Electroejaculation and Semen Characteristics of the Captive Hokkaido Brown Bear (*Ursus arctos yesoensis*)

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ABSTRACT. An electroejaculation technique was applied to the Hokkaido brown bear (*Ursus arctos yesoensis*) for semen collection and characterization of their seminal traits. Ten captive sexually mature bears were anesthetized and subjected to 21 electroejaculation trials during their mating season in 1995 and 1996. Spermic electroejaculates were recovered from 6 of the 10 bears (14 of 21 trials). The semen was characterized by serous fluid of semitransparent white color and a neutral pH. The mean values of ejaculate volume, sperm concentration, percentage of sperm motility, percentage of live spermatozoa, and percentage of pleiomorphic forms were 2.7 m*l*, 471.6 × 10^6 cells/m*l*, 80.2%, 89.7% and 21.8%, respectively. Although there was considerable variation among the seminal traits of the individual bears, the electroejaculation technique was effective in obtaining ejaculates from captive bears. — KEY WORDS: brown bear, electroejaculation, seminal trait.

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The Hokkaido brown bear (*Ursus arctos yesoensis*), a subspecies of the brown bear, is found only in Hokkaido Island, Japan, and recently their habitats have become fragmented and shrunken because of human activity. Tsuruga *et al.* [12] reported that the genetic diversity of this bear is generally low. When the effective population size is reduced and the genetic diversity is further depleted, an immediate loss of fitness will take place. Since some local populations have decreased in size and tend to be isolated, artificial breeding would be an effective technique to maintain the genetic resources of this species. However, no program or technique has been developed to maintain a captive breeding colony of Hokkaido brown bears as a genetic resource.

The mating season of the Hokkaido brown bear usually extends from early May to early July in captive conditions [10]. From macroscopic and histological observations of the testes of this bear, Tsubota and Kanagawa [11] reported that active spermatogenesis is sustained from February to September. However, there is less information about semen and live spermatozoa of bears, because only a few attempts have been made to collect semen, and the attempts have been only partially successful [4, 9]. Obtaining basic data on the semen collection and the semen characteristics is a first step in the application of artificial breeding techniques and in choosing appropriate conservation strategies for wild populations. The objectives of the present study were to determine the effectiveness of the electroejaculation technique for collecting bear spermatozoa and to document the seminal traits of captive Hokkaido brown bears.

The bears used in this study were maintained under captive conditions at the Noboribetsu Bear Park in Hokkaido, Japan. Ten adult males, aged 7–16 years, were used in this study. Twenty-one trials with these males were conducted from mid-May to mid-July, around the mating season, in 1995 and 1996 (Table 1). Commercial bear diets, domestic animal diets and some vegetables were provided, and the bears were housed in indoor/outdoor enclosures under the natural light cycle. All males were separated from females by a grid wall, but had visual, aural, and/or olfactory contacts during the experimental periods. A surgical plane of anesthesia was induced with an intramuscular (i.m.) injection of tiletamine HCl-zolazepam (Zoletil; 100 mg/m*l*, Virbac, Carros, France) at a dose of 3 mg/kg body weight by pole syringe in 19 trials. For male D (Trial no. 11), 0.01 mg/kg atropine sulfate (Atropine sulfate injection, Tanabe, Osaka, Japan) was given before the injection of tiletamine HCl-zolazepam. Male A (Trial no. 5) was preanesthetized by an i.m. injection of 0.25 mg/kg midazolam (Dormicum, Roche, Basel, Switzerland) and immobilized by an i.m. injection of 5.0 mg/kg ketamine HCl (Ketalar, Sankyo, Tokyo, Japan). With the anesthetic plane with midazolam and ketamine HCl, the animal was less immobilized than in the other trials.

Table 1. Dates of electroejaculation trials for each male Hokkaido brown bear in captivity

Bear	Date of the electroejaculation trial	
	1995	1996
A	July 2 & July 16	May 1, June 8, June 28 & July 16
B	June 18	May 21 & June 18
C	-	May 21
D	May 31	July 16
E	June 18 & July 2	-
F	July 16	-
G	-	May 13, June 8 & July 16
H	-	June 18
I	June 10	-
J	June 18	-

Electroejaculation was done immediately after the bears were immobilized. The electroejaculation method used for the giant panda (*Ailuropoda melanoleuca*) [6, 7] was employed with a slight modification in this study. The bipolar rectal probe used in this study was a plastic tube, 50 cm long and 2.0 cm in diameter, and has two copper ring electrodes (0.8 cm in width) on its tip. The distance between the electrodes was 2.0 cm. The probe was connected to a electroejaculator (1 channel type for mammals, Fujihira Industry Co., Ltd., Tokyo) (Fig. 1).

