Effects of hCG and salmon gonadoliberine analogue on spermiation in the Eurasian perch (Perca fluviatilis).

Daniel Zarski, Gergely Bernáth, Jaroslaw Król, Beata I Cejko, Zoltán Bokor, Sylvain Milla, Pascal Fontaine, Slawomir Krejszeff

Effects of hCG and salmon gonadoliberine analogue on spermiation in the Eurasian perch (Perca fluviatilis)

Daniel _Zarski [a], Gergely Bernáth [b], Jarosław Król [a], Beata I. Cejko [c], Zoltán Bokor [b], Katarzyna Palińska-Zarska [a], Sylvain Milla [d], Pascal Fontaine [d], Sławomir Krejszeff [a].

a :Department of Ichthyology, University of Warmia and Mazury, ul. Oczapowskiego 2, 10-719 Olsztyn, Poland
b : Department of Aquaculture, Szent István University, Páter Károly u. 1., 2100 Gödöllö, Hungary
c :Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Science, Olsztyn, Poland
d :UR AFPA, Université de Lorraine - INRA, 54505 Vandoeuvre-lès-Nancy, France

Published in Theriogenology. Available online: http://dx.doi.org/10.1016/j.theriogenology.2017.08.022

Keywords

Spawning agents, controlled reproduction, hormonalmanipulation, sperm quality, CASA

Abstract

This study analysed (i) the effect of human chorionic gonadotropin (hCG) and salmon gonadoliberine analogue (sGnRHa) on the effectiveness of induction of spermiation and (ii) the effect of latency time following the application of those spawning agents on the quantity and quality of the sperm of Eurasian perch, Perca fluviatilis, obtained during out-of-season spawning. For this study, pond-reared fish were used which had been acclimated to the controlled conditions. Three groups were distinguished which were treated with either saline (0.9% NaCl; control group), hCG (500 IU kg□1) or sGnRHa (100 mg kg□1). The fish were kept in a recirculating system at 12 °C throughout the study, during which sperm was collected every two days between the 2nd and 10th day following hormonal treatment. During the study, quantitative (e.g. sperm volume, total sperm production) and qualitative (measured with a computer-assisted sperm analysis system - i.e. CASA) parameters were monitored. The results of the study indicate that the hormonal treatment had a highly beneficial effect on the spermiation rate (100% in experimental groups from day 6 following injection) as well as quantity, which increased 50% in experimental groups (over 2200 x 109 of spermatozoa per kg of body weight) by day 4 following injection. For the sperm quality, both spawning agents tested had a rather positive effect, although sperm motility rate (MOT) was seen to be significantly reduced on day 10 following the application of hCG (MOT 1/4 72.8% ± 8.1), which was not observed after the application of sGnRHa (minimum mean MOT 81.7% ± 6.1). The results clearly indicate that hormonal treatment had a positive effect on spermiation in Eurasian perch, most apparent from day 6 following injection, regardless of the hormonal agent used. Though application of sGnRHa allowed a high volume of high quality sperm to be stripped for two days longer (up to day 10 post-injection) compared to the application of hCG

1. Introduction

Eurasian perch, Perca fluviatilis L., is one of the most promising candidate species dedicated to intensive aquaculture and is already in commercial-scale production in several European countries [1].

Despite huge progress in the production technology of this species recorded over the last two decades, further development of commercial production is still restricted due to the low possibility of control over the reproduction and high variability in gamete quality [2]. Therefore, in recent years, serious attempts have been undertaken to control the gonad maturation process [3] and spawning [4]. Until now, the main focus has been put on the development of efficient reproductive protocols for females, whereas very few studies

have been devoted to the aspects of spermiation in males,which is an equally important part of successful spawning operation [2]. This is particularly important when fish are intended to be spawned out of the spawning season, in which low spermiation rate, quantity and/or quality can very often be observed [5-7] or a high amount of sperm is needed for commercial-scale cryopreservation procedures [8].

To a great extent, controlled reproduction of percids is based on the application of hormonal treatment to synchronize spawning and enhance reproductive effectiveness [2]. For Eurasian perch females, it was found that the type of the spawning agent may have modulatory effects on quality [6,9] and proximate composition [10] of the eggs during out-of season spawning. For instance, the application of hCG (human chorionic gonadotropin) was found to affect the fatty acid profile of the eggs, which are crucial nutritional constituents for the developing embryo and larvae. Interestingly, this could be avoided by the application of salmon GnRH analog (sGnRHa) [10]. This probably stemmed from the fact that these two most popular spawning agents act at different levels of the hypothalamic-pituitary-gonadal (HPG) axis. The application of hCG directly stimulates the production of sex steroids by the gonads, whereas sGnRHa has a stimulatory effect on the pituitary, triggering the production of endogenous gonadotropins [11,12]. These two modes of action are probably responsible for the widelyobservable differences in the efficiency of different types of hormonal preparations in various finfish [2,13]. In freshwater finfish, hormonal stimulation is usually not necessary to collect sperm by regular stripping during the spawning season. However, without stimulation, low quantity and/or quality of the sperm is very often observed [5,6]. It has been reported that the type of spawning agent [14,15] as well as the latency time (the time period between hormonal injection and sperm collection) [16,17] are the two major factors directly effecting the quantity and quality of the sperm in freshwater teleosts. This was especially important for RAS-reared fish like the common barbel, Barbus barbus L., where hormonal therapy obtained two-fold more sperm of very high quality compared to the non-stimulated fish [18]. A similar observation was reported for yellow perch, Perca flavescens L., where the application of GnRH analog also obtained two-fold more sperm in February and March (before the spawning season) on the 2nd and 4th day post-injection [19]. However, these authors did not verify how the hormonal treatment affected sperm quality, which makes the recommendation of the most efficient protocol very difficult. To date, there have been no studies on the effect of hormonal therapy on sperm quality and quantity in percids which include a comparable analysis of different hormonal preparations acting at different levels of the HPG axis.

