Grzywaczewski 1999), as well as European catfish, *Silurus glanis* L. (Brzuska 2001, 2003, Ulikowski

2004) and African catfish, *Clarias gariepinus* (Burchell) (Brzuska et al. 1998, Brzuska 2004). Gonadotropin releasing hormone (GnRH), and especially the super active GnRH (GnRHa), have characteristics which can make them more effective in inducing gamete maturation than, for example, gonadotropic preparations (details in the paper by Zohar and Mylonas 2001).

The aim of the current experiment was to determine the possibilities of conducting the artificial spawning of wild pikeperch obtained from lakes during the pre-spawning period with the application of human chorionic gonadotropin (hCG) and mammalian GnRH analogue (Ovopel).

MATERIALS AND METHODS

SPAWNERS, TRANSPORT, MANIPULATION

The pikeperch spawners were obtained using trap gear (fyke-nets) in the pre-spawning season (late April) from Tałty and Tałtowisko lakes (Masurian Lakeland, northern Poland). The fish were transported within the hour in bags with oxygen (20 dm^3 water + 20 dm^3 oxygen; one fish per bag) to the Dgał Experimental Hatchery of the Inland Fisheries Institute (IFI) in Olsztyn (northern Poland). The anti-stress agent Propiscin (IFI Olsztyn, Polska; Kazuń and Siwicki 2001) was added to each bag at a concentration of 0.02 cm^3 dm^{-3}. Following transport, the spawners were transferred to tanks that were part of a recirculating system. Water temperature in the tanks (approximately 10.0°C) was close to that during transport. The fish were sorted by sex (males were identified by the appearance of semen when gentle pressure was applied to the abdomen), and body weight (BW ± 10.0 g) was determined. All manipulations were conducted after the fish had been anesthetized with Propiscin at a concentration of 2.0 cm^3 dm^{-3}. The females (30 individuals) were divided into five groups of six each. The various groups of females and males were held in separate tanks (volume 1.0 m^3) that were part of the same recirculating system. The average body weight of the females from the various groups ranged from 2.33 to 2.82 kg, while the average weight of the males was 1.33 kg (Table 1).

TABLE 1

Body weight of spawners, hormonal treatments, and the doses applied in the artificial spawning of wild pikeperch. Time interval between subsequent injections – 24 h

Group	Number of females	Body weight (kg)		Treatment	First injection	Second injection
		Mean	Range			
Group I	6	2.63	1.76 – 4.52	hCG (IU kg^{-1} BW)	200	200
Group II	6	2.43	1.90 – 3.32	hCG (IU kg^{-1} BW)	200	500
Group III	6	2.56	2.02 – 3.40	Ovopel (pellet kg^{-1} BW)	0.25	0.50
Group IV	6	2.33	1.73 – 3.20	Ovopel (pellet kg^{-1} BW)	0.25	1.00
Group V (control)	6	2.82	2.10 – 4.40	0,7% NaCl (cm^3 kg^{-1} BW)	0.20	0.50
Males	16	1.33	0.88 – 2.63	-	-	-

DETERMINING THE MATURITY STAGE OF OOCYTES AND HORMONAL STIMULATION

After the females had been anesthetized (2.0 cm^3 Propiscin dm^{-3}), oocyte samples were taken from each fish with a catheter (Brzuska and Bieniarz 1977). They were placed in glass test tubes with a clearing solution (Serra fluid – ethyl alcohol 96% : formalin : glacial acetic acid, 6:3:1, v/v). These oocytes were then moved to a glass plate and the maturity stage was determined macroscopically on a sample of 30 oocytes based on a four-stage maturity scale. The main evaluation criteria were the location of the germinal vesicle (GV) and the coalescence of the oil droplet. Oocytes classified as maturity stage I had GV in a central position and many small oil droplets. The germinal vesicle of stage II oocytes had shifted less than a half of the radius, and those with GV positioned on the periphery (near the egg membrane) were classified as stage III (in which the oil droplet has coalesced). Oocytes without GV, in which the process of GV breakdown (GVBD) had begun, were classified as stage IV. The maturity of the oocytes was checked every 24 h, with the first check prior to the first injection.

