Original Article

Stimulation Patterns for Erection and Ejaculation using Electroejaculator in Black Marsh Turtles

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Abstract

Semen collection by an electroejaculator was studied in black marsh turtles. High responses were recorded when electrical probe was inserted to the depth of 5.9±0.1 cm from the anus or 5.2±0.2 cm from posterior tip of the carapace. The depth position was approximately the center of the second carapace from the rear. The intensity of responses increased with increasing voltage and reached a plateau at 7.0 volts. The depth of the probe inserted to yield highest responses had higher relationship with carapace length when measured against the anus (r= 0.81, p= 0.0088) than the posterior end of carapace (r= 0.06, p= 0.8825). Stimulation voltages have been applied as pattern 1: 0.0 to 7.0 volts in 20 second, held for 20 second, return to 0.0 and rest 20 sec; pattern 2: 0 to 7.0 volts in 30 sec, held for 30 sec, return to 0.0 and repeat stimulus; pattern 3: 0.0 to 7.0 volts in 15 sec, held for 40 sec, return to 0.0 and rest for 5 sec. Frequencies of penile protrusion and ejaculation were similar among the 3 patterns of stimulation tested. Frequency of erection, however, was higher in pattern 2 (77.8%) than those in pattern 1 and 3 (38.9 and 50.0 %, respectively, p< 0.05). Time spent to the occurrence of penile protrusion (36.9±5.7, 25.3±4.7 and 33.4±5.6 min for pattern 1, 2 and 3 respectively, p> 0.05), penile erection (42.7±5.2, 31.7±4.5 and 35.0±5.8 min for pattern 1, 2 and 3 respectively, p> 0.05) and ejaculation (35.3± 6.4, 34.5±5.0 and 28.1±6.0 min for pattern 1, 2 and 3 respectively, p> 0.05) did not significantly differ. Ejaculations, however, were observed without penile erection. Electrical exposure times spent to achieve penile protrusion and ejaculation were not different among stimulation patterns studied. Erection, on the other hand, needed longer exposure time in pattern 3 (20.8±3.1 min) that those in pattern 1 and 2 (15.2±1.9 and 16.0±2.2 min, respectively, p< 0.05). In conclusion, electroejaculator can be used to stimulate penile erection and ejaculation in black marsh turtles.

Keywords: black marsh turtles, electroejaculation, ejaculation, penile erection, semen collection

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บทคัดย่อ

รูปแบบการกระตุ้นการแข็งตัวและการหลั่งน้ำเชื้อด้วยเครื่องกระตุ้นการหลั่งน้ำเชื้อไฟฟ้าในเต่าดำ

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ศึกษาการเก็บน้ำเชื้อในเต่าดำ (black marsh turtles) ด้วยเครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า เต่ามีปฏิกิริยาต่อการกระตุ้นสูงสุดเมื่อสอดแท่งกระตุ้นลึก 5.9±0.1 ซม. จากทวารหนัก หรือ ลึก 5.2±0.2 ซม. จากปลายของกระดองเต่าด้านท้ายของตัว ความลึกนี้เมื่อเปรียบเทียบกับตำแหน่งบนกระดองเต่าอยู่ประมาณกึ่งกลางกระดองแผ่นที่สองจากด้านท้าย การตอบสนองเพิ่มขึ้นตามความแรงของกระแสไฟฟ้าที่เพิ่มขึ้น และเกิดสูงสุดเมื่อกระแสไฟฟ้าสูงถึง 7.0 โวลท์ ความลึกของแท่งกระตุ้นเมื่อวัดจากทวารหนักมีความสัมพันธ์กับความยาวกระดอง (r= 0.81, p= 0.0088) มากกว่าวัดจากส่วนปลายของกระดอง (r= 0.06, p= 0.8825) เต่าได้รับการกระตุ้น 3 รูปแบบคือ แบบที่ 1 จาก 0.0 ถึง 7.5 โวลท์ ใน 20 วินาที คงไว้ 20 วินาที ลดลงมา 0.0 โวลท์ ทันที พัก 20 วินาที แบบที่ 2 จาก 0.0 ถึง 7.5 โวลท์ ใน 30 วินาที คงไว้ 30 วินาที ลดกลับมาที่ 0.0 โวลท์ และกระตุ้นรอบใหม่ทันที และแบบที่ 3 จาก 0 ถึง 7.5 โวลท์ ใน 15 วินาที คงไว้ 40 วินาที ลดลงมาที่ 0.0 โวลท์ พัก 5 วินาที ความถี่ของการยื่นออกขององคชาต และการหลั่งน้ำเชื้อเต่าดำ จากรูปแบบการกระตุ้นทั้ง 3 แบบคล้ายคลึงกัน อย่างไรก็ตามความถี่ของการแข็งตัวขององคชาตสูงสุดในรูปแบบที่ 2 (ร้อยละ 77.8) มากกว่ารูปแบบที่ 1 และ 3 (ร้อยละ 38.9 และ 50.0 ตามลำดับ p< 0.05) ระยะเวลากระตุ้นจนเกิดการยื่นขององคชาต (36.9±5.7, 25.3±4.7 และ 33.4±5.6 นาที ในรูปแบบที่ 1, 2 และ 3 ตามลำดับ, p> 0.05) การแข็งตัวขององคชาต (42.7±5.2, 31.7±4.5 และ 35.0±5.8 นาที ในรูปแบบที่ 1, 2 และ 3 ตามลำดับ, p> 0.05) และการหลั่งน้ำเชื้อ (35.3±6.4, 34.5±5.0 และ 28.1±6.0 นาที ในรูปแบบที่ 1, 2 และ 3 ตามลำดับ, p> 0.05) ไม่แตกต่างกัน การแข็งตัวขององคชาตมีระยะเวลาได้รับกระแสไฟฟ้านานที่สุดในรูปแบบที่ 3 (20.8±3.1 นาที) นานกว่ารูปแบบที่ 1 และ 2 (15.2±1.9 และ 16.0±2.2 นาที ตามลำดับ (p< 0.05) ผลจากการศึกษานี้แสดงให้เห็นว่าการกระตุ้นด้วยเครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้าสามารถทำให้เกิดการแข็งตัวขององคชาตและเกิดการหลั่งน้ำเชื้อในเต่าดำได้

