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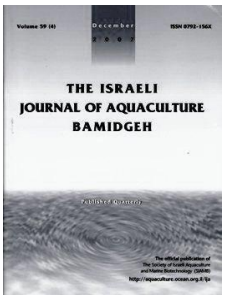

Role of antibiotics on chilled storage sperm motility of waigieu seaperch *Psammoperca waigiensis* (Cuvier and Valencienes, 1828)

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Abstract

The objective of the present study was to determine the effect of different antibiotics on chilled storage sperm motility of Waigieu seaperch *Psammoperca waigiensis*. Collected semen was diluted with an extender at a ratio of 1:3 (semen: artificial seminal plasma ASP) in 1.5ml Eppendorf tubes. Experiments were conducted by adding different antibiotics including: neomycin, gentamycin, or penicillin plus streptomycin at concentrations of 200, 400 and 600 ppm. All treatments were refrigerated at 2^{o}C. The results showed that sperm motility was best with 200 ppm gentamycin. Sperm with gentamycin remained motile up to 36 days.

Introduction

Short-term storage of sperm is a useful bio-technique that facilitates hatchery operations. It reduces the need of frequent collection of milt from males, enables transportation of sperm to distant locations and prevents problems related to asynchrony in gamete production between males and females. Semen storage is affected by extenders, dilution ratio, temperature, and antibiotics (Le *et al.* 2011; Lim *et al.* 2006; Lim *et al.* 2005). The presence of microorganisms in stored samples may endanger valuable germplasm by decreasing fertilization and lowering cell and viability (Segovia *et al.* 2000). To limit these potential problems, antibiotics are commonly added to chilled, stored, semen.

Waigieu seaperch, *Psammoperca waigiensis,* is a tropical marine fish of high economic value. Currently, not many studies have been conducted on this species. Nguyen *et al.* (2003) and Shimose and Tachihara (2006) studied the reproductive biological characteristics and artificial seed production of this species but the quantity of seed production so far is still limited. To be able to carry out mass seed for aquaculture requirements, studies on artificial insemination are essential. Additionally, this species is hermaphroditic, with asynchronous male and female phase. Recently, interest in the research of the culture of this species has risen (Pham *et al.* 2007a, b; Pham *et al.* 2009; Pham *et al.* 2012; Pham *et al.* 2010; Shimose and Tachihara 2006). A study of sperm properties of this species was carried out by Le *et al.* (2013). However, no studies aimed at determining the suitable antibiotic for the extended viability of the chilled sperm of this species have been published. The aim of this study was to identify such as suitable antibiotic for this purpose.

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Method and materials

All experiments were carried out at the Laboratory of the Faculty of Aquaculture, Nha Trang University.

Fish handling and semen collection

Male fish were caught from the wild and kept in sea cages fed trash fish rate of 5% body weight. Later they were anesthetized with Methylene glycol (Merk) at a concentration of 200 ppm. Semen was collected by abdominal massage and put it into a 1.5 ml Eppendorf tubes. Special attention was paid to avoid contamination with urine and feces in samples designated for chilled storage as these can lead to the activation of spermatozoa. The semen was placed on crushed ice immediately after collection.

Evaluation sperm motility

Motility was estimated by placing 1µl semen onto a glass slide, activating it with 99µl artificial seawater and observing under a microscope at 400X magnification. The microscope was connected to a camera. Only samples with at least 85% moving cells were chilled and stored. Motility was analyzed by using CASA (computer aided for sperm analysis) software.

Effect of antibiotics on sperm Waigieu seaperch motility

To determine optimal antibiotic for chilled storage of Waigieu seaperch sperm, the semen was diluted in Artificial Seminal Plasma (ASP) (containing: 0.5 NaCl, 0.02 NaH_2PO_4, 0.01 $NaHCO_3$, 0.04 KCl, 0.01 $CaCl_2.2H_2O$, 0.02 $MgCl_2.6H_2O$, with pH 8.1, osmolality 320 $mOsm.kg^{-1}$) at a ratio of 1:3 combined with antibiotics (neomycin and a combination of penicillin and streptomycin, gentamycin) at concentrations of 200, 400 and 600 ppm respectively. All samples were replicated 3 times and stored in a refrigerator at 2°C. The percentage of motile sperm in each tube was tested at 6-12 day intervals until sperm motility ceased.

Data analysis

Data were expressed as mean ± standard error (SE). Statistical evaluation was performed by one-way ANOVA using SPSS version 16.0. Differences with a probability value (P) of 0.05 ($P<0.05$) were considered significant.

Results

Sperm motility and velocity were highest when Neomycin at a concentration of 200ppm (9.44±0.53% and 63.11±1.45 $\mu m.s^{-1}$, respectively) was added to the sperm. It was significantly different than at other concentrations ($P<0.05$). Sperm stored in the above conditions that contained 600 ppm Neomycin had the lowest motility and velocity (2.89±0.06% and 52.78±1.20 $\mu m.s^{-1}$, respectively) (Figure 1).

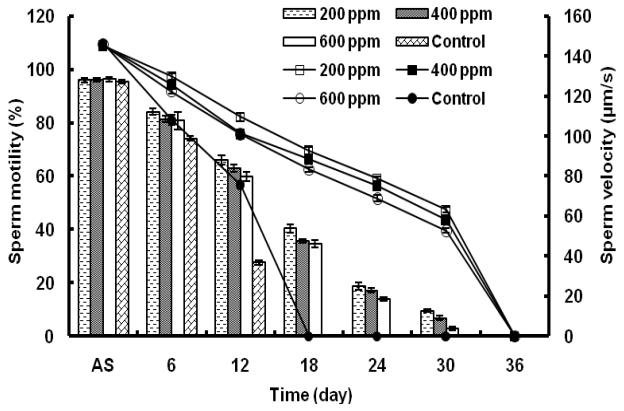

Figure 1. Effect of Neomycin at different concentrations on Wagieu seapearch sperm motility and velocity after storage at 2°C in 1:3 (semen: ASP); control = no antibiotic; n=3, AS: after storage.