After determining the maturity stage, the fish were given intraperitoneal injections. The fish from the experimental group were stimulated with the hCG (Biogonadyl®, Biomed, Lublin, Poland) or mammalian GnRH analogue with a dopamine inhibitor (Ovopel – 1 pellet of an average weight of 25 mg contained 18-20 μg D-Ala^6 Pro^9NEt-m GnRH and 8-10 mg metoclopramide, Horváth et al. 1997). The fish were injected twice at 24 h intervals. Females from group I received a total of 400 IU hCG kg^{-1} BW and those from group II – 700 IU hCG kg^{-1} BW (Table 2). The spawners from groups III and IV were stimulated with Ovopel at respective doses of 0.75 pellet kg^{-1} BW or 1.25 pellet

kg^{-1} BW. The control group of females (group V) was injected twice with a 0.7% saline solution (0.2 and 0.5 cm^3 NaCl kg^{-1} BW; Table 1). Following the first injection, the water temperature was raised over 12 hours to 14.5°C.

DETERMINING THE BIOLOGICAL QUALITY OF SEMEN

Since all the males exhibited full spawning readiness, none of them was subjected to hormonal stimulation. However, one day following the hormonal stimulation of the females, from 1.1 to 3.0 cm^3 of milt was collected from the males. The parameters that characterize the quality of the fish semen, i.e., concentration (1×10^9 cm^{-3}) and sperm motility (%), were determined. The concentration of sperm was determined with the spectrophotometric method (Ciereszko and Dabrowski 1993). The milt was diluted with 0.7% NaCl at a ratio of 1:4000, and a Beckam DU-640 spectrophotometer (Germany) was used to measure absorbance at a wavelength of 530 nm. Sperm concentration was read using a standard curve that was devised based on the cytometric method (Bielański 1979). Microscopic methods were used to determine sperm motility by placing approximately 1 mm^3 of semen on a glass slide. The sperm were activated with 0.5% NaCl solution (30 mm^3 of solution per semen portion). The percentage of motile sperm was determined at a maximum value of 95%. Only sperm exhibiting progressive motility were considered; those exhibiting circular or vibrating movements were not. The males used in spawning had sperm motility higher than ≥ 70%.

FERTILIZATION AND INCUBATION OF EGGS

The eggs were weighed (± 0.1 g) and then fertilized with milt from three males using the dry method (100 g of eggs was fertilized with 1cm^3 of milt). Following fertilization, adhesiveness was removed from the eggs with a talc and sodium chloride solution (Steffens et al. 1996). Following this, three samples of 100 eggs each were collected from each female and placed in experimental incubation apparatuses (rings constructed of PCV tubing with a diameter of 75 mm and a height of 25 mm sealed at one end with 0.2 mm mesh netting) installed in an incubation tank in a recirculating system. The remaining eggs were incubated in standard Weiss jars. After the eggs had been placed in the apparatuses, the water temperature was raised over the course of one day to 17.0°C. The moment the eyed-egg stage was reached, survival was determined based on a sample of 30 embryos collected from each experimental incubation apparatus.

STATISTICAL ANALYSIS

Statistical analysis of the data was done with the Statistica program (StatSoft® Polska Sp. z o.o.). The impact the applied hormones had on the ovulation time and the survival of eggs (percentage data were transformed prior to analyses with arcsin function) was analyzed with one-way analyses of variance (ANOVA). When statistically significant differences among groups were detected ($P \leq 0.05$), further statistical analyses was conducted with the LSD test.

RESULTS

SEMEN PARAMETERS

The concentration of sperm in the semen ranged from 16.21 to 25.42 $\times 10^9$ cm^{-3} (mean 19.78 $\times 10^9$ cm^{-3}). Sperm motility ranged from 50 to 90% (average 77.5%). Thirteen of the 16 males (81.3%) tested has semen with a motility rate ≥ 70% (these were used in the experiment to fertilize the eggs). The sperm motility rate in the group chosen to fertilize the females was 83.0 ± 8.5%, and the concentration was 19.56 ± 2.27 $\times 10^9$ cm^{-3}.

FEMALE MATURITY

On the day of the injection, the females had oocytes exclusively in maturity stage I or in stages I and II, of which stage II comprised from 5 to 50%. Significant progress was noted in oocyte maturation in the group of females stimulated with hCG 24 hours following the first injection. The specimens tested exhibited stage II (100%), II and III (50-95%, 5-50%) or stage III exclusively. In the same period, the oocytes of females from the control group were in stages I (20-90%) and II (10-80%). After 48 hours (24 hours following the second injection) the most mature oocytes – exclusively stage III, stages III and IV (10-30%, 70-90%), or only stage IV, were noted in the group that had been stimulated with hCG. In the group of females stimulated with Ovopel (from groups III and IV), the oocytes matured more slowly and less synchronously. This was especially true of the fish in group III, in which oocytes in the I, II and III stage (5-10%, 30-80%, 5-20%) were noted. A similar phenomenon was observed in the control group.