คำสำคัญ: เต่าดำ เครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า การแข็งตัวขององคชาต การหลั่งน้ำเชื้อ การรีดน้ำเชื้อ

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Introduction

Black marsh turtle (*Siebenrockiella crassicollis*) is a freshwater, small to medium-sized turtle and is classified as vulnerable by the International Union for Conservation of Nature Red List of Threatened Species (Asian Turtle Trade Working Group, 2000). It is also listed on the Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix II and is protected from exploitation under the Wild Animals Reservation and Protection Act B.E. 2535 (CITES, 2012). Despite the risk of being traded for food and traditional medicine, and being lower in population, very little reproductive physiology information is known. Over the last decades, substantial efforts aiming to understand the reproductive physiology of turtles have been presented. Semen collection would be the initial process of the attempts to fulfill the reproductive physiology of turtles.

Electroejaculation is an alternative means for semen collection in both human and animals. Men suffering from spinal cord injury can benefit from this technique (Utida et al., 2005). It has been successfully used in domestic animals such as cats (Chatdarong et al., 2006; Zambelli et al., 2010), boars (Martin et al., 1994), rams (Cochran et al., 1985; Marco-Jimenez et al., 2008), bulls (Palmer et al., 2004) and camels (Tingari et al., 1986). It has been applied to other species such as Japanese black bears (Okano et al., 2006), Asiatic black bears (Chen et al., 2007), Iberian red deer (Martinez et al., 2008), bongo antelope (Wirtu et al., 2008), coyotes (Bruss et al., 1983), Spanish ibex (Santiago-Moreno et al., 2009), agouti (Mollineau et al., 2008), coatis (Barros et al., 2009), collared peccaries (Souza et al., 2009), Malayan tapirs (Tipkantha et al., 2011) and armadillos (Serafima et al., 2010). Although the semen collection has been documented in sea turtles (Wood et al., 1982; Sahatrakul et al., 2007; Tanasanti et al., 2007; Suttiyotin et al., 2012), little is known about semen collection in black marsh turtles which are much smaller in size compared to the sea turtles. It is known that the voltages and the stimulation patterns used for electroejaculation vary among species. These influences may be due to anatomical differences and

voltage resistance among species. The objectives of the present study were to investigate the possibility of semen collection by electroejaculation and to evaluate stimulation patterns in black marsh turtles.

Materials and Methods

Animals: Black marsh turtles (n= 9) were retrieved from Nakhon Pathom province in the central part of Thailand. They were kept in a standard facility at the faculty of Veterinary Science, Mahidol University and were fed commercial diet and vegetables. They were regularly checked to assure good health by veterinarian during the time at the facility. Natural light was provided during daytime from 6 AM to 6 PM.