The aim of this studywas to investigate the effect of two types of the most widely-used spawning preparations (hCG and sGnRHa) in controlled reproduction of percids on the effectiveness of induction of spermiation in Eurasian perch during the out-of-season spawning (i.e. three months before the spawning season; also referred to as ‘advanced spawning’). Additionally, the effect of latency time following the application of these preparations on quantity and quality of the sperm obtained was also investigated.

2. Material and Methods

The study was conducted according to the European and national legislation for fish welfare and approved by the Local Ethical Committee in Olsztyn, Poland (permission No. 76/2013).

2.1. Fish origin and broodstock management prior to experiment

In this study, pond-reared Eurasian perch [n = 200, with an average weight of 75 g (±25 SD)], originating from the _Zurawia Fish Farm (Central Poland) were used. Fish were caught from the ponds in late October when the average water temperature decreased to 10 °C, which is a standard procedure when wild or pond-reared Eurasian perch were being prepared for out-of-season spawning [20]. Just after being caught, the fish were transported (in polyethylene bags containing 30 L of water; approx. 60% of total volume of the bag consisted of mostly pure oxygen atmosphere) to the laboratories of University of Warmia and Mazury in Olsztyn, Poland, where they were placed in 1000 L tanks connected to a recirculating aquaculture system (RAS) with controllable temperature (with an accuracy of 0.5 °C). The fish were then subjected to a wintering period according to the following temperature regimes: 7 days at 10 °C; 14 days at 8 °C; 40 days at 6 °C; 14 days at 8 °C, 7 days at 10 °C (as described by _Zarski et al. [20]). During this period, the fish were kept in a constant dimness (light intensity below 10 lx).

The oxygen level in the tank was always above 85% of saturation (between 8.9 and 10.5 mg L-1, which were the lowest values recorded at 12 and 6 °C, respectively) and the pH ranged between 7.6 and 8.2 throughout the study. Total ammonia nitrogen and nitrites did not exceed 0.4 and 0.02 mg L-1, respectively.

2.2. Hormonal stimulation and identification of the groups

The fish were assigned to three different groups -one control and two experimental groups. The control group was treated with 1 ml per kg of body weight of saline (0.9% NaCl, control group). Experimental groups were injected either with sGnRHa (Syndel, Canada) at a dose of 100 mg kg-1 or with hCG (Argent, USA) at a dose of 500 IU kg-1. The spawning agents were injected intraperitoneally (at the base of the left ventral fin). All the spawning agents were dissolved in saline (0.9% NaCl) in the way so that the fish were always injected with 1 ml of solution per kg of body weight. The doses chosen were the most commonly reported doses in percid aquaculture [2].

2.3. Broodstock management during the experiment and sperm sampling

After the wintering period, fish were anesthetized in an MS-222 solution (Argent, USA) at a dose of 150 mg L-1 and for further procedures only males were selected. The sex was

every time confirmed by catheterization (as described by Ross [21]), since none of the males was seen to spermiate at that time. 90 recognized males [average weight 57.4 g (±18.5 SD)] were randomly assigned to one of the three groups (30 fish per group), which were then treated with different spawning agents (see above). Fish from each group separately were then placed in individual 300 L tanks connected to the same RAS with a water temperature of 12 °C, which is the typically used spawning temperature for Eurasian perch [2]. From the moment of injection, the photoperiod was immediately changed to 14L:10D with an intensity of 80-120 lx, measured with a luxmeter at the water surface.

Every two days after injection (day 2, 4, 6, 8 and 10), six randomly-chosen fish were taken from each group and anesthetized (in MS-222 solution at a dose of 150 mg L-1). Next, the genital pore was wiped dry and sperm from each male (if possible) was stripped using a flexible catheter (as described for pikeperch by Sarosiek et al. [22]) directly into a dry Eppendorf tube, which was then immediately placed on melting ice (4 °C) prior to further analyses, which were done within 60 min following sperm collection. During the sampling, both the number of spermiating males and the total volume of sperm collected were recorded. The males from which the sperm was collected were removed from the experiment

2.4. Sperm analysis

The collected sperm was divided into three sub-samples. The first sub-sample (0.5 ml) was subjected to pH analysis with an Orion Star A211 pH benchtop meter (Thermo Fisher Scientific, Waltham, Massachusetts, USA) following the method described by Cejko et al. [23]. This sub-sample was then centrifuged (10000xg for 10 min) to obtain seminal plasma subjected to an osmolality analysis with a Vapor Pressure Osmometer 5600 (Wescor, Logan, Utah, USA). A second sub-sample (0.5 ml) was subjected to estimation of sperm concentration with the spectrophotometric method [24]. A third sub-sample (0.2 ml) was used for evaluation of spermatozoa motility parameters.

