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MONITORING OF REPRODUCTION AND INFLUENCE OF FECAL PROGESTERONE ON REPRODUCTIVE CYCLICITY IN CAPTIVE SRI LANKAN SAMBAR DEER (*RUSA UNICOLOR UNICOLOR)*

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This study examines the length of the estrous cycle in 16 Sambar deer hinds in National zoological gardens in Dehiwala and Kegalle, Sri Lanka (NZGDK) assessed with the use of changes in progesterone concentrations, along with the changes in the profile of this hormone and by the visual estrus manifestations. The objectives of the present study were to characterize ovarian activity throughout the estrous cycle and the non-pregnant luteal phase of captive sambar deer in Sri Lanka. These objectives were achieved with the use of radioimmunoassay (RIA) to measure fecal concentrations of progesterone and visual estrus manifestation. Fecal samples were collected from non-pregnant sambar deer hinds (aged 2–4 years)over the period of six months on daily basis, both during breeding and non-breeding seasons. Estrous cycles were recorded in non-pregnant females, based on fecal progesterone concentrations. The average estrous cycle length was 26.1±2.08 days (mean ± SEM) and 2.10 ± 0.51 days in the inter-luteal phase.The average fecal progesterone concentrations attained the peak mid-luteal values of 2.74 ng mL^{-1}. There appeared to be variation in fecal progesterone amplitude between animals and between dates, but the low frequency of sampling prohibited confirmation of trends. Behavioral estrus was detected only when the average progesterone concentrations were less than 0.07 ng mL–1. However, not all periods of depressed progesterone secretion were associated with the observed estrus. Behavioral estrus was detected in hinds when progesterone concentrations were less than 0.07 ng mL–1; a subsequent rise in progesterone indicated ovulation taking place at this time. ***Keywords:*** *Sambar deer hinds; estrus, progesterone; estrous cycle, Sri Lanka.*

МОНИТОРИНГ ВОСПРОИЗВОДСТВА И ВЛИЯНИЯ ФЕКАЛЬНОГО ПРОГЕСТЕРОНА НА РЕПРОДУКТИВНУЮ ЦИКЛИЧНОСТЬ ЦИКЛОВ САМБАРСКОГО ОЛЕНЯ ШРИ-ЛАНКИ (RUSA UNICOLOR UNICOLOR)

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В этом исследовании изучается продолжительность эстрального цикла у 16 самбарских оленей в Национальных зоологических садах в Дехивала и Кегалле, Шри-Ланка (NZGDK), оцениваемая с использованием изменений концентрации прогестерона, а также изменений профиля этого гормона, и визуальные проявления течки. Цели настоящего исследования состояли в том, чтобы охарактеризовать активность яичников

в течение эстрального цикла и небеременной лютеиновой фазы оленей самбара в неволе в Шри-Ланке. Эти цели были достигнуты с помощью радиоиммуноанализа (РИА) для измерения фекальных концентраций прогестерона и визуальных проявлений течки. Образцы фекалий были собраны у небеременных самок оленей самбар (в возрасте 2–4 лет) в течение шести месяцев ежедневно, в том числе в периоды размножения и отсутствия размножения. У самок регистрировали эстральные циклы небеременных особей на основании концентраций прогестерона в кале, их средняя продолжительность эстрального цикла составляла 26,1 ± 2,08 дня (среднее значение ± стандартная ошибка среднего) и 2,10 ± 0,51 дня в межлютеиновой фазе. Средние концентрации прогестерона в кале достигли пикового средне-лютеинового значения 2,74 нг / мл. Оказалось, что амплитуда прогестерона в фекалиях варьируется между животными и между датами, но низкая частота отбора проб не позволяла подтвердить тенденции. Поведенческий эструс выявлялся только тогда, когда средняя концентрация прогестерона была менее 0,07 нг / мл. Однако не все периоды пониженной секреции прогестерона были связаны с наблюдаемой течкой. Поведенческая течка выявлялась у самок, когда концентрация прогестерона была менее 0,07 нг / мл; последующее повышение прогестерона указывало на то, что в это время произошла овуляция. ***Ключевые слова:*** *самка самбарского оленя, течка, прогестерон, половой цикл, Шри-Ланка.*

