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Research Article

Ejaculate characteristics of captive felidae in conservation programs

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Abstract - Many carnivore species in the Felidae family, hold conservation significance according to the International Union for Conservation of Nature (IUCN) due to its rapid decline in natural habitats. The objective of this study was to optimize semen evaluation through electroejaculation and examine ejaculate characteristics in captive Felidae for conservation purposes. The totaling nine animals were anesthetized with medetomidine (0.02 mg/kg) and tiletamine-zolazepam (1.5 mg/kg) before semen collection, which was performed using electroejaculation. Semen characteristics, including volume, pH, sperm concentration, total sperm motility, and progressive sperm motility, varied among different species. In jaguars, the semen traits were as follows: volume 1248.57±696.29 µl, pH 6.29±0.33, sperm concentration 150±31.32 x10^6/ml, total sperm motility 70±3.42%, and progressive sperm motility 64±3.57%. Black leopards exhibited

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the following semen traits: volume 22.50±9.46 µl, pH 6.25±0.25, sperm concentration 35±7.36 x10^6/ml, total sperm motility 25.03±14.98%, and progressive sperm motility 12.50±2.50%. In leopards, semen traits were observed as volume 24.75±0.71 µl, pH 6.50±0.71, sperm concentration 49.5±0.71 x10^6/ml, total sperm motility 24.50±0.71%, and progressive sperm motility 11±1.41%. Asiatic golden cats demonstrated semen traits as volume 8.50±1.50 µl, pH 6.75±0.25, sperm concentration 17.50±2.50 ×10^6/ml, total sperm motility 12.50±2.50%, and progressive sperm motility 12.00±2.00%. Golden tigers exhibited the following semen traits: volume 105±14.14 µl, pH 6.25±0.35, sperm concentration 49.95±0.07 x10^6/ml, total sperm motility 60.25±0.35%, and progressive sperm motility 19.90±0.14%. The results of this study serve as guidelines for storing frozen semen and conducting artificial insemination in the Felidae family to aid in breeding and increasing the population for further conservation.

Keywords: Felidae, semen profile, sperm motility, electroejaculation

1. Introduction

The Felidae family, a group of carnivorous mammals, holds conservation value according to the International Union for Conservation of Nature (IUCN) due to its rapidly declining populations in the wild. Among its notable members, the jaguar (*Panthera onca*), which stands as the sole representative of the *Panthera* genus in the Americas, is listed as near-threatened on the IUCN Red List (Quigley et al., 2017). Similarly, the leopard (*Panthera pardus*), which has a widespread distribution across southern Africa, is categorized as vulnerable to extinction (Swanepoel et al., 2016). Another remarkable member, the golden tiger, first observed in the jungles of India, exhibits a distinct blonde coloration with pale golden fur and red-brown stripes instead of the typical black (Xu et al., 2017). Additionally, Asiatic golden cats (*Catopuma temminckii*), inhabiting forests across mainland and insular tropical Asia, face a near-threatened status on the IUCN Red List (Petersen et al., 2021). To safeguard the Felidae family and other imperiled felid species, effective conservation strategies are imperative, including the implementation of captive breeding and genetic management programs. These programs can utilize various assisted reproductive technologies (ART) such as genome resource banking, *in vitro* fertilization (IVF), embryo transfer (ET), and artificial insemination (AI) (Thiangtum et al., 2006). Artificial breeding emerges as a promising technique to preserve the genetic diversity of these species (Chen et al., 2007). Such endeavors are crucial for the maintenance and sustainability of these invaluable genetic resources. The initial step in applying artificial breeding techniques to wild populations involves obtaining fundamental semen characteristics (Ishikawa et al., 1998; Chen et al., 2007). Electroejaculation under surgical anesthesia has become the standard collection technique in the vast majority of wild mammalian species because many of them are intractable. The technique was used successfully in an extensive range of species (Prieto et al., 2014). Numerous investigations have explored the artificial collection of semen via electroejaculation, spanning various species such as the fishing cat (*Prionailurus viverrinus*) (Thiangtum et