From several motility parameters measured using the CASA system (Sperm Class Analyzer v. 4.0.0. by Microptic S.L., Barcelona, Spain), spermatozoa motility (MOT, %), beat cross frequency (BCF, Hz), curvilinear velocity (VCL, mm s-1), amplitude of lateral head displacement (ALH, mm), straight linear velocity (VSL, mm s-1) and linearity of movement (LIN, %) were chosen as the most widely-used quality indicators of sperm quality [8,14]. Sperm was activated on the Makler chamber (Sefi-Medical Instruments ltd., Israel) using a solution composed of 75 mM NaCl, 2 mM KCl, 1 mM MgSO4$7H2O, 1 mM CaCl2$2H2O, 20 mM Tris, pH 8 (modified Lahnsteiner's activating solution, as described by Bernath et al. [25]). All chemicals were purchased from POCH (Gliwice, Poland). The activating solution additionally contained 0.01 g ml-1 BSA (Bovine Serum Albumin). Motility was recorded between 10 and 12 s following activation.

2.5. Data analysis and statistics

All of the data presented are expressed as mean ± SD. Based on total sperm volume and sperm concentration, the relative total sperm production (rTSP) [number of spermatozoa (x 109) obtained from 1 kg offish] was calculated as described by Cejko et al. [23]. The data were expressed in percentage and arcsine transformed prior to statistical analysis and were then checked for normal distribution with a Shapiro-Wilk test. Whenever the normal distribution criterion was met, the data were analyzed using a one-way ANOVA test followed by Tukey's HSD post hoc test with a 5% significance level ($p < 0.05$). Non-parametric data were analyzed with a Kruskal-Wallis test ($p < 0.05$).

3. Results

In the control group, a maximum 50% of males (on day 8 after temperature increment) were found to spermiate. On the contrary, in both experimental groups, regardless of the type of the spawning agent, after day 2 following injection, sperm could be obtained from more than 60% of the fish. From day 6 onward, all the males were spermiating in both experimental groups (Fig. 1a). Regardless the spawning agent applied, the volume of sperm increased and reached a plateau from day 6 following injection, which was significantly higher ($p < 0.05$) than the volume recorded in the control group at these sampling times (Fig. 1b). The higher volume of the sperm did not correspond with the value of the pH recorded, which was stable throughout the study (Fig. 1c). The average osmolality values of the seminal plasma were always above 290 mOsm kg-1 (with 276 mOsm kg-1 being the lowest value recorded in sGnRHa treated group on day 2 after injection). The highest ($p < 0.05$) values were recorded on day 6 following injection. No differences were found between the experimental and control groups (Fig. 1d). Among the experimental groups, the highest sperm concentration was recorded on day 2 following injection, which then decreased to reach the lowest values ($p < 0.05$) on day 6. On day 10, another significant ($p < 0.05$) increase in the sperm concentration could be observed compared to day 6. Interestingly, the highest values of this parameter were recorded in control group, with the highest values observed on days 8 and 10 after the temperature increment (Fig. 1e). The reduction of the concentration values did not affect the rTSP in experimental groups in which the highest values were recorded on days 4 and 6 onward, in fish treated with hCG and sGnRHa, respectively. In the control group, the rTSP was lower ($p < 0.05$) than the experimental groups on days 8 and 10 after injection (Fig. 1f). Additional data were also presented in Table S1.

The motility rate (MOT) of spermatozoa was higher in all of the spermiating fish (in all the groups) for the first four days of the experiment. In the control group, motility was found to be significantly lower ($p < 0.05$), compared to the experimental groups, between day 6 and 8. Higher ($p < 0.05$) MOT was still observed on day 10 only in the group treated with sGnRHa. In the experimental groups, the peak of MOT was recorded on day 6 following injection, which was then found to decrease on day 8 and 10 in sGnRHa- and hCG-treated fish, respectively (Fig. 2a). For BCF, the highest values were always recorded on day 2 of the experiment for the experimental groups. In contrast, BCF in the control groups was similar throughout the experiment (Fig. 2b). In terms of VCL, no differences were found in any of the groups between day 2 and 8 of the experiment. The lowest values of VCL in experimental groups were recorded on day 2 following injection (Fig. 2c). The tendency observed in terms of VCL

corresponded to the tendency recorded for ALH (Fig. 2d) and VSL. For the latter, the highest values were recorded in the sGnRHa-treated group two days after injection, which then remained stable throughout the experiment. In sperm obtained from hCG-treated fish, no clear tendency was observed, i.e. after the initial significant increment ($p < 0.05$) (until day 4) much lower (<0.05) values (on day 6) were again recorded. In the control group, similar VSL were recorded throughout the experiment. No differences in VSL from day 8 onward were recorded in any of the groups (Fig. 2e). The spermatozoa obtained after the application of sGnRHa were characterized by significantly higher linearity of movement between day 4 and 8 after injection, compared to the spermatozoa obtained after the application of hCG. In the control group, stable LIN was recorded throughout the entire experiment (Fig. 2e). The remaining motility parameters were presented in Table S1.

