



Scientific Papers

SEMEN COLLECTION BY ELECTROEJACULATION OF THE GREEN TURTLE, *CHELONIA MYDAS*

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SUMMARY

Electroejaculation was used to obtain motile sperm from farm reared green turtles, *Chelonia mydas*. at the breeding facilities of Cayman Turtle Farm Ltd. Motility and volume was variable depending upon time and frequency of ejaculation and the individual male used. Total sperm counts up to 66×10^8 were observed with percent motility as high as 90%. Of over 70 attempts 60% yielded motile sperm. This technique was employed with males nine to 14 years old and weighing 110 to 175 kg. Semen collection is the first step in a continuing programme to develop a successful technique of artificial insemination.

INTRODUCTION

Since 1973, mating and subsequent nesting has occurred among the captive breeding colony of green turtles *Chelonia mydas* at the facilities of Cayman Turtle Farm. Ltd., a commercial sea turtle farm in the British West Indies (Wood & Wood. 1980). A programme to develop the means to collect semen and artificially inseminate turtles on the farm was initiated in 1978. This programme was designed to compliment the on-going reproductive programme by providing information on the sperm production capabilities of the males, By enabling artificial insemination of nesting females whose first nests in a season show no signs of embryonic development, and by eventually allowing for controlled and selective breeding among the farm's stock. Initial collection attempts and description of the turtle spermatozoa morphology have been previously presented (Platz. Mengden. Quinn. Wood & Wood. 1980).

MATERIALS AND METHODS

Male green turtles hatched and reared to maturity at Cayman Turtle Farm Ltd. were maintained in one of two excavated ponds each containing approximately one million gallons of constantly flowing sea water. These turtles ranged in age from nine to 14 years and weighed from 110 to 175 kg. The turtles were fed a commercial high protein (35%) pelleted diet. The males were kept with the females in the pond from March through November for the breeding season. Female to male ratio varied from 4.3:1 to 8.6:1 depending on the year. During the remaining months, the males

were kept separated either in a fenced section of the pond or in stock tanks. Data presented were collected from April 1979 through July 1981.

The following procedure was collection and evaluation of ejaculate:

1. The male was removed from the water and lifted onto a metal rack. Approximately 50 cms. high, and secured to the rack with ropes from all four flippers. The tail was allowed to hang unsupported from the edge of the rack. Anaesthesia was not used in the collections. The tail was rinsed with seawater to remove any sand or particular matter.
2. The electroejaculator (constructed by C Platz) was an AC 60 Hz stimulator. The rectal probe consisted of a PVC tube 70 cms. long and 2.5 in diameter containing three stainless steel electrodes, 13 cm long running within 1.0 cm parallel to each other at the end of the probe. The probe was coated with petroleum jelly and inserted through the cloaca into the rectum 40-60 cm, depending upon the size of the male.
3. Normal stimulus consisted of 15 stimuli, 4-8 volts in intensity. Effective amperage 0.15 - 0.40 amperes. Stimulus duration was 3-4 seconds from 0 to peak voltage to 0 again without pause at peak voltage. Approximately 2 seconds rest was allowed between stimuli. The series of 15 stimuli was repeated at four to five depths, as the probe was withdrawn 8 to 10 cms. at a time. Following removal of the probe the tail and penis if extended was milked manually to aid in removal of any remaining ejaculate. The turtle was then returned to the water.
4. The semen was collected in 5 ml of normal saline and examined immediately for percent motility, progressive motility and total sperm. (Platz, & Seager 1977, Platz, Wildt & Seager, 1978)

RESULTS AND DISCUSSION

Table I summarizes 74 attempted collections. Ejaculate containing sperm was obtained in 5 attempts. Fourteen of these 55 collections had no motile sperm or the total count was so low as to indicate that the ejaculate would be inadequate for fertilisation of a female. No abnormal spermatozoa were observed in samples which were examined at high magnifications. The ejaculate was often quite viscous and frequently contained clumps of material, which when manually broken apart contained millions of spermatozoa. The volume of ejaculate varied between 1 to 2 ml up to 100 ml. The physical response of the male to stimulus was variable among the males. In some instances the penis would be extended as the male was secured to the rack. In other instances, the penis was extended following the initial stimulus.

