Electroejaculation and semen evaluation in olive ridley turtle (*Lepidochelys olivacea*) and hawksbill turtle (*Eretmochelys imbricata*) in Thailand

MANITA TANASANTI[1], CHANITA SUJARITTHANYATRAKUL[1], KAMOLPORN DHANARUN[1], KOMSIN SAHATRAKUL[1], PARINYA SAKORNCHAROUN[1], SONTAYA MANAWATTHANA[2], PORNCHAI SANYATHITISEREE[3] AND KAITKANOKE SIRINARUMITTR[4]

[1] *Fifth Year Student, Faculty of Veterinary Medicine, Kasetsart University, Thailand.*

[2] *Phuket Marine Biological Center, P.O. Box 60, Phuket 83000, Thailand*

[3] *Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen campus, Nakorn-Pathom 73140, Thailand.*

[4] *Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkhaen campus, Bangkok 10900, Thailand.*

E-mail: fvetkns@ku.ac.th

ABSTRACT

Electroejaculation and semen evaluation were successfully performed on the olive ridley turtles (*Lepidochelys olivacea*) and hawksbill turtles (*Eretmochelys imbricata*) at Phuket Marine Biological Center (PMBC), Phuket province and Eastern Marine and Coastal Resources Research Center (EMCOR) Rayong province, in Thailand. Six male Olive Ridley turtles aged approximately 30 years old and weighing between 28.0 – 35.4 kilograms and 3 male Hawksbill turtles aged approximately 16 years old and weighing between 40.0-49.0 kilograms were used. The study on the Olive Ridley turtle was performed in May (Summer season) and on the Hawksbill turtle was performed in October (Rainy season). Before electroejaculation, each turtle was sedated and an electrical rectal probe was applied into its cloaca and stimulated with 2-6 volts electrical stimuli in 4 cycles. After electrical stimulation, manual stimulation was continued for complete penile erection. The results of semen evaluation found that, an average semen volume was 1 ml (range 0.01 to 2.2 ml) for Olive Ridley turtle (n=6), and 4.4 ml for Hawksbill turtle (n=1). Seminal fluid had turbidity, opalescence, viscosity and mucoid appearance. An average sperm motility was 28.25 % (range 0 to 98 %) for Olive Ridley turtle, and 60 % for Hawksbill turtle. An average sperm concentration was 67.3 million/ml (range 11.5 to 150 million/ml) for Olive Ridley turtle, and 512 million/ml for Hawksbill turtle. The sperm had narrow heads and moved in a spiral movement. There were also some motile lobulated appearances in seminal fluid which were of unknown function. Sperm viability in external milieu was approximately 90 minutes. Several staining agents used in the study were not suitable for sperm morphology evaluation. In conclusion, this study was the first report of successful semen collection using electroejaculation in olive ridley and hawksbill turtles in Thailand. The semen collection method developed here is a useful technique for an artificial insemination in order to conserve the threatened and endangered sea turtle species in the future.

KEYWORDS: semen collection, electroejaculation, semen evaluation, olive ridley turtle, hawksbill turtle

INTRODUCTION

Marine turtles that have been found in Thai waters are the leatherback turtle *(Dermochelys coriace*a), the green turtle *(Chelonia mydas),* the hawksbill turtle *(Eretmochelys imbricate),* the loggerhead turtle *(Caretta caretta)* and the olive ridley turtle *(Lepidochelys olivacea).* All sea turtles are considered in serious decline of numbers in each year. The data for olive ridley's nesting in Thailand has decreased from the past until now (Kittiwattanawong, 2004). Phuket Marine Biological Center (PMBC), at Phuket province and Eastern Marine and Coastal Resources Research Center (EMCOR) at Rayong province are Thai government centers for sea turtle conservation, especially the olive Ridley turtles and hawksbill turtles. However, the data of reproductive performance of sea turtle is not available, especially semen collection and semen evaluation in male turtles. The objectives of the study were to find the method for semen collection and semen evaluation in Olive Ridley and Hawksbill turtles.