Introduction. A standard program was needed for the conservation of the deer population at a manageable level in Sri Lanka [1]. Conservation in situ located in Sri Lanka's National Zoological Gardens with the collection of sambar deer and individual bucks encloses would be a good example of a controlled domestication process. Domestication program by performing intensive management such as cutting and carrying system feeding, tracking sexual activity, ongoing health examination, and disease prevention including well-supplied drugs and additive supplements (vitamins) can ensure basic daily welfare required for a safe sambar deer life [2]. For long-term goals, such efforts will avoid the extinction of the preserved species [2, 3].

In Sambar deer the reproductive status was ordinarily monitored by visual estrus manifestation such as apparent reddening and external genital swelling [4]. Currently, sambar deer breeding management is primarily based on observation and; however, behavioral data may not accurately reflect endocrine status, and there is a lack of accurate and precise physiological data concerning estrus and pregnancy tracking in this species [4, 5]. A series of studies have shown that steroid hormones in wild animal feces can be used to determine cyclicity and pregnancy with the development of the fecal steroid measuring technology [6, 7]. Other studies have shown, however, that estradiol (E2) is not expressed reliably in some species ' feces and urine, and thus this method cannot always be used to accurately determine reproductive physiology status [8, 20, 21].

So far there is only a limited number of researches have been performed using physiological parameters to investigate the estrous cycle and pregnancy in Sambar deer hinds. Several studies have used levels of fecal progesterone to effectively describe the estrous cycle in several types of cervids such as moose *(Alces alces)[9]*, reindeer *(Rangifer tarandus)*[10], elk*(Cervus canadensis)*[11], sika deer *(Cervus nippon)* [12]; brown brocket deer (*Mazama gouazoubira*) [12]; and Okapi (*Okapia johnstoni) [13]*. The present study, therefore, examined the estrous cycle and pregnancy status of captive Sambar deer hinds by assessing the rates of fecal steroid hormones (progesterone) to assess whether this approach can be used effectively in this species to control the breeding.

Materials and Method. Selection of Animals. For the experiment, 20 individually identified hinds in enclosures were selected from the National zoological gardens in Dehiwala and Kegalle (NZGDK). Selected hinds were individually identified by identifying names and codes given by NZGDK. Selected hinds estrous cycle, visual estrous manifestation, gestation period, lactation period, and the behavior and mating period of the bucks were recorded [14].

At the beginning of the experiment, 16 adult hinds and 3 adult bucks with hard antlers were in the same encloses of NZGDK. Selected sambar deer belonged to ~6–8-year old and the selected bucks were with auditory and olfactory contact with conspecific bucks and hinds. Selected sambar deer in enclosers were exposed to normal fluctuations in photoperiod. They were fed ad libitum with a diet in NZGDK and water was available in the enclosure all the time.

Observation of Lengths of the estrous cycle, gestation, Calving interval

The collected reproductive records included the reproductive parameters such as length of the estrous cycle and gestation period, calving interval, the mating, and calving dates and the weaning dates [4]. The length of the estrous cycle was detected by visual estrous manifestation and analysis of fecal progesterone [13–15]. Gestation was described as the intervals, respectively, between two successive matings [16]. The final day of mating and the date of calving was considered as the gestation period [16, 17]. Successful mating behaviors were displayed by eight individual hinds over 2.6 years and a total number of eight calves were born. The Interval of calving was calculated as the time between two consecutive calvings [17, 18]. The period of lactation was considered to be the time from calving to natural weaning [19].

