ROLE OF ANTIBIOTICS IN CHILLED STORAGE OF SPERM IN GRASS CARP *(Ctenopharyngodon idella)*

Le Minh Hoang[1], Dinh Van Khuong[1]

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ABSTRACT:

The objective of the present study was to evaluate the effect of antibiotics on chilled storage sperm motility of grass carp (Ctenopharyngodon idella). The extenders were used in this study were HBSS (Hanks' balanced salt solution), Modified HBSS, CCSE-2 (common carp sperm extender), Kurokuda-1 and Kurokuda-2. The dilution ratios were 1:1, 1:3 and 1:5 (sperm:extender). Two antibiotics Cephalexin and Amoxcelin were used in this study at the concentration of 50, 100 or 150 ppm. The experiments were conducted in a refrigerator at the temperature of 4ºC. The results showed that the sperm motility was the highest and activated to day 9 when Kurokuda-2 was used as the extender at the dilution ratio of 1:3. The sperm motility can be maintained until day 13 by adding 25ppm Cephalexin combined with 25ppm Amoxceline to extender.

Keywork: Grass carp, Ctenopharyngodon idella, sperm, chilled storage, extender, antibiotic.

I. INTRODUCTION

Chilled storage of fish sperm is a useful biotechnique that facilitates hatchery operations. It reduces the need of frequent collection of sperm from males, enables transportation of sperm to distant locations and prevents problems related to asynchrony in gamete production between males and females. Sperm chilled storage is affected by extenders, dilution ratio, temperature, and antibiotics (Le et al. 2011; Le et al. 2014). However, the presence of microorganisms in chilled samples may decrease fertilization and lower cell and viability (Segovia et al. 2000). To address this issue,, antibiotics are commonly added to chilled storage of sperm, but the effect of antibiotics on the chilled sperm storage of the grass carp, an important freshwater species in aquaculture, has not been tested.

Grass carp has a rapid growth rate and a low requirement for protein from food. The production of grass carp has a low cost compared to other freshwater fish. Grass carp can be cultured in integration to land farm to maximize the use of resources such as food, wastes and water. Grass carp is a favorite fish of many Asian countries. In response to the need of aquaculture of the grass carp, artificial seed production of this species has been investigated (FAO, 2004-2017).

There has been many studies investigating on the preservation of fish sperm such as of common carp, *(Cyprinus carpio)* (Alavi et al., 2007), sturgion Acipenseridae (Alavi et al., 2006), trout and salmon (Billard et al., 1992). However, there has no study investigating the preservation of grass carp sperm. This was the reason we conducted the study "The role of antibiotics in chilled storage of sperm in grass carp *(Ctenopharyngodon idella)*.

II. Materials and methods

All experiments were carried out at the laboratory of the Department of Fisheries Biology, Institute of Aquaculture, Nha Trang University.

1. Fish handing and sperm collection

Sperm was collected from grass carps during the spawning season between March and May 2018 without hormonal stimulation. The males were anesthetized with Methlylene glycol mono ester (Merk, Germany) at the concentration of 200 ppm before sperm collection. Sperm was collected by abdominal massage and put it into a 1.5 ml dry Eppendorf tubes. Handling was done with care to avoid contamination with urine and feces in samples designated for chilled storage as these can lead to the activation of spermatozoa. The samples were immediately placed on crushed ice until

[1] Institute of Aquaculture, Nha Trang University

use for experiment after collection.

2. Evaluation sperm motility

The sperm motility was immediately determined after sperm collection. The percentage of sperm exhibiting rapid, vigorous, forward movement was estimated under the microscope by diluting the sperm in distilled water at a ratio of 1:100 (sperm: distilled water). Only samples with motility equal to or greater than 80% were used for experiments. Motility was checked using a light microscope at 400× magnification and was expressed as percentage of motile spermatozoa. An activating medium of distilled water was used to estimate motility. Sperm was diluted in distilled water at the ratio of 1:100 (1µl sperm to 99 µl distilled water). Then, 1µl was put on a glass slide without a cover glass and observed at 400× magnification under a microscope.

3. Effect of extenders on motile sperm

To determine the optimal extender, sperm was diluted at a ratio of 1:3 (sperm:extender) with Hanks balanced solution (HBSS), Common carp sperm extender (CCSE-2), Kura Kuro's 1 (Ku1), Kura Kuro's 2 (Ku2) and Modified (Table 1). Diluted sperm was stored in a refrigerator at 4ºC, storage treatments were replicated three times. The percentage of motile sperm in each tube was tested at 2-4 day intervals until sperm stopped moving.