4. Discussion

The application of hormonal agents in percid aquaculture is a method allowing considerable improvement of gamete quality as well as the predictability of ovulation and/or synchronization of spawning [2]. This is especially important for out-of-season spawning, when non-stimulated males often release unsuitable amounts of sperm or do not spermiate at all [5,6]. The results obtained in our study clearly indicate that it is possible to collect sperm from non-hormonally manipulated fish during “advanced spawning”, as was reported by Alavi et al. [5]. However, as in the current study, in such a case, only a small amount of sperm was possible to be stripped, although it was characterized by satisfactory motility parameters (MOT over 80%). It is therefore clear that hormonal treatment had a highly beneficial effect at least on the sperm volume in Eurasian perch males during out-of-season (advanced) spawning.

From the perspective of controlled reproduction, the quantity and quality of sperm are both of major importance. A higher amount of sperm obtained from a single male reduces the number of males in the broodstock and/or improves the broodstock management, in which a high amount of sperm is needed throughout the reproductive period [7]. Finally, the interest in obtaining a high amount of high-quality sperm is crucial in selective breeding where preserving the traits exhibited by a particular male is of high importance. Therefore, when reconsidering the effectiveness of a particular hormonal treatment protocol, both quantity and quality should be taken into account. The results of our study indicate that both parameters were significantly improved by hormonal treatment, especially from day 6 of the experiment, regardless of the spawning agent used. In terms of quantity, a higher amount of sperm, depending on the spawning agent used, was possible to be obtained on day 4 (in GnRH group) and day 6 (in hCG treated group) following injection, while on day 6 the sperm volume possible to be obtained increased two-fold in both groups. The increment in volume could certainly be attributed to the increased production of seminal fluid. This is shown by decreased sperm concentration and stable, high osmolality values, which allowed us to reject the possibility of urine contamination, which is usually heralded by lowered osmolality of seminal plasma [22]. The increased volume of semen and seminal fluid is a typical phenomenon following hormonal stimulation [26,27] and is a required phenomenon as it usually facilitates sperm handling and fertilization procedure [7,28]. Interestingly, the application of hCG caused significantly higher production of seminal plasma on day 6 (highlighted by significantly lower sperm concentration), which can be due to the direct involvement of hCG in the production of sex steroids [11] and, thus, probably a more agonistic effect on the production of seminal fluid under the control of 17a,20b-dihy-droxy-4-pregnen-3-one (DHP) [29,30]. Nonetheless, both hormonal stimulations obtained similar rTSP throughout the experiment, which suggests that both spawning agents could be considered for the stimulation of spermiation in Eurasian perch.

Spermatozoa motility was found to be a reliable quality indicator of sperm. It not only considers the MOT, being one of the most important parameters, but also the remaining parameters indicating the character of the movement of sperm cells [31]. The results of this study clearly indicate that both tested hormonal treatments have a significant effect not only on the amount of sperm to be stripped, but also on the motility parameters. It should be emphasized that from among the hormonally treated groups, a similar trend could be observed in most of the motility parameters. Interestingly, MOT following sGnRHa treatment remained high throughout the experiment, whereas the application of hCG caused a reduction of MOT on day 10. Thus, the results suggest that both spawning agents are suitable to stimulate spermiation in Eurasian perch. In other freshwater teleosts, e.g. common dace, Leuciscus leuciscus (L.) [26], motility parameters such as VCL and VSL were affected by the application of different hormones. However, in other freshwater teleosts, e.g. in crucian carp, Carassius carassius (L.) [27], from among the motility parameters only ALH was positively affected by the type of the spawning agent used (by the application of a mixture of sGnRHa and domperidone - a dopamine receptor antagonist), but highly considerable differences in the obtained volume of sperm were recorded. Therefore, it can be generalized that hormonal stimulation in freshwater teleosts primarily affects the increment of the sperm volume by indirect involvement in final maturation processes of the spermatozoa through the HPG axis. It can then be suggested that the type of spawning agent may affect the spermatozoa quality to a limited extent, with a rather positive effect. However, we must not rule out some negative effect of those treatments on spermatozoa motility, as illustrated by the lower VCL and ALH on day 2 and VSL on day 6 relative to the control group, which remains unclear and to be more closely studied in the future. However, in the current study a considerable improvement was generally found in enhanced sperm production. This allows the application of hormonal manipulation of spermiation in males of Eurasian perch to be recommended, regardless of the type of the spawning agent, which would allow a high amount of high quality sperm to be obtained without any risk.

The effective latency period (ELP) may be defined as the time period following the hormonal treatment during which higher quality and/or quantity sperm is possible to be collected, when compared to non-treated fish. The ELP was observed to be usually dependent on the type of the spawning agent used and species studied (as reviewed by Mylonas et al., 2016). Among the freshwater teleosts, in the

most extreme cases the ELP was reported to occur between 12 h and 36 h following injection in common barbel, Barbus barbus (L.) [17], and between 1 and 11 days in whitefish, Coregonus lavaretus (L.), [32]. These differences could stem from the temperature regimes applied in both studies, since the common barbel were injected with spawning agents at 19 °C, whereas the whitefish were kept in variable thermal conditions ranging between 2.2 °C and 6.3 °C. In another cyprinid species, the ide, Leu-ciscus idus (L.), which was hormonally treated at 12 °C (as in the current study), the ELP was found to occur between 60 and 84 h (2.5-3.5 days) [16]. However, the temperature seems not to be the main determinant affecting ELP, since in the smelt, Osmerus eperlanus (L.), injected at a lower temperature (9.5 °C), the ELP was shorter and ranged between 24 h and 72 h following injection [15]. In this context, we can conclude that Eurasian perch belongs to a very rare group of fish species with relatively long ELP which, at 12 °C, occurred between day 6 and 8 following injection with hCG and between day 6 and 10 following injection with sGnRHa.