Anesthesia: The turtles were weighed and withheld from food 6 hours before anesthesia was used. General anesthesia was induced with ketamine (40 mg/kg) intramuscular injection at front or rear leg region (Kimsakulvech and Suttiyotin, 2012). At 15 min after ketamine injection, the turtles were transferred and rested on top of a cylinder box approximately 12 cm in diameter and 15 cm in height. Before any attempt to insert electrical probe or to stimulate with electric current, the turtles were observed for fully sedation to avoid mistreatment of conscious animals. After testing, all turtles were observed for fully recovery before being returned to their facilities. Turtles were considered full recover from anesthesia when they could swim and immediately response to stimuli.

Experimental design

Experiment 1, Depth of the probe insertion: Depth of probe insertion in relation to electrical stimulation effect was investigated in nine black marsh turtles. General data related to anatomical region at the rear end of the turtles were recorded (Wyneken, 2001). These criteria included body weight, carapace width, carapace length, plastron width, plastron length, body circumference, tail length and length from tail tip to anus. After sedation, the turtles were placed on a cylinder box approximately 12 cm in diameter and 15 cm in height (Fig 1). This allowed them to rest on their plastron with their legs hanging along the box. The setting provided free movement of the legs (if any) and no restraint was required. The turtles were observed until they were well sedated before the beginning of stimulation. Electroejaculator used in the study (Suttiyotin et al., 2012) consists of adjustable output power connected with a rectal probe (Fig 2). A small electrode probe was made to suit the size of the black marsh turtles. The probe measured 0.5 cm in diameter and 30 cm in length with 2 ring electrodes, 1 cm apart. K-Y jelly was applied to the probe before gentle insertion into the rectum to reach designed distances. The depth of insertion started at 1 cm from the anus with 1 cm increment in each depth. Electrical stimulation was applied at the voltage of 1.0 volt, increased by 1.0 volt every 5 sec to the maximum of 10.0 volts. Rear leg retraction was used as an indicator for response to the stimuli. The depth at which the turtle expressed highest intensity of muscle

Figure 1 Handling of Black Marsh turtles during electroejaculation. Note that the penis was partially protruded from the anus.

Figure 2 Electroejaculator used for semen collection from black marsh turtles.

contraction was recorded. To compare the depth of insertion to the distance from the turtle's rear, the probe was marked and measured against the carapace.

Experiment 2, Stimulation patterns: Two series of 3x3 Latin square were applied for stimulation pattern test. Nine male black marsh turtles weighing between 0.6 to 1.2 kg were allocated to 3 groups. The stimulatus was divided into 3 periods (9.00 to 12.00, 12.00 to 15.00 and 15.00 to 18.00). Three patterns of stimulation were random into 3 groups and repeated with different pattern at 1 week interval. The turtles were sedated by intramuscular injection of ketamine (40 mg/kg) and manipulated as described above. Sedation was induced in all turtles within 15 min and they were rechecked for unconsciousness prior to stimulation. Electrical probe was inserted into the rectum to the depth at which the first electrode was positioned at the center of the second carapace from the rear. Pre-measurement from the center of second carapace to the opening of the anus was made to ensure correct position. Stimulation was performed in one of the following patterns:

Pattern 1: The voltage was gradually increased from 0.0 to 7.5 volts in 20 sec, held at 7.5 volts for 20 sec and immediately returned to 0.0 volt. The gap between stimuli was allowed for 20 sec. The pattern was consecutively repeated for 5 times to complete 1 stimulus cycle. The turtles rested for 5 min before the next cycle of stimulation started. A total of 5 stimulation cycles was achieved in each turtle.

Pattern 2: The voltage was gradually increased from 0.0 to 7.5 volts in 30 sec, held at 7.5 volts for 30 sec and immediately returned to 0.0 volt. The pattern was consecutively repeated for 3 times to complete 1 stimulus cycle. The turtles rested for 3 min

before the next cycle of stimulation started. A total of 10 stimulation cycles was performed in each turtle.

Pattern 3: The voltage was gradually increased from 0.0 to 7.5 volts in 15 sec, held at 7.5 volts for 40 sec and immediately returned to 0.0 volt. The gap between stimuli was allowed for 5 sec. The pattern was consecutively repeated for 3 times to complete 1 stimulus cycle. The turtles rested for 2 min before the next cycle of stimulation started. A total of 12 stimulation cycles was performed in each turtle.

Spermatozoa were collected from semen adhered to the probe or erected penis. Semen sample was smeared on a microscope slide, fixed in absolute methanol for 1 minute and air dried. Spermatozoa were stained with Wright-Giemsa stain for 60 sec. The slide was then examined for spermatozoa under a light microscope at magnification of 400x.

