Motile Parameters of Spermatozoa in Yellow Croaker

*Larimichthys polyactis*: Effect of Varying Sugars

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Abstract

The objective of this study was to find the optimal sugar level for motile parameters of spermatozoa of the yellow croaker *Larimichthys polyactis*. The spermatozoa were immotile in distilled water and motile in solutions containing different sugars. The highest spermatozoa velocity, motility, and duration of spermatozoa motility were obtained in solutions containing 0.5 M glucose, sucrose, fructose, or mannitol. This study provides baseline knowledge on the sensitivity of yellow croaker spermatozoa varying types and concentrations of sugar.
Introduction

Yellow croaker *Larimichthys polyactis*, also called *Pseudosciaena polyactis*, is a teleost that belongs to perchlike Sciaenidae and is an important traditional commercial fish in Korea. It migrates to the East China Sea in winter and returns to the Yellow Sea to spawn in spring (Kim et al., 1997; Lim et al., 2010).

Spermatozoa of freshwater and marine fish species are immotile in the testes and seminal plasma. Spermatozoa become motile after they are released into an aqueous environment during natural reproduction or into a diluent during artificial reproduction (Darszon et al., 1999; Alavi and Cosson, 2006; Cosson et al., 2008ab). Spermatozoa motility determines semen quality and fertilizing capacity (Alavi et al., 2004; Alavi and Cosson, 2005ab; Abascal et al., 2007) and is influenced by factors such as temperature (Williot et al., 2000; Alavi and Cosson, 2005b), pH (Ingermann et al., 2002, Alavi and Cosson, 2005ab; Zuccarelli et al., 2007), cations (Darszon et al., 1999; Linhart et al., 2003; Cosson, 2004; Alavi and Cosson, 2006; Alavi et al., 2007), osmolality (Linhart et al., 2003; Cosson, 2004; Alavi and Cosson, 2006; Alavi et al., 2007; Zuccarelli et al., 2007), dilution ratio (Alavi et al., 2004, 2007; Alavi and Cosson, 2005ab), and sugars (Lim, 1998; Chang, 2001).

The reproductive cycle (Lim et al., 2010), life-history information (Trewavas, 1991), semen properties and spermatozoan structure (Le et al., 2011a), storage of semen (Le et al., 2011b), semen cryopreservation (Le et al., 2011c), and the effects of pH, temperature, cations, and semen/diluent ratio on spermatozoa motility (Le et al., 2011d) of yellow croaker have been studied. In this study, we examined the effects of sugars (glucose, fructose, sucrose, mannitol) in different concentrations on the velocity, motility, and duration of sperm motility in yellow croaker.

Materials and Methods

Fish and semen collection. The experiment was carried out at the Aquaculture Management Division of the National Fisheries Research and Development Institute, Korea, during June 2009, which coincided with the peak spawning activity for yellow croaker (Lim et al., 2010). Broodfish were raised in a 16-m³ tank. Ten males were randomly selected and anesthetized in 100 ppm ethyl 3-aminobenzenoate methanesulfonate salt (Sigma, USA) and fish length and weight were recorded before semen collection. These fish were held in a separate 2-m³ spawning tank with flow-through seawater (32 psu salinity, 15.0–17.0°C) at a flow rate of 0.2 l/s, supplemented by an air-stone under simulated natural photoperiod (12 h dark/12 h light). They were fed a commercial feed (Suhyup Feed Co. Ltd., Uiryeong, Korea) once a day. Semen was collected by gentle abdominal massage from the anterior portion of the testes towards the genital papilla using a 1.5-ml Eppendorf tube to prevent contamination by urine, mucus, or blood. Semen was separately held for each fish on ice for up to 30 min before measurement of the semen properties (Le et al., 2011bc).

Spermatozoa analysis. The percentage of spermatozoa exhibiting rapid, vigorous, and forward movement was determined under a microscope by diluting the semen into artificial seawater composed of 27 g NaCl, 0.5 g KCl, 1.2 g CaCl₂, 4.6 g MgCl₂, and 0.5 g NaHCO₃ in one liter of distilled water at the ratio of 1:100. Samples with a movable spermatozoa ratio of more than 90% were pooled and used for further study.

Concentrations (0, 0.5, 1.0, 1.5, 2.0 M) of sugar solutions (glucose, fructose, sucrose, mannitol) were prepared by dissolving the sugar in distilled water. Semen samples were activated with a solution of 1:100 (1 µl semen sample:99 µl sugar solution), then 1 µl was put on a slide (Teflon Printed Glass Slide; 21 wells with 4-mm diameter; Funakoshi Co., Japan) without a cover slide and observed immediately at 200X magnification under a microscope (Axioskop 2 plus Zeiss, Germany). Velocity, motility, and duration of motility were quantified using a video camera (Carl Zeiss, Germany) and timer (VTG-55B, Germany) connected to a video recorder and player (Samsung VHS, SVG1000, Korea). The spermatozoa heads were recorded by video tape at the same time with video timer. The recorded tape was played and the motile parameters were observed and calculated. Velocity (μm/s) was determined by the distance covered by the
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In general, the highest velocity, motility, and duration were obtained in solutions containing 0.5 M glucose, fructose, sucrose, and mannitol (Fig. 1).

![Fig. 1. Effect of (a) glucose, (b) fructose, (c) sucrose, and (d) mannitol concentration on velocity, motility, and duration of motility of spermatozoa of yellow croaker Larimichthys polyactis. Different superscripts indicate significant differences (p<0.05).]
Discussion

Glucose supports motility and capacitation in human spermatozoa (Williams and Ford, 2001). Likewise, sugars stimulate the motility of fish spermatozoa. The motile parameters of spermatozoa of marbled brown sole, starry flounder, olive flounder, and black porgy were best at concentrations of 0.9 M glucose, fructose, sucrose, or mannitol (Lim, 1998; Chang, 2001). Glucose concentration (0-5%) has a determining effect on viability and activation of fresh sperm in bocachico Prochilodus magdalenae (Martínez et al., 2011). Fructose provides energy for sperm while the absence of fructose may indicate a problem with seminal vesicles (Ponglowhapan et al., 2004). Approximately 80% of the sperm of Java carp Puntinus javanicus, goby Oxyeleotris marmorata, and catfish Clarias batrachus showed motility of 10-100 mOsm/kg in a mannitol solution (Morita et al., 2006). The highest sperm velocity of Perca fluviatilis was activated by a sucrose solution of 200 mOsm/kg (Alavi et al., 2007).

In the present study, the effects of various sugars on the motile parameters of spermatozoa of yellow croaker were estimated. The highest velocity, motility, and duration of motility were achieved in solutions containing 0.5 M glucose, fructose, sucrose, or mannitol. Therefore, the suitable concentration of glucose, fructose, sucrose, and mannitol differs from one fish species to another.

The present study provides baseline knowledge of the sensitivities of yellow croaker spermatozoa under the effects of varying sugars. Understanding the effects of these sugars is helpful to the aquaculture industry as it allows for the development of optimal methods for artificial reproduction and short and long-term semen preservation. Further tests of the motility of fish sperm activated by glucose, fructose, sucrose, or mannitol solutions at different concentrations are necessary.

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References

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