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Observational Study on Reproductive Behavior in Semi-Wild Sambar Deer (*Rusa unicolor*) for Wildlife Conservation and Assisted Reproductive Management

Aiman Murad[1], Hartini Ithnin[2], Tengku Rinalfi Putra[3], Cosmas Ngau[4], and Wan-Nor Fitri[5*]

[1]*Department of Farm and Exotic Animals Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Ex-situ Conservation Division, Department of Wildlife and National Parks (DWNP), Peninsular Malaysia, KM 10 Jalan Cheras, Kuala Lumpur, Malaysia*
[3]*Department of Veterinary Pre-Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Pusat Konservasi Hidupan Liar (PKHL), 35600 Sungkai, Perak, Malaysia*
[5]*Department of Farm and Exotic Animals Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

*Corresponding author's Email: wannorfitri@upm.edu.my

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ABSTRACT

Understanding the reproductive behaviors of different wildlife species is essential to unravel their reproductive strategies, ecological adaptations, and conservation requirements. This study delved into the reproductive biology of the sambar deer (*Rusa unicolor*), with a focus on promoting assisted reproductive technology for wildlife conservation and investigating the reproductive behaviors of male and female sambar deer. The study was conducted at Pusat Konservasi Hidupan Liar (PKHL) Sungkai, Perak, Malaysia. The observation focused on one male and two female sambar deer. Direct observations of the deer were conducted for 14 days in September 2022. The direct observations were performed in the morning (Session 1= 8-10 am), afternoon (Session 2 = 10-12 pm), and evening (Session 3= 3-5 pm), using the instantaneous sampling method. A total of 75 behavior instances were recorded, in which male deer exhibited the most reproductive behavior at 58 instances (77.3% of the total reproductive behavior). Successful mating was observed on day 6, elucidating a crepuscular preference in the male animal in exhibiting reproductive behavior. The female's reproductive behavior lasted for a short period, from 24 hours for Female 2 and 72 hours for Female 1. In conclusion, there was a distinct behavior between the male and female deer during the rutting season. Understanding the reproductive behavior to estimate the length of estrus can be useful as a non-invasive tool to detect heat and can be considered to improve breeding management and implement assisted reproductive technology.

Keywords: Breeding, Conservation, *Ex-situ*, Release program, Wildlife

INTRODUCTION

In the study of animal behavior, it is important to understand the species' reproductive strategies, ecological adaptations, and conservation needs. The reproductive biology of the sambar deer (*Rusa unicolor*), an ecologically significant species as a prey source for the critically endangered Malayan tiger (*Panthera tigris jacksoni*) remains to be obscured (Ten et al., 2021). Further decline in the population of sambar deer in their natural habitat increases the urgency of conserving the species (Kawanishi et al., 2014). There has been a growing interest in employing assisted reproductive technology in deer, which highlights a progressive step in wildlife conservation reproductive management (Rola et al., 2021). This initiative aligns with the Malaysian government's dedicated efforts to elevate the genetic standards of the nucleus herd population, thereby enhancing the overall resilience and viability of these deer for subsequent release programs into their natural habitat (Munisamy et al., 2022).

Assisted reproductive technology, such as artificial insemination, is an important technique to explore wildlife species. The technique has been proven to be an effective tool in improving the genetic exchange in livestock (Van Doormaal and Kistemaker, 2003). However, before this technology can be applied, it is important to understand the reproductive biology and behavior of the sambar deer. Due to the limitation in obtaining new genetic resources for breeding, deer was shown to be predisposed to inbreeding, which has been shown to contribute to fertility decline (Yahaya et al., 2020). Poor reproductive performance in deer was also associated with the low exchange of genetic materials (Zakaria et al., 2016). Stress and reproductive behavior studies of the sambar deer with fecal metabolites analysis have been performed (Abdul Hamid et al., 2022; Selvarajah et al., 2022). It is important to collate this specific knowledge in the reproductive biology of the sambar deer due to the species-specific differences that can limit the efficiency of assisted reproductive technology (Morrow et al., 2009).