Observation of visual estrus manifestations

Estrous manifestation of16 hinds in NZGDK was observed. Hinds' visual estrus manifestations included the apparent reddening and swelling of the external genitalia, loss of appetite, and a natural tendency of the hind to approach the buck [19]. Each estrus manifestation was recorded on a standard sheet twice a day from August 2019 to April 2020. During this period both the locations (NZGDK) were undergone for the dry

season and a rainy season. Sexual behaviors of the buck included vocalization, flehmen, penile erection (excluding copulation), chasing, mounting, copulation, and fighting [4].

Fecal Sample Collection

Fecal samples were collected twice a day over six months within half an hour of voidance from sambar deer hinds enclosed in NZGDK. Fecal samples were collected from 16 individually identified hinds. Selected hinds were ~6–8 years old and during the selection of hinds pregnant hinds were excluded. To identify the age records in NZGDK were used. Collected fecal pellets were labeled with the name based on individual hind identification, and collected samples were kept in ziplock bags. Ziplock bags with collected fecal pellets were stored in an icebox as soon as they were collected from the field and transferred in to -20^{o}C storage until extraction and submission to the Radioimmunoassay (RIA) analysis [7].

progestogen extractions and radioimmunoassay

Frozen fecal specimens were dried in a standard oven, and each sample was carefully powdered and blended. A subsample weighing 0.2 g was mixed in a test tube with 5 ml of 90% ethanol and briefly vortexed. Tubes were then boiled in a water bath (90° C) for 20 minutes, adding ethanol to prevent dry cooking [7, 15]. The extract was preboiled with 90% ethanol and centrifuged for 20 minutes at 1500 rpm. The extraction was then poured into another storage vial, and a further 5 ml of 90% ethanol was added to the remaining fecal powder and vortexed for 30 seconds and centrifuged for 15 minutes at 1500 rpm. Combined and dried down, the first and second extracts were reconstituted in one ml of methanol and vortexed for a short time. The methanol samples were placed at -20° C until the RIA assessment using the PROGESTERONE [I-125] RIA KIT (Ref: RK-460CT) .

Results. Length of the estrous cycle, gestation, and Calving interval

Based on the complete observations of 23 estrous cycles recorded in 12 individual adult sambar deer hinds have found a mean estrous length of 18.36 ± 0.96 days (Figure 1). Estrous cycles were observed all year-round and included dry seasons and rainy seasons of the year. Adult sambar deer hinds mean gestation period was 260.6 ± 13.48 days (Figure 1). Data were recorded for eight individual calving occasions, calving time, and the mean calving interval was 297 ± 19.31 days (Figure 1).

Lactation period

A lactation cycle was observed in eight individual adult hinds during 2.6 years (January 1^{st,} 2018 to July 1st, 2020). The mean number of days of lactation was taken by the period from calving to weaning, and that was taken as 85.67 ± 5.02 days (Figure 1).

