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Semen characteristics of Bornean sun bear (*Helarctos malayanus euryspilus*)

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A R T I C L E I N F O

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A B S T R A C T

The Bornean sun bear (*Helarctos malayanus euryspilus*), endemic to Borneo island, is the subspecies of the Malayan sun bear. The species is at risk, not just because of anthropogenic threats, but also slow reproduction in the wild. In captivity, due to poor reproductive performance, assisted reproductive technology is deemed a fundamental tool to propagate the depleting numbers of the Bornean sun bear. This is a pioneer study that presents the semen characteristics of the Bornean sun bear via conventional semen evaluation methods. Forty two semen samples from ten sun bears were collected via electroejaculation and evaluated. The electroejaculator probe (2.5 cm in diameter and 7.0 cm in length) was inserted rectally and positioned dorsal to the prostate gland. The optimum voltage used to obtain semen differed with each individual, but all showed hindlegs contraction and penile erection before ejaculation. The average combined testes volume in the Bornean sun bear was 23.37 ± 5.09 cm^3 . The mean semen volume was 617.30 µL, with sperm concentration of 1034.40 × 10^6 sperm/mL and pH 7.79. Sperm viability was 80.19% with a general motility of 79.13% and progressive movement of 70.20%. There were high sperm abnormalities at 70.67%. Sun bear sperm length was 61.28 ± 2.46 µm and consisted of an oval head, midpiece, and tail. From this study, good semen donors were identified from the captive Bornean sun bear population in Sabah, Malaysia. The fresh semen baseline data established in this study will provide crucial reference for assisted reproduction programs in the Bornean sun bear.

Introduction

The Malayan sun bear (*Helarctos malayanus*), which is predominantly found in mainland Southeast Asia, is the world's smallest and least-known bear species [1]. The subspecies in Borneo island, the Bornean sun bear (*Helarctos malayanus euryspilus*) is characterized by a smaller head and body size compared to its mainland counterparts [1,2]. The sun bear is omnivores, feeding primarily on invertebrates (termites, ants, beetles, etc), plants (figs, fruits) and small vertebrates [3–5]. Sun bears can live up to 30 years in captivity [1]. Sun bear is known to be solitary in the wild [3], however in captivity, peer rearing is common practice. The gestation ranges from 95 to 107 days, and usually only one cub is birthed, rarely twins [6,7]. Like other bear species, the cub leaves the mother after becoming full grown at the age of two to three [7,8]. In the past three decades, the global population of sun bear is estimated to have declined at a rate of 30% [9]. In Borneo, this species is threatened by habitat loss, rampant poaching for traditional Asian medicine, and illegal pet trading [1,10].

Seven of the eight ursid species in the world are seasonal breeders [8]. The Malayan sun bear, nevertheless, is an exception. Longitudinal monitoring of fecal testosterone in seven male sun bears proved that the testosterone level peaked during mating, which was closely associated with female estrus behavior [11]. In another hormonal study, the female sun bear remained receptive all year long [6]. Sun bear evolved to adopt more flexible reproductive strategies that are not affected by photoperiods but are likely influenced by rainfall and food availability in lowland tropical forests [6,11]. In the wild, a female sun bear faces both natural and anthropogenic disturbances, and may produce only one or

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Table 1
Descriptive analysis of the 10 Bornean sun bears' semen characteristics (n = 30).

Semen characteristics	Mean	SD	Median	IQR	Range
pH	7.79	0.67	8.00	0.63	6.00–8.80
Volume (μL)	617.30	558.31	480.08	677.50	50.00–2525.00
Concentration (10^6 sperm/mL)	1034.40	1489.72	319.40	1348.81	59.00–7620.00
Total sperm (10^6)	642.59	1240.43	166.46	412.27	14.25–5418.65
Sperm viability (%)	80.19	10.57	81.35	16.40	59.30–94.30
Sperm motility (%)	79.13	13.59	80.00	20.00	45.00–95.00
Progressive sperm (%)	70.20	16.27	70.00	23.75	30.00–90.00
Normal sperm (%)	28.25	11.93	24.90	13.00	11.00–57.00

two cubs per lifetime [1,6]. Poor reproductive performance of captive sun bear is reported due to sensitivity to environmental variables [6,12,13]. An alternative to natural breeding i.e. an *ex-situ* breeding program is therefore essential to increase the sun bear population size [14]. As in many wildlife species, semen cryopreservation is a crucial initiative to conserve genetic resources for species recovery [15].