Statistical analysis: Data were expressed as mean±SE. Pearson's correlation coefficients were used to determine the relationships between body weight and carapace width, carapace length, plastron width, plastron length and body circumference. To determine positions of probe with anatomical parameters, Pearson's correlation coefficients were also used to determine the association between depth of insertion and body weight, carapace width, carapace length, plastron width, plastron length and body circumference. Correlation coefficients were considered significant at the level of $p< 0.05$. In Experiment 2, frequencies and percentages of penile protrusion, erection and ejaculation, and electrical exposure times were analysed, with 2 replications, by one way analysis of variance. The model was $Y_{ijk} = \mu + T_i + S_j + E_{ijk}$. Where: Y_{ijk}: dependent variables, μ: populated mean, T_i: effect of treatment, S_j: effect of square, and E: residual error. All statistical analysis was performed using SPSS version 17.0. Differences between means were tested using Duncan's multiple range test and were considered significant when $p< 0.05$.

Results

Depth of probe insertion: Mean values of body weight, carapace width, carapace length, plastron width, plastron length, body circumference, tail length and length from tail tip to anus are presented in Table 1. When electrical stimuli were applied at different position along the rectum, a pattern of response was observed. The intensity of response increased with depth of insertion to a certain position, then decreased until no response was observed. Mean value of position at which highest intensity of response observed was 5.9±0.1 cm anterior to anus or 5.2±0.2 cm from posterior tip of carapace (Table 1). The turtles started to show responses when the voltage reach 6.0 volts. The intensity of response did not increase when higher than 7.0 volts was applied. When the penis protruded and erected, it measured 2.2±0.2 cm in width and 3.5±0.3 cm in length.

There were positive correlations between body weight of Black marsh turtles and carapace width (r= 0.91, p= 0.0006), carapace length (r= 0.97, p= 0.0006), plastron width (r= 0.92, p= 0.0004), plastron length (r= 0.96, $p< 0.0001$) and body circumference (r = 0.88, p = 0.0018). The correlation between the position of the probe that showed the highest response gave better result by measuring the distance from the anus than from the posterior end of carapace. The results revealed that the depth from anus to the tip of the probe positively correlated with body weight (r= 0.77, p= 0.0158), carapace length (r= 0.81, p= 0.0188) and plastron length (r= 0.71, p= 0.0331). There were, however, no relationships between the depth from anus to the tip of the probe and carapace width (r= 0.53, p= 0.1471), plastron width (r= 0.54, p= 0.01369) and body circumference (r= 0.38, p= 0.1342). The depth from the posterior end of carapace to the tip of probe did not correlate with body weight (r= 0.14, p= 0.7253), carapace width (r= 0.32, p= 0.4048), carapace length (r= 0.06, p= 0.8825), plastron width (r= 0.05, p= 0.9060), plastron length (r= 0.18, p= 0.6509), and body circumference (r= 0.53, p= 0.1463).

Table 1 Anatomical characteristics of black marsh turtles.

Parameters	n	Mean ± SE
Body weight (kg)	9	0.9 ± 0.1
Carapace width (cm)	9	16.8 ± 0.4
Carapace length (cm)	9	20.2 ± 0.5
Plastron width (cm)	9	8.0 ± 0.2
Plastron length (cm)	9	14.1 ± 0.3
Body circumference (cm)	9	32.6 ± 0.6
Tail length (cm)	9	6.4 ± 0.4
Length from anus to tail tip (cm)	9	2.5 ± 0.2
Depth of probe insertion from anus (cm)	9	5.8 ± 0.1
Depth of probe insertion from posterior tip of carapace (cm)	9	5.2 ± 0.2
Penis width (cm)	8	2.2 ± 0.2
Penis length (cm)	8	3.5 ± 0.3

Stimulation patterns: The number of penile protrusions was not affected by patterns of stimulation (Table 2, Fig 3). Out of 18 stimulations, there was a trend that pattern 2 gave a little higher frequency of penile protrusion (15 times, 83.3%) compared to those of pattern 1 (11 times, 61.1%) and pattern 3 (12 times, 66.7%). The difference was, however, not significant (Table 2). The erection responses to stimulation patterns followed the same trend but with more distinct differences. The frequency of penile erection of pattern 2 (14 times, 77.8%) was higher than those of pattern 1 (7 times, 38.9%, $p < 0.05$) and pattern 3 (9 times, 50.0%, $p < 0.05$). There was, however, no significant effect of stimulation patterns on frequency of ejaculation (ranged 8-10 times, 11.4-55.6%, Table 2).

Table 2 Frequencies of penile protrusion, erection and ejaculation of black marsh turtles after electroejaculation attempts.