The bear was placed in a lateral position for the electroejaculation. The rectal probe was inserted into the rectum and the electrodes were placed to a depth of 14–22 cm from the anus. The depth was adjusted to produce a strong contraction of the posterior legs and good penile erection during the electrical stimulation, because the ejaculates were elicited when such a response was observed in our previous study. The electrical stimulation with 60 Hz alternative current, sine wave and 5 V was repeated as a cycle of a 4-sec stimulation period and a 10-sec off period for 10 to 20 shocks. When these stimulations did not produce an ejaculation, the stimulations were resumed after a 1–2 min pause, for a maximum of 80 shocks. The ejaculate was collected into a 50 m*l* plastic tube which was warmed at 37°C in advance and covered the penis.

The ejaculates were evaluated immediately after collection. The ejaculate color was noted, the total volume was measured, and the pH was assessed using colorimetric pH paper (Toyo Roshi, Tokyo). The sperm concentration was determined using a standard hemocytometer method [13]. Raw, undiluted aliquots were assessed microscopically (× 400) at 37°C for calculating the sperm motility percentage. The percentage of live spermatozoa was assessed using an eosin-nigrosin staining method [1] with a slight modification. The modified stain consisted of 1 g water-soluble eosin, 2 g water-soluble nigrosin, and 100 m*l* of 2.9% sodium citrate solution. The mixture of the stain and semen was smeared on glass slides at 37°C and air-dried, and two hundred and fifty spermatozoa/ejaculate were counted under microscopy (× 400) for the calculation of the rate of white-head spermatozoa, which were defined as live spermatozoa. Other smears of the semen were air-dried, fixed with methanol and stained with 5% Giemsa's solution for morphological analysis [3]. Microscopy (× 400) was used to assess 300 spermatozoa/ejaculate for the incidence of pleiomorphic forms including macrocephalic, microcephalic, abnormal acrosome, bicephalic, biflagellate, abnormal or bent midpiece, coiled or bent flagellum, bent neck and cytoplasmic droplets. The total number of motile spermatozoa/ejaculate was calculated as follows: the sperm concentration/m*l* of ejaculate × the ejaculate volume × the percentage motility value.

The electroejaculation results are shown in Table 2. Ejaculates containing motile sperm were recovered from 14 of 21 trials (Trial nos. 1–13 and 15). No fluid was obtained from 2 trials (Trial nos. 18 and 21). In the other 3 trials (Trial nos. 16, 19 and 20), only a small amount of urine was

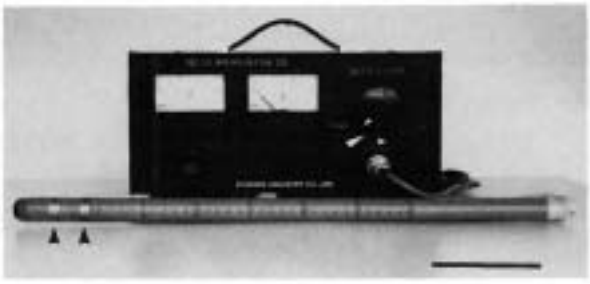

Fig. 1. Electroejaculator and rectal ejaculatory probe used for semen collection from Hokkaido brown bears. Arrow heads indicate copper ring electrodes. Bar = 10 cm.

elicited. In the remaining 2 trials (Trial nos. 14 and 17), the ejaculates contained only non-motile sperm.

The ejaculate containing motile sperm was characterized by a serous fluid of semitransparent white color. More milky fluid was observed when epithelial cells and white blood cells contaminated the sample. The average and range values for various characteristics of the semen containing motile spermatozoa are provided at the bottom of Table 2. Although there was considerable variation in the semen volume, sperm concentration and motile sperm number/ejaculate, the sperm motility rate and the percentage of live spermatozoa were relatively consistent. The motility rate for 11 of the 14 ejaculates was 70–99%, and the percentage of live spermatozoa for 10 evaluated ejaculates was more than 80%. The ranges of ejaculate volume and motility rate observed in the present study (2.7 ± 2.6 m*l* and 80.2 ± 23.6%) were similar to those of electroejaculated giant pandas (2.3–3.6 m*l* and 45–75%) [7]. The mean values of ejaculate volume and sperm concentration (2.7 ± 2.6 m*l* and 471.6 ± 429.2 × 10^6 cells/m*l*) were similar to those in a previous report about electroejaculated brown bears (*Ursus arctos lasiotus*) (3.46 ± 2.13 m*l* and 366 ± 258 × 10^6 cells/m*l*) [4]. However, the mean values of motility and the ejaculate pH (80.2 ± 23.6% and 7.4 ± 0.3) were considerably greater than the comparable values reported in brown bears (63 ± 14% and 6.14 ± 0.45) [4].

Since the urine of the bears showed an acid pH less than 5.8 in the present study, the urine contamination might decrease the pH of the ejaculates and the motility of the sperm. In the present study, ejaculates were recovered in several series during the electrical stimulations in each trial to prevent urine contamination. Urine contamination was more likely occured in the later series of electrical stimulations. The suppression of urine contamination is thought to be the key to keep a neutral pH in the electroejaculates and high sperm motility rate.