Among the ursids, the giant panda is considered a model species that has achieved conservation success as the captive population size has increased with help from assisted reproductive technology (ART) [16]. In the giant panda, knowledge has been accumulated for four decades in male reproductive biology, particularly in semen traits and cryopreservation methods that were the determinants for successful artificial insemination (AI) [16–18]. Meanwhile, semen collection and cryopreservation have been initiated in other bear species, such as the Asiatic black bear [19,20], the brown bear [21], the American black bear [22], and the polar bear [23]. However, there is no published data available on semen characteristics of the Bornean sun bear [24].

This is the first report on semen collection via electroejaculation (EE) in the Bornean sun bear. The objective of this study is to obtain baseline data on spermatozoa and semen characteristics of the Bornean sun bear, thus providing valuable groundwork for semen cryopreservation and aiding further ART efforts.

Materials and methods

Ten male sun bears from two zoological facilities (Bornean Sun Bear Conservation Centre (n = 8) and Lok Kawi Wildlife Park (n = 2), in Sabah, Malaysia) were involved in this study, with sampling conducted from 2017 to 2020. A total of 42 EE trials were performed on the ten selected male bears. The animals' age and body weight ranged from three to 18 years and 35–72 kg respectively. Nine of the captive bears were rescued from illegal pet trades and were hand-raised before being brought into the facilities. One adult male bear (Bear A) was a wild bear, and was temporarily held in captivity for wound treatment. There was a wide range in the period of captivity for the ten bears (11 days to 18 years). The animals were fed with rice porridge, a variety of fruits and vegetables, and commercial dog kibbles. This study was approved by the Animal Ethics Committee Universiti Malaysia Sabah (AEC0002–2021).

Animals were fasted for at least 12 h prior to procedure. Two anesthesia protocols for semen collection were used, i) 29 EE with ketamine 4 mg/kg (Ilium Ketamil 100 mg/mL, Troy Laboratories, Australia), medetomidine 0.04 mg/kg (Medetomidine 40 mg/mL, Kyron, South Africa) and butorphanol 0.05 mg/kg (Butorphanol 50 mg/mL, Kyron, South Africa), or ii) 13 EE with tiletamine-zolazepam 3 mg/kg (Zoletil 100 mg/mL, Virbac, Australia) and medetomidine 0.04 mg/kg (Medetomidine 40 mg/mL, Kyron, South Africa). Drugs were administered intramuscularly via a CO_2 powered blowpipe system (Vario 1 V, Telinject, GmbH, Germany) in a 3 mL two-chambered compressed air dart with 1.50 × 20 mm Ø plain needle. Bear was intubated with a size 9–10 I.D. cuffed endotracheal tube. During anesthesia, vital parameters of pulse rate, peripheral capillary oxygen saturation, breathing rate and $ETCO_2$ were monitored. Anesthesia depth was assessed by jaw tone, anal tone, palpebral reflex, pupillary light reflex, and withdrawal reflex. A supplemental dose of ketamine was titrated to effect intravenously in maintaining anesthesia when required.

Bears were placed in lateral recumbency and the dimensions of each testis were measured in cm for length (L), width (W), and height (H) with the aid of an ultrasound (M-Turbo, USA). The volume (V) of each testis was calculated as V = L x W x H x 0.71 and total testes volume was determined by combining the volume of the left and right testes [25]. The methodology for EE was a modification from the procedure in the giant panda [18] and the Asiatic black bear [20]. A cleansing enema was performed to remove fecal matter and the genital area was then cleaned with sterile saline. Once the animal was in the surgical plane of anesthesia, semen collection was conducted using an electroejaculator (Seager®, Dalzell Medical Systems, USA) consisting of three 4.6 × 56.8 mm electrodes with a rectal probe (2.5 cm in diameter and 7 cm in length). The probe was positioned dorsal to the bulbous prostate upon digital palpation, approximately 6–8 cm from the anus. Stimulation was given for two to three seconds with a five-second interval before the next stimulation, at increasing voltages starting from one volt. The maximum voltage used in each subject was based on the animal's response to the stimulus, manifested by symmetrical hindlegs contraction and full penile erection. A repeated stimulation cycle was conducted up to a maximum of three times with ten minutes of rest if insufficient semen was produced. Each semen fraction was collected in a 50 mL conical tube and placed on a rack in a 37 °C water bath. The bear was then returned to the cage, positioned in lateral recumbency, and reversed with atipamezole (Alzane 5 mg/kg, Zoetis, Spain) intramuscularly at a dose five times that of medetomidine. The EE procedure took about an hour.