Patterns	n	Protrusion	Erection	Ejaculation
Pattern 1	18	11	7[b]	8
Pattern 2	18	15	14[a]	9
Pattern 3	18	12	9[b]	10
Total	54	38	30	27
P value		0.315	< 0.05	0.801

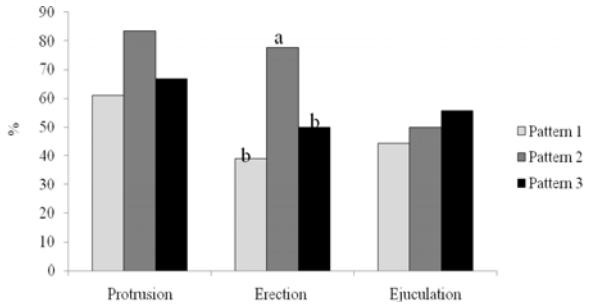

Figure 3 The percentages of penile protrusion, penile erection and ejaculation resulted from electrical stimulation in black marsh turtle. [a,b] Significant difference between patterns within erection category (p< 0.05).

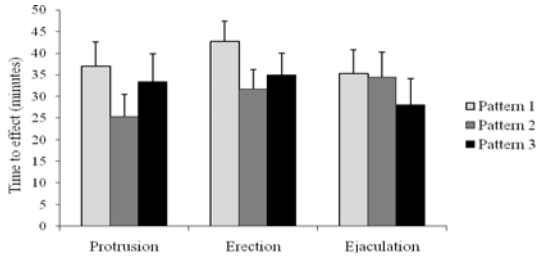

Figure 4 Time from stimulation to penile protrusion, penile erection and ejaculation after stimulation with 3 patterns in black marsh turtles.

Time from stimulation to animal responses (penile protrusion, penile erection and ejaculation) did not differ among the 3 stimulation patterns tested (Fig 4). Penile protrusions were observed in 36.9±5.7, 25.3±4.7 and 33.4±5.6 min in pattern 1, 2 and 3 of stimulation respectively (p> 0.05). Penile erections occurred after protrusions and were recorded at 42.7±5.2, 31.7±4.5 and 35.0±5.8 min after stimulation with pattern 1, 2 and 3 respectively (p > 0.05). Ejaculations were observed with or without penile erection and were measured 35.3±6.4, 34.6±5.0 and 28.1±6.0 min after stimulation with pattern 1, 2 and 3 respectively (p> 0.05).

Electrical exposure time from the beginning of stimulation to animal responses (resting time excluded) in each pattern of stimulation was also considered. There was a trend that electrical exposure time to achieve penile protrusion in pattern 3 (17.6±3.3 min) was higher than those of pattern 1 (12.8±1.9 min) and pattern 2 (12.0±2.4 min). The differences, however, were not statistically significant (p> 0.05, Table 3). To express penile erection, the turtles exposed to longer time of electrical stimulation in pattern 3 (20.8 ± 3.1 min) than those of pattern 1 and pattern 2 (15.2±1.8 and 15.9±2.2 min respectively,

Table 3 Electrical exposure time (min) to effect in 3 patterns of ejaculation in black marsh turtles.

Patterns	n	Protrusion	Erection*	Ejaculation
Pattern 1	18	12.8 ± 1.9	15.2 ± 1.8[b]	12.2 ± 2.0
Pattern 2	18	12.0 ± 2.4	15.9 ± 2.2[b]	16.9 ± 2.6
Pattern 3	18	17.6 ± 3.3	20.8 ± 3.1[a]	15.5 ± 3.3
P value		0.053	< 0.05	0.186

*Different superscript differs significantly at p< 0.05.

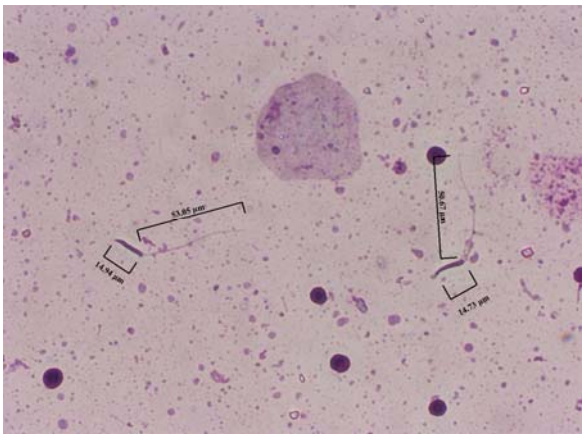

Figure 5 Spermatozoa harvested form electroejaculation in black marsh turtles.

p < 0.05). The exposure times of which ejaculation occurred were 12.2±2.0, 16.9±2.6 and 15.5±3.3 min in pattern 1, 2 and 3 respectively (p> 0.05).

