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Sodium affects the sperm motility in the European eel

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ABSTRACT

The role of seminal plasma sodium and activation media sodium on sperm motility was examined by selectively removing the element from these two media, in European eel sperm. Sperm size (sperm head area) was also measured using an ASMA (Automated Sperm Morphometry Analyses) system, in the different conditions. Intracellular sodium $[Na^+]_i$ was quantitatively analyzed by first time in the spermatozoa from a marine fish species. Measurement of $[Na^+]_i$ was done before and after motility activation, by Flow Cytometry, using CoroNa Green AM as a dye. Sperm motility activation induced an increase in $[Na^+]_i$, from 96.72 mM in quiescent stage to 152.21 mM post-activation in seawater. A significant decrease in sperm head area was observed post-activation in seawater. There was a notable reduction in sperm motility when sodium was removed from the seminal plasma, but not when it was removed from the activation media. Sodium removal was also linked to a significant reduction in sperm head area in comparison to the controls. Our results indicate that the presence of the ion Na^+ in the seminal plasma (or in the extender medium) is necessary for the preservation of sperm motility in European eel, probably because it plays a role in maintaining an appropriate sperm cell volume in the quiescent stage of the spermatozoa.

1. Introduction

The spermatozoa of species which exhibit external fertilization are generally immotile in the seminal plasma and initiate their motility immediately upon the dilution in freshwater or seawater at spawning (Morisawa, 1985). The mechanism for sperm activation has been widely studied in mammals and in sea urchin (*Strongylocentrotus purpuratus*, see review of Espinal et al., 2011). However, little is known about this mechanism in fish sperm. In marine teleosts, spermatozoa are immotile due to the iso-osmolality with the seminal plasma (Morisawa and Suzuki, 1980; Stoss, 1983; Alavi and Cosson, 2006). Then, the hyperosmotic shock faced by the spermatozoa when they are released into the marine environment leads to a rapid flux of ions and water across the spermatozoa membrane which activates the cells' motility (Morisawa, 2008). However, few studies have been conducted in marine fish species about the changes in the concentrations of ions inside the sperm cells before and after motility activation.

The main ions present in marine fish seminal plasma are: Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^- (Suquet et al., 1993; Asturiano et al., 2004). In a previous study on European eel the ionic composition of seminal plasma was linked to sperm motility. It was observed that the concentration of some seminal plasma ions (K^+, Ca^{2+}, Mg^{2+}) changed in a progressive way from the low motility sperm samples to the high motility sperm samples. For instance, seminal plasma K^+ concentrations increased during the improvement in sperm quality, while Ca^{2+} and Mg^{2+} concentrations showed a progressive reduction in correlation with the improvement in sperm quality (Asturiano et al., 2004). Na^+ was also present in European eel seminal plasma, but the concentrations of this ion were almost constant, between 110 and 120 mM, irrespective of the motility category. The selective elimination, one by one, of the main ions (Na^+, K^+, Ca^{2+}, H^+) present in the seminal plasma could be key to determining the role they play in the further sperm activation process due to hyperosmotic shock, or, in other words, in maintaining, in quiescent stage, the capability for further sperm activation.

In a preliminary study on European eel, Gallego et al. (2014) observed that intracellular concentrations of Ca^{2+} and K^+ in sperm increased after hyperosmotic sperm activation, with a progressive decrease in intracellular pH suggesting a flux of these ions through the spermatozoa membrane during sperm activation. Also, it has been demonstrated the presence of a Na^+/Ca^{2+} exchanger involved in the sperm motility of European eel. The inhibition of this exchanger by bepridil induced a suppression of the increase in $[Na^+]_i$ and was linked to a notable reduction in sperm motility (Pérez et al., 2016). Although Na^+ is one of the main constituents of fish seminal plasma (Suquet et al., 1993; Asturiano et al., 2004), Na^+ fluxes during sperm activation in marine fish sperm have been poorly studied.

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Regarding the involvement of Na^+ in sperm motility, several studies have demonstrated the importance of this ion in the sperm cells from mammals and sea urchin (Escoffier et al., 2012; Espinal et al., 2011). In mammalian sperm, the hyperpolarization associated with capacitation involves a decrease in $[Na^+]_i$ mediated by an inhibition of epithelial Na^+ channels (ENaC; Escoffier et al., 2012). Na^+/H^+ exchangers (NHEs) are also present in the sperm membranes of mammals and sea urchin, and are known not only to participate in the regulation of intracellular pH, but also in water absorption across epithelia, and cell volume regulation (Martins et al., 2014; Nomura and Vacquier, 2006). However, information about sodium channels in marine fish sperm is restricted to the use of a sodium channel inhibitor, amiloride, which inhibits sperm motility in Atlantic croaker (*Micropogonias undulatus*; Detweiler and Thomas, 1998). However, in other marine species, the Pacific herring (*Clupea pallasi*; Vines et al., 2002), it is the decrease in external sodium (not the increase) that appears to be involved in sperm activation. Therefore, the ion sodium could play species-specific roles in sperm motility in marine species. For this reason, the present study tries to analyze and understand the role of the ion Na^+ in the sperm motility of a marine species, the European eel.

There are several methods that can be used to measure the intracellular ion concentrations in sperm. In sea urchin (*S. purpuratus*; Rodriguez and Darszon, 2003), the intracellular concentrations of Na^+, Ca^{+2} and pH_i were measured by spectrophotometry, while Márian et al. (1997) was the first to use flow cytometry to quantify intracellular ions in sperm cells from a freshwater fish. However, Márian et al. (1997) and her group quantified some ions, including $[Na^+]_i$ by indirect methods, through the measurement of the fluorescence emitted by a pH-indicator dye in the presence of nigericin, which equals the $[H^+]_i = [Na^+]_i$. The present work shows for the first time the intracellular sodium concentrations measured by a direct method (through the intensity of the fluorescence emitted by ion sodium) and by flow cytometry (through the intensity of fluorescence emitted cell by cell) in the sperm of a teleost species.