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The sambar deer exhibit a distinct sexual dimorphism. Physically, male and female deer can be distinguished by the presence of antlers in the male animal (Leslie, 2011). The function of antlers and reproductive performance has long been an enigma and center of attention in deer reproductive physiology studies (Monfort et al., 1993). The breeding selection through fitness and genetic superiority in the antler confirmation contribute to the gene being passed down the generation and the survivability of the offspring (Kruuk et al., 2002). The dynamic antler cycle and social dominance also mean varying opportunities for the male to breed (Savanth et al., 2011). The drop of the antler signifies the end of the breeding activity in an Elk deer; however, a different male may continue the mating activity (Johnson et al., 2005). Thus, the behavioral patterns and sexual preferences require a further understanding in elucidating the main component contributing to reproductive success. This study aimed to investigate the sambar deer reproductive behaviors, aiming to shed light on contrasting patterns exhibited by male and female individuals in promoting active conservation management of the species.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Department of Wildlife and National Parks (DWNP), Peninsular Malaysia, with approval number JPHLTN.600-6/1/4 JLD (78) and Institutional Animal Care and Use (IACUC), Universiti Putra Malaysia, Serdang, Selangor, Malaysia, approval number UPM/IACUC/AUP-U041/2022.

Study site

The study was conducted at Pusat Konservasi Hidupan Liar (PKHL) Sungkai, Perak, Malaysia (4.0353° N, 101.3658° E) including a total of 2468 acres of lowland dipterocarp forest. It is one of the 35 protected areas managed by the Department of Wildlife and National Parks (DWNP), Peninsular Malaysia, and is situated on the western side of Perak in the Batang Padang district.

Management of sambar deer

The sambar deer at the conservation center were managed under a semi-wild system. They were all kept in the paddocks with shelter and provided with food and water daily. The deer were fed with cut and carried various leaves, including *charcoal tree*, *Trema orientalis*, and turn-in-the-wind, *Mallotus paniculatus* given *ad libitum*. The pasture was planted with Koronivia grass, *Brachiaria humidicola*, and guinea grass, *Megathyrsus maximus*. Besides that, the deer were fed with concentrates, mineral lick blocks (Solsel®, European Salt Company [ESCO], Hannover), and multivitamins (Vita-Stress, Sunzen, Malaysia) every two weeks. The grass was provided *ad libitum*. The grass quality varies with season; thus, the concentrates fed were adjusted based on the performance of the body condition score which was assessed weekly. The deer were fed once daily in the morning, and the keeper utilized this time to observe the animals for any sign of poor health as well as to check the perimeter of the fence for integrity. The average temperature in the district during the study period was 27˚C, the temperature during the observation was 28.5˚C ranging from 23˚C to 34˚C. There was no artificial lighting provided at the conservation center.

Ethogram behavioral observation

In this study, the deer were managed in a herd of mixed ages (2-6 years old) and varied distribution of sexes (male = 4, female = 22, and juvenile = 4). The observation focused on one male and two females. The sambar deer is housed in a paddock enclosure that is 75 meters long and 36 meters wide. A preliminary behavioral observation for three days was conducted to confirm that the deer were all healthy. Direct observations of the deer were conducted for 14 days, in September 2022. The direct observations were separated into three sessions: in the morning (Session 1= 8-10 am), afternoon (Session 2 = 10-12 pm), and evening (Session 3 = 3-5 pm), using the instantaneous sampling method (Altmann, 1974). In brief, during each session, the observations were done in 15 minutes sub-session time block, which is equivalent to eight slots over a span of two hours. Only a single frequency will be noted for each behavior under a one-time slot. Each slot only corresponds to a single occurrence of behavior despite multiple repetitions. The observation sheet to collect the behavioral data was described in the ethogram (Table 1). The ethogram was created from a slight modification following a preliminary observation period of three days before data collection (Abdul Hamid et al., 2022; Selvarajah et al., 2022).

Data analysis

The data is presented in frequency. The percentage was derived by dividing the number of reproductive behaviors by the total number of reproductive behaviors exhibited by the individuals. Preparation, tabulation, and presentation of data and graphs were made using Microsoft Excel Version 2310 from Microsoft 365 Apps for Enterprise.

