UNIVERSITI PUTRA MALAYSIA

SEXUAL BEHAVIOUR DURING BREEDING PERIODS IN CAPTIVE SAMBAR DEER (RUSA UNICOLOR) IN PUSAT KONSERVASI HIDUPAN LIAR, SUNGKAI PERAK

MUHAMMAD AIMAN AKMAL BIN MURAD

SEXUAL BEHAVIOUR DURING BREEDING PERIODS IN CAPTIVE SAMBAR DEER (*RUSA UNICOLOR*) IN PUSAT KONSERVASI HIDUPAN LIAR, SUNGKAI PERAK.

MUHAMMAD AIMAN AKMAL BIN MURAD

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

October 2022

ACKNOWLEDGEMENT

I would like to give my warmest thanks to my supervisor, Dr. Wan Nor Fitri Wan Jaafar for his guidance, tolerance, and endless support throughout this project and Dr Tengku Rinalfi Putra Tengku Azizan and Puan Hartini Ithnin as my co-supervisor for their time and advice.

I would also like to express my appreciation to Pusat Konservasi Hidupan Liar Sungkai Center Chief, Encik Cosmas Ngau and staff, En Azrul Ahmadi Athan for their guidance and support in allowing me to conduct my final year project in their premises. Not to mention Puan Mala, who provided me with accommodation at the National Wildlife Rescue Center during my two-week study.

I am also grateful to all the lecturers, staff and my classmates, especially Nimallan A/L Balakrishnan and Nur Intan Hazimah bin Abd Razak who were directly or indirectly involved in my project. I also want to dedicate special thanks to Nur Aliah Batriesha binti Eleshamsor for her help and company through out this study.

Lastly, I would like to thank Allah and my parents. It would have been impossible to finish this study without their unwavering support over the past few months.

CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

PKHL	Pusat Konservasi Hidupan Liar
R. u. brookei	*Rusa unicolor brookei*
R. u. equina	*Rusa unicolor equina*
R. u. cambojensis	*Rusa unicolor cambojensis*
R. u. dejeani	*Rusa unicolor dejeani*
R. u. hainana	*Rusa unicolor hainana*
R. u. swinhoii	*Rusa unicolor swinhoii*
R. u. unicolor	*Rusa unicolor unicolor*
ARTs	Assisted reproductive technologies
AI	Artificial insemination
IVF	In vitro fertilization
CL	Corpus luteum
E2	Estradiol
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
DWNP	Department of Wildlife and National Parks
WSC	Water soluble carbohydrate

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD 4999 - Projek Ilmiah Tahun Akhir.

TINGKAH LAKU SEKSUAL RUSA SAMBAR (*RUSA UNICOLOR*) DI DALAM KURUNGAN SEMASA MUSIM MENGAWAN DI PUSAT KONSERVASI HIDUPAN LIAR, SUNGKAI PERAK

oleh

MUHAMMAD AIMAN AKMAL BIN MURAD

Desember 2022

Penyelia: Dr Wan Nor Fitri Wan Jaafar

Penyelia bersama: Dr Tengku Rinalfi Putra Tengku Azizan

Puan Hartini Ithnin

Rusa sambar telah disenaraikan sebagai lemah ancaman dengan bukti terkini mendapati terdapat risiko kepupusan spesies tersebut yang akan berlaku di Malaysia. Pelbagai usaha telah dilakukan untuk meningkatkan populasi rusa di habitat asal. Walau bagaimanapun, keperluan untuk menangani secara langsung isu populasi rusa sambar yang rendah dengan strategi pembiakan pemuliharaan harus dilakukan. Sebelum itu, status pembiakan rusa perlu dikenalpasti dan ini boleh dilakukan dengan pemerhatian tingkah laku seksual. Penyelidikan

ini bertujuan untuk mengkaji tingkah laku seksual rusa sambar di dalam kurungan. Pemerhatian langsung dan kaedah persampelan serta merta digunakan untuk merekodkan tingkah laku yang hanya memfokuskan kepada seekor jantan dan dua ekor betina. Pemerhatian telah dilakukan selama dua minggu iaitu selama 84 jam dengan tiga sesi setiap hari dengan dua jam diperuntukkan bagi setiap sesi - Sesi pertama pada 8 pagi, sesi kedua pada 10 pagi dan sesi ketiga pada 3 petang. Terdapat perbezaan kekerapan tingkah laku seksual yang direkodkan dalam tiga sesi pengumpulan data. Rusa sambar menunjukkan tingkah laku seksual lebih kerap pada sesi ketiga dengan 51% berbanding sesi pertama dan kedua dengan masing-masing 40% dan 9%. Kekerapan dalam sesi yang berbeza menunjukkan bahawa rusa paling aktif pada waktu petang berbanding waktu tengah hari. Hasil dapatan daripada kajian ini, tempoh estrus untuk rusa sambar telah dikenalpasti iaitu antara satu hingga dua hari. Oleh itu, tidak mustahil untuk memantau kitaran biang dan menganggarkan tempoh estrus rusa sambar melalui pemerhatian secara langsung terhadap tingkah laku seksual. Hasil daripada kajian ini akan bermanfaat dalam program pembiakan di masa hadapan menggunakan pengesanan biang secara manual untuk teknologi pembiakan berbantu (ART).

Kata kunci: pemerhatian tingkah laku seksual; kitaran biang; konservasi; pembiakan; kepupusan

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 - Final Year Project.

SEXUAL BEHAVIOUR DURING BREEDING PERIODS IN CAPTIVE SAMBAR DEER (*RUSA UNICOLOR*) IN PUSAT KONSERVASI HIDUPAN LIAR, SUNGKAI PERAK

by

MUHAMMAD AIMAN AKMAL BIN MURAD

December 2022

Supervisor: Dr Wan Nor Fitri Wan Jaafar

Co-supervisors: Dr Tengku Rinalfi Putra Tengku Azizan

Puan Hartini Ithnin

Sambar deer is listed as vulnerable with recent evidence of impending risk of extinction in Malaysia. Various efforts to increase the population in wild habitat were in place. However, there is a need to directly address the low sambar deer population using the conservation breeding strategy. Prior to that, the animal reproductive status needs to be established and can be performed by sexual behaviour observation. This investigation was aimed to study the sexual behaviours of captive sambar deer. Direct observations and instantaneous sampling

methods were used to record behaviours focusing only on one male and two females. The observation was performed for two weeks at a total of 84 hours with three sessions each day and two hours allocated for each session - session one at 8 pm, session two at 10 am, and session three at 3 pm. There are differences in frequency of sexual behaviours recorded within three different session of data collection. The sambar deer exhibit more sexual behaviour in the third session with 51% compared to first and second session with 40% and 9% respectively. The frequency in different sessions suggest that deer are most active in the evening and least active in the afternoon. From this study, the estrus period in sambar deer was established as ranging from one to two days. Therefore, it is possible to monitor the oestrus cycle and to estimate the estrus period of sambar deer via direct observation of sexual behaviour. The result from this study will be useful in future breeding programs using manual heat detection for assisted reproductive technology (ART).