The results of this study clearly indicate that hormonal treatment had a highly beneficial effect on the spermiation rate as well as the quantity of the sperm obtained from Eurasian perch males during advanced spawning. Considering our results, it should be highlighted that sperm from Eurasian perch should be stripped not earlier than on day 6 following injection, regardless of the type of hormonal treatment used. However, some positive effect could be seen even after day 2 following injection. In effect, it can be recommended to use both tested hormonal agents, with the restriction that the application of sGnRHa allows a high volume of quality sperm to be stripped for at least two days longer (up today 10 post-injection) compared to the application of hCG.

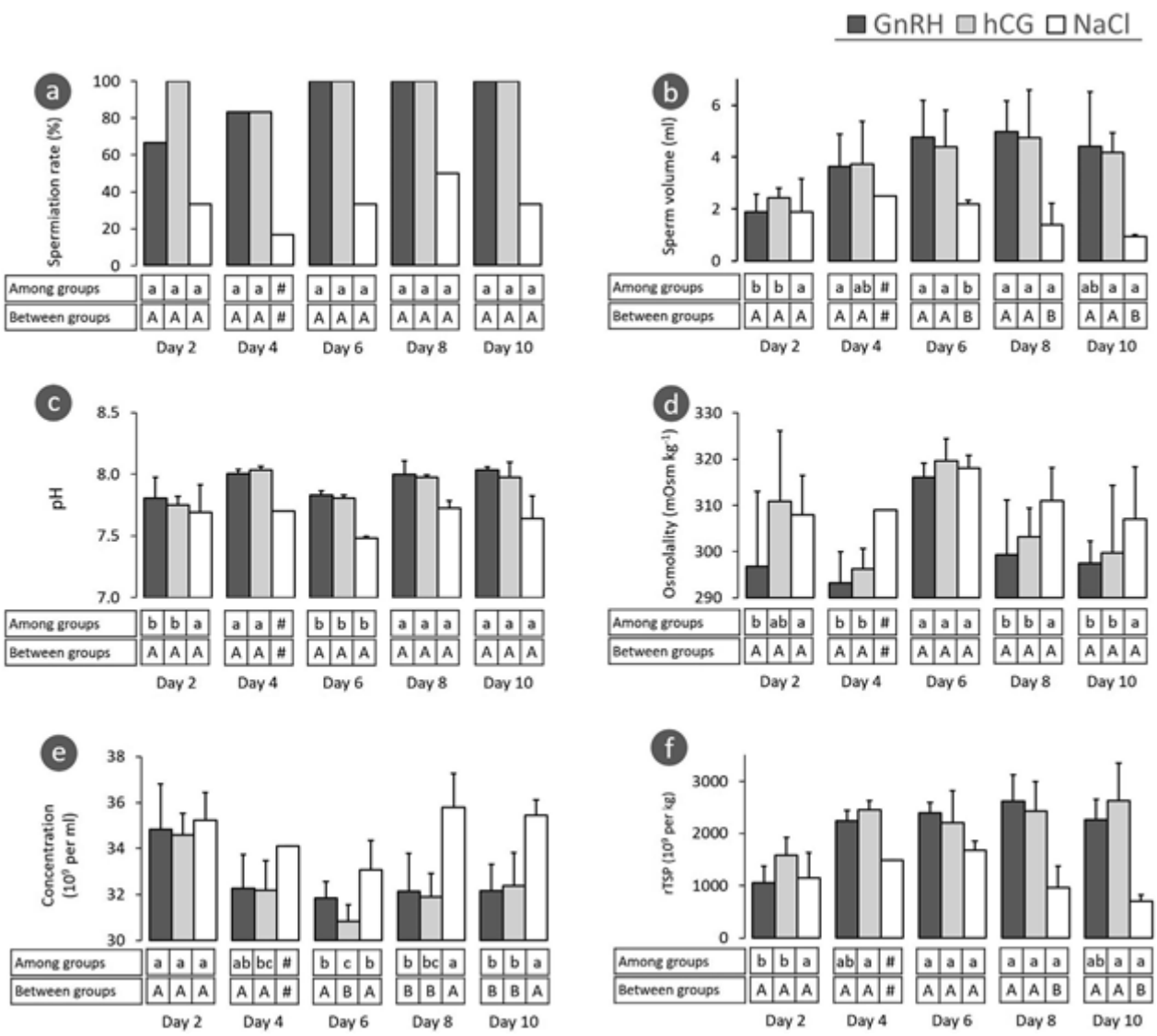

Figure 1.