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Table 1. Ethogram of sambar deer (*Rusa unicolor*) reproductive behavior in Pusat Konservasi Hidupan Liar Sungkai during 2 weeks of observational study in September 2022

Behavioral grouping	Behavioral subgrouping	Code	Description
Reproductive behavior (Male-specific)	Follow	FO	Male follow female
	Smelling of female urine or feces	SM	Smelling urine or feces, followed by flehmen response
	Anal sniffing	AS	Male smells female vaginal area
	Flehmen response	FR	Male raised their head and curled his upper lip
	Chin resting	CR	Male rests its chin on the rump of the female
	Grooming	GR	Male licking the female's body
	Mounting	MO	Male climbs on the female's back with the thorax resting on her rump. Front limbs hang and the hind limbs on the ground
	Mating	MA	Male executes successive movements of flexion and extension of the vertebral spine while mounting on the female.
Reproductive behavior (Female specific)	Follow	FO	The female follows the male
	Smelling of male urine or faeces	SM	Smelling urine or feces, followed by flehmen response
	Anal sniffing	AS	The female smell male rectal/penis area
	Flehmen response	FR	The female raised her head and curled her upper lip
	Chin resting	CR	The female rests its chin on the rump of the male
	Grooming	GR	Female licking the male's body

FO: Follow, SM: Smelling of urine or feces, AS: Anal sniffing, FR: Flehmen response, CR: Chin resting, GR: Grooming, MO: Mounting, MA: Mating

RESULTS AND DISCUSSION

A total of 84 hours of behavioral observations were made during the fourteen days, capturing the reproductive behavior of three sambar deer individuals (M1, F1, and F2). Out of the recorded observations, M1 exhibited reproductive behavior in 58 instances, while F1 and F2 displaced reproductive behavior in 14 and 3 records, respectively. The frequency of reproductive behavior is represented in Graph 1. Notably, the male (M1) showed a high frequency of sexual behavior, accounting for 77.33% of the total incidences, compared to F1 and F2 at 18.67% and 4%, respectively. Further analysis revealed that M1 primarily engaged in anal sniffing (AS), which constituted the highest observed frequency at 32.8%. Conversely, both females, F1 and F2, exhibited a high frequency of grooming (GR) behavior at high frequencies of 50% and 66.7%, respectively.

Due to the high frequency of reproductive behavior shown by the male sambar deer, deeper insights were needed to assess the activity pattern across different observation sessions. The reproductive behavior of male sambar deer is summarized in Table 2. It was found that the male displayed reproductive behavior preference towards the crepuscular periods during the evening and morning as compared to the afternoon session. Out of the total 58 reproductive behaviors displayed, 51% occurred during the evening session (3 pm to 5 pm), followed by 40% during the morning session (8 am to 10 pm), while only 9% during the afternoon (10 am to 12 pm).

Throughout the 14-day observation period, the individual sambar deer reproductive behavior was visualized (Graph 2). The male consistently displayed active reproductive behavior throughout the observation period. However, two peaks in reproductive behavior were observed during days 6 and 10. Additionally, successful mating occurred on day 6, during the S1 morning observation session between M1 and F1 (Figure 1). The F1 reproductive behavior steadily increased from zero on day 3 to day 5, peaked on day 6, and quickly reduced on day 7 until it finally ceased activity on day 8. The reproductive behavior decreased and remained plateaued post-mating. In the case of F2, reproductive behavior remains at zero, with a peak activity observed on day 10. However, no successful mating was observed and the reproductive behavior reduced on day 11 and finally ceased for the following observation. The reproductive behavior in the females lasted for a short period, from 24 hours for F2 and 72 hours for F1. The male sexual behavior displayed a