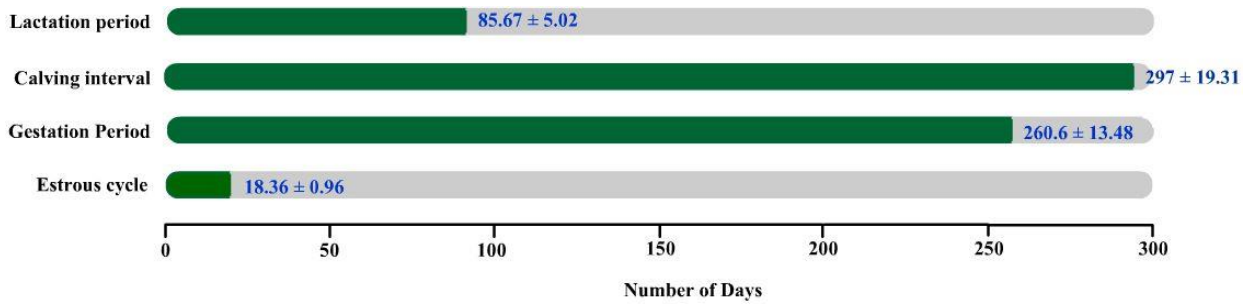

Figure 1 -Average reproductive parameters ± SD of sambar deer hinds in captivity

Estrous cycle

Individual visual estrus of a sambar deer hinds was observed daily throughout this study period. The mean number of days that visual estrous was observed was 2 ± 0.52 (Table 1). In this study, on the day of her visual estrus manifestation (apparent reddening and swelling of the external genitalia), stags approach the and mounting behavior was displayed. One important characteristics of tropical deer is that they can breed throughout the year. The findings of this study indicated that breeding of sambar deer in Sri Lanka (*Rusa unicolor unicolor*) may take place at any time of the year. The results are covering a dry season and a rainy season. This is in compliance with a previous finding of Chan *et al*. (2009) in Forman Sambar deer *(Cervus unicolor swinhoei)*.

Table 1 - The length of the estrous cycle (days) and visual estrus (days) in individual sambar deer hinds in captivity from 1st of August 2019 to 1st of May 2020

Visual Estrous	Hinds								Mean ± SD
	A	B	C	D	E	F	G	H	
1-2	1	2	2	2	1	2	1	2	2 ± 0.52
2-3	1	2	2	1	2	2	2	1	
3-4	2	2	1	2	2	1	2	1	
5-6	1	2	2	2	1	1	2	2	
6-7	1	2	2	2	1	2	1	2	

In all months of the year stag mounting on hinds was observed, indicating that the attending stag had mated with adult hinds. Figure 2 shows mean descriptive profiles of fecal progesterone of 15 hinds individually identified from DKNZG. Mean progesterone concentration of the selected hinds (n=15) increases to a peak of 1.2 ± 0.02 ng g^{-1} around day 13. The mean minimum progesterone concentration recorded limiting adjoining cycles was 0.11 ± 0.02 ng g^{-1}. The mean basal level during the lowest value between the two peaks was 0.1 ± 0.02 ng g^{-1} (Figure 2). To start the progesterone concentration from 0.12 ± 0.01 ng g^{-1} at day 1 and to decline to 0.2 ± 0.03 ng g^{-1} it was taken 18.36 ± 0.96 days (Figure 2 and Figure 3).

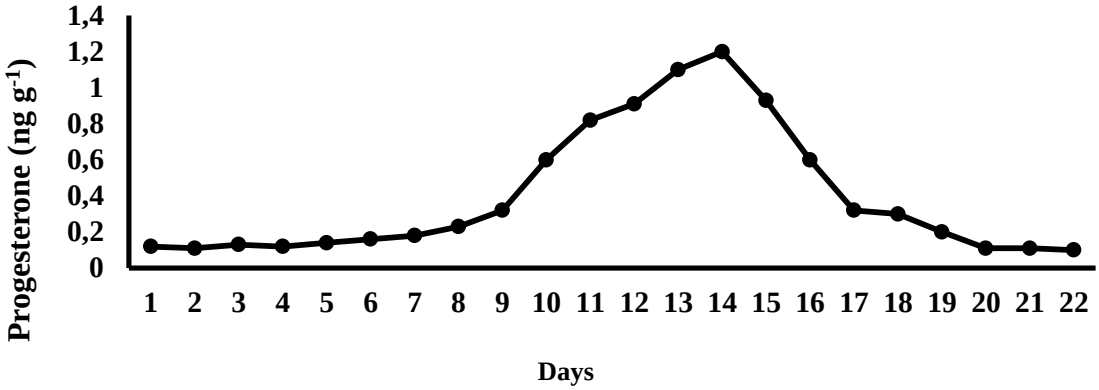

Figure 2 - Profil of mean Fecal progesterone values (ng g^{-1}) during the estrous cycle