Semen evaluation was performed primarily by one lab technician immediately after semen collection at the sampling site. Semen was observed visually for color and consistency, and pH was evaluated with pH paper. Urine contaminated semen fractions characterized by thin yellowish semen and acidic pH were discarded. A volume of 5 μL semen of each fraction was evaluated subjectively for percentage of general motility and forward progressive sperm motility under a phase-contrast microscope (Olympus Microscope CX43). Semen fractions that contained motile sperm were pooled and transferred into 2 mL vials floated on a styrofoam rack in a 37 °C water bath. Sperm concentration was determined with a Neubauer hemocytometer. Thin semen smears stained with Eosin Nigrosin and Diff-Quik were examined for sperm viability and morphology at 400x magnification. At least 200

Table 2
Semen characteristics of the 10 Bornean sun bears.

Bear ID (name)	Age (years)	No. of EE	Semen pH	Volume (µL)	Semen concentration ($x10^6$ sperm/mL)	Total sperm ($x10^6$)	Sperm viability (%)	Sperm motility (%)	Sperm progressive (%)	Normal sperm (%)
A (Ace)	> 10	2	7.7 (7.5–7.8)	1317.5 (110–2525)	2295.0 (2146–2444)	2843.7 (268.8–5418.6)	83.6 (78.9–88.2)	85.0 (80.0–90.0)	80.0 (70.0–90.0)	22.7 (21.40–24.0)
B (Along)	8	7	8.0 (6.0–8.5)	450.0 (50–710)	308.8 (154–2140)	177.5 (29.7–963.0)	85.3 (67.9–94.3)	90.0 (70.0–95.0)	90.0 (60.0–90.0)	24.7 (11.0–57.0)
C (Bermuda)	17	4	8.2 (7.5–8.5)	745.0 (50–1330)	134.8 (35–285)	56.3 (14.3–108.0)	72.5 (57.5–88.8)	65.0 (35.0–70.0)	50.0 (25.0–50.0)	31.6 (15.6–47.1)
D (Linggam)	13	5	7.25 (6.0–8.0)	600.0 (300–2000)	148.2 (45.5–240)	94.7 (23.7–160.7)	85.9 (81.2–89.8)	70.0 (10.0–90.0)	45.0 (0–85.0)	26.6 (19.0–69.0)
E (Om)	13	4	6.0 (5.8–6.0)	1422.0 (110–4090)	214.4 (6.88–1241.3)	62.2 (0.8–1489.6)	50.4 (46.3–71.3)	2.5 (0–10.0)	0	7.5 (5.0–17.1)
F (Ronnie)	12	3	8.5 (7.8–8.8)	800.0 (435–1280)	87.0 (59–513)	75.5 (69.6–223.2)	76.3 (68.5–86.9)	70.0 (38.0–80.0)	54.3 (33.0–70.0)	27.7 (17.1–33.5)
G (Sunbearo)	3	5	7.7 (5.8–8.0)	690.0 (330–1570)	123.1 (15.4–176.8)	58.3 (23.8–100.3)	77.4 (46.2–86.4)	45.0 (0–80.0)	38.0 (0–70.0)	23.2 (14.3–38.7)
H (Sigalung)	9	6	7.3 (6.5–8.0)	140.0 (30–300)	836.5 (0–2120)	60.26 (0–530.0)	79.2 (0–93.3)	85.0 (0–95.0)	75.0 (0–90.0)	24.0 (13.9–46.9)
I (Anton)	8	3	8.0	500.0 (460–960)	1625.0 (886.5–7620)	851.0 (747.5–3810.0)	91.3 (70.9–92.5)	85.0 (81.0–90.0)	76.0 (70.0–78.0)	31.6 (22.6–54.0)
J (Diego)	14	3	8.5	1230.0 (1110–1700)	985.0 (250–2450)	1674.5 (307.5–2719.5)	67.3 (61.8–68.1)	80.0 (53.0–80.0)	70.0 (53.0–76.0)	26.3 (23.4–29.5)