Results showed that sperm stored in ASP at ratio of 1:3 with gentamycin at 200ppm had motile spermatozoa and sperm motility was significantly active for longer periods than the others (Figure 2) ($P<0.05$). The maximum duration was 36 days while that stored with 600 ppm Neomycin remained motile for only 30 days.

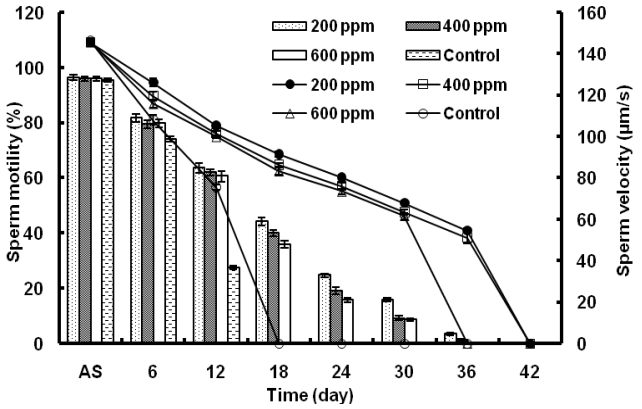

Figure 2. Changes of motile spermatozoa and sperm velocity of Waigieu seaperch semen after cold storage at 2°C in 1:3 (semen: ASP) and Gentamycin at different concentrations; control = no antibiotic; n = 3, AS: after storage

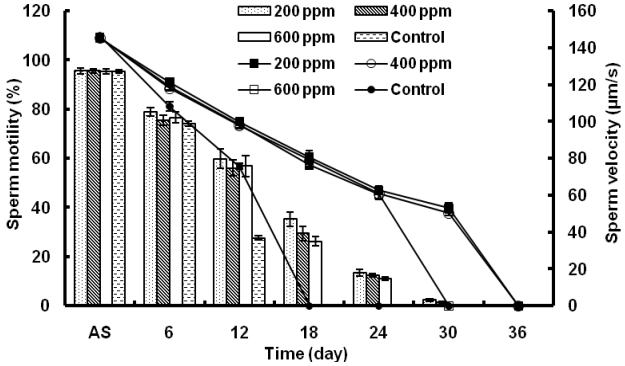

Figure 3. Motility and velocity of Waigieu seaperch sperm (%) after chilled storage at 2°C in 1:3 (semen: artificial seminal plasma) and Penicillin+Streptomycin at different concentrations, control=no antibiotic, n=3, AS: after storage.

Figure 3 shows that with the addition of penicillin plus streptomycin at a concentration of 200 ppm sperm remained mobile for up to 30 days and were significantly different from other concentrations ($P<0.05$). Sperm stored with 200ppm penicillin plus streptomycin retained movable sperm and sperm velocity for 24 days.

The results indicate that the optimal condition for chilled storage Waigieu seaperch semen is artificial seminal plasma extender diluted at 1:3 (semen: ASP), supplemented with 200ppm gentamycin and stored at 2°C.

Discussion

The motility and velocity of sperm of waigieu seaperch changed in chilled storage, indicating changes in sperm quality. Sperm quality is most accurately measured by fertilization percentage (Lim *et al.*, 2005; Le *et al.*, 2011). This method could not be used in our experiments because females of Waigieu seaperch did not produce mature eggs until the experiments were completed. When fertilization trials could not be performed, motility and velocity of sperm were used as an indicator of sperm quality (Lim *et al.*, 2005, 2006; Le *et al.,* 2011).

Addition of antibiotics to undiluted semen and extender improve storage duration (Billard *et al.* 2004; Bobe and Labbe 2009; Le *et al.* 2011). According to Chao *et al.* (1992), sperm motility of the grouper *Epinephelus malabaricus* lasted for up to 8 days when treated with Ringer's solution for marine fish with 500 ppm streptomycin. The sperm of Atlantic halibut (*Hippoglossus hippoglossus*) stored in Modified Hanks' Balanced Salt Solution at a ratio of 1:9 with penicillin plus streptomycin at a concentration of 200 mU/ml, maintained motility sperm for up to 70 days (Igor *et al.* 2006). A concentration of 50 IU/ml bipenicillin + 50 µg/ml streptomycin used for chilled storage of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) semen improved motility and fertilization capacity (DeGraaf and Berlinsky 2004). In Atlantic salmon (*Salmo salar*),

higher antibiotics concentrations (125 IU/ml penicillin + 125 µg/ml streptomycin) were not toxic, and provided improvement of storage duration (Stoss 1983). Paddlefish (*Polyodon spathula*) sperm storage duration was also improved by addition of the antibiotic combination penicillin/streptomycin (Brown and Mims 1995). According to Le *et al.* (2011), yellow croaker *Larimichthys polyactis* semen stored in artificial seminal plasma at a dilution ratio of 1:3 at 0^{o}C and supplemented 600 ppm of gentamycin, or 200 ppm of neomycin had the longest duration storage retaining motility up to 26 days. In this study, the motility spermatozoa and sperm velocity were also prolonged by addition of antibiotics.

Conclusion

The highest movable sperm and sperm velocity were obtained after chilled storage at 2^{o}C in a dilution ratio of 1:3 (semen: ASP) containing 200 ppm gentamycin.

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