EFFECT OF HORMONAL STIMULATION

The effect of hormonal stimulation, expressed as the percentage of females from which eggs were obtained, was highly varied among the groups. The highest percentage

of ovulating females was obtained in the group stimulated with hCG (group I – 83.3% (5/6), group II – 100% (6/6); Table 2).

TABLE 2

Effects of the artificial spawning of wild pikeperch with the application of human chorionic gonadotropin (hCG) and mGnRHa with a dopamine inhibitor (Ovopel) (mean values ± SD). Data refer to female size in subsequent groups and the applied injection (treatment type and doses) – see Table 1

Group	Ovulation (%)	Latency time (h)	Weight of eggs*		Survival to eyed-egg stage (%)*	Female survival (%)**
			% of female BW	g kg^{-1} female BW		
Group I	83.3	51 ± 7	10.6^{a} ± 2.1	106.1^{a} ± 20.6	72.4^{a} ± 5.8	83.3
Group II	100	53 ± 5	11.8^{a} ± 2.6	118.9^{a} ± 25.7	68.0^{a} ± 7.1	100
Group III	0	-	-	-	-	50.0
Group IV	50.0	70 ± 9	8.9^{a} ± 4.2	89.3^{a} ± 41.4	3.2^{b} ± 3.6	66.7
Group V (control)	50.0	76 ± 17	11.7^{a} ± 2.4	116.8^{a} ± 23.8	70.5^{a} ± 4.3	83.3

** – data in columns with the same letter indices do not differ significantly statistically (P > 0.05)*
*** – female survival was determined 96 h following the first injection*

The percentage of ovulating females in the group stimulated with Ovopel was significantly lower. None of the females in group III ovulated, while the percentage of ovulating fish was the same in the control group and group IV (Table 2). The most synchronized spawning was noted in the group stimulated with hCG, and the least in the control group, in which eggs were obtained in a period from 64 to 88 hours after the first injection of saline solution (Table 2). The weight of the eggs obtained, expressed as the percentage of body weight, ranged from 8.9% (group IV) to 11.8% (group II), and the difference among groups was not statistically significant ($P > 0.05$, Table 2). The lack of significant inter-group variation of this index resulted from high intra-group variation in group IV. However, the hormone had a significant impact on egg survival. In the group stimulated with Ovopel, it was significantly lower ($P < 0.05$) and was only 3.2%. The dose (400 vs. 700 IU kg^{-1} BW) of hCG was not noted to have had a significant impact on the survival of the eggs, which was similar to that of the control group at approximately 70% (Table 2). During artificial spawning and shortly after it, mortality among the stimulated females was observed, especially among those stimulated with Ovopel (groups III and IV). No mortality was recorded among the males.

DISCUSSION

The quality of gametes (sperm and eggs) determines the biological value of the offspring. We did not find any data in the available literature describing the quality of

wild pikeperch semen, thus no comparison could be made between the current results and the general standards. However, we can conclude that the semen of the pikeperch used in the present study were of high biological quality. This is confirmed by the relatively high volume of ejaculate, the high sperm motility in the semen used to fertilize the eggs, and survival to the eyed-egg stage (in the group stimulated with hCG and in the control group) as well as the high sperm concentration. The average sperm concentration of about 20×10^9 cm^{-3} noted in the current study was not significantly higher than that noted in males reared from larvae under strictly controlled conditions (recirculating systems) on artificial feed (Zakęś et al., unpublished data). Generally, the concentration of semen in pikeperch is lower than that of other percid fishes – walleye (mean 38.6×10^9 cm^{-3}), European perch, *Perca fluviatilis* L. ($27.7 – 32.6 \times 10^9$ cm^{-3}), or yellow perch (48.5×10^9 cm^{-3}) (Brown and Moore 1996, Glogowski et al. 1999, Król 2002).

The impacts of hCG and Ovopel on the maturation of males were not investigated in the current study. This was because during the spawning migration, these fish were fully ready for spawning and gentle pressure on the abdomen was sufficient for milt to be released. This is confirmed by the observations of Lappalainen et al. (2003), who maintain that male pikeperch migrate to spawning grounds and achieve full maturity earlier than females do. Hormonal stimulation in such mature fish is generally unnecessary (this study; Zakęś, unpublished data). However, it is known that hCG has a positive impact on the maturation and spermiation of pikeperch males both in the natural spawning period and outside of it (single injection 200 IU kg^{-1} BW; Zakęś and Szkudlarek 1998, Zakęś and Szczepkowski 2004).