Keywords: behavioural observation; oestrus cycle; conservation; breeding; extinction

1.0 INTRODUCTION

Sambar deer, *Rusa unicolor* (Leslie, 2011) is the largest oriental deer. There are seven different subspecies of sambar deer, which can be found in a variety of habitats and altitudes from India to Sri Lanka and other parts of Southeast Asia. There are two subspecies of sambar deer found in Malaysia; *Rusa unicolor equina* in peninsular Malaysia and *Rusa unicolor brookei* in Borneo. Over the years, the population of sambar deer in the wild keeps decreasing. The International Union for Conservation of Nature Red List classifies this species as vulnerable (Timmins et al., 2015) due to severe population declines in the wild across its geographic range, primarily due to hunting, deforestation, and overexploitation. In Peninsular Malaysia, sambar deer have been listed as endangered, along with other species such as Malayan tapir and Malayan gaur, due to a more than 50% decline in the previous ten years (PERHILITAN, 2018). A study by Steinmetz et al. (2010) stated that illegal poaching of large ungulate species (> 5kg) for meat, antlers, and other body parts to meet local and regional demand resulted in many site-level extinctions in the region. This situation is especially relevant to the sambar deer in Peninsular Malaysia. The population of sambar deer will continue to decline if their natural habitat is exploited, no action is taken for illegal poaching, and lack of effective ex-situ breeding programs for large-scale restocking of this species. To boost the number of sambar deer, a proactive plan needs to be done not only by the government agencies but also by all related stakeholders.

One of the efforts in boosting the population of the sambar deer is by conducting an ex-situ breeding program. Include an introduction to sambar deer reproductive performance. A major objective of the program is to produce as many captive-bred offspring as possible and to provide them with adequate nutrition, as well as a proper management system with minimum human contact before reintroducing them into the wild. Despite this, there is little information on its conservation status, behaviour, ecology, genetics, and reproduction

physiology, which would assist in better conservation and management of the species. Since 1970, Pusat Konservasi Hidupan Liar (PKHL) Sungkai, has established an ex-situ breeding program for many species including sambar deer, and many have found their way back into the wild over the decades. Though the deer have been released into the wild, there appears to be a very small improvement in terms of the sambar population. Because of this situation, it is obvious that further interventions and research are needed for this breeding program to succeed. Before that, the reproductive status of the animal needs to be established.

In recent decades, non-invasive methods of monitoring the reproductive status of animals became popular among the research community. It is beneficial to utilize a non-invasive procedure over an invasive one since it does not disturb or risk the animals through restraint or anaesthesia, in addition to preventing stress caused by improper handling (Gholib et al., 2021). Especially in wildlife, unnecessary and excessive stress can lead to the mortality of the animal. In deer, mortality is often related to capturing myopathy. Capture myopathy is a condition that causes significant morbidity and mortality in wild animals all over the world. This condition results from prolonged or brief intense pursuits, captures, restraints, or transportations of wild creatures that are stressful and physically exhausting (Breed et al., 2019). The observation of sexual behaviour is one of the quickest and most non-invasive methods that can be used to determine the animal's reproductive status. By observing sexual behaviour, the oestrus cycle can be monitored and the estrus period can be estimated. Therefore, this study aims to identify the best time of the day to monitor the estrus signs and to estimate the estrus period of sambar deer.

2.0 LITERATURE REVIEW

Sambar deer morphology

Sambar deer, *Rusa unicolor* (Leslie, 2011) has seven subspecies namely*; R. u. brookei, R. u. equina, R. u. cambojensis, R. u. dejeani, R. u. hainana, R. u. swinhoii, and R. u. unicolor*. The largest subspecies will be the sambar deer in India and Sri Lanka which is *R. u. unicolor* (Leslie, 2011) and the smallest is *R. u. swinhoii* native to Taiwan and known as Formosan deer. The body mass and antler length of Rusa unicolor vary greatly from west to east. Sambar deer exhibit sexual dimorphism in terms of body size and the presence of antlers in males. In general, the body weight of the male is 225-320 kg while females weigh less than 225 kg. The length of head and body are 162-246 cm, the tail length up to 30 cm, and the shoulder height is up to 160 cm. The antlers of males are rough, corrugated, and three-tined (Leslie, 2011). The antlers can get as long as one meter and serve mainly as a defence mechanism against predators and to fight especially during mate selection and establishing dominance over other males (Clutton-Brock, 1982). The coat colour varies from yellowish brown to brown (Blanford 1888; Leslie 2011); the belly is frequently same colour as the rest of the body, or darker, but occasionally whitish on the inside of the buttocks and the undersides (Lydekker 1915; Leslie 2011); females and juveniles typically more brightly coloured; juveniles not spotted, except for sambar deer species in Malaysia (Lydekker 1898). Males also develop a dense and bushy mane around their necks (Schaller 1967; Leslie 2011). Sambar deer have four scent glands which are preorbital glands, metatarsal glands, caudal glands, and dermal glands or “sore spot” located on the throat (Dixon, 2018).

Distribution of sambar deer

According to Corbet and Hill (1992), the sambar deer is the most common deer in Asia. It is found from southern Nepal, India, Sri Lanka, and Burma through southern China and Southeast Asia to the Pacific coast and the islands of Borneo, Hainan, and Taiwan (Leslie, 2011). Aside from the Asia region, sambar deer also can be found in other parts of the world. In Australia, feral populations are created by deer intentionally brought in for hunting or by deer that have escaped from deer farms (Yamada et al. 2003). The sambar deer has been classified as an invasive species that cause damage to forest plantations, ornamental gardens, and farm fences and gates. Due to its size and crepuscular behaviour, it can compete with livestock for grazing land and poses a serious threat to road traffic (Home, n.d.). Sambar deer are also under the Biosecurity Act of 2014, which prohibits moving, keeping, feeding, giving away, selling, or releasing them into the environment. Report all sightings within 24 hours to authorities (Fisheries, 2017). The Introduced populations can also be found in New Zealand, California, Florida, and Texas.