MATERIALS AND METHODS

The first study was performed at PMBC during Summer season (in May). Six olive ridley turtles

(*Lepidochelys olivace)* aged approximately 30 years old and weighing between 28.0 – 35.4 kilograms were used in the study. The second collection was performed at EMCOR during the Rainy season (in October). Three hawksbill turtles aged approximately 16-year-old and weighing between 40.0-49.0 kilograms were used. Each turtle was measured for the Curved Carapace Width (CCW), Curved Carapace Length (CCL), Straight Carapace Width (SCW) and Straight Carapace Length (SCL). Each Olive Ridley turtle was sedated with Ketamine HCl (25 mg/kg, IM), and each Hawksbill turtle was sedated with Ketamine HCl (2.5 mg/kg, IV), and Detomidine (0.015 mg/kg IV) (McArthur, 2004; Carpenter, 2005). After sedation, the turtle was placed on the table and the location of gonad was located by measurement from the cloaca to the last vertebral scute. Then the tail and cloaca were washed with sea water and wiped out. The electrical probe was lubricated with K-Y jelly (Johnson & Johnson, New Brunswick, Newjersey, USA) and then was introduced into the cloaca by leading the stainless steel electrodes dorsally. The gonad was stimulated three times with 2-6 volts electrical stimuli (1 cycle) and a total of 4 cycles were made (Komsin Sahatrakul et. al., 2007). After electrical stimulation, manual stimulation was continued for about 15-30 minutes for complete penile erection (Platz, et al. 1980; Wood, et al. 1982). Then, the cloaca was cleaned with normal saline, and the turtle was left on the table for thirty minutes to one hour for the recovery period.

After collection, semen was evaluated for color, appearance, volume, and motility. Sperm morphology was evaluated, by staining with Diff-quik, William's staining, Gential violet, Toluidiene blue and Eosin-nigrosin. Sperm concentration was evaluated by using Hematocytometer (Komsin Sahatrakul et. al., 2008).

RESULTS

From six male olive ridley turtles, eight ejaculations were attempted (two turtles that were ejaculated twice), whilst there was only one hawksbill turtle was successful in semen collection. Results of all semen evaluations were presented (Table 1). The turtle's sperm had narrow heads and moved in a spiral movement. Besides spermatozoa, there were some motile lobulated appearances in seminal fluid (Figure1), which were of unknown function.

In this study, there was no suitable staining for sperm morphology evaluation because the staining created swollen head or defects of the tail (Figures 2, 3, 4, 5, 6). Eosin-nigrosin staining was used to evaluate live and dead sperm. Dead sperm was stained with pink or purple color at the head part. Live sperm had no staining.

Olive ridley turtle's sperm viability in external milieu was approximately 90 minutes and in canine extender at 4 degree celcius was about 16-22 hours.

DISCUSSION

This study is the first report of successful semen collection using electroejaculation and semen evaluation of sea turtles in Thailand. Several staining agents used in the study were not suitable for sperm-morphology evaluation, and also suitable extender should be considered for preparing chilled semen. The semen collection method developed here is a useful technique for planning of complete semen evaluation, chilled semen preparation, frozen semen and artificial insemination in order to conserve the threatened and endangered sea turtle species in the future. However, the method of semen collection here needs to anesthetize the turtles and uses electroejacultion, other methods of using only manual stimaution without any anesthetization should be considered. Nevertheless, the best ways to conserve sea turtles are to protect their environment in order to protect their natural population and their habitat.

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Table 1 The data shows the results of semen evaluations from six male olive ridley turtles (from 8 ejaculations), and from one hawksbill turtle. There data is presented as average and range.

Parameters	**olive ridley turtle (n=6)**	**hawksbill turtle (n=1)**
Appearance	Turbid, mucous opalescence, viscosity	Turbid, mucous opalescence, viscosity
Volume (ml)	1 (0.01 – 2.22)	4.4
pH	6 (5-7)	5.5
% Motility	28.25 (0-98)	60
% Progressive motility	17.75 (0-60)	30.5
Concentration of sperm ($x\ 10^6$) /ml	67.3 (11.5-150)	512
Total number sperm ($x\ 10^6$) /ejaculation	40.9 (1.2-82.4)	1510.4

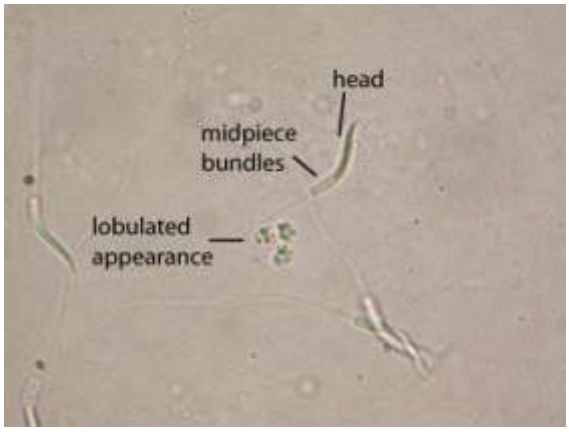

Fig.1 Shows the normal morphology of olive ridley turtle's sperm (1000 x), and lobulated appearance in the collected semen.

