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Administration of 17α-hydroxyprogesterone into mature male Japanese eel reduces sperm motility by decreasing potassium ion concentrations in the seminal plasma

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ABSTRACT

Our objective was to evaluate the effects of hormonal treatments on the milt quality during the induction of spontaneous spawning in male eels. We measured the changes in milt characteristics (sperm motility, milt pH, seminal plasma ionic concentration) during the course of hormonal treatments intended to induce spontaneous spawning, and before and after spawning. Male eels received weekly injections of hCG (1 IU/g BW/wk) to maintain spermiation. To induce spontaneous spawning, males received another priming injection of hCG (1 IU/g BW) two days before spawning, followed 24 h later by an injection of 17α-hydroxyprogesterone (OHP: 1 μg/g BW). Three males, treated as described above, and one female, that had received hormonal injections to induce vitellogenesis and final maturation, were transferred to a tank to spawn. In Experiment 1, we measured the characteristics of milt (1) before administering the priming dose (1–2 days after the weekly injection of hCG), (2) before injection with OHP, (3) after spawning (24 h after the OHP injection), and (4) before weekly injections with hCG (4–5 days after OHP injection). To evaluate the effects of hormonal treatment on the characteristics of milt we administered the same treatments but did not allow spawning (Experiment 2). In both experiments, the percent motility remained high prior to OHP injection, decreased significantly 24 h after OHP injection, then recovered by the beginning of weekly hCG injection. The changes in potassium ion concentration were similar to those in sperm motility (%) but there was no change in pH and sodium ion concentrations. When the results were analyzed by individual in Experiment 1 ($n = 34$), the relationship between sperm motility (%) and milt pH showed significant correlations without 24 h after the OHP injection. The relationship between sperm motility (%) and potassium ion concentration showed a highly significant correlation 24 h after the OHP injection. Thus, both pH and potassium ion concentration regulate motility in Japanese eel spermatozoa during hormonal treatment. Furthermore, administration of OHP into spermiating males reduced motility by decreasing the potassium ion concentration in the seminal plasma.

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1. Introduction

The Japanese eel (*Anguilla japonica*) is widely cultured in Japan, but does not mature when held in captivity. To overcome this, maturation is typically induced by hormonal treatment (Tanaka et al., 2003). In males, spermatogenesis and spermiation can be induced by weekly injections of human chorionic gonadotropin (hCG) (Ohta et al., 1996a). Similarly, continuous administration of hCG via an osmotic pump appears to be effective at inducing maturation (Kagawa et al., 2009). We confirmed that weekly injections of hCG following a six week period of continuous treatment with hCG resulted in long-term spermiation for up to 3 months after the commencement of spermiation in each male (Imaizumi et al., Unpublished data). In females, vitellogenesis is induced by between 8–13 weekly injections of salmon pituitary extract (SPE). Final maturation and ovulation can then be induced by a follow up injection of SPE (priming dose) and, after 24 h, an injection of 17, 20β-dihydroxy-4-pregnen-3-one (DHP: Kagawa et al., 1997; Ohta et al., 1996b) or 17α-hydroxyprogesterone (OHP: Unuma et al., 2011, 2012), a DHP precursor. Using this approach, ovulated eggs can be obtained within 12–18 h after the final injection.

Fertilized eggs are typically obtained by one of two methods in eels. The first is by artificial fertilization in which ovulated eggs and spermatozoa are mixed in seawater (Ohta et al., 1997b; Tanaka et al., 2003). The second is by spontaneous spawning in which a female and a few males are administered hormonal treatment to induce final maturation and spermiation and then held together in a tank (Dou et al., 2007, 2008; Satoh et al., 1992). Horie et al. (2008) compared fertilization success

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between the two methods and concluded that the spontaneous spawning method yielded better success. The authors suggested that the difference in fertilization success may be explained by the shorter time lag between ovulation and fertilization when using the spontaneous spawning method, as egg quality decreases rapidly after ovulation (Nomura et al., 2013; Ohta et al., 1996b, 2003).

Although the first attempt to artificially induce spontaneous spawning in the Japanese eel was conducted more than 30 years ago (Satoh, 1979; Satoh et al., 1992), little effort has been made since to improve the methodology. This is primarily because the majority of research has focused on improving egg quality. In those works, the fertilization rates were essential to compare the egg quality which resulted from artificial fertilization using good quality milt. (e.g., Furuita et al., 2004; Kagawa et al., 1997; Unuma et al., 2004, 2005).

According to the few recent reports in which researchers have attempted to induce spontaneous spawning in the eels (Dou et al., 2007; Horie et al., 2008; Van Ginneken et al., 2005), females receive a conventional hormonal treatment before spawning. This consists of repeated injections of piscine pituitary extract to induce vitellogenesis, followed by an injection of pituitary extract (priming dose), followed by a final injection of DHP (Horie et al., 2008; Van Ginneken et al., 2005), or hCG and OHP (Dou et al., 2007). In males, spermatogenesis and spermiation are induced by repeated injections of hCG and final inducer consisting of a high dose (1000 IU/male) injection of hCG (Dou et al., 2007), or both hCG and OHP (Horie et al., 2008). Miura et al. (1991) reported that injections of DHP in the early spermiation period significantly raised milt volume, the percentage of motile sperm, and the duration of sperm motility in the Japanese eel. Thus, as with females, DHP or OHP are potential candidates for the final inducer in male eels.