Sexual behaviour

The reproductive process should always comprise reproductive behaviour. Reproductive behaviour is the systematic progression of behavioural actions related to reproduction (Greene et al., 2022). There are three stages of sexual behaviour in males; precopulatory, copulatory, and postcopulatory. Meanwhile, female sexual behaviour serves as; attractivity, proceptivity, and receptivity. In three stages of sexual behaviour, precopulatory behaviour consists of the search for a partner, erection, and penile protrusion from the prepuce. The second stage is the copulatory stage which consists of mounting, intromission of the penis into the vagina, and successful ejaculation. Finally, the postcopulatory stage includes the dismount of the male from the female, the refractory period, and memory (Senger, 2012).

Oestrus detection

Oestrus detection is one of the crucial parts in determining the successfulness of the assisted reproductive technology. The adoption of artificial insemination in the deer breeding program requires the need for detecting the receptivity of the female before the insemination. This is to make sure that ovulation has occurred before the semen being inseminated to increase the chance of conception (Belstra et al., 2007). Some protocols ensuring the successful heat detection are; 1. Tracking the individual animal using a numbering system, 2. A good record keeping system, 3. The duration and time for monitoring, 4. Use heat detection tools wisely and effectively, 5. Hoof management needs to be done regularly, and 6. synchronization protocol with hormones (Rao et al., 2013).

Reproductive performance

Reproductive performance can be divided into mating, fawning, and rutting patterns. Rutting is closely related to breeding behaviour (Clements et al. 2010). Rutting began with the rubbing of antlers leading to the shedding of velvet followed by other sexual behaviour including the stag spraying the sharp-smelling urine, restlessness, fighting other stags, and low feed intake (Dahlan & Dawend, 2013). Many studies were done on the sambar deer specifically that lived in tropical regions and found that there were no definite reproductive patterns for this species which means they are polyoestrous and can reproduce all year round with the highest mating activities in November (Putranto et al., 2010). The sambar deer begin to be sexually active at age of 1.5 years until 12 years (Garsetiasih, 2016). The sambar deer have an estrus period of 1 to 3 days in 18 to 37 days of the oestrus cycle (Putranto et al., 2010). The mean age of fawning is 32 months and the mating interval was 11 months (Dahlan & Dawend, 2013). The conception rate of sambar deer in captivity is generally low with an average of 48.8% and with mean gestation length of 240 days (SEMIADI et al., 1970).

Assisted reproductive technology

Assisted Reproductive Technologies (ARTs) refer to a wide range of treatments and procedures that include the alteration of reproductive cycles, gametes, or embryos (Themes, 2017). For domestic animals, various types of ARTs have been developed such as artificial insemination (AI), in vitro fertilization (IVF), multiple ovulation embryo transfer, and cloning (Themes, 2017). In deer species, cryopreservation in combination with AI is the most widely used ART procedure (Asher et al., 2000). The AI conception rates in deer are typically in the 50-80% range (Pintus & Ros-Santaella, 2014). In wildlife, it has been suggested as one method of overcoming some of the issues associated with managing small, isolated populations of species with varying degrees of reproductive success (Wildt, 2003). Many benefits can be achieved from these technologies, for instance, they will need fewer genetic resources and can maintain the diversity of genetics over time (Lees & Wilcken, 2009). However, there will be a risk in performing this technique with limited knowledge, especially in wildlife. Some restrictions were to capture and transport the wildlife from the wild to the breeding centre, determination of oestrus phase, restraining protocol, and proper protocol of collecting semen and inseminating into the female (Herrick, 2019). Hence, a further understanding of reproductive biology through non-invasive method highlights the importance of this study for the development of assisted reproductive technology in deer.

Antler cycle

Sambar deer can grow their antlers up to 100 cm and only males have them (Leslie, 2011). The antlers undergo an annual cycle and will be in full growth before the rutting season, and cast after the season ends. Antlers are cast and regrown from a blastema each year into three-branched structures of cartilage and bone that are utilized for sparing and attractiveness. (Weerasekera *et al.*, 2019). There were 7 stages of the antlers cycle namely, (a) cast, (b) growing 1: single spike, (c) growing 2: antler fork into a Y-shaped as the second tine appears, (d) growing 3: velvet begins to harden as the third tine appears, (e) growth completed: velvet shedding begins, (f) hard antler, and (g) casting (Weerasekera *et al.*, 2019). The antler cycle

was controlled by testosterone. Testosterone secretion was influenced by changes in day length and is highly correlated with reproductive status (Stewart et al., 2018). The testosterone level remained constant in the growing phase, peaking during antler calcification which also caused the shedding of velvet and finally fell rapidly which then led to antler casting (Gomes et al., 2021). Male deer commonly shed their antlers between March and July and time for the velvet to hardens is around 103 days (Dahlan & Dawend, 2013b).

Threats to sambar deer in Malaysia

The population of sambar deer in Peninsular Malaysia has been classified as endangered with an estimated population of 1000 animals in the wild (PERHILITAN, 2018). The main threats to this species were poaching and habitat loss (Kawanishi et al., 2014). The sambar deer meat, also known as venison has a very high demand by all ethnicities in Malaysia, unlike wild boar which is prohibited in the Muslim community (Kawanishi et al. 2003). The sambar deer are hunted for trophies, sales to restaurants, personal consumption, and for medicinal purposes. For decades, the antler, specifically the velvet, was believed to be an effective medicine. For instance, as anti-cancer drugs (Chonco et al., 2021) and thought to be beneficial to men's sexual function (Conaglen, 2003).

Due to agriculture, industry, and human settlements, Peninsular Malaysia has already lost more than 90% of its lowland rainforest (DWNP 2010). The main loss is due to the land opening for agriculture, Malaysia is one of the largest producers of palm oil and rubber. In Malaysia, oil palms are grown on an area of 5.9 million hectares, yielding 19.86 tonnes of palm oil and 2.32 tonnes of palm kernel oil (Malaysian Palm Oil Industry, n.d.). This over-exploitation will reduce the habitat of the deer and lead to fewer food resources causing one of the factors in the extinction of the species. However, deer integration in oil palm is a type of mixed farming in which the mixtures of the two commodities can be synergized to best utilise the same plot of land. The livestock-crop integrated production system (LICRO) is considered both the most

efficient agricultural production system and the future direction for livestock production in Malaysia. Deer considered a good species of animal after cattle for the integration system due to their higher stocking rate (Dahlan, 1998)

Sambar deer as main diet for Malayan tiger, *Panthera tigris jacksoni*

The Sambar deer (*Rusa unicolor*) and the Barking deer (*Muntiacus muntjac*) were the main diet for Malayan tigers (Ten et al., 2021). The sambar is favourable to tiger predation due to its huge body size, crepuscular and nocturnal behaviour, the infrequency of browsing in open regions, frequent occurrence in small groups, and vulnerability due to non-aggressiveness even in deer with strong antlers. Unlike other animals such as wild boar and gaur which are very aggressive that can injure or kill tigers (A. J.T. Johnsingh, 2016). Thus, increasing the sambar deer population is one of the most crucial steps for Malayan tiger conservation (*Thailand Boosts Deer Population in Effort to Support Tigers*, n.d.)