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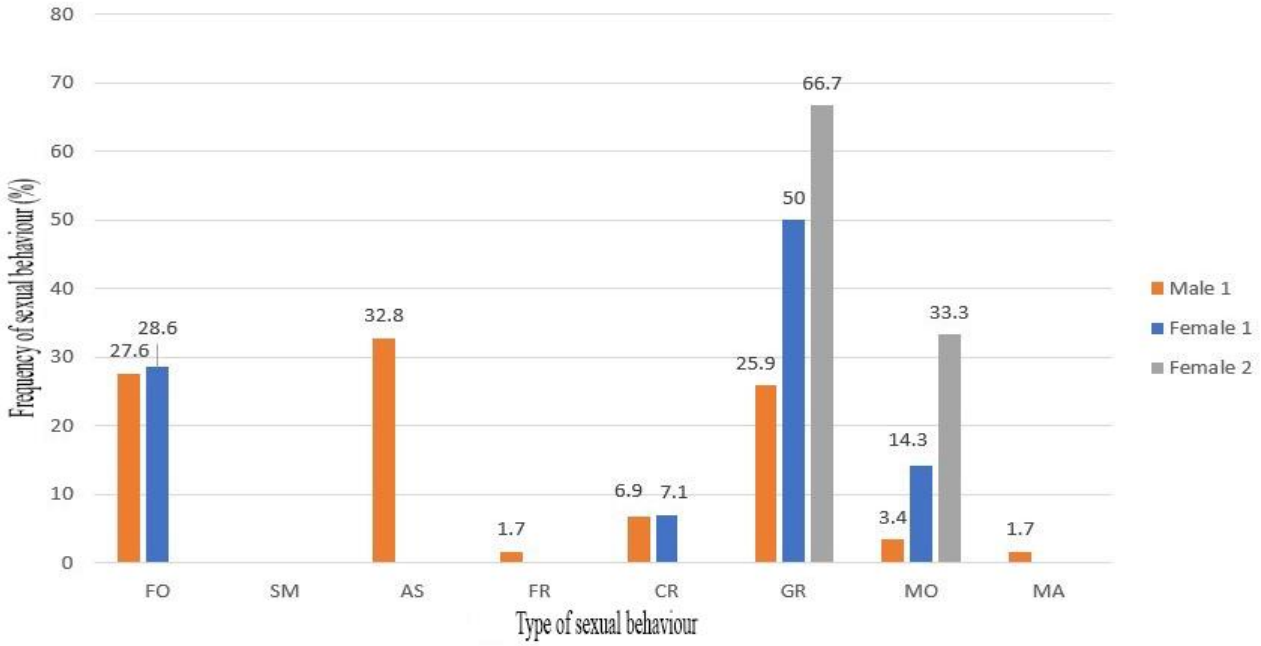
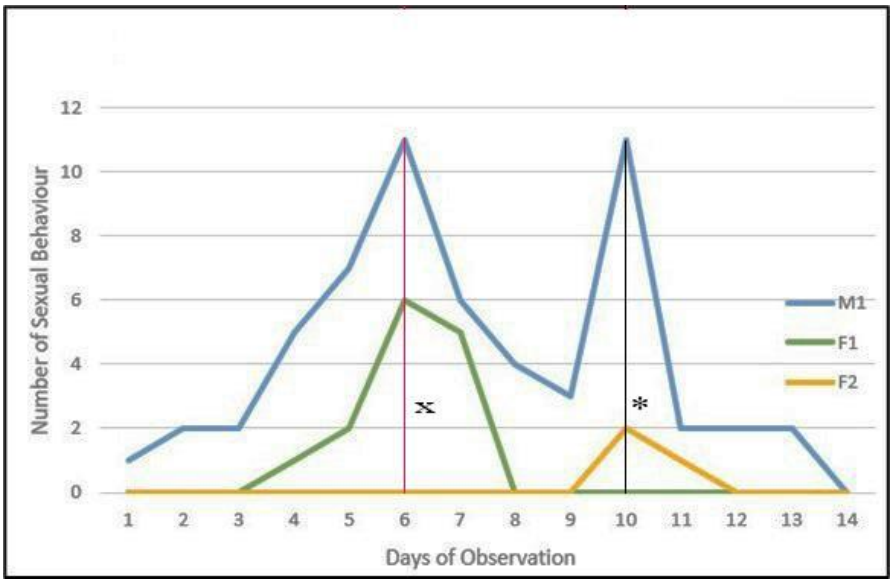
sharp decline from day 11 to zero reproductive behavior on day 14, coinciding with the shedding of the final and second sides of the antler on day 13.



Graph 1. The frequency of reproductive behavior was recorded from the three deer (*Rusa unicolor*) in Pusat Konservasi Hidupan Liar (PKHL) Sungkai during the two weeks of observational study in September 2022. FO: Follow, SM: Smelling of urine or feces, AS: Anal sniffing, FR: Flehmen response, CR: Chin resting, GR: Grooming, MO: Mounting, MA: Mating.