The effect of hormonal stimulation, expressed both as the percentage of females from which eggs were obtained, spawning synchronization, and egg survival was the most advantageous in the group stimulated with hCG. Our earlier study, also conducted during the natural pikeperch spawning period, indicated that the synchronization of spawning and the percentage of ovulating females after the application of hCG was significantly higher than with the application of carp pituitary extract (CPE; Zakęś and Szkudlarek 1998). The high effectiveness of hCG hormonal stimulation has been confirmed in subsequent studies whose aim was to develop techniques for the out-of-season spawning of this species (Zakęś and Szczepkowski 2004). These studies confirmed that the effect of out-of-season pikeperch spawning was independent

of the number or quantity of the doses of this hormone (range – 200-600 IU kg^{-1} BW). One-time stimulation of females (dose – 200 IU kg^{-1} BW) was just as effective as, for example, three injections (total dose – 600 IU kg^{-1} BW). Human chorionic gonadotropin is often administered in one injection at doses ranging from 100 to 4000 IU kg^{-1} BW (Zohar and Mylonas 2001). The high effectiveness of this hormone in stimulating and synchronizing fish reproduction is explained by its relatively long half-life in the bloodstream (Ohta and Tanaka 1997).

In the current study, the size of the hCG dose was not noted to have had an impact on the effectiveness of artificial pikeperch spawning. The application of a double injection of hCG resulted from the necessity of unifying the procedure followed with spawners in the various experimental groups. Double hormonal stimulation is recommended in the application of the second preparation tested in this experiment – Ovopel (Horváth et al. 1997). This application procedure in hormonal stimulation with super active GnRH analogues is due to the relatively short half-life of this type of hormone, which is about 23 minutes *in vivo* (Gothilf and Zohar 1991). The higher levels of luteinizing hormone (LH) that result following the injection of GnRHa are short and last from several hours to several days (Zohar et al. 1995). In most cases, this period is too short to elicit full oocyte maturity, ovulation, and spawning (Zohar and Mylonas 2001). This is why it is recommended to inject GnRHa twice. Double injections of this hormone are recommended in percid fishes such as walleye (Pankhurst et al. 1986), yellow perch (Dabrowski et al. 1994), European seabass, *Dicentrarchus labrax* (L.) (Carillo et al. 1995) and salmonids (Mylonas et al. 1992). Significantly, one injection of Ovopel (1 pellet kg^{-1} BW; Brzuska 2003, 2004) was effective in European and African catfish. Although the authors of the current study applied the super active GnRH analogue Ovopel in two injections of the recommended dosage (Horvath et al. 1997), its effectiveness was very low. In group III, which received the lower dose of Ovopel in the second injection (0.5 pellet kg^{-1} BW), no eggs were obtained from any of the females. Although significant progress in the maturation of the oocytes was noted following the first injection (0.25 pellet kg^{-1} BW) (increase in the percentage of stage II oocytes), the subsequent injection did not elicit further oocyte maturation. In group IV, which received a higher Ovopel dose (1 pellet kg^{-1} BW) in the second injection, the oocyte maturation process was faster, but in comparison with the fish stimulated with hCG, it was less synchronous (24 h following the second injection various females had oocytes in stages II, III, and IV). The resulting spawning in this group was less synchronized, and the eggs obtained were of very

low biological quality (average survival at the eyed-egg stage was 3.2%). It should be emphasized that in the groups stimulated with Ovopel there was a high rate of female mortality. In most studies of this preparation, this negative reaction was not noted (for example, Horváth et al. 1997, Brzuska and Grzywaczewski 1999, Brzuska 2003, 2004). These studies were performed primarily on domesticated pond fish, which are more resistant to all types of manipulation and the accompanying stress. The phenomenon of high mortality was noted with regard to wild spawners of ide, *Leuciscus idus* (L.), caught during the pre-spawning season in open waters and then stimulated with Ovopel (Kucharczyk et al. 1999). Hodson and Sullivan (1993) recommend using gonadotropins (for example, hCG) and not gonadotropin-releasing hormones in the reproduction of wild fish. They maintained that, in the case of wild striped bass, *Morone saxatilis* (Walb.), as a hormone that acts directly on the gonads, hCG has a decidedly more advantageous impact on the maturation of oocytes, ovulation, and spermiation. Gonadotropin-releasing hormones influence the hypothalamo-pituitary-gonadal (HPG) axis, which means that the latency period is longer than it is with GtH. High mortality in wild spawners, which is a severe reaction to stress, might be caused by the long latency period between injection and spawning (Zohar and Mylonas 2001). The oocyte maturation process and ovulation might be stopped as a reaction to the stress females are subjected to (Schreck et al. 2001). Higher levels of cortisol, which is a stress reaction, can affect the HPG axis leading to disturbances in the regulation of the hormonal maturation of oocytes and ovulation (Carragher et al. 1989).