al., 2006; Pothakam et al., 2023; Wittayarat et al., 2024), Amur leopard cats (Jeong et al., 2018), flat-headed cat (*Prionailurus planiceps*), Asiatic golden cat (*Catopuma temminckii*) (Wittayarat et al., 2024), peccaries (*Tayassu tajacu*) (Souza et al., 2009), serow (*Capricornis sumatraensis*) (Suwanpugdee et al., 2009), and Asiatic black bears (*Ursus thibetanus*) (Chen et al., 2007). Spermic ejaculates were recovered from all the species mentioned above, but sperm quantity and quality were variable among individuals. The objective of this study was to optimize semen evaluation through electroejaculation and characterize ejaculate traits in captive Felidae for conservation purposes.

2. Materials and methods

2.1 Animal

A total of nine mature males from the Felidae family were utilized in this study, comprising two jaguars, four black leopards, one leopard, one Asiatic golden cat, and one golden tiger (Table 1). These animals were housed in conservation captivity at the Chiang Mai Night Safari. Over the period from 1 November 2022 to 30 August 2023, a total of 18 trials were conducted involving these males.

Table 1. Details of Felidae family at Chiang Mai Night Safari

No	Animal	ID	Age (years)	Weight (kg)
1	Jaguar	6159	14	65
2				69.5
3				68
4				68
5		8920	18	95.5
6				104.3
7				103
8				107
9	Black leopard	2607	14	58
10		3500	15	58
11		2920	10	47
12		1171	9	50
13	Leopard	8188	5	39.55
14				39
15	Asiatic golden cat	3568	18	14.55
16				20.8
17	Golden tiger	8697	9	150
18				147

2.2 Anesthesia and recovery

The animals underwent a 12-hour fasting period prior to the commencement of the experiments. They were then immobilized through an intramuscular injection of a combination of medetomidine (Eurovet, Animal Health BV, Netherlands) and tiletamine-zolazepam (Zoletil®, Virbac, São Paulo, Brazil) at a dosage of 0.2 and 1.5 mg/kg, respectively. After the semen collection procedure and individual data collection, the animals were injected with atipamezole (ZooPharm) at a dosage five times the medetomidine dose (0.1 mg/kg) by intramuscular injection (Hartman et al., 2015).

2.3 Electroejaculation

Semen was collected from each male using a standardized electroejaculation procedure (Wildt et al., 1987). The animal was restrained in a lateral position, and the pubic region was cleaned. Subsequently, the penis was washed successively with sterile physiological saline before semen collection. Semen collection was conducted using an electroejaculator (Minitube electroejaculator, Germany). The electroejaculator probe used for jaguar, black leopard, leopard, and golden tiger measured 2.54 cm in diameter and 12.7 cm in length. For the Asiatic golden cat, a smaller probe measuring 0.99 cm in diameter and 6.35 cm in length was used. The probe was soaked with lubricating jelly (K-Y gel®) and completely inserted into the rectum of the animal. The stimulatory cycle comprised 16 stimuli at each voltage, starting from 0.5 V and increasing in steps of 0.5 V up to 8 V. These stimuli were maintained for a duration of 3 seconds from the beginning of the procedure. When the animal ejaculated, the electric value at that time was noted and this electric level was maintained until semen was no longer found.

2.4 Semen evaluation

Immediately after collection, semen was evaluated for color, volume, pH, sperm concentration and sperm motility. Ejaculate volume was estimated by aspirating the semen into a calibrated positive displacement pipette; pH was measured using pH test paper; sperm concentration was determined using a Neubauer hemocytometer; the total sperm motility was evaluated by placing a drop of semen on a pre-warmed slide at 37ºC without a cover slip, and the progressive sperm motility was conducted by observing spermatozoa in five different visual fields. Two experienced technicians then examined the slide under a binocular microscope (Olympus cx23, Japan) at 100X magnification, using standard techniques.

