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

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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 μg 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 × 10^9 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 = 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

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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 widely-observable 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 study was 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 Żurawia 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 Żarski 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 μg 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 (10000×*g*