Table 1. Composition of extenders for chilled storage of sperm of grass carp in 50ml distilled water

Composition	Extender			
	Ku2	HBSS	CCSE-2	Modified
NaCl (g)	0,22	0,4	0,175	0,18
KCl (g)	0,31	0,02	-	0,5
$NaHCO_3$ (g)	0,01	0,0175	-	0,01
$CaCl_2$ (g)	0,011	-	-	0,011
NaOH (g)	-	-	10,5	-
Glucose (g)	-	0,05		-
Sucrose (g)	-	-	1,72	-
$Na_2HPO_4.7H_2O$ (g)	-	0,003	-	-
$MgCl_2$ (g)	-	-		0,004
KH_2PO_4 (g)		0,003	-	-
$CaCl_2.H_20$ (g)	-	0,008	-	-
ASTT (mOsm/kg)	-	300	325	-
pH	-	-	7,7	-

Ku2: Kuro Kura's2

4. Effect of dilution ratio on sperm motility

To determine the optimal dilution, sperm was diluted in HBSS, CCSE-2, Ku1, Ku2, Modified at the ratio of 1:1, 1:3 và 1:5 (sperm:extender). Mixtures were placed in 1.5ml Eppendorf tubes and stored in a refrigerator at 4ºC. Treatments were replicated three times. The spermatozoa motility was tested at 2-4 day intervals until spermatozoa stopped moving. Sperm was not diluted with extender was used as the control samples.

5. Effect of antibiotics on sperm motility

To determine optimal antibiotics for chilled sperm storage of grass carp, the sperm was diluted in Kura Kuro's 2 at a ratio of 1:3 combined with antibiotics Cephalexin with Amoxcelin at the concentrations of 50, 100 or 150 ppm. All treatments had three replicates and stored in a refrigerator at 4ºC. The percentage of motile sperm in each tube was tested at 2-4 day intervals until sperm motility ceased. The sperm samples without antibiotic were used as the control treatment.

6. Data analysis

Data were expressed as mean ± standard error (SE). One-way ANOVA were performed

using SPSS version 22.0. Differences with a probability value (P) of 0.05 (P<0.05) were considered significant.

III. Results and discussion

1. Effect of extenders on sperm motility

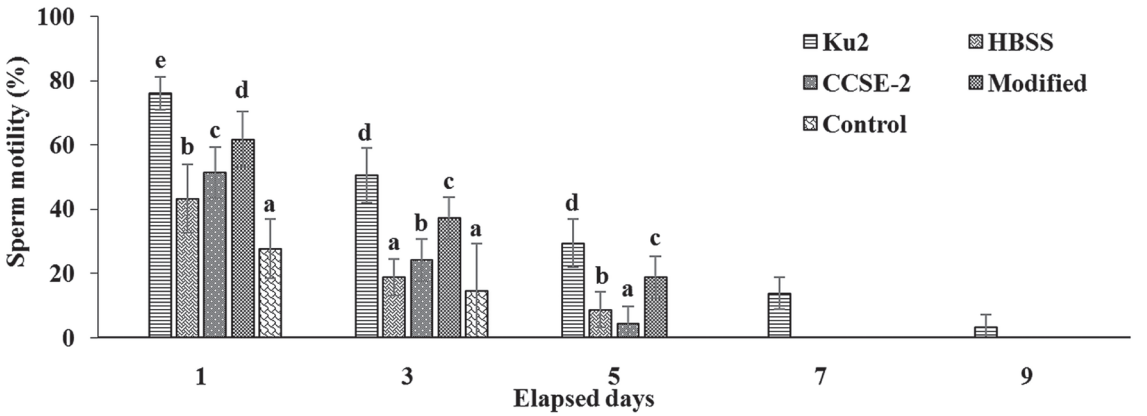

Figure 1. Sperm motility (%) in various extender Ku1, Ku2, HBSS, CCSE-2 and Modified

Control: No extender. Different alphabets indicate statiscial significance at p<0.05.

Sperm was stored in Ku2 retained its movable spermatozoa longer than other extenders (Figure 1). Specifically, Ku2 sperm remained motile for 9 days (3.22%), while sperm was stored in CCSE-2, HBSS and Modified remained motile only for 5 days with motility as 4.33%, 8.67% and 18.78%, respectively. Sperm stored in later extenders was immotile at the day 7. Sperm not stored in extender, on the other hand, was not active at the day 5.