Spermatozoa retrieved from ejaculation were composed of head and tail (Fig 5). The sperm head was narrow, curved and appeared as vermiform in shape. Dense homologous matrix was observed in the sperm head. Acrosome, however, could not be distinguished in these stained slides. The sperm tail was very long when movement observation was conducted in wet smear under a light microscope. On the other hand, the sperm tail looked a lot shorter in strained smears (Fig 5).

Discussion

Application of electroejaculation to facilitate semen collection has been achieved in both human (Brindley, 1981; Heruti et al., 2001; Utida et al., 2005), domestic (Palmer et al., 2004; Chatdarong et al., 2006) and exotic animals (Bruss et al., 1983; Busso et al., 2005; Howard et al., 1984; Serafima et al., 2010; Tipkantha et al., 2011). Attempts have been made to collect semen from sea turtles by electroejaculation (Wood et al., 1982; Sahatrakul et al., 2007; Tanasanti et al., 2007; Suttiyotin et al., 2012). To our knowledge, there has been no report in black marsh turtles. We successfully stimulated penile erection and ejaculation in black marsh turtles using a non-commercial electroejaculator. Electroejaculation induced stress as indicated by rising of peripheral blood cortisol concentration immediately after electrical stimulation at least in cats (Cater et al., 1984). The stress caused by this technique of semen collection needs attention to ensure minimal invasive operation to turtles. Patterns of stimulation in this study would be a basic knowledge for future applications in turtles.

This study indicated position that black marsh turtles expressed highest responses. The distances were measured against the anus of posterior tip of carapace. Since there were relationships between the depths of the probe from anus with body parameters but not between the depths from tip of carapace and body parameters, it would be more

precise to use the anus as a landmark. The depth of insertion, however, would depend on the size of the turtles. In our observation, the depth was measured approximately to the center of the second carapace from the rear. It is, thus, suggested that to maximize electrical stimulation in black marsh turtles, the probe should be insert to the center of the second carapace from the rear. The measurement may be against the anus or the posterior end of carapace, provided that the probe is inserted to the position indicated above.

Stimulation with pattern 2 gave better frequency of penile erection than pattern 1 and 3 while frequencies of penile protrusion and ejaculation were similar. This indicates that lower rate of voltage increment in pattern 2 (from 0 to 7.5 volts in 30 sec) creates more frequency of protrusion than higher rates (from 0 to 7.5 volts in 20 and 15 sec in pattern 1 and 3, respectively). Since turtles expressed similar frequencies of ejaculations in the 3 patterns, any of these patterns may be used in black marsh turtles. On the other hand, pattern 3 caused longer exposure time for turtles to produce penile erection. It would be the last choice for semen collection in black marsh turtles. In addition, if harvesting of semen from erected penis is to be addressed, pattern 2 may be more suitable. In comparison to sea turtles, patterns of stimulations vary among reports. Stimulation with 15 stimuli, increment of voltage from 0 to 4-8 volts in 3-4 sec was proved to be successful (Wood et al., 1982). Other reports applied 2-6 volts stimuli in sea turtles and yield good results (Sahatrakul et al., 2007; Tanasanti et al., 2007). We recently reported application of electrical stimuli from 0 to 7.5 volts in 20 sec with 4 ejaculates from 6 turtles stimulated (Suttiyotin et al., 2012). Although there is a difference in size between black marsh turtle and sea turtles, it seems that the voltage of up to 7.5 would be successfully used in both species.

Because of the small volume of semen obtained from electrical stimulation, it was difficult to evaluate semen quality and quantity. When compared to sea turtles, semen was harvested in bigger volume that can be evaluated and diluted in extenders to some extent (Wood et al., 1982; Sahatrakul et al., 2007; Tanasanti et al., 2007; Sirinarumitr et al., 2010).

In conclusion, semen could be collected from black marsh turtles with an electroejaculation. Pattern 2 of stimulation (voltage increment from 0.0 to 7.5 volts in 30 sec, held at 7.5 volts for 30 sec and immediately returned to 0.0 volt) may be appropriated for black marsh turtles. Further study may be needed to elucidate deeper details of semen collection method in black marsh turtles to yield higher semen volume enough for evaluation and processing.

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References

Asian Turtle Trade Working Group. 2000. *Siebenrockiella crassicollis*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. [Online]. Available: http://www.iucnredlist.org/apps/redlist/details/39616/0., Accessed June. 15, 2012

Barros, F.F.P.C., Queiroz, J.P.A.F., Filho, A.C.M., Santos, E.A.A., Paula, V.V., Freitas, C.I.A. and Silva, A.R. 2009. Use of two anesthetic combinations for semen collection by electroejaculation from captive coatis (*Nasua nasua*). Theriogenology. 71: 1261-1266.