Table 2. The three-session frequency of the male sambar deer (*Rusa unicolor*) exhibiting behavior reproductive behavior in Pusat Konservasi Hidupan Liar (PKHL) Sungkai during the two weeks of observational study in September 2022

Time of the day	Frequency of sambar deer exhibiting reproductive behavior
8 am - 10 am	40% (22/58)
10 am - 12 pm	9% (5/58)
3 pm - 5 pm	51% (31/58)



Graph 2. Frequency of sexual behaviors exhibited by all three deer (*Rusa unicolor*) in Pusat Konservasi Hidupan Liar (PKHL) Sungkai during the two weeks of observational study in September 2022. M1: Male 1, F1: Female 1, F2: Female 2. X: Successful mating observed, * shedding of the final and second side of the antler observed

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Figure 1. Successful mating involving the observation of successful penile insertion on day 6 in sambar deer (*Rusa unicolor*) in Pusat Konservasi Hidupan Liar Sungkai during 2 weeks of observational study in September 2022

The male sambar deer demonstrated a significantly higher frequency of sexual behaviors compared to the two female individuals. This aligns with previous studies indicating that male deer often exhibit more active and varied sexual behaviors as part of their reproductive strategies (Bazyan, 2013). In the present study, the male sambar deer predominantly engaged in anal sniffing (AS), which represented the most frequent sexual behavior observed. This behavior is known to play a crucial role in scent marking and gathering of olfactory information related to female receptivity and reproductive status (Apfelbach et al., 2015). The high frequency of AS exhibited by the male individual suggests its importance in male sambar deer's reproductive strategies.

Conversely, the females, F1 and F2, exhibited grooming (GR) behavior at notably high frequencies towards the male deer. Grooming behavior in the female sambar deer and its role in reproduction is poorly understood. A study on the sika deer found that there was no correlation in grooming following the rutting season, as it only reduces the tension in the group (Matsuno and Urabe, 1999). However, a recent study observed a high grooming frequency, suggesting this behavior is a pre-courting behavior for the male to assess female receptivity in sambar deer (Selvarajah et al., 2022). This further strengthens the finding in the present study suggesting that grooming behavior plays an important role during the mating process and is aligned with a successful mating event. Female deer grooming male deer may indicate their interest and availability for mating, contributing to courtship dynamics within the population. The present study suggests that female deer may groom male deer as a form of sexual attraction and to signal their reproductive readiness. The minimal sexual behaviors displayed by the female individuals, except during the peak periods, may indicate a more selective and receptive mating strategy (Ciuti and Apollonio, 2016). The minimal sexual behaviors exhibited by the female deer suggest the possibility of behavioral observation in females to estimate estrus reliably (Mahre et al., 2013)

Estimating the estrus period is crucial for successful reproductive management since the fertilization window is limited. The result from this study indicated that there are two distinct peaks in male sexual behavior, which coincided with the estrus behaviors of the female deer. The result from this study further enhances the findings suggested in another study on sambar deer, which found that the frequency of sexual behavior in males can be used to estimate the presence of females in estrous in sambar deer (Putranto et al., 2010). The observed peaks in reproductive behavior on days 6 and 10 further highlight the temporal dynamics of sexual activity in male sambar deer.

The female reproductive behavior in this study offers intriguing insights into the female receptivity pattern. Notably, the presence observations revealed a noteworthy occurrence. After successful mating in F1, there was a pronounced decline in her reproductive behavior. Simultaneously, the reproductive behavior of F2 remained absent until day 10, and even then, it diminished shortly afterward. This sequence coincided with the male shedding its final antler, and consequently, his reproductive behavior also ceased. From these observations, it becomes apparent that the females' estrus cycles might not synchronize. This reproductive strategy could potentially serve to stagger the timing of female receptivity, affording the male ample opportunity to fertilize multiple females. This adaptive strategy may enhance the chances of successful fertilization, contributing to the species' reproductive success. The present findings further strengthen the idea that the more dominant and older females mate earlier in the rut than the younger and less dominant

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females (Farrell et al., 2011). Further exploration of these intricacies could provide valuable insights into the underlying mechanisms of sambar deer reproductive behaviors.

