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Determination of differences in the biochemical properties of sperm activating and non-activating ovarian fluids and their influences on sperm motility in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Ovarian fluid (OF) of individual female fish can initiate and affect sperm motility characteristics differently. Although it is naturally expected that OFs should stimulate and prolong sperm motility, some OFs inhibit sperm motility. In this study, 7 of 41 samples the OFs of different female *Oncorhynchus mykiss* inhibited sperm motility, while the other OFs initiate sperm motility and dissimilarly affect the progressive motility percentage (%), the duration of progressive motility (s) of sperm. This study aimed to figure out the differences between the activating and non-activating OFs with respect to the concentrations of the major inorganic ions (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}), protein, cholesterol, glucose, osmolality, pH, catalase activities, and lipid peroxidation levels. The significant differences were found between all parameters, except Cl^-, of the sperm activating and non-activating OFs ($P < 0.05$). The concentrations of K^+, Ca^{2+} and Mg^{2+}, protein cholesterol, glucose and enzymatic activities in the non-activating OFs were higher, while their Na^+ and pH levels were lower than those in the activating OFs. The non-activating effect of OFs on sperm motility is mainly due to the ionic composition, especially K^+, rather than pH.

1. Introduction

Like many fish species with external fertilization, spermatozoa of salmonid species are immotile in the testis, and their motility is initiated when they are released into water. This initiation phenomenon has mainly been described based on a decrease in K^+ for Salmonidae and Acipenseridae species or changes in osmolality for Cyprinidae and marine fish species. These variations create stimulating signals for flagellar movement, following depolarization of the cell membrane (Morisawa et al., 1983a,b; Alavi and Cosson, 2006). Initiation of motility, as well as duration of motility, is highly affected by contents of the external media (Cosson et al., 2008; Dzyuba and Cosson, 2014) such as hatchery water, various experimental activation media, and ovarian fluid (OF). For instance, the increase in Ca^{2+} concentrations in the activation solution could prevent the inhibitory effect of K^+ on the motility of trout spermatozoa (Billard and Cosson, 1992).

Unlike the other teleost, the lack of oviducts is observed in salmonid species. The mature eggs are released from the follicles, and then discharged into the coelomic cavity (or the body cavity), and spawned out through the genital papilla (Henderson, 1976; Nagahama, 1983; Berndtson and Goetz, 1990). The coelomic epithelium cells do not have secretive functions while the one-layered cells of ovarian cavity are secretory-active epithelium in mature rainbow trout. Therefore, the term OF which bathes the eggs and constitutes 10–30% of the total egg volume can also refer to coelomic fluid or peritoneal fluid (Van den Hurk and Peute, 1979; Lahnsteiner et al., 1995; Lahnsteiner et al., 1999) in salmonids.

The OF is ordinarily a proper and distinguished medium for sperm motility in salmonids (Billard, 1983). In different salmonid species (*Salvelinus alpinus* Turner and Montgomerie, 2002; Urbach et al., 2005; *Salvelinus namaycush* Butts et al., 2012; Galvano et al., 2013; *Oncorhynchus mykiss* Dietrich et al., 2008; *Oncorhynchus tshawytscha* Rosengrave et al., 2009; *Salmo trutta caspius* Hatef et al., 2009; *Salmo trutta fario* Lahnsteiner, 2002), it has been shown that the OF improved spermatozoa motility characteristics such as swimming velocity, swimming trajectories, the duration of forward motility, and the percentage of motility. Apart from salmonids, the positive effects of OF on spermatozoa motility have also been observed in other fish species like *Perca fluviatilis* (Mansour et al., 2009), *Gadus morhua* (Litvak and Trippel, 1998), *Alburnus alburnus* (Lahnsteiner et al., 1997b), *Cottus gobio* (Lahnsteiner et al., 1997a), *Hemilepidotus gilberti* (Hayakawa and Munehara, 1998), *Gasterosteus aculeatus* (Elofsson et al., 2003). Besides various ions, proteins, nutrients, metabolites, and hormones, the OF also contains the conjugated steroids, some of which are used as pheromones, produced by fish gonad (Scott and Vermeirssen, 1994; Hirano

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et al., 1978; Lahnsteiner et al., 1995). The function of this specific composition is attributed to not only a prolong effect on spermatozoa motility but also a cryptic female choice, which refers that spermatozoa have different motility characteristics when activated by OFs from different females (Lahnsteiner et al., 1995; Liley et al., 2001; Yeates et al., 2013). The cryptic female choice is well explained in *S. alpinus* (Urbach et al., 2005), *O. tshawytscha* (Rosengrave et al., 2009), and *Poecilia reticulata* (Gasparini and Pilastro, 2011). Consequently, the OF has ability to mediate spermatozoa motility and to stabilize the micro-environment around the micropyle of egg (Billard, 1983; Lahnsteiner, 2002).

By examining the effects of the OFs from different female rainbow trout on the percentages (%) and the durations (s) of sperm progressive motility, this study aimed to determine the differences between the activating and non-activating OFs with respect to the concentrations of the major inorganic ions (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}), protein, cholesterol, glucose. Osmolality, pH, catalase activities, and lipid peroxidation levels were also determined.