Brindley, G.S. 1981. Electroejaculation: Its technique, neurological implications and uses. J Neurol Neurosurg Psychiatry. 44: 9-18.

Bruss, M.L., Green, J.S. and Stellflug, J.N. 1983. Electroejaculation of the coyote. Theriogenology 20: 53-59.

Busso, J.M., Ponzio, M.F., Chiaraviglio, M., Fiol de Cuneo, M. and Ruiz, R.D. 2005. Electroejaculation in the Chinchilla (*Chinchilla lanigera*): Effects of anesthesia on seminal characteristics. Res Vet Sci. 78: 93-97.

Carter, K.K., Chakraborty, P.K., Bush, M. and Wildt, D.E. 1984. Effects of electroejaculation and ketamine-HCl on serum cortisol, progesterone, and testosterone in the male cat. J Androl. 5: 431-437.

Chatdarong, K., Ponglowhapan, S., Manee-in, S. and Pongphet, K. 2006. The use of propofol for electroejaculation in domestic cats. Theriogenology 66: 1615-1617.

Chen, L.M., Hou, R., Zhang, Z.H., Wang, J.S., An, X.R., Chen, Y.F., Zheng, H.P., Xia, G.L. and Zhang M.J. 2007. Electroejaculation and semen characteristics of Asiatic Black bears (*Ursus thibetanus*). Anim Reprod Sci. 101: 358-364.

CITES. 2012. Consideration of proposal for amendment of appendices I and II. [Online]. Available: http://www.cites.org/eng/cop/12/prop/E12-P29.pdf., Accessed June. 15, 2012

Cochran, R.C., Judy, J.K., Parker, C.F. and Hallford, D.M. 1985. Prefreezing and post-thaw semen characteristics of five ram breeds collected by electroejaculation. Theriogenology 23: 431-440.

Heruti, R.J., Katz1, H., Menashe, Y., Weissenberg, R., Raviv, G., Madjar, I. and Ohry, A. 2001. Treatment of male infertility due to spinal cord injury using rectal probe electroejaculation: the Israeli experience. Spinal Cord. 39: 168-175.

Howard, J.G., Bush, M., Vos, V. and Wildt, D.E. 1984. Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants (*Loxodonta africana*). J Reprod Fert 72: 187-195.

Kimsakulvech, S. and Suttiyotin, P. 2012. Electrical stimulation produced erections and ejaculations in black turtles. Proceedings of the VPAT Regional Veterinary Congress 2012. May 13-16, 2012. pp. O35-O56.

Marco-Jimenez, F., Vicente, J.S. and Viudes-de-Castro, M.P. 2008. Seminal plasma composition from

ejaculates collected by artificial vagina and electroejaculation in Guirra ram. Reprod Dom Anim 43: 403-408.

Martin, P.A., Dooley, M.P., Hembrough, F.B. and Pineda, M.H. 1994. Retrograde flow of spermatozoa into the urinary bladder of boars during collection of semen by electroejaculation. Theriogenology 41: 869-877.

Martinez, A.F., Martinez-Pastor, F., Alvarez, M., Fernandez-Santos, M.R., Esteso, M.C., de Paz, P., Garde, J.J. and Anel, L. 2008. Sperm parameters on Iberian red deer: Electroejaculation and post-mortem collection. Theriogenology 70: 216-226.

McArthur, S. 2004. Anaesthesia, analgesia and euthanasia. In: Medicine and Surgery of Tortoises and Turtles. S. McArthur, R. Wilkinson and J. Meyer (eds) Oxford: Blackwell Publisher. 377-401.

Mollineau, W.M., Adogwa, A.O. and Garcia, G.W. 2008. A preliminary technique for electro-ejaculation of agouti (Dasyprocta leporine). Anim Reprod Sci. 108: 92-97.

Okano, T., Murase, T., Yayota, C., Komatsu, T., Miyazawa, K., Asano, M. and Tsubota, T. 2006. Characteristics of captive Japanese black bear (Ursus thibetanus japonicas) semen collected by electroejaculation with different voltages for stimulation and frozen-thawed under different conditions. Anim Reprod Sci. 95: 134-143.

Palmer, C.W., Amundson, S.D., Brito, L.F.C., Waldner, C.L. and Barth, A.D. 2004. Use of oxytocin and cloprostenol to facilitate semen collection by electroejaculation or transrectal massage in bulls. Anim Reprod Sci. 80: 213-223.