The effect of different spawning agents (either 500 IU kg~1 of hCG or 100 mg kg-1 sGnRHa were applied, both administered in a single injection at 12 °C) on (a) spermiation rate, (b) sperm volume, (c) pH of semen, (d) osmolality of seminal plasma, (e) sperm concentration and (f) relative total sperm production (rTSP) in comparison to non-treated fish (injected with NaCl solution) and in relation to time (in days) of sperm collection following injection. Data are expressed as mean (±SD). Every times six individuals were taken for sperm collection, however the real number of samples analysed depended on number of individuals spermiated (for details see Table S1). Statistical significance is indicated at the bottom of each individual graph. Data indicated with different lowercase letters indicates statistical differences among the groups and between the respective day of sperm collection ($P < 0.05$). Data indicated with different UPPERCASE LETTERS indicates statistical differences between the groups and among the respective day of sperm collection ($P < 0.05$). A hash (#) indicates the data excluded from the analysis because it represent only 1 sample of sperm which was possible to be obtained.

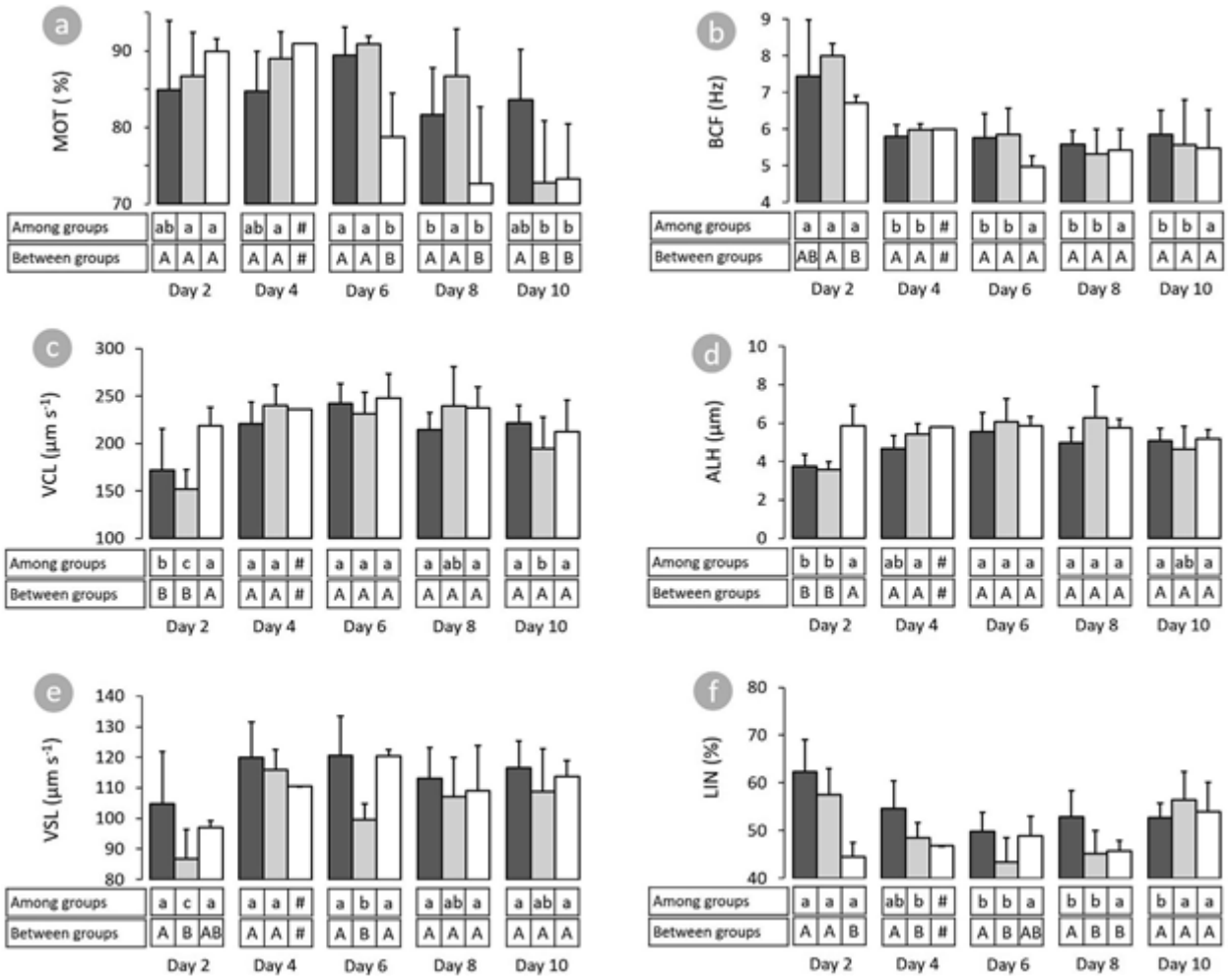

Figure 2.

The effect of different spawning agents (either 500 IU kg~1 of hCG or 100 mg kg-1 sGnRHa were applied, both administered in a single injection at 12 °C) on motility parameters of spermatozoa compared to non-treated fish (injected with NaCl solution) and in relation to time (in days) of sperm collection following injection. Data are expressed as mean (±SD). Every times six individuals were taken for sperm collection, however the real number of samples analyzed depended on number of individuals spermiated (for details see Table S1). Statistical significance is indicated at the bottom of each individual graph. Data indicated with different lowercase letters indicates statistical differences among the groups and between the respective day of sperm collection ($P < 0.05$). Data indicated with different UPPERCASE LETTERS indicates statistical differences between the groups and among the respective day of sperm collection ($P < 0.05$). A hash (#) indicates the data excluded from the analysis because it represents only 1 sample of sperm which was possible to be obtained and analyzed. MOT -motility rate, BCF -beat-cross frequency, VCL -curvilinear velocity, ALH -amplitude of lateral head displacement, VSL -straight-line velocity, LIN -linearity of movement

5. References

[1] Toner D. The market for Eurasian perch. In: Kestemont P, Da˛browski K, Summerfelt RC, editors. Biol. Cult. Percid fishes. Dordrecht: Springer Netherlands; 2015. p. 865-79. http://dx.doi.org/10.1007/978-94-017-7227-3_34.

