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Extender for Sperm Dilution in Olive Ridley Turtle *(Lepidochelys olivacea)* and Hawksbill Turtle (*Eretmochelys imbricata*) Semen

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ABSTRACT

The objective of the study was to find an extender to dilute and preserve sea turtle semen. Six adult olive ridley turtles (*Lepidochelys olivacea)*, and 4 adult hawksbill turtles (*Eretmochelys imbricata*) from Phuket Marine Biological Center and Eastern Marine and Coastal Resources Research Center in Thailand had semen collected using electroejaculator. The study was repeated twice in 2007 and 2008. After collection, each semen sample was divided and preserved in 8 different extenders, which were 1) refrigeration medium test yolk buffer, 2) Tyrode medium supplemented with albumin, lactate and pyruvate, 3) Beltsville poultry semen extender, 4) 3% Sodium citrate buffer, 5) Phosphate-buffered solution, 6) EEL extender, 7) 1% bovine serum albumin, and 8) HAM F-10 and kept at 4°C and evaluated for viability (motile sperm) at 0, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 hours. The results found that for 28% olive ridley turtles and 25% hawksbill turtles that their spermatozoa diluted in extender 1, and for 14% of both sea turtles that their spermatozoa diluted in extender 2 could survive for 24 hours. However, the motility from both sea turtles semen in both extenders was decreased by 50-80 %. Most sperm died after being diluted in the last 6 extenders. By conclusion, extender 1 and 2 were suitable extenders for sea turtle semen viability, however, adding other ingredients should be considered to enhance in viability.

KEYWORDS: semen, extender, olive ridley turtle, hawksbill turtle

INTRODUCTION

From our previous studies we found that sea turtle semen showed turbidity, opalescence, a viscose mucous appearance and semen volume was very low (Tanasanti, et al. 2007; Sahatrakul, et al. 2007; Sahatrakul, et al., 2008). Only 1 ml semen from olive ridley turtle, and about 4 ml from hawksbill turtles were collected from our previous report (Tanasanti, et al. 2007). From the characteristics of sea turtle semen, we found that it had a more viscose mucous appearance compared to semen from domestic animals which had less viscosity and was easily to evaluate without adding any extender. So, for complete semen evaluation and for other studies, such as chilled or freezing semen, sea turtle semen needs to be combined with some extenders in order to dilute and preserve semen.

The objective of the study was to find a suitable extender to dilute and to preserve semen of sea turtle.

MATERIALS AND METHODS

The study was performed at Phuket Marine Biological Center (PMBC), Phuket province, which is in the Southern part of Thailand, and at the Eastern Marine and Coastal Resources Research Center (EMCOR), Rayong province, where is in the Eastern part of Thailand. Semen from 6 adult olive ridley turtles (*Lepidochelys olivacea*) from PMBC, and 4 adult hawksbill turtles (*Eretmochelys imbricate*); 2 turtles from PMBC and 2 turtles from EMCOR were collected using an electroejaculator (Tanasanti, et al. 2007; Sahatrakul, et al. 2007; Sahatrakul, et al., 2008). The study was repeated 2 times in January and May 2008 (at PMBC) and in October 2007 and 2008 (at EMCOR). Data was reported as an average. After collection, each semen sample was divided, diluted and preserved in 8 different extenders which were reported as used for semen dilution in other animals. The extenders were Extender 1; refrigeration medium test yolk buffer (dog extender), Extender 2; Tyrode medium supplemented with albumin, lactate and pyruvate

(SP-TALP) (Umapathy, et al., 2005) Extender 3; Beltsville poultry semen extender (BPSE) (Giesen and Sexton, 1983), Extender 4; 3% Sodium citrate buffer (Sule, 2007), Extender 5; Phosphate-buffered solution (PBS) (Pagl, 2006), Extender 6; EEL extender (Asturiano, et al., 2007), Extender 7; 1% bovine serum albumin (1% BSA) (Yamashiro, et l., 2006), and Extender 8; HAM F-10 (Correa, et al. 1997) (Table 1). Each semen sample in each extender was kept at 4°C (Tanasanti, et al. 2007) and evaluated for sperm viability (motile sperm) at 0, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 hours.