The occurrence of successful mating on day 6 during the morning session indicated the importance of timing and synchrony between male sexual behavior and female receptivity. The mating frequency is unknown in the sambar deer. This study only observed one successful mating once during the whole observation period. Deer is known to mate only once during the rutting season, accounting for 75% of the breeding female population (Say et al., 2003). Hence, the successful mating status of F2 remains ambiguous, given its alignment with the male deer's antler-shedding period. While it remains a plausible scenario that successful mating transpired, it is possible that the decline in mating behavior could have been influenced by the male's breeding ability. Considering that breeding entails resource competition among females, the dynamics of female-female interactions and dominance hierarchies emerge as pivotal factors in evaluating female fitness for breeder selection. This aspect gains even greater significance when applied to breeding programs aimed at conserving and reintroducing wildlife into their natural habitats. By monitoring the frequency of sexual behavior and specific copulatory behaviors, the timing and length of the estrous period can be estimated (Higuchi et al., 2013). This information is essential for identifying the optimal time for insemination, as ovulation typically occurs 24 to 28 hours after standing heat is observed in deer (Asher, 1985).

The antler cycle of male deer also appears to influence sexual behavior (Malo et al., 2012). This study indicates an arrest in male sexual behavior after the second antler was cast down. The antler stage is useful for determining the breeding season in deer (Fitri et al., 2017). Testosterone levels were observed to peak during the period of highest semen quality and subsequently decrease following the casting of the antler (Jaafar et al., 2017). The sudden drop in testosterone concentration during the antler casting stage contributes to the reduced frequency of sexual behaviors in the male deer (Bartos, 2012). Antler shedding is a physically demanding process, requiring energy allocation and physiological changes, which may temporarily reduce the male's reproductive activity (Kavčić et al., 2019). This suggests a potential interplay between hormones, antler development, and sexual behavior in sambar deer.

The relationship between sexual behavior and feeding patterns is an interesting aspect of this study. Most male sexual behaviors occurred during the evening, coinciding with the time when grazing activities were the most predominant. This finding aligns with the crepuscular nature of sambar deer, which are most active during dawn and dusk (Comte et al., 2022). Additionally, environmental factors such as temperature and food availability may influence the timing of sexual behavior. Higher water-soluble carbohydrate concentrations in the grass during the evening hours may affect the grazing decisions of deer (Wang et al., 2020). The timing of sexual behavior is crucial as it may optimize reproductive success by synchronizing with the receptivity of females or minimizing predation risks (Pérez-Barbería and Walker, 2018). The higher frequency of sexual behaviors during the evening and morning sessions suggests that male sambar deer allocate their reproductive efforts during these periods, potentially reflecting optimal conditions for mating and courtship. This was observed in the population of jungle fowl (*Gallus gallus domesticus)*, which changed its preference to mate in the evening, particularly in male-biased groups (Løvlie and Pizzari, 2007). Therefore, late afternoon or evening could be considered the optimal period to observe estrous signs and monitor sexual behaviors in the sambar deer based on presence observation.

CONCLUSION

The present study offers valuable insights into the reproductive behaviors of the sambar deer, illuminating the distinct patterns displayed by male and female individuals. Notably, the present study highlights a crucial aspect of the estimation of female estrous. This estimation hinges on a composite analysis of factors including antler stage, male reproductive behavior, and the corresponding responses from female individuals. Given the fleeting receptivity of female oocytes to fertilization, present findings assume significance in refining the accuracy of reproductive interventions such as artificial insemination. The noteworthy decline in reproductive behavior observed during antler casting serves to deepen the comprehension of the antler cycle and mating ability. This, in turn, prompts essential considerations for semen collection procedures when not in the rutting season. The recommendation for future study is to consider performing estrous synchronization response and artificial insemination in sambar deer based on behavioral observation to evaluate the efficacy of the heat detection technique.

DECLARATIONS

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Availability of data and material
The data is available upon reasonable request from the authors.

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Authors' contribution

Aiman Murad was centered on the acquisition of data and fieldwork. Hartini Ithin was integral in the conceptualization of the sampling. Tengku Rinalfi Putra was involved in the manuscript revision and intellectual discussion. Cosmas Ngau was the key field person who ensured that the project ran smoothly and assisted immensely in data analysis and interpretation. Wan Nor Fitri contributed to the write-up of the manuscript and the overall form of the article. All authors have read and approved the final draft of the manuscript.

Ethical consideration

Ethical issues, such as the integrity of the data and permission to conduct and publish the study, have been checked by all the authors before publication in this journal.

Competing interests

The authors have no conflict of interest.

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