2.5 Statistical analysis

All semen characteristics were examined individually for mean and standard error of the mean (SEM) values.

3. Results and discussion

3.1 Semen collection

A total of 18 semen collections were conducted on nine males, with 1-3 ejaculates per male, spread approximately 3 months apart. Males were able to ejaculate semen 17 times, which is a success rate of 94.44%. Spermic ejaculates were obtained

from all males. All males responded to electrical stimuli with muscle contractions starting at 0.5 V and gradually increasing by 0.5 V increments until reaching 8.5 V (Table 2). The semen collection procedure was successful for every male across all species, but there was a wide variation in semen characteristics among the species and individual males. In the case of jaguars, ejaculates were obtained starting at 4 V (ranging from 4 to 7 V) stimulation. However, it has been demonstrated that jaguars store their semen using an electrical current between 5 to 9 V (Silva et al., 2019). The black leopard yielded ejaculates starting at 5 V (ranging from 5 to 7.5 V) stimulation. The leopard produced ejaculates beginning at 4.5 V (ranging from 4.5 to 6 V) stimulation. For the Asian golden cat, ejaculates were obtained starting at 5.5 V (ranging from 5.5 to 6 V) stimulation. However, it has been demonstrated that Asian golden cats store their semen using an electrical current between 2 to 5 volts (Wittayarat et al., 2024). The golden tiger yielded ejaculates starting at 8 V (ranging from 8 to 8.5 V) stimulation. Differences in response to electrical stimulation among Felidae (jaguars, black leopards, leopards, Asiatic golden cat, and golden tiger) found that golden tigers had the highest voltage requirement to trigger a response. The voltage required to trigger a response decreased in black leopards, jaguars, Asiatic golden cat, and leopards, respectively. Additionally, in the domestic cat, it has been reported that the volume and the number of sperm were different in each ejaculate when electroejaculation with stepwise increases in voltage (1, 2, 4, and 8 V) was used, and the semen volume increased as the electroejaculation trial was repeated (Fukui et al., 2013). Although, the response to the electroejaculation stimulus can vary not only between species or between males from the same species, but even between collections from the same male. Therefore, modifications to the protocol during the process, based on the male's responses, may be needed, even if one strives to follow the same stimulation protocol in all electroejaculation procedures (Wildt et al., 1987). It is important to develop a specific collection protocol for the target species, but it is no less important to have an adequate probe, sometimes specially designed, for each species collected from (Schmitt & Hildebrandt, 2000; Roth et al., 2005; Prieto et al., 2014). The general quality of seminal traits in the Felidae family and other wild animals in captivity (*ex situ*) was likely influenced by many factors such as nutrition, genetic heterozygosity, and age (Thiangtum et al., 2006; Chen et al., 2007)

Table 2. Result of electroejaculation in Felidae family

No	Animal	ID	Electro. (V)	Volume (µl)	pH	% Motility		Concentration (x10^6/ml)
						Total	Progressive	
1	Jaguar	6159	5.5	5000	6	80	70	260
2			4	1000	7	70	65	200
3			6	1300	5	75	70	220
4			6.5	300	7	75	70	65
5		8920	7	-	-	-	-	-
6			5.5	670	6	60	50	120
7			6.5	120	7	60	55	100
8			6.5	350	6	70	68	85
9	Black Leopard	2607	7.5	10	6	10	10	20
10		3500	7.5	10	6	10.05	10	25
11		2920	6.5	20	6	20	10	45
12		1171	5	50	7	40	20	50
13	Leopard	8188	4.5	25	7	25	10	50
14			6	24.5	6	24	12	49
15	Asiatic Golden	3568	5.5	7	7	10	10	15
16			6	10	6.5	15	14	20
17	Golden Tiger	8697	8	115	6	60	20	50
18			8.5	95	6.5	60.5	19.8	49.9