[2] Zarski D, Horv□ath A, Held JA, Kucharczyk D. Artificial reproduction of percid fishes. In: Kestemont P, Da˛browski K, Summerfelt RC, editors. Biol. Cult. Percid fishes. first ed. Dordrecht: Springer Netherlands; 2015. p. 123-61. http:// dx.doi.org/10.1007/978-94-017-7227-3_4.

[3] Fontaine P, Wang N, Hermelink B. Broodstock management and control of reproductive cycle. In: Kestemont P, Da˛browski K, Summerfelt RC, editors. Biol. Cult. percid fishes, Princ. Pract. Springer Netherlands; 2015. p. 958. http://dx.doi.org/10.1007/978-94-017-7227-3.

[4] Zarski D, Bokor Z, Kotrik L, Urbanyi B, Horv□ath A, Targo□nska K, et al. A new classification of a preovulatory oocyte maturation stage suitable for the synchronization of ovulation in controlled reproduction of Eurasian perch Perca fluviatilis L. Reprod Biol 2011;11:194-209. http://dx.doi.org/10.1016/S1642-431X(12)60066-7.

[5] Alavi SMH, Rodina M, Hatef A, Stejskal V, Policar T, Ham□a□ckov□a J, et al. Sperm motility and monthly variations of semen characteristics in Perca fluviatilis (Teleostei: Percidae). Czech J Anim Sci 2010;55:174-82.

[6] Szczerbowski A, Kucharczyk D, Mamcarz A, Łuczy□nski MJ, Targo□nska K, Kujawa R. Artificial off-season spawning of Eurasian perch Perca fluviatilis L. Archive 2009;17:95-8.

[7] Mylonas CC, Duncan NJ, Asturiano JF. Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. Aquaculture 2016. http://dx.doi.org/10.1016/j.aquaculture.2016.04.021.

[8] Bern□ath G, Bokor Z, _Zarski D, V□arkonyi L, Hegyi □A, Staszny □A, et al. Commercial-scale out-of-season cryopreservation of Eurasian perch (Perca fluviatilis) sperm and its application for fertilization. Anim Reprod Sci 2016;170:170-7. http://dx.doi.org/10.1016/j.anireprosci.2016.05.005.

[9] Targo□nska K, Szczerbowski A, _Zarski D, Łuczy□nski MJ, Szkudlarek M, Gomułka P, et al. Comparison of different spawning agents in artificial out-of-season spawning of Eurasian perch, Perca fluviatilis L. Aquac Res 2014;45: 765-7. http://dx.doi.org/10.1111/are.12010.

[10] Zarski D, Pali□nska-_Zarska K, Łuczy□nska J, Krejszeff S. The type of spawning agent affects the egg composition during out-of-season spawning but not during in-season spawning in Eurasian perch, Perca fluviatilis. Gen Comp Endocrinol 2017;245:19-29. http://dx.doi.org/10.1016/j.ygcen.2016.04.027.

[11] Mylonas CC, Fostier A, Zanuy S. Broodstock management and hormonal manipulations of fish reproduction. Gen Comp Endocrinol 2010;165:516-34. http://dx.doi.org/10.1016/j.ygcen.2009.03.007.

[12] Mylonas CC, Zohar Y. No title. Rev Fish Biol Fish 2000;10:463-91. http://dx.doi.org/10.1023/A:1012279814708.

[13] Kucharczyk D, Targo□nska K, _Zarski D, Kujawa R, Mamcarz AA. Review of the reproduction biotechnology for fish from the genus Leuciscus. Arch Pol Fish 2008;16:319-40. http://dx.doi.org/10.2478/s10086-008-0021-5.

[14] Cejko BI, Judycka S, Juchno D, Boro□n A, Leska A, Jabło□nska O, et al. Hormonal treatment affects sperm motility in the spined loach (Cobitis taenia, Pisces, Cobitidae). Aquac Res 2016:1-7. http://dx.doi.org/10.1111/are.13170.

[15] Kowalski RK, Hliwa P, Cejko BI, Kr□ol J, Stabi□nski R, Ciereszko A. Quality and quantity of smelt (Osmerus eperlanus L.) sperm in relation to time after hormonal stimulation. Reprod Biol 2012;12:231-46. http://dx.doi.org/ 10.1016/S1642-431X(12)60088-6.