At the day 9 the duration of sperm motility in extender Ku2 retained 51.67s. However, sperm which was stored in CCSE-2, HBSS and Modified had a duration of sperm motility of 18.89s, 54.56s and 58.22s, respectively at the day 5 (Figure 2).

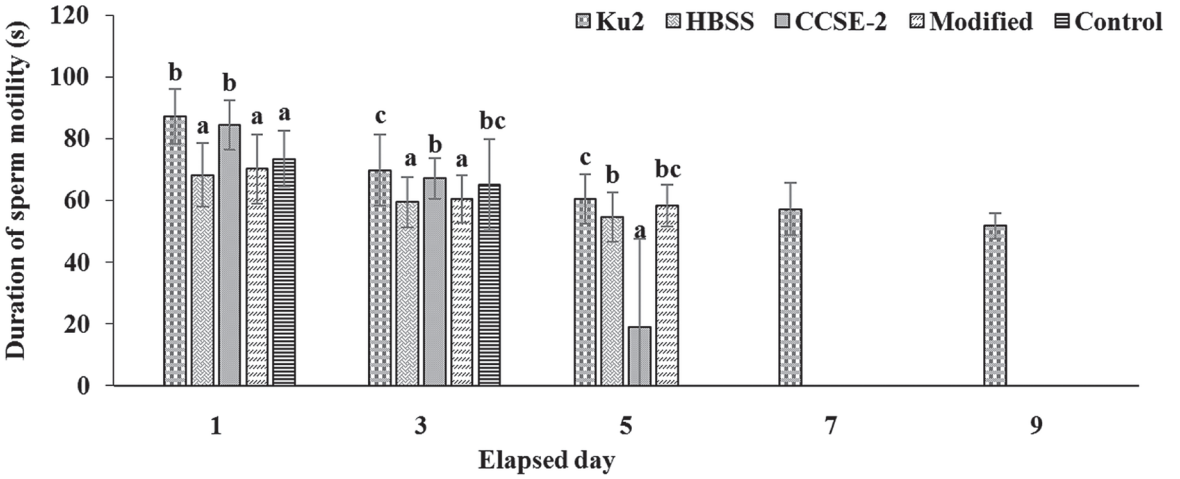

Figure 2. Duration of sperm motility (s) in various extenders Ku2, HBSS, CCSE-2 và Modified

Control: No extender. Different alphabets indicate statistical significance at p<0.05.

2. Effect of dilution ratios on sperm motility

The most motile sperm was observed when sperm stored in Ku2 at the ratio of 1:3 (9.67%), which remained motile for 9 days and 7 days at the ratios of 1:1 and 1:5 (9.67% and 10%, respectively) (Figure 3).

Sperm stored in Kura Kuro's 2 (Ku2) at the ratio of 1:3 remained the duration of sperm motility better than that of 1:1 and 1:5. The duration of sperm motility at the ratio 1:3 retained 51.67s at the day 9 and at the ratio of 1:1 and 1:5 reached at the day 5 was 59.33s and 51s, respectively (Figure 4).

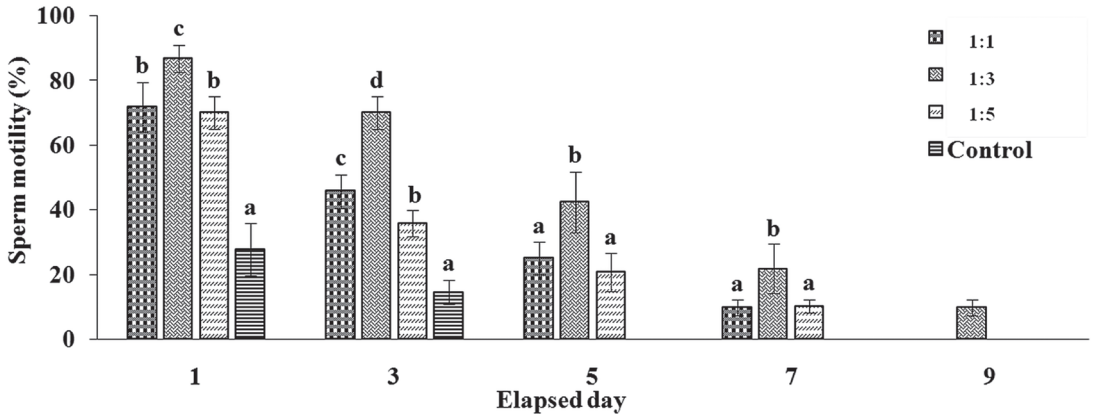

Figure 3. Sperm motility (%) at various dilution ratios in Kura Kuro's 2 extender
Control: No dilution. Different alphabets indicate statistical significance at p<0.05.