Oestrus cycle

The oestrus cycle is a cyclic pattern of ovarian activity that allows female animals to transition from a period of reproductive receptivity to non-receptivity, allowing pregnancy to occur after mating. The oestrus cycle has two distinct phases: the luteal phase and the follicular phase. The luteal phase can be further divided into metestrus and diestrus, while the follicular phase can be divided into proestrus and estrus. The luteal phase begins immediately at the point of ovulation until the corpus luteum (CL) is luteolysed towards the end of the oestrus cycle by the dominant hormone progesterone (Senger, 2012). The follicular phase is stimulated by the drastic drop in progesterone level due to luteolysis of the corpus luteum. The proestrus is distinguished by rising of Follicular stimulating hormone (FSH) which is responsible for follicle growth and increased estradiol (E2) concentrations, and the decline of FSH with the surge of Luteinizing hormone (LH) at the end of the phase (Skinner, 2018). Finally, the estrus phase is when the concentration of LH peaks, causing ovulation. Estrus,

also known as "heat", is the time when female animals become sexually receptive and indicate that they are ready for mating.

Preorbital gland opening

The preorbital gland has been discovered in a variety of ungulates, including cervids like deer and bovids like wildebeest and antelope (Ceacero et al., 2014). The preorbital gland is a pair of exocrine glands located in front of the eyes (Ceacero et al., 2015). The role of this gland is yet not fully understood. The function of this gland was frequently reported to mark the territories, during fighting, and establishing mother-offspring bonds (Rajagopal & Archunan, 2011). In addition, it has been reported that the opening of the preorbital gland in red deer calves signals to the mother when her calf is hungry (Bartos et al. 2005). Further, the preorbital gland opening is also associated with alert and stressful conditions such as during restraining for ear tagging and weighing (Bartošová-Víchová et al., 2007).

3.0 MATERIALS AND METHODS

3.1 Background of study site

The study was conducted at Pusat Konservasi Hidupan Liar Sungkai Perak, Malaysia (4.0353° N, 101.3658° E) includes 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) and is situated in the western portion of Perak. The conservation centre conducts breeding and conservation programmes for various animal species, including sambar deer, Malayan gaur, pangolin, and a few bird species such as Great argus. A permit for this study has been issued by DWNP with the reference number JPHLTN.600-6/1/4 JLD (78).

3.2 Management of sambar deer

The sambar deer at the conservation centre were managed under an intensive system. They were all kept in the paddocks with shelter and provided with food and water every day. The deer were provided with a variety of leaves, including *Trema orientalis* and *Mallotus paniculatus*, as well as unfettered access to the paddock's native vegetation. Besides feeding on greens, their diet was also supplemented with goat concentrates, mineral lick blocks and multivitamins that are given every two weeks. Every day, the deer were fed once in the morning, and the keeper utilises this time to watch the animals for any abnormalities and check the perimeter of the paddocks for any fence breakage.

3.3 Ethogram behavioural observation

In this study a herd of deer of mixed ages and sexes (Male= 4, Female= 22, and Juvenile= 4) were managed in a herd. The observation focused on one male and two females. The male was identified by its long and hard antlers. In female 1, there is a 15 cm scar on the right rump while in female 2, there is no scar anywhere on the body. The sambar deer is housed in an enclosure that is 75 metres long and 36 metres wide (Figure 1). They were all born in captivity, and throughout the study it was confirmed that they were all healthy. Direct observations of the animals were made over the course of fourteen days. The direct observations were done in the morning (S1= 8 am-10 am), afternoon (S2= 10 am-12 pm), and evening (S3= 3pm-5pm), using the instantaneous sampling method (Altmann, 1974). There were 8 slots in every session and each slot represented 15 minutes of observation. Each slot corresponds to a single occurrence of a behaviour. If a behaviour is displayed more than once in a single slot, the total number of instances is added together and considered as one behaviour. To prevent the behaviour of the sambar deer from being disturbed by the presence of the observer, the behaviour was observed from outside the paddocks. The observation sheet was used to record all data and details of the animals' behaviours described in the ethogram (Table 1) during the sampling period. The ethogram was created by making various modifications after a preliminary observation period, which was carried out before data collection.

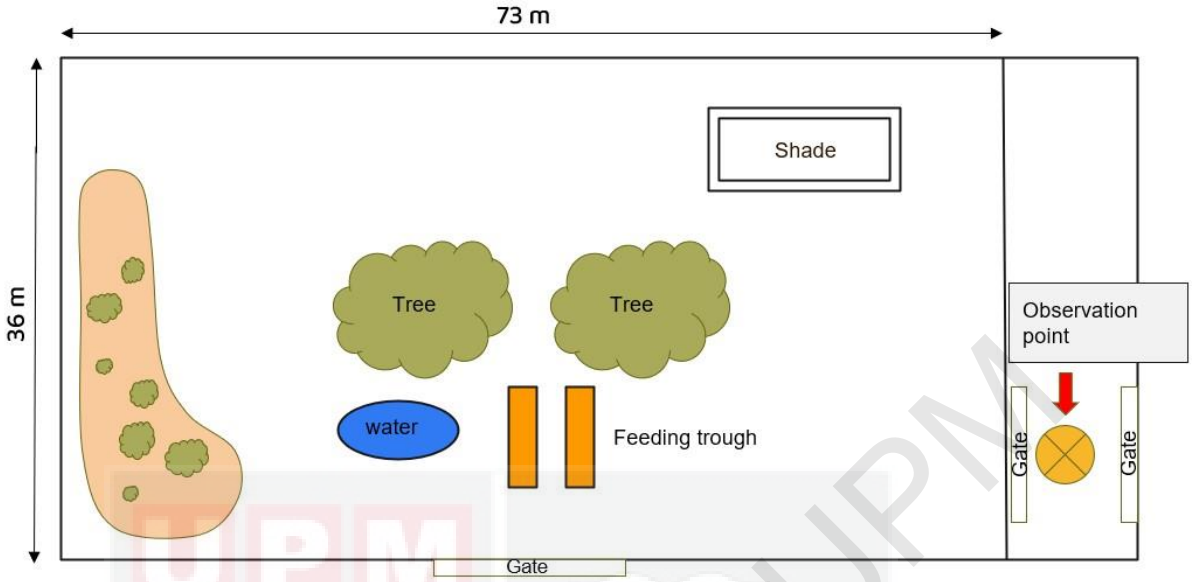

Figure 1. The layout of the sambar deer paddocks in PKHL Sungkai

Table 1

Ethogram of sambar deer sexual behaviour

Behavioural grouping	Behavioural subgrouping	Code	Description
Reproductive behaviour (Male specific)	Follow (Figure 3)	FO	Male follow female
	Smelling of female urine or faeces (Figure 4)	SM	Smelling urine or faeces, following by flehmen response
	Anal sniffing (Figure 5)	AS	Male smells female vaginal area
	Flehmen response (Figure 6)	FR	Male raised their head and curled upper lip
	Chin resting (Figure 7)	CR	Male rest its chin on the rump of the female
	Grooming (Figure 8)	GR	Male licking the female's body