2. Materials and methods

2.1. Fish and gamete collection

Female and male rainbow trout breeders were maintained in a commercial fish hatchery located in Muğla, Turkey. All fish were fed with the same diet. Gametes were obtained in the beginning of December 2013 from 2-year-old fish by manual abdominal stripping while avoiding any contamination from water, blood, urine, or feces. The ovulation of females was not hormonally induced. Starting in the middle of November, the fish were examined by gentle manual pressure on their abdomen if they had already ovulated. When the female fish could be stripped, the experiment was started. All fish randomly selected from the same strain and the broodstock pool. The batches of eggs from each female were stripped to the sterilized glass beakers which were placed 210 μm meshes on the bottom of them, allowing the OF to drain from the eggs. In this way, OF was separated from the eggs and then pipetted out of the beaker and into screw-cap tubes to minimize air equilibration, especially avoiding a decrease of pH of OF (Rosengrave et al., 2009). We also paid attention on the turbidity of OF which could be affected by broken eggs to prevent the changes of pH in OF (Dietrich et al., 2007; Lahnsteiner, 2000). The OF samples which will be used for biochemical analysis were stored at −80 °C, and the measurement of pH and sperm motility characteristics was performed within an hour.

2.2. Analytical procedures and measurement of sperm motility

To avoid an increase in pH due to the loss of CO_2 (Rosengrave et al., 2009), the pH of OFs was measured with a pH meter (WTW 3110 GmbH, Germany) immediately. Osmolarity measurements were performed with a Gonotec Osmomat 030 cryoscopic osmometer (Gonotec, Berlin, Germany). All OF samples from were stored at −20 °C for ionic and biochemical analyses. The parameters of OF (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, cholesterol and glucose) were measured using an Abbott-Aeroset autoanalyzer (Chicago, IL, USA) using original kits. The protein concentrations were determined by the Bradford method (Bradford, 1976). Catalase activities (CAT) of OFs were measured by the method previously described by Goth (1991). The lipid peroxidation levels in the OF samples were measured with a thiobarbituric acid (TBA) reactive substance assay which monitors malondialdehyde (MDA) production (Buege and Aust, 1978).

The effects of OF on sperm motility characteristics (percentage (%) and duration (s) of progressive sperm motility) were examined on a pooled sperm sample activated in the OF from each female ($n = 41$). The pooled sperm sample was constituted by using sperm samples with more than 90% motility percentage from six males. A saline activation medium containing 125 mM NaCl, 30 mM glycine, and 20 mM Tris–HCl, adjusted at pH 9.0 (Billard, 1983) was used for examining the activation of motility. 1 μL sperm was thoroughly mixed with 399 μL of the activation solution (accepted as the control) or OFs. The motility of sperm was recorded, in triplicates, with a video camera (AxioCam ICc 5, Germany) mounted on a phase-contrast microscope (Zeiss Axio Scope A1, Carl Zeiss Microscopy, Germany) at 400× until the spermatozoa trajectories become tight concentric circles (Rurangwa et al., 2004). The video records were scanned to determine the percentages of progressive motility (%) and the durations of progressive motility (s). The sperm motility percentages were estimated as the percentage of cells that exhibited progressive forward movement (Billard and Cosson, 1992; Horvath et al., 2003), and the durations of motility were determined as the times until forward movement stopped and circular movement began. The percentages of sperm motility were assessed using an arbitrary scale with 10% interval increments in which non-motility was recorded as 0% (modified from Borges et al., 2005).

2.3. Statistical analysis

All values are represented mean ± standard deviation. Because of the unequal variance and sample size, non-parametric Mann–Whitney U tests were used (Mann and Whitney, 1947) and <0.05 was taken to indicate significant differences between the parameters of the OFs which activate spermatozoa and cannot activate spermatozoa. Relationships between the OF parameters and sperm motility characteristics were shown by Pearson correlation coefficients.

3. Results

3.1. Percentage and duration of progressive sperm motility in OF samples

The effects of OFs from different female rainbow trout on the percentage and duration of progressive sperm motility are shown in Fig. 1. Sperm were motile in 34 OF samples, and were immotile in the other 7 OFs. The average motility percentages and durations of the sperm activated by the activation solution were 96.7 ± 5.8% and 21.3 ± 0.6 s respectively. The duration of sperm motility activated by the OFs ranged from 21 to 37 s while the percentage of sperm motility in the activating OFs was at least 30%.

3.2. OF parameters and their relationship to sperm motility characteristics

The osmolality, the pH values, the protein concentration, the metabolite and ionic composition of the OFs together with MDA and CAT values are shown in Table 1. The data were represented in the different columns as the OF parameters which can activate ($n = 34$) and cannot activate spermatozoa ($n = 7$). Na^+ as the dominant basic ion and Cl^- as the dominant acidic ion were the components in both the activating and non-activating OFs. There were statistically significant differences between the two groups in terms of all parameters, but not of Cl^- ($P < 0.05$). The concentrations of the constituents and enzymatic activities in the activating OFs were much more than those in the non-activating OFs, but except for the concentrations of Na^+ and the values of pH. The values of these two parameters in the activating OFs were lower than those in the non-activating OFs. However, a fivefold increase for K^+ and a sixfold increase for Mg^{2+} were noticed in the non-activating OFs on the basis of their concentration mean values. Also, MDA and CAT increased in the non-activating OFs, compared to those in the activating OFs.

There was a significant correlation between pH-motility percentage and pH-duration of motility ($R = 0.81$ and $R = 0.85$, respectively, $n = 41$, $P < 0.05$). The most important negative significant correlations were, however, found between K^+-motility percentage, K^+-duration of motility, Mg^{2+}-motility percentage and Mg^{2+}-duration of motility ($R = -0.80$, $R = -0.84$, $R = -0.86$ and $R = -0.91$, respectively, $n = 41$, $P < 0.05$). Besides, MDA and CAT have shown negative