Sahatrakul, K., Dhanarun, K., Sujaritthanyatrakul, C., Sakorncharoun, P., Tanasanti, M., Manawatthana, S. Sanyathitiseree, P. and Sirinarumitr, K. 2007. Semen collection by electroejaculation and semen evaluation of olive ridley turtle (*Lepidochelys olivacea*). Abstract Book of Department of Marine Coastal Resources (DMCR) Seminar Conference. 78-79.

Santiago-Moreno, J., Coloma, M.A., Dorado, J., Pulido-Pastor, A., Gomez-Guillamon, F., Salas-Vega, R., Gomez-Brunet, A. and Lopez-Sebastian, A. 2009. Cryopreservation of Spanish ibex (Capra pyrenaica) sperm obtained by electroejaculation outside the rutting season. Theriogenology 70: 1253-1260.

Serafima, M.K.B., Lira, R.A., Costab, L.L.M., Gadelhab, I.C.N., Freitas, C.I.A. and Silva, A.R. 2010. Description of semen characteristics from six-banded armadillos (*Euphractus sexcinctus*) collected by electroejaculation. Anim Reprod Sci. 118: 362-365.

Sirinarumitr, K., Patthong, Y., Jaimjaturong, P., Woonwong, Y., Petchsamut, W., Limpasuntisin, P., Manawatthna, S., Sirinarumitr, T., Sanyathitiseree, P., Kornkaewrat, K.and Suithunmapinunta, P. 2010. Extender for sperm dilution in olive ridley turtle (*Lepidochelys olivacea*) and hawksbill turtle (*Eretmochelys imbricata*) semen. Proceedings of the 5th International Symposium on SEASTAR2000 and Asian Bio-logging Science (The 9th SEASTAR2000 workshop). pp. 7-10.

Souza, A.L.P., Castelo, T.S., Queiroz, J.P.A.F., Barros, I.O., Paula, V.V., Oliveira, M.F. and Silva, A.R. 2009. Evaluation of anesthetic protocol for the collection of semen from captive collared peccaries (*Tayassu tajacu*) by electroejaculation. Anim Reprod Sci. 116: 370-375.

Suttiyotin, P., Nakthong, C., Kimsakulvech, S., Sirimanapong, W. and Jarearnkul, D. 2012. Management of medium sized animal farms with reproductive biotechnology approach. The complete research report. Mahidol University, Nakorn Pratom. 136 pp.

Tanasanti, M., Sujaritthanyatrakul, C., Dhanarun, K., Sahatrakul, K., Sakorncharoun, P., Manawatthana, S., Sanyathitiseree, P. and Sirinarumittr, K. 2007. Electroejaculation and semen evaluation in olive ridley turtle (Lepidochelys olivacea) and hawksbill turtle (Eretmochelys imbricata) in Thailand. Proceedings 4th Int Symp SEASTAR2000 and Asian Bio-logging Science (The 8thSEASTAR2000 workshop). December 15-17, 2007. Royal Phuket City Hotel, Phuket, Thailand. p 29-32.

Tingari, M.D., El-Manna, M.M., Rahim, A.T.A., Ahmed, A.K. and Hamad, M.H. 1986. Studies on camel semen. I. Electroejaculation and some aspects of semen characteristics. Anim Reprod Sci. 12: 213-222.

Tipkantha, W., Pukazhenthi, B., Siriaroonrat, B., Thongphakdee, A., Maikaew, U, Thomas, W., Tongthainan, D., Thaeonoen, J., Kamolnorranath, S., Bush M. and Thongtipsiridech, S. 2011. Ejaculate characteristics of captive Malayan tapirs (*Tapirus indicus*). Thai J Vet Med. 41(4): 499-508.

Utida, C., Truzzi, J.C., Bruschini, H., Simonetti, R., Cedenho, A.P., Spougi, M. and Ortiz, V. 2005. Male infertility in spinal cord trauma. Int Braz J Urol. 31(4): 375-383.

Wirtu, G., Pope, C.E., MacLean, R.A., Godke, R.A., Paccamonti, D.L. and Dresser, B.L. 2008. Reversal of motility loss in bongo antelope (*Tragelaphus eurycerus isaaci*) spermatozoa contaminated with hyposmotic urine during electroejaculation. Anim Reprod Sci. 103: 392-397.

Wood, F., Platz, C., Critchley, K. and Wood, J. 1982. Semen collection by electroejaculation of the green turtle, Chelonia mydas. British J Herpetology. 6: 200-202.

Wyneken, J. 2001. The Anatomy of Sea Turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 172 pp.

Zambelli, D., Iacono, E., Raccagni, R. and Merlo, B. 2010. Quality and fertilizing ability of electroejaculated cat spermatozoa frozen with or without Equex STM Paste. Theriogenology 73: 886-892.