Mounting (Figure 9)	MO	The male climbs onto the female's back with the chest resting on the rump. The hind limbs lie on the ground and the front limbs hang down.
Mating (Figure 10)	MA	While mounting, the male performs successive flexion and extensions of the vertebral spine.
Preorbital gland opening (Figure 11,12 & 13)	PGO	The opening of an elongated slit that is located in front of each eye.

Table 1 *(Continue)*

Behavioural grouping	Behavioural subgrouping	Code	Description
Reproductive behaviour (Female specific)	Follow	FO	Female follow male
	Smelling of male urine or faeces	SM	Smelling urine or faeces, following by flehmen response
	Anal sniffing	AS	Female smells male rectal/penis area
	Flehmen response	FR	Female raised their head and curled upper lip
	Chin resting	CR	Female rests its chin on the rump of the male
	Grooming	GR	Female licking the male's body

Figure 2. Follow

Figure 3. Smelling of female urine or faeces

Figure 4. Anal sniffing

Figure 5. Flehmen response

Figure 6. Chin resting

Figure 7. Grooming

Figure 8. Mounting

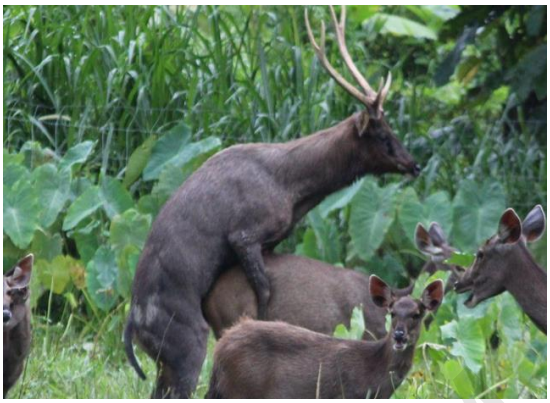

Figure 9. Mating

Figure 10. Preorbital gland opening on male

Figure 11. Preorbital opening on female

Figure 12. Preorbital gland opening associated with aggressive behaviour

3.4 Behaviour statistical analysis

The data is presented in frequency. Throughout two weeks of research, the frequency of each behaviour done by one male and two female sambar deer was in the form of a percentage (Figure 13). The percentage was derived by dividing the number of one specific behaviour exhibited with the total number of sexual behaviour exhibit by each individual. As a result, all frequency values were calculated on an individual basis.

4.0 RESULTS

4.1 Behavioural observations

A total of 84 hours of behavioural observations were made, consisting of 58 records of sexual behaviour by M1, 14 records by F1, and 3 records by F2. Some of the behaviours that were seen throughout three separate sessions (S1, S2 & S3) differ from one another (Figure 13). Figure 13 illustrated the frequency of sexual behaviour recorded from three deer (M1, F1 and F2). Males show a high frequency of sexual behaviour with 77.3% compared with two females, F1 and F2 both with 18.6% and 4% respectively. In the male, anal sniffing (AS) was exhibited the most with 32.8% out of a total of 8 sexual behaviours observed. Meanwhile, in females, both exhibited high frequency of grooming (GR) behaviour.

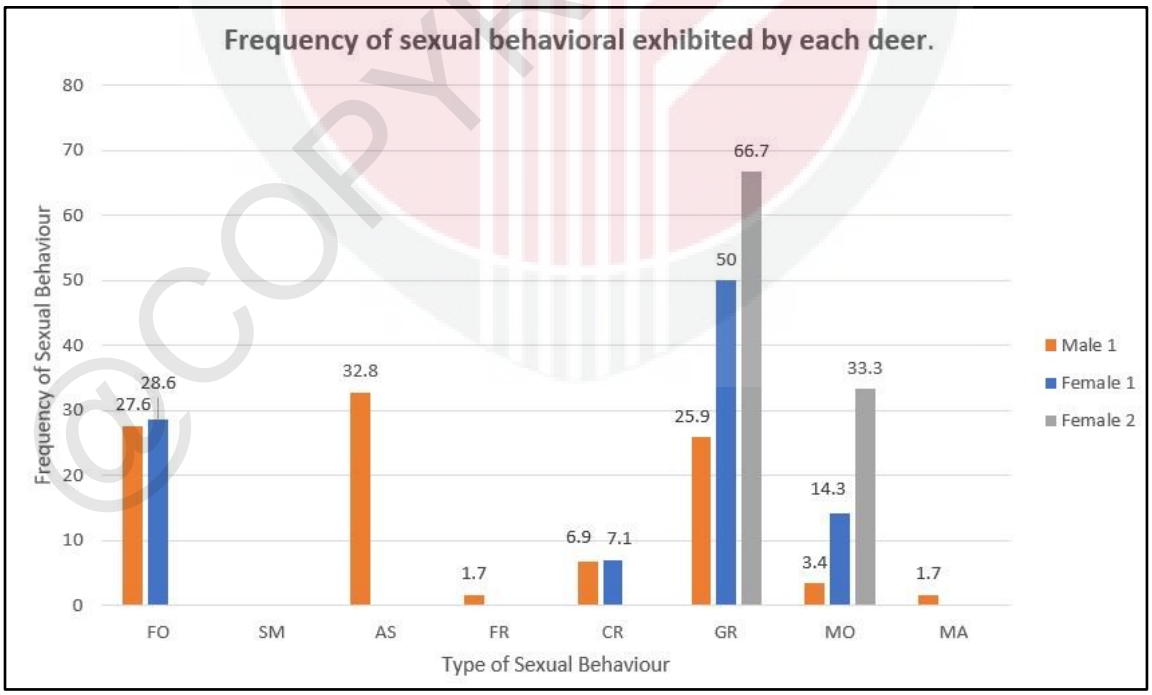

Figure 13. The frequency of sexual behaviour recorded from three deer

Table 2 shows the time of the day when sambar deer exhibit sexual behaviour. According to the three sessions of observation, sambar deer in PKHL are more likely to exhibit their sexual behaviour in the evening as compared to the morning and afternoon as illustrated by (Table 2). From this study, as shown in Table 2, the male deer exhibited a total of 58 sexual behaviours were exhibited by the male and from that 51% of the event happens at the evening which is from 3 pm to 5 pm followed by morning session which is 40% and the least which in the afternoon at 4%.