Eel spermatozoa are immotile in an isotonic solution such as seminal plasma. Motility is initiated when osmolality is increased (Ohta and Izawa, 1996). The potential for motility in eel spermatozoa is dependent on extracellular potassium and bicarbonate ion concentrations in an isotonic solution (Ohta et al., 1997a, 2001). The pH, which changes corresponding with bicarbonate concentrations *in vivo* (Morisawa and Morisawa, 1988), is also known to control sperm motility in the teleost sperm duct (Lahnsteiner et al., 1998; Morisawa et al., 1993). However, the relationship between changes in potassium ion concentrations in the seminal plasma and sperm motility is poorly understood. Asturiano et al. (2004) observed the effect of changes in the ionic composition of the seminal plasma on sperm quality in artificially matured European eel. The authors noted a tendency for K^+ to increase as motility increased, but the correlation was not significant. Thus, there is no reliable evidence that potassium ion concentration has any effect on sperm motility in the sperm duct.

Our objective was to evaluate the effects of hormonal treatments on the milt quality during the induction of spontaneous spawning in male eels. We measured the changes in milt characteristics (sperm motility, milt pH, and seminal plasma potassium and sodium ion concentration) during the course of hormonal treatments intended to induce spontaneous spawning, and before and after spawning.

2. Materials and methods

2.1. Fish and hormonal treatment

All of the experiments were conducted at the Shibushi Laboratory, National Research Institute of Aquaculture, Fisheries Research Agency, Japan.

We obtained female eels ($n = 14$, body weight 541.4 ± 27.0 g) that had been reared from glass-eels in fresh water ponds at the laboratory, or were purchased from a commercial supplier of wild freshwater eels. The eels were acclimated to sea water (20 °C) for a period of 4–5 days. During the hormonal treatments, the eels were held in-doors in 400 L flow-through seawater tanks under a natural photoperiod. The fish were marked individually by freeze branding (Sorensen et al., 1983).

Female eels were injected intraperitoneally with salmon pituitary extract (SPE: 30 μg/g BW) once a week and their body weight was measured each Monday morning. After the 5th injection, the temperature of the rearing water was decreased from 20 °C to 15 °C over a period of 36 h, then held constant until administration of the priming injection (Unuma et al., 2011, 2012). Following each of the 9th–17th injections, when the body weight of the female exceeded 110% of the initial weight, we measured the oocyte diameter of the largest group in the ovary on a Monday using the cannula method (Ohta et al., 1996b). When the mean egg diameter exceeded 830 μm, the female was injected with a priming dose of SPE (30 μg/g BW) the next day (Tuesday). If the diameter was between 750–829 μm, the priming dose was given on Wednesday. After the priming dose, the rearing water temperature was gradually raised from 15 °C to 20 °C by the next morning. At 24 h after receiving the priming dose, the females were injected intraperitoneally with 17α-hydroxyprogesterone (OHP; 2 μg/g BW; Sigma-Aldrich Japan, Tokyo, Japan). All injections were administered at ~09:00 of each day (Fig. 1).

Male eels ($n = 57$, body weight $= 303.4 \pm 3.28$ g) were purchased from commercial eel suppliers in Kagoshima Prefecture, Japan. Male eels were treated with 330 IU/week/male human chorionic gonadotropin (hCG; "Gonatropin"; Aska Pharmaceutical Co., Ltd., Tokyo, Japan) for a period of 6 weeks using an osmotic pump (Type 1002; Alzet Osmotic Pumps Co., Cupertino, CA, USA; diameter = 6 mm, length = 15 mm), following the methods described by Kagawa et al. (2009). The osmotic pump releases 5 μl of solution per day for approximately 45–50 days when the fish are maintained at a water temperature of 20 °C. Beginning 6 weeks after implantation of the osmotic pump, the male eels were administered weekly intraperitoneal injections of hCG (1 IU/g BW) until the end of the experiments (1–10 times per male). At the same time a female received a priming injection of SPE (two days before spawning), we randomly selected three males from the pool of males that were expressing sufficient milt, and these individuals received an additional priming injection of hCG (1 IU/g BW). At 24 h after the injection with hCG (at the time of OHP injection in the females), the males also received an injection of 1 μg/g BW OHP (Fig. 1). The OHP was dissolved in 99.5% ethanol and diluted 1:1 with 0.9% NaCl just before the injection. The rearing water temperature for males was maintained at 20 °C until the OHP injection.

2.2. Spontaneous spawning

Following injection with OHP, the female and three males were transferred to a tank (450 L) containing seawater (22 °C) to induce spontaneous spawning (Horie et al., 2008). At 11 and 24 h after the OHP injection, the occurrence of spawning was confirmed by the existence of fertilized eggs in a net (50 L) placed over the tank outflow. When eggs were present in the net, they were gently stirred. We then collected a 10 mL water sample using a pipette to estimate the number of eggs. In addition, we collected a random sample of >100 eggs to determine the fertilization rate. All of the females were dissected after spawning. Milt was collected from all of the males at 24 h after injection with OHP (following spawning), and the males were returned to their original tanks (20 °C).

2.3. Experimental design

2.3.1. Experiment 1

We measured the changes in sperm motility (%), milt pH, and seminal plasma potassium and sodium ion concentrations during the course of hormone injections and before and after spontaneous spawning. We induced spawning in 14 females and 42 males (3 males per female). We collected ~ 0.5 mL of milt from each male using a micro pipette by the application of gentle pressure on the abdomen to examine the milt characteristics at several time points (Fig. 1): 1) at the time the priming

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