Table 1 Eight extenders were tested for olive ridley turtle and hawksbill turtles semen dilution and preservation.

Extender 1	Refrigeration medium test yolk buffer (dog extender),
Extender 2	Tyrode medium supplemented with albumin, lactate and pyruvate (SP-TALP)
Extender 3	Beltsville poultry semen extender (BPSE)
Extender 4	3% Sodium citrate buffer
Extender 5	Phosphate-buffered solution (PBS)
Extender 6	EEL extender
Extender 7	1% bovine serum albumin (1% BSA)
Extender 8	HAM F-10

RESULTS

Motility of fresh sperm from olive ridley turtles and hawksbill turtles were 8.33±15.70%, and 14±15.57 %, respectively. After extender dilution, most spermatozoa from both olive ridley turtles and hawksbill turtles survived and then started to decrease viability within 48 hours. Within 3 hours of extender dilution, motility of sperm from olive ridley turtle in dog extender, BPSE and 3%Na citrate extenders were decreased to 6.43±6.27%, 2.14±3.93% and 0.83±2.04%, respectively. Only spermatozoa diluted in SP-TALP, motility was increased to 17.14±23.43%, and then decreased after 6 hours of dilution. The motility of sperm from hawksbill turtle at 0.5 hours in dog extender, SP-TALP, BPSE and 3%Na citrate extenders were decreased to 13.13±13.07%, 10±9.57%, 3.33±2.89% and 5%, respectively. In the other extenders, most spermatozoa from both olive ridley and hawksbill turtles were non-motile (Figure 1 and 2).

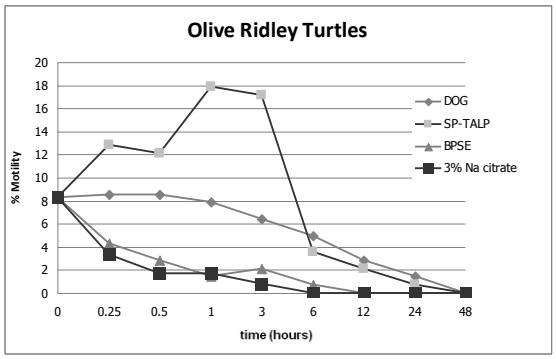

Fig. 1 Motility of spermatozoa from olive ridley turtles before and after being diluted and preserved in Extender 1; refrigeration medium test yolk buffer (dog extender), Extender 2; Tyrode medium supplemented with albumin, lactate and pyruvate (SP-TALP) Extender 3; Beltsville poultry semen extender (BPSE), Extender 4; 3% Sodium citrate buffer were shown. For the other extenders including Extender 5; Phosphate-buffered solution (PBS), Extender 6; EEL extender, Extender 7; 1% bovine serum albumin (1% BSA), and Extender 8; HAM F-10, spermatozoa were non- motile after dilution (not shown in the figure).

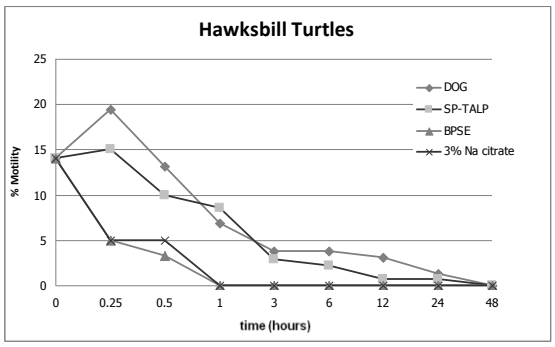

Fig. 2 Motility of spermatozoa from hawksbill turtles before and after being diluted and preserved in Extender 1; refrigeration medium test yolk buffer (dog extender), Extender 2; Tyrode medium supplemented with albumin, lactate and pyruvate (SP-TALP) Extender 3; Beltsville poultry semen extender (BPSE), Extender 4; 3% Sodium citrate buffer were shown. For the other extenders including Extender 5; Phosphate-buffered solution (PBS), Extender 6; EEL extender, Extender 7; 1% bovine serum albumin (1% BSA), and Extender 8; HAM F-10, spermatozoa were non- motile after dilution (not shown in the figure).