The low effectiveness of Ovopel noted in the current experiment is probably related to the great sensitivity of wild pikeperch spawners, their susceptibility to stress, the relatively long latency period (longer than with GtH), and the possibility of a reaction between the stress hormone (cortisol) and the sex hormones. Further studies that test the impact of Ovopel on the maturation process, ovulation, spermiation, and the hormonal profile and stress reaction of wild and domesticated pikeperch are warranted.

The results of the current experiment, in combination with those of the authors' earlier work, indicate that human chorionic gonadotropin can be recommended to stimulate maturation, ovulation, and spermiation in European pikeperch. In addition to this preparation's practical advantages (effectiveness, simplicity of application, precise dosing), it is readily available and relatively inexpensive, especially in light of the small dose required to successfully stimulate pikeperch reproduction.

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STRESZCZENIE

SZTUCZNY ROZRÓD SANDACZA (*SANDER LUCIOPERCA* (L.)) STYMULOWANY HORMONALNIE LUDZKĄ GONADOTROPINĄ KOSMÓWKOWĄ (HCG) I SSACZYM ANALOGIEM GNRH Z INHIBITOREM DOPAMINOWYM

Celem eksperymentu było określenie możliwości stymulacji hormonalnej rozrodu sandacza przy użyciu ludzkiej gonadotropiny kosmówkowej (hCG) i ssaczego analogu GnRH (D-Ala^6 Pro^{9}NEt-mGnRH) z inhibitorem dopaminowym (metoclopramid) – Ovopelem. Tarlaki sandacza pozyskano sprzętem pułapkowym (żaki) w okresie przedtarłowym z jeziora Tałty i Tałtowisko (Pojezierze Mazurskie, północna Polska). Po godzinnym transporcie ryby umieszczono w basenach wchodzących w skład obiegu recyrkulacyjnego, a następnie posortowano według płci. Samice podzielono na pięć grup doświadczalnych, każda po 6 sztuk (tab. 1). Ryby iniekowano dwukrotnie, co 24 h: hCG (grupa I – 200 i 200 IU kg^{-1} masy ciała (BW); grupa II – 200 i 500 IU kg^{-1} BW), Ovopelem (grupa III – 0,25 i 0,50 granulki kg^{-1} BW; grupa IV – 0,25 i 1,0 granulka kg^{-1} BW) lub 0,7% roztworem NaCl (grupa V, kontrolna – 0,2 i 0,5 cm^3 kg^{-1} BW). W poszczególnych grupach efekty stymulacji hormonalnej wyrażone odsetkiem owulujących samic, stopniem synchronizacji tarła i przeżywalnością embrionów do stadium zaoczkowania były bardzo zróżnicowane. Najwyższy procent wytarcia uzyskano w grupach stymulowanych hCG – 83,3% (grupa I) i 100% owulujących samic (grupa II) (tab. 2). U ryb z tych grup rozwój oocytów przebiegał szybko i synchronicznie, co rzutowało na skrócenie i stosunkowo niewielkie zróżnicowanie czasu latencji (47-57 godzin od pierwszej iniekcji; średnia ≈ 51 godzin) u poszczególnych samic. Nie stwierdzono wpływu dawki hCG (400 wobec 700 IU kg^{-1} BW) na czas latencji i jakość gamet. Ovopel nie wpłynął korzystnie na proces dojrzewania oocytów i owulację sandacza. Żadna ryba z grupy III nie oddała ikry, a w grupie IV, podobnie jak w grupie kontrolnej, ikrę pozyskano jedynie od trzech (50%) samic. W przeciwieństwie jednak do grupy V, ikra samic stymulowanych Ovopelem miała niską jakość biologiczną – przeżywalność na etapie zaoczkowania mieściła się w przedziale 0-8%. Zauważono również zwiększoną śmiertelność samic, zwłaszcza w grupie III. Przeprowadzony eksperyment wykazał dużą skuteczność stymulacji hormonalnej sandacza przy użyciu hCG, podczas gdy efekty stymulacji Ovopelem okazały się zaskakująco niskie.