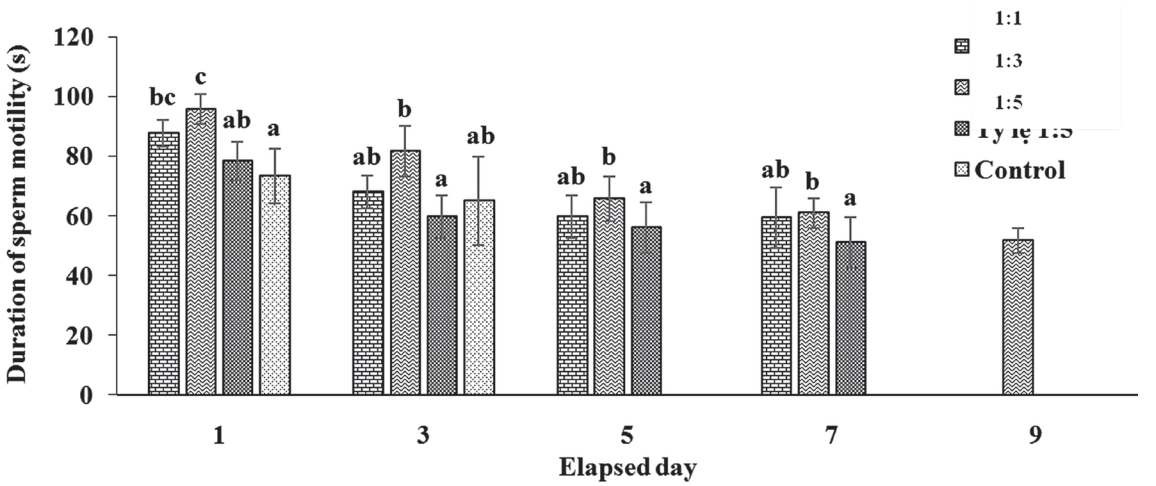

Figure 4. Duration of sperm motility (s) at various dilution ratios in Kuro's 2 (Ku2) extender.
Control: No dilution. Different alphabets indicate statistical significance at p<0.05.

3. Effect of antibiotics on sperm motility

Sperm stored in Kura Kuro's 2 at the ratio of 1:3 and with an addition of 25ppm Cephalexin and 25ppm Amoxcelin had a higher motility than those stored in other extenders and the controls (no extender and without antibiotic). It remained motile for 13 days (6.89%), whereas the treatment without antibiotic sperm was immobile after 9 days (Figure 5).

The duration of sperm motility in the treatment of combination between 25ppm Cephalexin and 25 ppm Amoxcelin reached 3.59s at the day 13. However, it remained 3.07 s and 3.19s at the day 11 in the treatment of only Cephalexin or Amoxcelin respectively (Figure 6).

The addition of antibiotics either to the undiluted sperm or to the storage diluent usually improves storage duration, and this addition can be one of the most important parameters for chilled storage of sperm (Billard et al., 2004; Bobe et al., 2009). According to previous studies, a combination of 50 IU/ penicilin and 50 IU/streptomycin for carp semen without dilution at 4ºC showed that motile and fertilization capacity of sperm can be remained more than 18 days (Saad et al. 1988). With same concentration, similar results were obtained for sperm storage of atlantic cod *Gadus morha* and haddock *Melannogrammus aeglefinus* (DeGraaf and Berlinsky, 2004). Paddlefish *Polyodon spathula* sperm storage