[16] Cejko BI, Kowalski RK, Kucharczyk D, Targo□nska K, Krejszeff S, _Zarski D, et al. Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L. Aquac Res 2010;41:804-13. http:// dx.doi.org/10.1111/j.1365-2109.2009.02357.x.
[17] Cejko BI, Kowalski RK, _Zarski D, Dryl K, Targo□nska K, Chwaluczyk R, et al. The influence of the length of time after hormonal treatment with [(d-Ala6, Pro9 NEt)-mGnRH+metoclopramide] i.e. Ovopel on barbel Barbus barbus (L.) milt quality and quantity indicators. J Appl Ichthyol 2012;28:249-53. http:// dx.doi.org/10.1111/j.1439-0426.2011.01923.x.
[18] Cejko BI, _Zarski D, Judycka S, Kucharczyk D, Sarosiek B, Kowalski RK. Effect of two commercial preparations containing different GnRH analogues with dopamine antagonists on barbel Barbus barbus (L.) sperm quantity and quality. Aquac Int 2014;22:97-109. http://dx.doi.org/10.1007/s10499-013-9657-2.
[19] Dabrowski K, Ciereszko A, Ramseyer L, Culver D, Kestemont P. Effects of hormonal
treatment on induced spermiation and ovulation in the yellow perch (Perca flavescens).
Aquaculture 1994;120:171-80. http://dx.doi.org/ 10.1016/0044-8486(94)90231-3.
[20] Zarski D, Horv□ath □A, Bern□ath G, Krejszeff S, Rad□oczi J, Pali□nska-_Zarska K,
et al. Controlled reproduction of wild Eurasian perch. Cham: Springer International Publishing; 2017. http://dx.doi.org/10.1007/978-3-319-49376-3.
[21] Ross RM. Catheterization: anon-harmful method of sex identification for sexually monomorphic fishes. Prog Fish-Cult 1984;46:151-2. http:// dx.doi.org/10.1577/1548-8640(1984)46<151:C>2.0.CO;2.
[22] Sarosiek B, Dryl K, Krejszeff S, _Zarski D. Characterization of pikeperch (Sander lucioperca) milt collected with a syringe and a catheter. Aquaculture 2016;450:14-6. http://dx.doi.org/10.1016/j.aquaculture.2015.06.040.
[23] Cejko BI, Krejszeff S, Judycka S, Sarosiek B, Dietrich M, Kucharczyk D, et al. Sperm
quality and selected biochemical markers of seminal plasma at the beginning of the reproductive period of common carp, Cyprinus carpio L. Aquac Int 2014;22:111-22. http://dx.doi.org/10.1007/s10499-013-9672-3.
[24] Ciereszko A, Dabrowski K. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using a spectrophotometric technique. Aquaculture 1993;109:367-73. http://dx.doi.org/10.1016/0044-8486(93) 90175-X.
Bern□ath G, _Zarski D, Krejszeff S, Pali□nska-_Zarska K, Bokor Z, Kr□ol J, et al.
[25] Optimization of conditions for the cryopreservation of Eurasian perch (Perca fluviatilis
Linnaeus, 1758) sperm. J Appl Ichthyol 2015;31:94-8. http:// dx.doi.org/10.1111/jai.12740.
[26] Cejko BI, Targo□nska K, Kowalski RK, _Zarski D, Sarosiek B, Kucharczyk D, et al.
The effectiveness of hormonal preparations (Ovopel, Ovaprim, LHRHa, hCG and CPE)
in stimulating spermiation in dace Leuciscus leuciscus (L.). J Appl Ichthyol 2012;28:873-7. http://dx.doi.org/10.1111/jai.12054.
[27] Cejko BI, _Zarski D, Krejszeff S, Kucharczyk D, Kowalski RK. Effect of hormonal stimulation on milt volume, number of sperm, and sperm motility in the crucian carp, Carassius carassius (L.). Isr J Aquac - Bamidgeh 2013:65.
[28] Clearwater SJ, Crim LW. Gonadotropin releasing hormone-analogue treatment increases sperm motility, seminal plasma pH and sperm production in yellowtail flounder Pleuronectes ferrugineus. Fish Physiol Biochem 1998;19: 349-57. http://dx.doi.org/10.1023/A:1007759620936.
[29] Ohta H, Kagawa H, Tanaka H, Okuzawa K, Hirose K. Milt production in the Japanese
eel Anguilla japonica induced by repeated injections of human chorionic gonadotropin.
Fish Sci 1996;62:44-9.
[30] Schulz RW, de França LR, Lareyre JJ, LeGac F, Chiarini-Garcia H, Nobrega RH, et al. Spermatogenesis in fish. Gen Comp Endocrinol 2010;165:390-411. http://dx.doi.org/10.1016/j.ygcen.2009.02.013.
[31] Fauvel C, Suquet M, Cosson J. Evaluation of fish sperm quality. J Appl Ichthyol 2010;26:636-43. http://dx.doi.org/10.1111/j.1439-0426.2010.01529.x.
[32] Wojtczak M, Ku□zmi□nski H, Dobosz S, Mikołajczyk T, Dietrich GJ, Kowalski R, et al. Milt characteristics in European whitefish (Coregonus lavaretus) in relation to s season and hormonal stimulation with a gonadotropin-releasing hormone analogue, azagly-nafarelin. Adv Limnol 2007;60:171-85.

6. Acknowledgements

This study was financed by the EUREKA project “Development of an induce spawning technology and hatchery manual for the Eurasian perch (Perca fluviatilis), (E! 8028 PERCAHATCH) and by the grant from Lorraine Region for post-doctoral position of DZ_ at University of Lorraine. Additional support was also obtained from COST Action (FA1205 AQUAGAMETE).