Animal Reproduction Science 138 (2013) 305–313

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Motility of sturgeon spermatozoa can sustain successive activations episodes

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ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 15 February 2013
Accepted 16 February 2013
Available online 6 March 2013

Keywords:
Sturgeon
Spermatozoa
Motility signaling
Osmolality
Calcium

ABSTRACT

Here we report for the first time the possibility of sequential sperm motility activation in sturgeon (sterlet, *Acipenser ruthenus*), a fish with external fertilization, through changes either in osmolality (global solute concentration) or in the Ca^{2+} concentration of the medium surrounding the spermatozoa. Sperm motility was initiated in any of three solutions containing buffer and sucrose at 80, or 40 or 10 mM (called S80, S40, S10, respectively); S80 is hypertonic relative to sterlet seminal fluid, while S40 is isotonic and S10 is hypotonic. After cessation of sperm movement at the end of this first motility period, a second and then a third, subsequent motile phase were observed. The second motility period was induced at cessation of motility in S80 by imposing a two-fold decrease in osmolality. After arrest of motility in this half-diluted S80, a third motility period could be initiated by addition of $CaCl_2$ to 1 mM final concentration. At the end of a first motility period in either S40 or S10, subsequent motility re-activation episodes were achieved only by addition of 1 mM $CaCl_2$. Depending on conditions in which sperm samples were activated, significant differences in curvilinear velocity, percent motile spermatozoa, motility duration time, and specific external features of spermatozoa flagella were observed. Altogether, these observations on the ability of sturgeon spermatozoa to sustain sequential activation episodes by experimental adjustment of their environmental conditions represent a potent model for deeper investigations on the sperm motility activation mechanisms.

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1. Introduction

Activation of sperm flagellar motility occurs differently according to animal species, either progressively during a "maturation" process or as a response to a specific signal perceived by the spermatozoa (Darszon et al., 2001). In the case of animals with external fertilization such as fish, several sperm activating factors have been described in fish of differing taxa, with changes in osmolality and/or ion composition of the environment being considered the most common (Cosson, 2004; Alavi and Cosson, 2006).

The study of sperm motility by varying the activation media has been applied to several species of the order Acipenseriformes (sturgeon and paddlefish) because of its potential applications for artificial propagation. Almost all representatives of these taxa have a high market demand but an endangered status (Billard and Lecointre, 2001). Sturgeon sperm immotility in seminal plasma, along with immediate activation at transfer into fresh water, are considered common features of most freshwater spawning fish species. The ability of sturgeon and paddlefish spermatozoa to be activated in both hypo- or

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http://dx.doi.org/10.1016/j.anireprosci.2013.02.011

hyper-tonic (relative to seminal fluid) activating media (Alavi et al., 2006) can be considered a specific property of the activation mode of sperm motility in this group of species. The well-known suppression of sperm motility in the presence of a high potassium ion (K^+) concentration in activating medium, which can be abolished by the presence of calcium (Ca^{2+}) ions (Linhart et al., 2002, 2003), has been summarized as "antagonism between K^+ and Ca^{2+} ions" (Cosson, 2004, 2010). This suggests that sturgeon and paddlefish possess an ionic mode of sperm motility activation. Moreover, the participation of Ca^{2+} in the sequence of cellular events leading to sperm motility in fish possessing an ionic mode of activation has also been extensively documented in salmonid fishes (Cosson et al., 1989; Boitano and Omoto, 1992). Furthermore, changes in internal Ca^{2+} concentrations are also thought to be an essential step of motility activation in tilapia (Morita et al., 2003). However, the involvement of environmental osmotic changes in sterlet sperm motility activation has also been suggested (Alavi et al., 2011).

In the spermatozoa of several fish species with an osmotic mode of motility activation, motility can be stopped and immediately reactivated by applying successive alterations in osmotic pressure (Boryshpolets et al., 2009; Hu et al., 2009; Takai and Morisawa, 1995). The same result can be achieved by, for example, changes in K^+ (in salmonids (Benau and Terner, 1980)) or CO_2 (in flatfish species (Inaba et al., 2003)) concentrations, which alter sperm metabolic conditions and are considered the main activating signals for motility control. So, investigation of the ability of fish sperm to sustain successive activation episodes could be considered as a tool for a more precise study of sperm motility activation mechanisms.