In the present study, the European eel was used as the experimental organism, as it is easy to produce high quantities of good quality sperm after a 6–7 weeks of treatment with weekly injections of hCG, and to maintain spermiation for several weeks (Gallego et al., 2012). The present study focuses on the importance of the Na^+ present in the seminal plasma and in the activation media in the sperm motility activation. The involvement of cell volume changes in sperm motility has also been studied by measuring the sperm head area. Moreover, this study looks at the $[Na]_i$ before and after sperm activation of the European eel to determine whether Na^+ fluxes participate in the sperm motility of this species.

2. Material and methods

2.1. Chemicals and solutions

The Na ionophore Monensin (M5273), amiloride inhibitor (A7410), EDTA, and Bovine Serum Albumin (BSA) were purchased from Sigma Aldrich Co. (St. Louis, MO). CoroNa Green AM (C36676), and Propidium Iodide (R37108) fluorochroms were purchased from Invitrogen and Molecular Probes respectively (Life Technologies, Madrid-Spain). Salts were of reagent grade.

A stock solution of 20 mM of monensin was diluted in DMSO, aliquoted and kept at −20 °C until use. An aliquot of the stock solution to be used with the sperm, was thawed only once and mixed with the sperm to reach a final concentration of 20 μM.

In the same day, a stock solution of 50 mg/ml of amiloride was diluted in ultrapure hot water and mixed with the sperm to a final concentration of 2 mM. DMSO stock 1 mM CoroNa Green AM was prepared and used as described in Section 2.7.

2.2. Fish maintenance and hormonal treatment

A total of 40 adult male eels (mean body weight 115 ± 8 g) were transferred to our facilities at the Universitat Politècnica de València (Spain) from the fish farm Valenciana de Acuicultura, S.A. (Puzol, Valencia; East coast of Spain). The fish were distributed into two 90-L aquaria (approximately 20 male eels per aquarium) equipped with separated recirculation systems, thermostats, and coolers, and covered with black panels to maintain constant darkness. The animals were gradually acclimatized to seawater (salinity 37.0 ± 0.3 g/L) over the course of 1 week, and were then maintained in seawater at 20 °C until the end of the experiment, as in previous works (Peñaranda et al., 2009; Gallego et al., 2013). Water renewal was 1/3 of the volume of each aquarium per week.

Once the fish were in seawater the hormonal treatment with hCGrec (recombinant hCG; Ovitrelle, Merck Serono, Madrid) was initiated. Once a week, the animals were anesthetized with benzocaine (60 ppm) and weighed before receiving the intraperitoneal injection of hCGrec (diluted in NaCl 0.9%) at a dose of 1.5 IU/g fish.

During the experiment the fish were starved, and were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC).

2.3. Sperm collection and sampling

Sperm samples were collected once a week, 24 h after the administration of the hormone, to obtain maximum sperm quality (Pérez et al., 2000). Sperm was collected in Falcon tubes by gentle abdominal pressure, after fish anesthetization. The genital area had been previously cleaned with distilled water, and dried, in order to avoid sample contamination with feces, urine and seawater. The sperm samples were kept refrigerated (4 °C) until the motility analyses, which took place within the first hour after collection.

2.4. Sperm motility evaluation

The sperm motility activation was carried out as per the method described by Gallego et al. (2013), by mixing 1 μl of diluted sperm (dilution 1/25 in control extender, Table 1, Peñaranda et al., 2009) with 4 μl of artificial seawater (ASW; Aqua Medic Meersalz, 37 g/l, with 2% BSA (w/v), pH adjusted to 8.2). The mixture was made in a SpermTrack-10® chamber, 10 μm depth (Proiser R + D, S.L.; Paterna, Spain) and observed in a microscope Nikon Eclipse 80i, with a 10× lens (Nikon phase contrast 10 × 0.25, Ph1 BM WD 7.0). Motility was recorded 15 s after the sperm was mixed with ASW, using a high-sensitivity video camera HAS-220 and the ISAS software (Proiser R + D, S.L.; Paterna, Spain). The frame rate used was 60 fps. For each motility test, samples were evaluated in triplicate. Both the sperm and the ASW were maintained at 4 °C in a water bath during the sperm motility evaluation. The best samples (>50% total motility) were selected for the studies.

The sperm motility parameters considered in this study were: total motility (MOT, %); progressive motility (MP, %), defined as the percentage of spermatozoa which swim forward in an essentially straight line; the percentage of fast (FA; average path velocity, VAP > 100 μm/s); curvilinear velocity (VCL, in μm/s), defined as the time/average velocity of a sperm head along its actual curvilinear trajectory; straight line velocity (VSL, μm/s), defined as the time/average velocity of a sperm head along the straight line between its first detected position and its last position; VAP (μm/s), defined as the time/average of sperm head along its spatial average trajectory; straightness (STR, %), defined as the linearity of the average spatial path, VSL/VAP; ALH, amplitude of the lateral movement of the sperm head and cross beating frequency (BCF; beats/s), defined as the average rate at which the curvilinear sperm trajectory crosses its average path trajectory. Spermatozoa were considered immotile if their VCL was <10 μm/s (Martínez-Pastor et al., 2008).