Data presented in median (range).

spermatozoa were examined for the assessment. Twenty normal spermatozoa from each of the ten bears were measured for sperm head width, head length, midpiece length, principal tail length, and end piece length from Diff-Quik stained slides under a phase-contrast microscope at 400x magnification with ToupTek ToupView software (Version: x64).

Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using RStudio Version 1.4.1103. Data distributions were tested for normality with the Shapiro Wilk test. The relationship of testicular volume and semen characteristics with independent variables (individual bear, anesthesia protocols, bear weight and age) were examined with generalized linear model. All semen parameters were log transformed to improve the normality of the data use in the generalized linear models. P values < 0.05 showed statistical significance.

Results

The total testicular volume of the ten bears was 23.37 ± 5.09 cm^3. Testicular volume did not differ with age and body weight statistically ($P > 0.05$).

Semen color and consistency was thin colorless to creamy white. Inter-individual variations of the semen characteristics is shown in Table 2. From the generalized linear model, Bear E had significant lower value in semen motility, progressive movement and semen pH ($P < 0.02$). On the other hand, bears C, D and J had significant higher percentage in normal sperm ($P < 0.04$). Bear H had lower semen volume and concentration ($P < 0.05$). Increased age factor statistically reduced the normal sperm percentage ($P = 0.04$). There was no association between semen characteristics with bear weight and different anesthesia protocols. Table 1 summarizes the data of sun bear semen characteristics derived from 30 good quality samples. A total of 12 samples were excluded due to urine contamination (n = 3), non-motile sperm samples (n = 5), and outliers with extremely low sperm motility and progressive movement (n = 4).

Morphology of the Bornean sun bear sperm is typical of most mammals, comprising an oval head, midpiece, and tail (Fig. 1). The sperm biometric measurements were head length (4.99 ± 0.64 µm), head width (3.63 ± 0.52 µm), midpiece (9.71 ± 1.33 µm), principal tail length (42.47 ± 2.42 µm), end piece length (4.10 ± 1.20 µm) and total sperm length (61.28 ± 2.46 µm). Sperm abnormality was 70.67 ± 14.31%, predominant by bent midpiece (29.57 ± 8.84%) and bent tail (28.98 ± 9.81%). Other sperm defects include bicephaly, misshapen head, macrocephaly, microcephaly, tightly coiled tail, coiled tail, double tail, proximal and distal cytoplasmic droplets (Table 3) (Fig. 2).

Discussion

Several modifications of the EE techniques were employed to facilitate semen collection in the Bornean sun bear. One important adaptation done was to use a smaller electroejaculator probe due to the small build of the species. The sun bear's body mass is two to three times smaller than the Asiatic black bear and the giant panda [26]. In this study, the rectal probe size was 2.5 cm in diameter and 7.0 cm in length, which is shorter in length than the one utilized in the giant panda (4.5 cm in diameter and 56.5 cm in length [18], 3.0 cm in diameter and 32.0 cm in length [27]) and in the Asiatic black bear (2 cm in diameter and 50 cm in length [28], 2.60 cm in diameter and 32 cm in length [20]). Apart from that, the identification of the prostate (6–8 cm from the anus) as a landmark for probe placement increased the success of the EE. The probe insertion depth was arbitrarily suggested to be at 15–20 cm in the Asiatic black bear [20], 18 cm in the giant panda [27], and 14–22 cm in the Hokkaido brown bear [29] because digital palpation of the landmark is difficult in large bears, probably limited by its anatomical location and the small anal orifice. In the Asiatic black bear,

Fig. 1. Normal sperm of *Helarctos malayanus euryspilus* with Diff-Quik stain at 400x magnification.

Table 3
Sperm abnormalities in the 10 Bornean sun bears.