3.2 Semen characteristic

Semen was collected using electroejaculation. The mean (±SEM) seminal characteristics in jaguars were as follows: volume 1,248.57±696.29 µl, pH 6.29±0.33, sperm concentration 150±31.32 ×10^6/ml, total sperm motility 70±3.42%, and progressive sperm motility 64±3.57%. Consistent with the report by Araujo et al. (2018), jaguars exhibited a semen concentration of 347.2±295.6 µl (ranging from 2 to 800 µl), and progressive sperm motility of 77 ±11.4% (ranging from 50% to 90%). In black leopards measurements were 22.50±9.46 µl, 6.25±0.25, 35±7.36 ×10^6/ml, 25.03±14.98%, and 12.50±2.50%, respectively. For the leopard measurements were 24.75±0.71 µl, 6.50±0.71, 49.5±0.71 ×10^6/ml, 24.50±0.71%, and 11±1.41%, respectively. For the Asiatic golden cat measurements were 8.50±1.50 µl, 6.75±0.25, 17.50±2.50 ×10^6/ml, 12.50±2.50%, and 12.00±2.00%, respectively. Moreover, it has been reported that Asiatic golden cat ejaculate volume and sperm concentration

were 136.0 µl, and 0.1×10^6 spermatozoa/ml, respectively (Wittayarat et al., 2024). In the golden tiger measurements were 105±14.14 µl, 6.25±0.35, 49.95±0.07 $\times10^6$/ml, 60.25±0.35%, and 19.90±0.14%, respectively (Table 3). However, it has demonstrated that Siberian tiger characteristics of semen in each ejaculate volume were 5.8±2.7 ml (range: 2.3-11.5), and motile sperm were 82.4±11.4 % (range: 50.0-95.0) (Fukui et al., 2013). Once semen is obtained, and before its use for assisted reproduction techniques (ART), the evaluation of sperm quality is a necessary step to know whether the sample of semen is suitable for conservation treatment. Sperm collection in combination with artificial insemination is a common and widely used assisted reproduction technique (ART) in animal reproduction. The methods are mostly complemented by semen cryopreservation, ART may support conservation programs for the Felidae family (jaguars, black leopards, leopards, Asiatic golden cat, and golden tiger).

Table 3. Comparison of semen characteristics of wild captive Felidae by electroejaculation

Characteristic	Jaguar (n=2)	Black Leopard (n=4)	Leopard (n=1)	Asiatic Golden (n=1)	Golden Tiger (n=1)
Volume (µl)	1248.57±696.29	22.50±9.46	24.75±0.71	8.50±1.50	105±14.14
pH	6.29±0.33	6.25±0.25	6.50±0.71	6.75±0.25	6.25±0.35
concentration ($\times10^6$/ml)	150±31.32	35±7.36	49.5±0.71	17.50±2.50	49.95±0.07
total sperm motility (%)	70±3.42	25.03±14.98	24.50±0.71	12.50±2.50	60.25±0.35
sperm progressive (%)	64±3.57	12.50±2.50	11±1.41	12.00±2.00	19.90±0.14

Data represent Mean±SEM

4. Conclusion

From this study, it can be concluded that the Felidae responded to electrical stimuli at about 4-8.5 V. The semen characteristics in Felidae were as follows: volume ranged from approximately 8.5 to 1248.57 µl, pH ranged from approximately 6.25 to 6.75, sperm concentration ranged from approximately 17.50 to 150 $\times10^6$/ml, total sperm motility ranged from approximately 12.50 to 70%, and progressive sperm motility ranged from approximately 11 to 64%. Based on the results of semen collection via electroejaculation from the Felidae family (including the jaguars, black leopards, leopard, Asiatic golden cat, and golden tiger), guidelines for storing frozen semen and artificial insemination within the Felidae family can be established to aid in breeding efforts and population augmentation for conservation purposes. The electroejaculation method shows promise and can be further refined for application in other wildlife species, contributing to sustainable wildlife conservation efforts.

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