Ejaculates containing viable sperm were also obtained without the penis ever being exposed. Urination was also variable, some urinating before or after stimulation and others not at all. In a few instances the turtle defecated on partial insertion of the probe. in which cases. the turtle was released because clean samples were unable to be obtained afterwards.

In addition to the 74 attempted collections summarized in **Table I**, several males were secured on the rack, but insertion of the probe caused minor bleeding and the turtle was released. These turtles showed no continued bleeding or ill effects when observed in the water following the attempts.

TABLE 1. Summary of semen collection.

Number of attempted collections	74
Number of collections with sperm	55
Volume of ejaculate, range	1-100 ml
% motility, range	0-90%

Average motility (55 collections)	36
Total sperm count in ejaculate, range	0.6600 x 10 ⁶
Average count (55 collections)	470x 10 ⁶
Age of males (28 males)	9-14 years
Size of males (28 males)	110-1750 kg

Although the normal electroejaculation procedure consisted of a series of stimuli in some instances, the male would ejaculate without any electrical stimulus. This occurred during six separate attempts when the male ejaculated as the probe was inserted. In approximately 10% of the collections, an ejaculate was obtained after only a few stimuli. To minimise stress to the male, no further stimulus other than manual milking of the tail and penis was applied once an ejaculate containing motile sperm was obtained.

Muscular response of the males to electrical stimulus consisted of curling of the rear flippers, tensing of the neck, and a sharp intake of breath. More active movement against the restraints of the ropes and the position would occur with or without electrical stimulation. In initial electroejaculation trials described previously by Platz *et al.* (1980) higher voltages up to 30 volts (0.90 amperes) were used. However with successive trials, the lower voltages appeared to be sufficiently effective and to have less chance of harming the turtle.

TABLE II. Successive electroejaculation of three males

	Day				
	1	2	3	4	5
Male 118					
% Motility	0	5	0	0	5
Total Sperm count, x 10 ⁶	0	5	30	30	33
Male 124					
% Motility	5	25	65	0	0
Total Sperm count, x 10 ⁶	48	1	182	15	0
Male 253					
% Motility	10	65	80	0	35

Total Sperm count, x 10 ⁶	645	487	450	88	530
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Observations on the farm's breeding stock show that the turtles mate for hours and days at a time. Three males were electroejaculated 5 days in succession to determine if sperm production is limited or time dependent over such an interval. **Table II** lists the results of this trial. Obviously, the variability among males is considerable, but for male 253, viable sperm with reasonable motility was collected four of five successive days.

In addition, the same male is often observed to mate throughout the season with several different females. Two males electroejaculated in successive months during 1979 demonstrated continued sperm production until October. Males nos. 288 and 10 showed respective total sperm counts (and percent motiles) in April, May, July, and October of 130 x 10⁶ (20%), 350 x 10⁶ (10%), 1900 x 10⁶ (40%), 0 (0%); and 280 x 10⁶ (80%), 100 x 10⁶ (50%), 20 x 10⁶ (5%), 0 (0%). Although these two males showed no sperm production in October (which is beyond the mating season of April through July), other males yielded ejaculates in October with good motility and high counts as summarized in **Table III**.

TABLE III Seasonal production of sperm.
Number in parentheses is actual number of attempted collections.

	February	April	May	June	July	August	October
Number of Collections	1 (1)	8 (9)	15 (18)	7 (8)	12 (16)	2 (3)	11 (19)
% Motility							
Average	45	20	27	62	34	35	52
Range	-	5-80	0-80	0-90	5-60	5-70	0-80
Total Sperm count, x 10 ⁶							
Average	1355	81	198	313	491	260	1035
Range	-	10-280	1-645	1-1176	10-1900	1-520	10-6600

Data reported in this paper were obtained using males nine to 14 years of age. Only two of the 28 different males failed to produce an ejaculate containing sperm. Green turtle males of this age would appear physiologically capable of fertilizing the female. The data presented also indicated that one male would be capable of fertilizing several females in one season. There is considerable variability encountered in the electroejaculation attempts among the different males used and among the different times of the season when the collections were made. Further investigations are necessary to determine if the variability is due to technical, environmental or physiological parameters.

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