The success of the present procedure was presumed to be due to several factors. An appropriate anesthetic and electrical stimulation are necessary for the collection of high-quality ejaculates. In the present study, the bears were anesthetized with tiletamine HCl-zolazepam or the combination of midazolam and ketamine HCl, and high-quality spermic ejaculates were recovered. We therefore

Table 2. Electroejaculation trial results in ten Hokkaido brown bears

Trial No.	Bear	Date	Anesthesia[a)]	Class[b)]	Ejaculate				Percentage of sperm		
					Volume (m*l*)	pH	Sperm conc. ($\times 10^6$ cells/m*l*)	Motile sperm no. ($\times 10^6$ cells/ejaculate)	Motile	Live	Abnormal
1	A	95/7/2	Z	M	2.4	7.6	125.0	290.4	96.8	100.0	19.0
2		95/7/16	Z	M	1.0	7.6	440.5	436.1	99.0	90.9	N.D.
3		96/5/13	Z	M	3.2	7.6	140.8	307.2	69.3	81.2	17.7
4		96/6/8	Z	M	3.8	7.4	427.8	1488.1	91.5	89.3	15.6
5		96/6/28	Mi/K	M	7.8	7.2	518.1	3839.2	95.0	95.4	9.2
6		96/7/16	Z	M	8.3	7.3	911.8	7046.6	93.7	87.3	14.5
7	B	95/6/18	Z	M	1.3	N.D.	274.7	316.6	88.6	87.1	40.8
8		96/5/21	Z	M	1.1	6.9	378.8	319.4	77.4	88.8	17.5
9		96/6/18	Z	M	0.1	7.8	N.D.	N.D.	50.0	N.D.	N.D.
10	C	96/5/21	Z	M	0.2	7.4	1570.0	298.3	95.0	92.1	10.3
11	D	95/5/31	Z	M	3.0	N.D.	276.5	746.6	90.0	N.D.	43.0
12		96/7/16	Z	M	1.6	7.3	123.1	170.6	88.3	85.0	19.4
13	E	95/6/18	Z	M	1.3	7.7	N.D.	N.D.	76.2	N.D.	32.9
14		95/7/2	Z/A	NM	5.5	5.8	116.0	0.0	0.0	N.D.	29.6
15	F	95/7/16	Z	M	N.D.[d)]	6.6	N.D.	N.D.	12.5	N.D.	N.D.
16	G	96/5/13	Z	U	N.D.	<5.8	0.0	–	–	–	–
17		96/6/8	Z	NM	2.0	6.0	64.0	0.0	0.0	82.0	42.7
18		96/7/16	Z	NF	0.0	–	–	–	–	–	–
19	H	96/6/18	Z	U	N.D.	<5.8	–	–	–	–	–
20	I	95/6/10	Z	U	4.0	<5.8	0.0	–	–	–	–
21	J	95/6/18	Z	NF	0.0	–	–	–	–	–	–
Mean ± S.D[c)]					2.7 ± 2.6	7.4 ± 0.3	471.6 ± 429.2	1387.2 ± 2160.7	80.2 ± 23.6	89.7 ± 5.3	21.8 ± 11.7

a) Z: Zolazepam and Tiletamine
Mi: Midazolam
K: Ketamine
A: Atropine

b) M: Motile sperm
NM: Non-motile sperm
NF: No fluid
U: Urine

c) The data of urine, no fluid, and the ejaculates containing non-motile sperm (trial nos. 14, 16–21) were excluded.
d) N.D.: Not determined

feel presumed that tranquilization with a benzodiazepine complex (such as zolazepam or midazolam) is suitable for the electroejaculation of bears.

The rectal probe design in the present study was a modification of that used for the giant panda [6]. While the probe used for the giant panda has 8 electrodes, the number of electrodes was reduced from 8 to 2, and both electrodes were placed at the tip of the probe in the present study. These modifications enabled the stimulation of a narrow area of the rectum and might have helped to obtain spermic ejaculation without urine.

Whether electroejaculation techniques can be applied or not depends on the mating system [5], the anatomical characteristics of the reproductive organs of the species [8] and the endocrinological status of the individual animals [2]. In the present study, it was not clarified where of the rectum was electrically stimulated. Further study on the anatomical characteristics of the male bear genital tract will improve the semen collection technique. In addition, behavioral and endocrinological studies on the male bear might help understanding the considerable variation in the responses to electrical stimulation and the seminal traits among the male bears.

This study is the first report regarding the electroejaculation technique which was effective in obtaining ejaculates from the captive Hokkaido brown bear. Although considerable variation in ejaculate traits was observed among the individual males and trials, the present study offers basic data regarding the electroejaculation techniques used in the bears and provides the characteristics of their semen. The results could be useful for the collection of valuable spermatozoa for future artificial breeding and for research studies on male bear reproduction. To obtain high-quality semen consistently, some modification of the technique might be needed. Additional studies of the relationships between seminal traits and endocrinological status may elucidate the variation in seminal traits.

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