Morphological defects	Fresh semen abnormality (%)
Bicephaly	0.07 ± 0.21
Misshapen head	0.40 ± 2.23
Macrocephaly	0.28 ± 0.86
Microcephaly	2.75 ± 3.69
Bent midpiece	29.57 ± 8.84
Tightly coiled tail	4.72 ± 8.27
Double tail	0.36 ± 0.59
Bent tail	28.98 ± 9.81
Coiled tail	2.95 ± 3.81
Proximal droplets	0.04 ± 0.22
Distal droplets	0.12 ± 0.07
Total	70.67 ± 14.31

the depth of the prostate gland is approximately 32–36.50 cm from the anus [30]. In this study, low voltage (1–3 V) was sufficient to produce ejaculates in some bears, which is in contrast to Okano's study that reported high-quality ejaculates of the Japanese black bear that were yielded with high voltage (10 V) [19]. The optimum electrical stimulus to produce semen was best judged from the animal's response manifested by bilateral symmetrical hindlegs contractions and full penile erection, consistent with the suggestion in the Asiatic black bear [20].

The semen traits obtained in this study were compared to other bear species. The ejaculate pH of the Bornean sun bear (pH 7.79) was slightly more alkaline compared to the Asiatic black bear (pH 7.00–7.70) [19,20,28,30,31], the brown bear (pH 7.30–7.40) [29,34], and the giant panda (pH 6.40 – 7.20) [17]. Urine contamination characterized by acidic pH and thin yellow semen was commonly reported in bear ejaculates collected via EE [19,20,29]. In this study, low occurrence (7.14%) of urine contamination was observed as compared to the Asiatic black bear (65.0%) [20], the Japanese black bear (45.8%) [19], and the Hokkaido brown bear (35.5%) [29], possibly due to fractioned semen collection, low stimulation used during EE [20], and shorter insertion depth of rectal probe [33]. Total sperm and semen volume produced by the Bornean sun bear (642.59×10^6, 0.62 mL) were more than the Asiatic black bear ($100.9–502.8 \times 10^6$, 0.38 – 0.61 mL) [19,20,28,30,31], but much reduced compared to the giant panda (830×10^6, 2.20 mL) [27] and the brown bear (1387.2×10^6, 2.7 – 3.7 mL) [29,34]. The Bornean sun bear produced much more concentrated semen (1034.4×10^6 sperm/mL) as compared to the Asiatic black bear ($316 – 659 \times 10^6$ sperm/mL) [31] and the brown bear (590×10^6 sperm/mL) [34], but was on par with the giant panda's during breeding (1100×10^6 sperm/mL) [35]. These differences are species specific and possibly influenced by differences in electrical stimuli [20,33] and testicular size [33]. As the Bornean sun bear's body size is far smaller than other bear species, bilateral testicular volume ($23.37 cm^3$) is reasonably smaller compared to the giant panda ($1360.20 cm^3$) [27] and the Asiatic black bear ($78.10 cm^3$) [30] at peak breeding. Generally testicular size is positively correlated with body mass, as larger testicles can meet the greater hormone demand and to produce a larger amount of sperm [36]. It is interesting to note that semen production in the Bornean sun bear was far greater as compared to the Asiatic black bear that is two to three times bigger in body size. In terms of sperm viability and motility, the Bornean sun bear in this study recorded good results (80.19%, 79.13%) that were comparable to the peak semen quality of the Asiatic black bear (89.3%, 82.9%) [31], the giant panda (84.2%, 76.1%) [35] and the brown bear (86.7%, 94.5%) [34] during breeding season.