In sturgeon, the changes in motility parameters under various conditions of osmolality and ionic composition of the activating media have been described (Alavi et al., 2004, 2008), but the extent of sperm reactivation abilities, whether only once or multiple times, is still unclear. A more complete description of environmental conditions for motility activation and reactivation is required in order to understand the basis of sperm motility signaling in fish of Acipenseriformes order.

In the present study we hypothesized that factors influencing motility activation of sturgeon sperm such as environment osmolality and Ca^{2+} concentration could operate separately and that relationships between them can be elucidated by studying the ability of spermatozoa to exhibit successive motility periods. The sterlet, *Acipenser ruthenus*, was used as a model sturgeon species.

2. Materials and methods

2.1. Fish rearing conditions and sperm collection

The study was carried out at the hatchery of the Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic. Broodstock of sterlet (3–5 years old, 0.67–1.18 kg body weight) were kept during the natural spawning season in 4 m^3 outdoor plastic tanks with a constant pond water flow rate of 20 L min^{-1} and temperature of 8–12 °C. Prior to hormone treatment, fish were moved to a closed water recirculation system, with water temperature gradually elevated to 15 °C over the course of the subsequent 24 h. Males were injected intramuscularly with carp pituitary extract (CPE, product of Rybníkářství Pohořelice a.s., Czech Republic) at 4 mg kg^{-1}. Thirty-six hours post-injection, the urogenital tract was emptied by aspiration using a plastic catheter (4 mm diameter). Thirty-nine hours post-injection, semen was collected from the urogenital papilla by catheterization into 50 mL plastic vials and stored on ice for experimentation within 3 h (Podushka, 2003).

2.2. Chemicals

Sucrose, $CaCl_2$, Tris, ethylene glycol tetra-acetic acid (EGTA) were purchased from Sigma-Aldrich Co. Prague, Czech Republic.

The pH of 10 mM EGTA stock solution was adjusted to 8.0 by addition of NaOH.

The free Ca^{2+} concentration in media used in the study was calculated using shareware Macintosh software, taking into account concentrations of sucrose, Tris, EGTA, and $CaCl_2$ (Saudrais et al., 1998). Concentration of free Ca^{2+} in S80, S40, and S10 was calculated as 8, 4, and 1 μM respectively and in S80, S40, and S10 containing 0.1 mM EGTA was calculated as 5×10^{-5}, 2×10^{-5}, and 6×10^{-6} μM respectively.

2.3. Estimation of seminal fluid and activating media osmolalities

Seminal fluids (SF) were obtained after sperm sample centrifugation at 16,000 × *g* for 10 min. Osmolalities of SF and media used in these experiments were evaluated using a Vapor Pressure Osmometer 5520 (Wescor, USA), and expressed as mOsm kg^{-1}.

2.4. Evaluation of sperm motility parameters

Immediately following dilution, sperm motility was recorded in each mode of treatment until motility arrest, using a CCD video camera (Sony, SSCDC50AP) mounted on a dark-field microscope (Olympus BX50, ×200) and illuminated with a stroboscopic lamp (Chadwick-Helmut, 9630, USA) set to a flash frequency of 50 Hz. Video recordings were made using a DVD recorder (Sony, DVO-1000 MD). Video recordings were analyzed to estimate spermatozoa curvilinear velocity (VCL, length of sperm head track divided by the time of the track, μm s^{-1}), percent motile spermatozoa (motility, %), and motility duration (s). To compute the VCL and percent motile spermatozoa at 10 sec post-activation, five successive frames were analyzed by image analyzer (Olympus Micro Image 4.0.1 for Windows, Hamburg, Germany). Ten to 50 spermatozoa were evaluated in each frame. Spermatozoa with velocity lower than 3 μm s^{-1} were considered as immotile and excluded from further analysis. Only motile spermatozoa

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