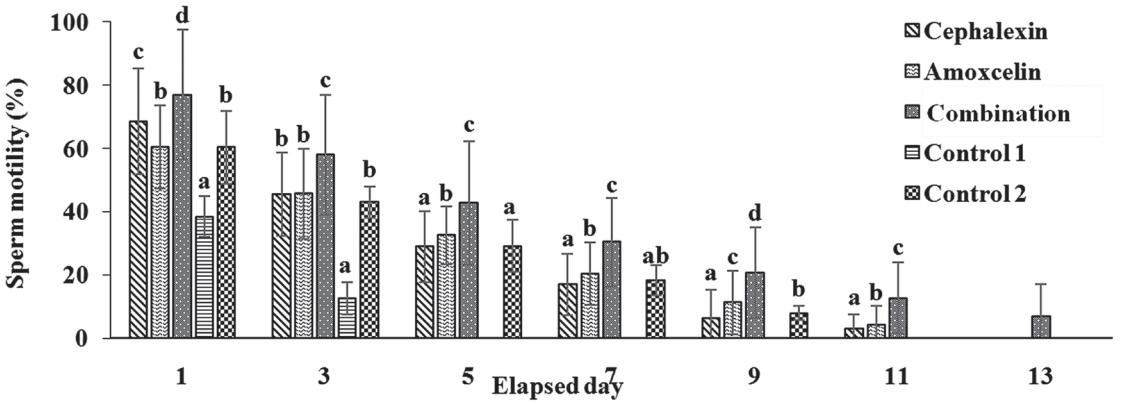

Figure 5. Sperm motility (%) at the different antibiotics such as Cephalexin, Amoxcelin and thei combination. Control 1: No extender, *Control 2: Without antibiotic Different alphabets indicate statistical significance at p<0.05.*

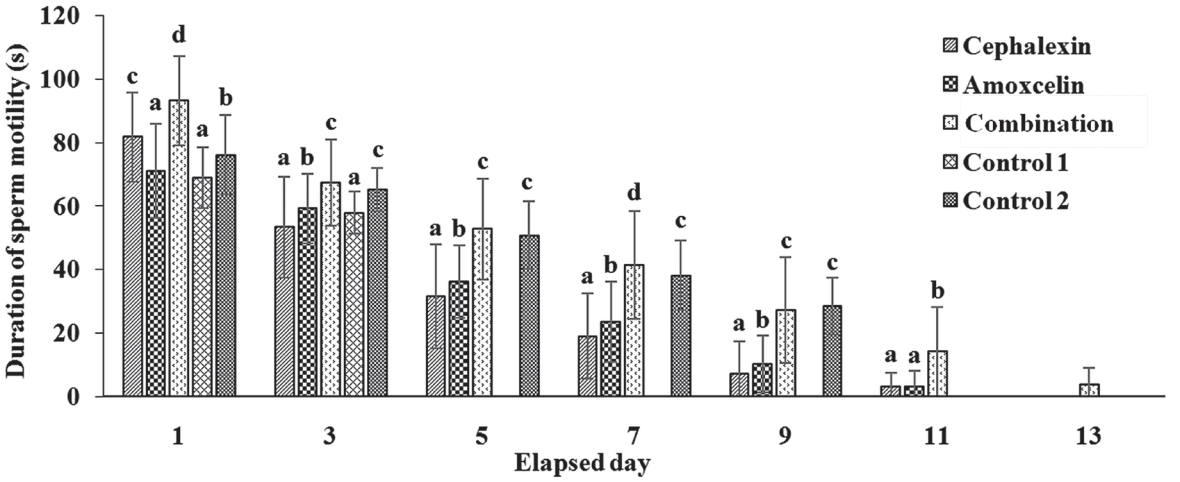

Figure 6. Duration of sperm motility treated with different antibiotics as Cephalexin, Amoxcelin and their combination. *Control 1: No extender, Control 2: Without antibiotic. Different alphabets indicate statistical significance at p<0.05.*

was also improved by adding a combination of antibiotic penicilllin/streptomycin (Brown and Mims, 1995). In African catfish *(Clarias gariepinus)*, however, addition of 25 to 50 IU/ml penicillin + 25 to 50 µg/ml streptomycin did not improve sperm quality during short term storage and doses of 100 IU/ml + 100 µg/ml were toxic for the cells whereas addition of gentamycine sulfate at 1 mg/ml did not improve the motility of these stored sperms (Christensen and Tiersch, 1996).

IV. CONCLUSION AND RECOMMENDATION

1. Conclusion

The highest sperm motility and duration of sperm motility were obtained after chilled storage at 4ºC in a dilution ratio of 1:3 (sperm:Ku2) containing 25 ppm Cephalexin + 25 Amoxcelin. It reamained the lifetime until the day 13.

2. Recommendation

In this study, addition of two commonly used antibiotics Cepalexin and Amoxcelin prolonged the survival of sperm for three days compared to untreated sperms. It remains to be tested whether using other antibiotics may improve the chilled sperm storage of the grass carp for a longer duration.

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