For number of turtles that their spermatozoa could survive in each extender, we found that most olive ridley turtles had semen that could survive for the first 3 hours of incubation and then started to decrease motility. After 24 hours of incubation, there were 28% of olive ridley turtles and 25% of hawksbill turtles that their spermatozoa

could survive in dog extender, and 14 % of both sea turtles that their spermatozoa could survive in SP-TALP. In BPSE, only 14% of olive ridley turtles that their spermatozoa could survive for 6 hours, and 66 % of hawksbill turtles that their spermatozoa could survive only 0.5 hours. In 3% Sodium citrate buffer, only 16% of olive ridley turtles that their spermatozoa could survive for 3 hours, and 100 % of hawksbill turtles sperm could survive only 0.5 hours. Most sperm died after being diluted in the last 4 extenders (Figure 3 and 4).

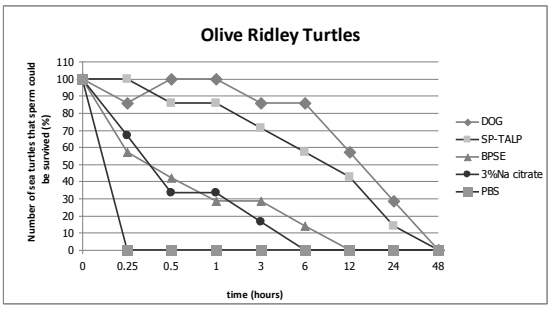

Fig. 3 Percentage number olive ridley turtles that their spermatozoa could be diluted and preserved in Extender 1; refrigeration medium test yolk buffer (dog extender), Extender 2; Tyrode medium supplemented with albumin, lactate and pyruvate (SP-TALP) Extender 3; Beltsville poultry semen extender (BPSE), Extender 4; 3% Sodium citrate buffer were shown. For the other extenders including Extender 5; Phosphate-buffered solution (PBS), Extender 6; EEL extender, Extender 7; 1% bovine serum albumin (1% BSA), and Extender 8; HAM F-10, spermatozoa from all olive ridley turtles that did not survive (Extender 6,7 and 8 not shown in the figure).

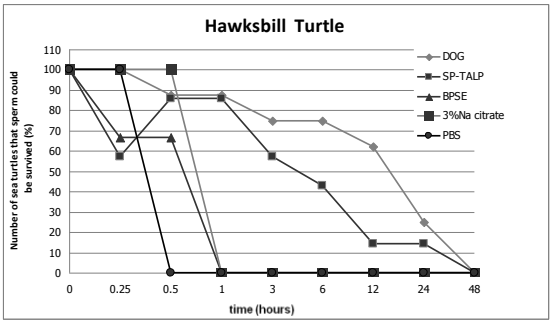

Fig. 4 Percentage number hawksbill turtles that their spermatozoa could be diluted and preserved in Extender 1; refrigeration medium test yolk buffer (dog extender), Extender 2; Tyrode medium supplemented with albumin, lactate and pyruvate (SP-TALP) Extender 3; Beltsville poultry semen extender (BPSE), Extender 4; 3% Sodium citrate buffer were shown. For the other extenders including Extender 5; Phosphate-buffered solution (PBS), Extender 6; EEL extender, Extender 7; 1% bovine serum albumin (1% BSA), and Extender 8; HAM F-10, spermatozoa from all hawksbill turtles did not survive (Extender 6,7 and 8 not shown in the figure).

DISCUSSION AND CONCLUSION

Motility of fresh spermatozoa from olive ridley and hawksbill turtles was low compared to semen from other domestic animals which are about 70 % motility, and this affects the motility and viability of sperm after being diluted and preserved in different extenders. The season may also affect semen ejaculation and semen quality. The appropriate season for semen collection from each research center should be investigated. Spermatozoa from both olive ridley and hawksbill turtles can survive in both dog extender and SP-TALP extender. Both extenders seem to be suitable extenders for sea turtle semen preservation. However, adding some other ingredients should be considered to enhance an increase in motility and survival rate.

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