Table 2. Time of the day when sambar deer exhibit sexual behaviour

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

Next, figure 14 shows a frequency of sexual behaviours exhibited by all three; male 1, female 1 and female 2 throughout fourteen days of observation. As illustrated in (Figure 14) the male's behaviour had two peaks. Day 6 of the observation was the first time, and day 10 was the second. Additionally, successful mating was noted on day 6. The event was observed in the morning, S1. The frequency and length of the sexual behaviours displayed by the two females, on the other hand, were minimal and only lasted one to two days. (Figure 14) also demonstrates a sharp decline in male sexual behaviour that began on day thirteen, on the same day when the second antler was cast.

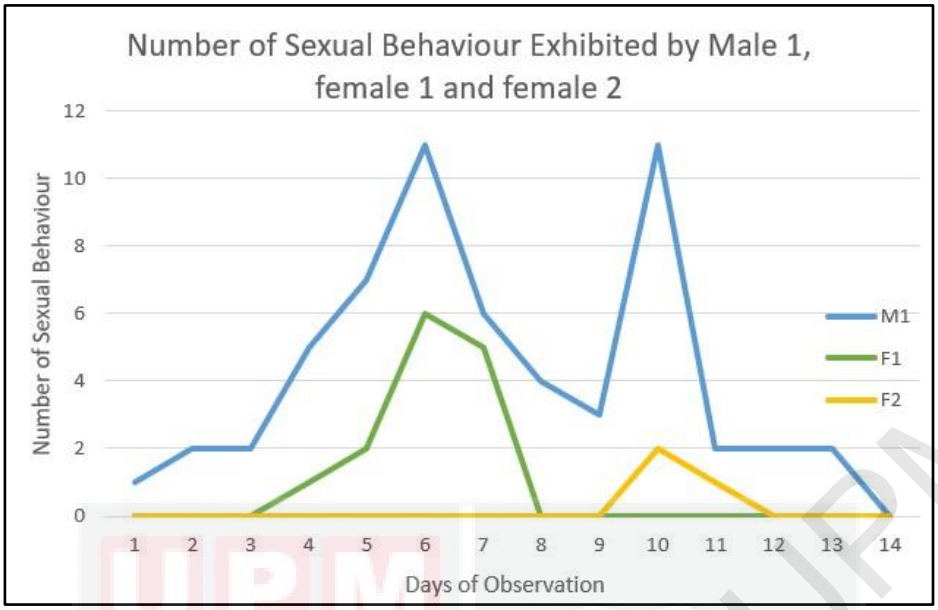

Figure 14. Frequency of sexual behaviours exhibited by all three; male 1, female 1 and female 2 throughout fourteen days of observation.

Moreover, there were incidental events during the observation. The incidental finding was an opening of preorbital scent glands. The preorbital gland is a pair of exocrine glands located in front of each eye. The openings were observed three times, twice by the male and once by the female and all were on the same day, 26th September. The first event was associated with sexual behaviour which was mounting. The opening was observed when the male mounted the female and closed a few seconds once the male dismounted the female (Figure 10). The second event was also observed in the same male. Differing from the first time, this preorbital gland opening was associated with agonistic behaviour. The gland was open throughout the event where the dominant male chased the younger male (Figure 11). The third event was observed in the female and was associated with sexual behaviour (Figure 12). The preorbital gland opened when the female sniffed other female genitalia.

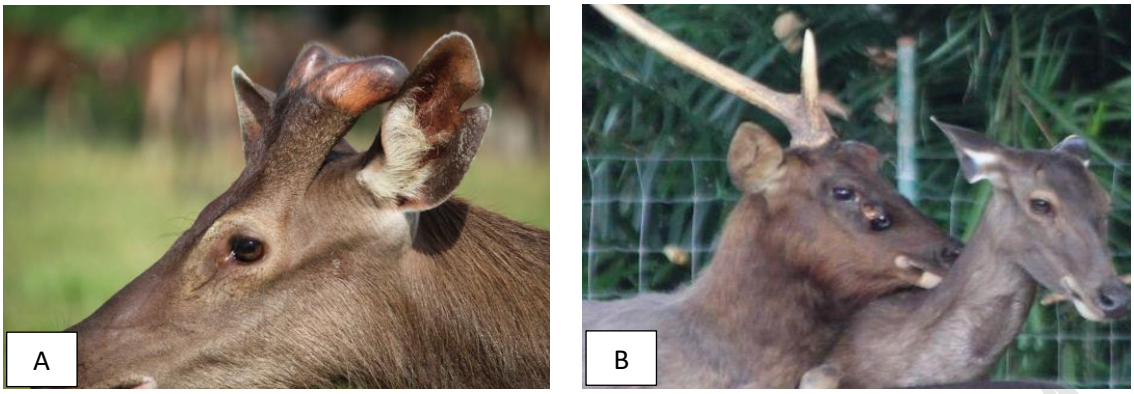

Figure 15. The preorbital gland. A: The preorbital gland close, appears as a slit extending in front of the eye. B: The preorbital gland open, shows a pinkish inner side.

5.0 DISCUSSIONS

Sexual behaviour observation

Based on the results, Figure 13 shows that the number of Follow is highest in male deer, whereas the number of Grooming is highest in female deer. The Follow behaviour shown by male is consistent with the precopulatory stage of sexual behaviour establish by Senger (2012). The crucial part of the precopulatory stage is to search for suitable sexual partners. The male is the one who is responsible for the search for the receptive and sexually ready female. Thus, the behaviour of Follow which is described as the male lowering and extending his head while following the female by both walking or chasing and maintaining a distance of fewer than 2 m will be seen frequently. Unlike males, females will send olfactory signals to attract possible sexual partners. On the other hand, female deer tend to approach males during or near the estrus period. A study also shows that allogrooming intersex increase during the breeding season (Forand & Marchinton, 1989).