Diff-Quik stain is recommended by the WHO in andrology for sperm morphology examination [37]. This study demonstrated the use of Diff-Quik stain to visualize the morphology of sun bear sperm, and potentially apply it in other bear species. The spermatozoa from the Bornean sun bear was bigger in all dimensions compared to a Malayan sun bear that recorded a mean sperm head area of $14.48 \mu m^2$, midpiece length of 7.90 µm and tail length of 44.06 µm [38]. However, the only available data from Kanatiyanont's study was not representative of the species as the value was obtained from a single Malayan sun bear. The head length and width of spermatozoa in the Bornean sun bear is smaller than the American black bear (6.57 µm and 4.76 µm) [22], the Japanese black bear (6.30 µm and 4.50 µm) [31], and the brown bear (6.09 µm and 4.42 µm) [39]. The total tail length is longer in the American black bear (75.34 µm) [22], the Japanese black bear (69.60 µm) [31], the sloth bear (72.50 µm), the spectacled bear (73.10 µm), and the brown bear (73.80 µm) [22,36]. Sperm head length in giant panda is similar to the Bornean sun bear but the giant panda has a broader head, shorter midpiece and tail (4.20 µm, 7.20 µm and 39.00 µm respectively) [18].

Sperm abnormalities ranged from 37.9% to 45.6% in the Asiatic black bear [20,30], and 3.6–9.3% in the giant panda [27]. The abnormality of sperm in this study was considerably high (70.67%) and was consistent in all individuals. Natural breeding among the subjects in Okano's sampling site was frequently sighted. Therefore Okano suggested that high sperm abnormalities (60.1–73.7%) could be a normal feature for the Japanese black bear, or the phenomena was aggravated by the collection technique used i.e. electroejaculation [31]. Such a high abnormal rate in sperm morphology is also noted in felid species, including the Sunda clouded leopard (68.8% and 75.8%) [40]. High sperm abnormalities are also related to inbreeding in cheetah [41] and domestic dogs [42], but this is not likely the case in our study as the subjects originated from various locations spread throughout the state, and not from a single fragmented forest. High occurrence of minor defects, including bent tail and bent midpiece was consistent with observation in the giant panda [18,43] and the Asiatic black bear [28,31]. This is commonly caused by hypoosmotic stress during procedure handlings, such as prepucial washing with normal saline or urine