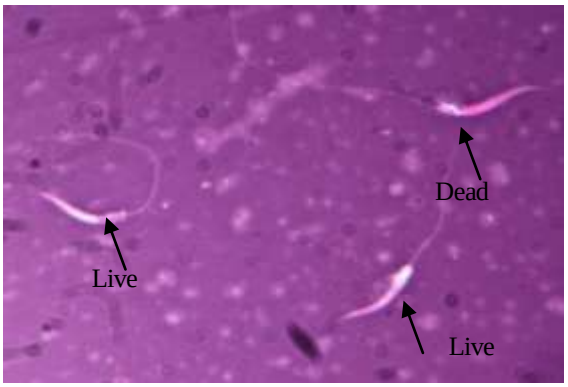

Fig.2 Live and dead sperm from olive ridley turtle's semen using eosin-nigrosin staining (1000x). The dead sperm was stained with pink or purple color. The live sperm was unstained.

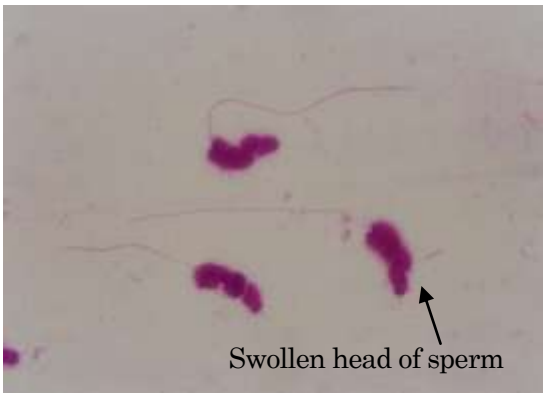

Fig.3 Morphology of sperm from olive ridley turtle's semen using Diff-quik staining (1000x). The staining created swollen head of sperm.

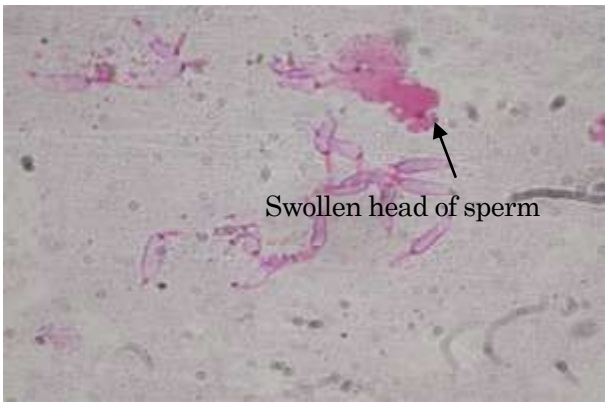

Fig.4 Morphology of sperm from olive ridley turtle's semen using William's staining (1000x). The staining created swollen head of sperm.

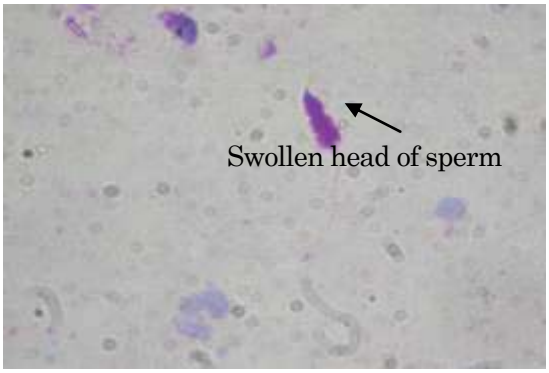

Fig.5 Morphology of sperm from olive ridley turtle's semen using Gential violet staining (1000x). The staining created swollen head of sperm.

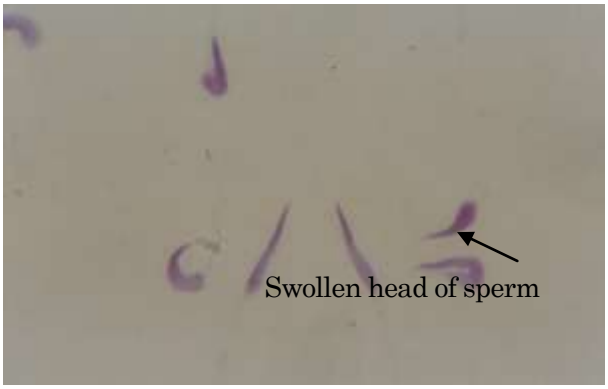

Fig.6 Morphology of sperm from olive ridley turtle's semen using Toluidiene blue staining (1000x). The staining created little swollen head of sperm.