Estimating the estrus period

There were two periods of time where the male sexual behaviour peaks as illustrated in Figure 14. These peaks correspond with the females' estrus period. According to the graph, F1 exhibits a rise in sexual behaviour beginning on day 4 and reaching a peak on day 6, which coincides with the first peak of male behaviour. On the other hand, F2 also exhibits a rise in sexual behaviour on day 10 corresponding with the second peak of male behaviour. Based on this data the estrus period of the sambar deer can be estimated. The estimation could be made solely by observing male deer or observing both simultaneously. The estimation of the estrus length also could be done based on the frequency of the sexual behaviour exhibited and the specific copulatory behaviour such as chin resting and mounting on female. The increase in sexual behaviour from 2 times on day 3 to 11 times on day 6 in male indicated that there are females in the herd entering estrus. The high frequency of behaviour that range for two to four days in male is consistent with a study published in 2010 by Putranto et al., stating

that the estrus cycle of sambar deer was 2.00 ± 0.41 days (range = 1 - 3 days). The estrus period must be determined in order to identify the best time to inseminate the female to increase the chances of pregnancy. Due to the ovulation process that occurs 24 to 28 hours after the standing heat, it is best to inseminate the deer during this period of time (Asher, 1985).

Sexual behaviour associated with feeding pattern

As shown in (Table 2), 51% of male sexual behaviours occur in the evening, between 3 pm and 5 pm, followed by 40% in the morning, and 4% in the afternoon. Based on observation, feeding behaviour was more frequently accompanied by sexual behaviour. The majority of the grazing activities take place in the late afternoon or evening. Ungulates are most active at dusk and dawn and spend most of their noon resting (Gregorini, 2012). Most behaviours begin at dawn, then decrease throughout the day, then pick up again as dusk approaches. This statement is consistent with the natural behaviour of the deer which is classified as crepuscular to nocturnal (Leslie, 2011). Other than that, environmental temperature also influences the behaviour of the deer. During the midday, the temperature could reach up to 34°C, the deer became less active and preferred to sleep under the large tree to avoid the scorching sun. A study by Wang et al., (2020) stated that water soluble carbohydrate (WSC) concentrations in the grass were higher in the evening from 3pm to 7 pm compared to morning and afternoon which could influence the grazing decision by the deer. WSC which includes fructans, sucrose, glucose and fructose are important for ruminants as a source of energy. Thus, this data enables us to come to the conclusion that late afternoon or evening is the optimal period to observe estrus signs. Monitoring sexual behaviour is crucial in determining the estrus period of the animal. In implementing ART such as artificial insemination, it is crucial to understand what to look for and when to look for the behaviours so the animals can breed efficiently. Successful recognition of the signs of oestrus for mating, just before the time of ovulation, can increase the conception rate of the flock.

Sexual behaviour associated with antler cycle

Additionally, figure 14 also illustrates the rapid decrease in male sexual behaviour on day 13 that is related to the antlers casting stage. The male antlers were cast at different times during the fourteen-day observation period. The first antler was cast on day 6, and the second antler was cast on day 13. The stags shed their antlers by fighting or bumping into a tree or other hard structure. Commonly, deer will cast both of their antlers on the same day and some have a gap of 2 to 3 days depending on the testosterone level deficit which differs between individuals. There were no differences in the frequency of sexual behaviour displayed after the first antler was cast down; however, sexual behaviour decreases significantly after the second antler is cast down. The event of antlers being cast down and the dropping in the sexual behaviour frequency show the relationship between the casting stage of the antlers and the sexual behaviour. According to Weerasekera et al. (2019), the faecal testosterone level was highest during the hard stage of the antlers and lowest during the cast antler stage. The study found that antler shedding is caused by a sudden drop in testosterone concentration. On the other hand, sexual behaviour is controlled by the testosterone hormones which are also responsible for the development of male reproductive tissue, and secondary male characteristic and promotes aggressive behaviour. This suggested that testosterone is the hormone in control of both sexual behaviour and the antler cycle.

Sexual behaviour associated with the preorbital gland opening

There were incidental findings from the male deer during the two weeks of this behavioural study. The discovery was a preorbital scent gland opening. There were two occasions where the scent glands opening was observed. First, during mounting behaviour where the dominant male mounts on the female, then again during aggressive behaviour where the dominant male chases the younger male. In deer, they have a few specialized scent glands all over their body including the preorbital gland, metatarsal gland, preputial gland, and interdigital and tarsal gland. The function of scent glands is to produce and secrete a chemical

compound to convey a message to other individuals. In a report paper by Ceacero *et al.*, (2015) they found that on the Rusa deer there was a preorbital scent gland opening associated with agonistic interaction, stressful events, and alert behaviour. However, in this case, the scent gland opening during sexual behaviour is a discovery, and the function behind it is not fully understood. As a result, more research is needed to fully understand the relationship between preorbital scent gland opening and the reason for it.

6.0 RECOMMENDATIONS

However, two weeks of observation is insufficient and yields very little information. To improve the study's reliability, the observation period should be extended for at least two complete oestrus cycles concerning many environmental factors that can influence the outcome. Besides the period of observation, the sample size also should be increased. By monitoring more than one herd of deer, a comparison can be made between the herd. Furthermore, when monitoring crepuscular and nocturnal animals, it is best to conduct the observation at night. Although some wild captive animals adapt and change their behaviour in response to the management system, we cannot deny that sambar deer are more active at night than during the day. In addition, the behavioural study can be combined with the endocrine study, as hormonal analysis can support the behavioural study's findings.

7.0 CONCLUSIONS

In the efforts of conservation of wildlife species, all agencies, organizations, and individuals need to work together side by side to figure out the best strategy for achieving the best outcomes. Besides enforcing the law and restoring the habitat, the breeding program is also an important strategy to increase the population of sambar deer. This study contributes to the behavioural elements of sambar deer that can benefit the effort on the conservation of this species.

The non-invasive approach in this study is beneficial to avoid unnecessary stress to the deer as the natural behaviour of the deer is shy, cryptic, and crepuscular. This study yielded some useful information that can help in the breeding program. Nevertheless, without any hormonal analysis, the best time to monitor estrus and estimation of the estrus period can be made and are useful especially in assisted reproductive technology. Finally, the goal of this research is to serve as a starting point for reproductive behaviour. This study hopes that this overview will intrigue readers' interest and lead to new research approaches that will aid in understanding the complexities of sexual behaviour in the effort to conserve this valuable species.