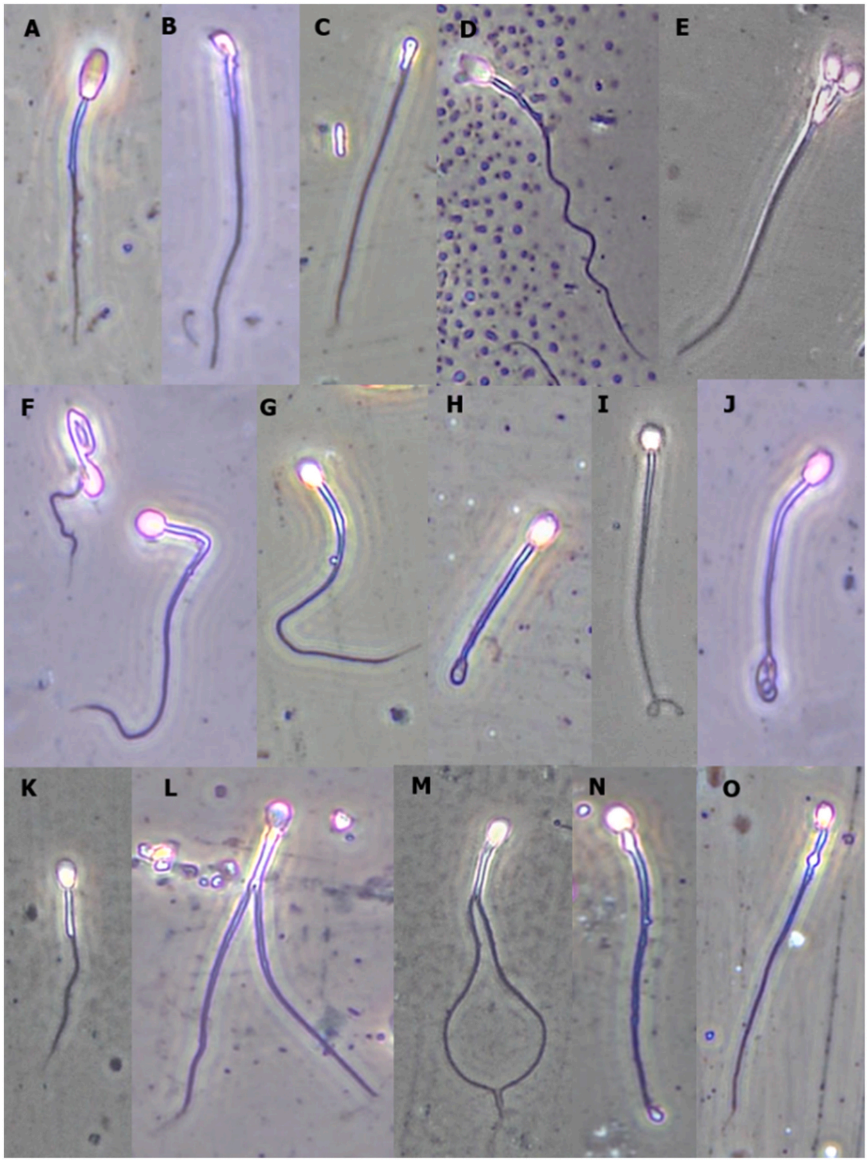

Fig. 2. Abnormal sperm morphology in the Bornean sun bear with Diff-Quik stain at 400x magnification. (A) macrocephaly, (B) microcephaly, (C) absence of head, (D) misshapen (triangular) head, (E) bicephaly, (F) bent midpiece, (G) bent tail, (H-I) coiled tail, (J) tightly coiled tail), (K) short tail, (L-M) double tail, (N) proximal droplet, (O) distal droplet.

contamination upon collection [31]. In this study, low level of urospermia may present and contribute to these tail abnormalities. Therefore, urea and creatinine tests are suggested to diagnose urospermia, in addition to assessment of semen color, pH and odor [44]. Our subjects did not show any indication of serious clinical testicular health problems, evident by the homogenous testicular ultrasound finding and low occurrence of primary defects (i.e. head deformities, double tail and proximal droplets).

The age of sexual maturity in male bears varies from two to seven years among the ursids, determined by an increase in testicular sizes and the presence of sperm in the epididymis [45]. Sexual behaviors, however, may be manifested in the later years of maturation [16]. Reported maturity estimation was 5.5 years in the giant panda [45], four years in the Japanese black bear [28], 3.5 years in the brown bear [46,47], and six years in the polar bear [48,49]. In this study, the youngest bear (Bear G) that produced average sperm quality was approximate three-year-old. In dog, age is found significantly associated to semen parameters such as decreased total sperm count, increased sperm abnormality, decreased sperm viability and progressive motility [50]. Within the 30 years life span in captivity, male sun bears continue to breed until 25-years-old [51]. Successful reproductive performance was observed in male brown bears aged over 20 years [47]. In a 30-year-old Asiatic black bear, reduced sperm concentration (20–30 $\times 10^6$ sperm/mL), poor sperm motility (30%) and increased sperm abnormality (81.7%) especially in coiled and bent tail was reported [20]. In this study, the oldest subject (Bear C) produced semen with average quality, suggesting that senescence in sun bear occurred much later than 17-years. Age albeit not affecting sperm quality in this study, increased risk of EE procedure in older bears is likely associated with other underlying health concerns [16,45].

Conclusion

This is the first report of semen characteristics in the Bornean sun bear, and provides comparative data to other Ursidae species. The baseline data of semen characteristics established in this study serves as a guideline to select semen donors for future ART work such as AI using fresh semen. Compared to cryopreserved semen, the fertilization rate of

AI using standardized good quality fresh ejaculates in the giant panda [16] is much higher. In this study, even though under multiple captive influences, several captive male bears in this study (Bear B, Bear H, and Bear I) were identified as possible semen donors for future AI. This is valuable information that can support the conservation value of captive wildlife. The findings are encouraging as it indicates the ex-situ care (rearing protocols, diet and husbandry, etc.) of the Bornean sun bear is on the right path for at least the male reproductive function to be sustained. Concurrent study on reproductive biology and semen cryopreservation should be carried out to enhance ART work in this species.

CRediT authorship contribution statement

Boon Nie Yeoh: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Zainal Zahari Zainuddin:** Conceptualization, Project administration, Writing – review & editing. **Mark Wen Han Hiew:** Writing – review & editing**. Siti Aisyah Sidik:** Writing – review & editing**. Siew Te Wong:** Resources. **Rosa Sipangkui:** Resources. **Abdul Hamid Ahmad:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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