8.0 REFERENCES

A. J.T. Johnsingh. (2016). *The Corbett Foundation Blog | Conserve sambar, conserve tigers*. http://corbettfoundation.org/articles/2016/04/21/sambar-conservation-is-tiger-conservation/

Abdul Hamid, N. H., Mohd Nadzir, M. N. H., Omar, J., Annavi, G., Wan Jaafar, W. N. F., & Salleh, A. (2022). Non-Invasive Measurement of Progesterone and Cortisol Metabolites in the Faeces of Captive Female Rusa unicolor at Zoo Negara, Malaysia and Its Reproductive and Stress Behaviour. *Pertanika Journal of Science and Technology*, *30*(2), 1583–1599. https://doi.org/10.47836/pjst.30.2.40

BartošováVíchováJ., BartošL., & ŠvecováL. (2007). Technical note: Preorbital gland opening in red deer (Cervus elaphus) calves as an indicator of stress1. *Journal of Animal Science*, *85*(2), 494–496. https://doi.org/10.2527/jas.2006-446

Breed, D., Meyer, L. C. R., Steyl, J. C. A., Goddard, A., Burroughs, R., & Kohn, T. A. (2019). Conserving wildlife in a changing world: Understanding capture myopathy—a malignant outcome of stress during capture and translocation. *Conservation Physiology*, *7*(1). https://doi.org/10.1093/conphys/coz027

Ceacero, F., Landete-Castillejos, T., Bartošová, J., García, A. J., Bartoš, L., Komárková, M., & Gallego, L. (2014). Habituating to handling: Factors affecting preorbital gland opening in red deer calves1. *Journal of Animal Science*, *92*(9), 4130–4136. https://doi.org/10.2527/jas.2014-7716

Ceacero, F., Pluháček, J., Komárková, M., & Zábranský, M. (2015). Pre-orbital gland opening during aggressive interactions in rusa deer (Rusa timorensis). *Behavioural Processes*, *111*, 51–54. https://doi.org/10.1016/j.beproc.2014.11.017

Chonco, L., Landete-Castillejos, T., Serrano-Heras, G., Serrano, M. P., Pérez-Barbería, F. J., González-Armesto, C., García, A., de Cabo, C., Lorenzo, J. M., Li, C., & Segura, T. (2021). Anti-tumour activity of deer growing antlers and its potential applications in the treatment of malignant gliomas. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-020-79779-w

Clutton-Brock, T. H. (1982). The Functions of Antlers. *Behaviour*, *79*(2-4), 108–124. https://doi.org/10.1163/156853982x00201

Conaglen, H. M. (2003). Effect of deer velvet on sexual function in men and their partners: a double-blind, placebo-controlled study. *Archives of Sexual Behavior*, *32*(3), 271–278. https://doi.org/10.1023/a:1023469702627

Dahlan, I., & Dawend, J. (2013). Growth and reproductive performance of sambar deer in Sabal Forest Reserve of Sarawak, Malaysia. *Tropical Animal Health and Production*, *45*(7), 1469–1476. https://doi.org/10.1007/s11250-013-0383-6

Fisheries, A. and. (2017, April 6). *Sambar deer*. Www.business.qld.gov.au. https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/biosecurity/animals/invasive/restricted/sambar-deer

Forand, K. J., & Marchinton, R. L. (1989). Patterns of Social Grooming in Adult White-tailed Deer. *American Midland Naturalist*, *122*(2), 357. https://doi.org/10.2307/2425923

Garsetiasih, R. (2016). Daya Cerna Jagung dan Rumput sebagai Pakan Rusa (Cervus Timorensis). *Buletin Plasma Nutfah*, *13*(2), 88. https://doi.org/10.21082/blpn.v13n2.2007.p88-92

Gomes, M. A., Ditchkoff, S. S., Zohdy, S., Gulsby, W. D., & Newbolt, C. H. (2021). Patterns of testosterone in male white-tailed deer (Odocoileus virginianus): Seasonal and lifetime variation. *Ecology and Evolution*, *11*(10), 5320–5330. https://doi.org/10.1002/ece3.7423

Greene, E. S., Deviche, P., & Dridi, S. (2022). Reproductive behavior. *Sturkie's Avian Physiology*, 1111–1135. https://doi.org/10.1016/b978-0-12-819770-7.00052-9

Herrick, J. R. (2019). Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. *Biology of Reproduction*, *100*(5), 1158–1170. https://doi.org/10.1093/biolre/ioz025

Home. (n.d.). Www.nillumbik.vic.gov.au. https://www.nillumbik.vic.gov.au

LEES, C. M., & WILCKEN, J. (2009). Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook*, *43*(1), 6–18. https://doi.org/10.1111/j.1748-1090.2008.00066.x

Malaysian Palm Oil Industry. (n.d.). Malaysian Palm Oil Council. https://mpoc.org.my/malaysian-palm-oil-industry/

Mary, E., & Balakrishnan, M. (1984). A study on olfactory communication signals in sambar deer,Cervus unicolor. *Proceedings: Animal Sciences*, *93*(1), 71–76. https://doi.org/10.1007/bf03186229

Rajagopal, T., & Archunan, G. (2011). Histomorphology of preorbital gland in territorial and non-territorial male blackbuck Antelope cervicapra, a critically endangered species. *Biologia*, *66*(2). https://doi.org/10.2478/s11756-011-0015-4

SEMIADI, G., ADHI, I. G. M. J., & TRASODIHARTO, A. (1970). Calving pattern on captive sambar deer (Cervus unicolor) in East Kalimantan. *Biodiversitas Journal of Biological Diversity*, *6*(1). https://doi.org/10.13057/biodiv/d060112

Senger, P. L. (2012). *Pathways to pregnancy and parturition*. Washington Current Conceptions.

Skinner, M. K. (2018). *Encyclopedia of reproduction*. Elsevier, Academic Press.

Ten, D. C. Y., Jani, R., Hashim, N. H., Saaban, S., Abu Hashim, A. K., & Abdullah, M. T. (2021). Panthera tigris jacksoni Population Crash and Impending Extinction due to Environmental Perturbation and Human-Wildlife Conflict. *Animals*, *11*(4), 1032. https://doi.org/10.3390/ani11041032

Thailand boosts deer population in effort to support tigers. (n.d.). Tigers.panda.org. Retrieved November 27, 2022, from https://tigers.panda.org/news_and_stories/stories/thailand_boosts_deer_population_in_effort_to_support_tigers/

Themes, U. F. O. (2017, September 27). *Assisted reproductive technologies used in domestic species*. Veterian Key. https://veteriankey.com/assisted-reproductive-technologies-used-in-domestic-species/

WILDT, D. E. (2003). The role of reproductive technologies in zoos: past, present and future. *International Zoo Yearbook*, *38*(1), 111–118. https://doi.org/10.1111/j.1748-1090.2003.tb02070.x