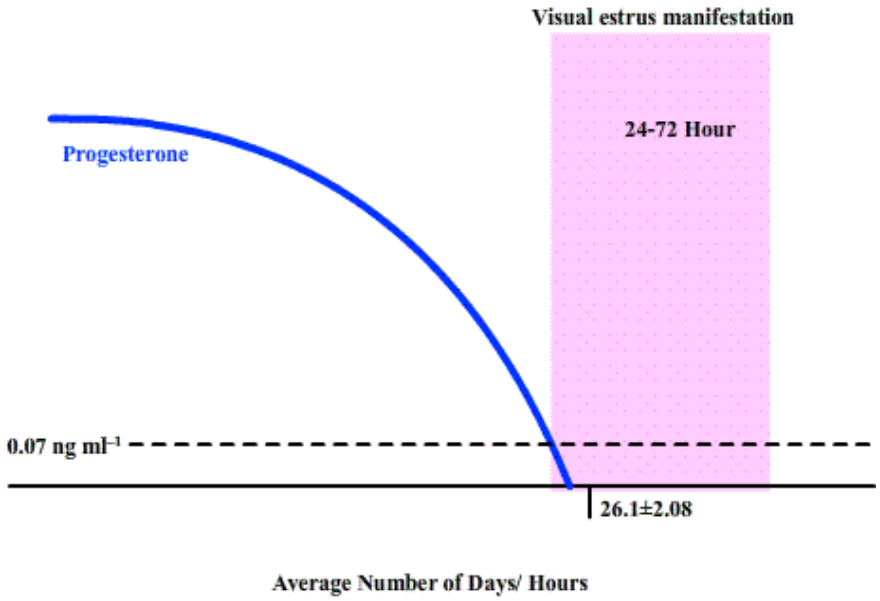

Figure 3 - Fecal progesterone concentration and visual estrus manifestation

Discussion. The length of the estrous cycle can be roughly divided into two forms in many other cervid species: a short cycle of 10-12 days and a longer cycle of 18-25 days [20]. Scientific proof of intermittent elevations of progesterone levels associated with corpus luteum indicated that at the beginning of the breeding season [21]. silent ovulation regular follows in cervids such as fallow deer, Alaskan reindeer, Formosan sika deer, red deer, and wapiti deer in the estrous cycle. During the breeding season, some cervids, such as red deer, fallow deer, reindeer, Pere David's deer, and Eld's deer, had an estrous period ranging from 19.5 to 22.4 days, showing that this pattern could be linked to their affable features [22]. similarly according to our findings, sambar deer in Sri Lanka had a typical pattern of estrous cycle lengths in comparison to larger body species such as wapiti, red deer, and fallow deer. It was proposed that sambar deer stags could suit throughout each month of the year, resulting in calving occurring almost throughout the year. Nevertheless, according to findings from HPNP Sri Lanka, the hard antler stag production was seasonal [7].

Stags presence with a rough antler has a positive effect on the beginning of the breeding season and the period of the postpartum hinds. The pattern of the estrous cycle in hinds may have been preferred throughout the year and mating may occur through the presence of hard antler in stags [2, 7]. The mean length of the estrous cycle in sambar hinds, based on plasma progesterone concentration, is closely associated with an earlier estimation of luteal cycle length in sambar deer [5]. The mean length of gestation of 260.6 ± 13.48 days was compatible with Semiadi *et al.*,1994 who proposed that sambar deer gestation

ranged from 249 to 284 days. Mean calving interval of 297 ± 19.31 days in this study was obtained from eight individual hinds but previous findings of sambar deer hinds calving interval were 329 29.7 days.

According to the findings of chan *et al*. 2009, the total average of estrus length and the estrous cycle was 2.00 ± 0.41 days (range = 1–3 days, n = 8) and 25.00 ± 5.22 days (range = 18– 37 days,n = 4), respectively, estrus length of a hind in this study was last for 24 to 72 hours every ~25 days in 4 consecutive cycles. It is agreed to a previous study that generally hind was in heat for 24 hours every 28 days for 2 to 3 consecutive cycles, 24 to 48 hours every 20 days in Java Rusa deer *(Rusa timorensis)* and 20 to 25 hours every 17– 18 Calamian deer *(Axis calamianensis)* In this study, there were four visual estrus manifestations, such as the apparent reddening, swelling of external genitals, loss of appetite and the doe's natural tendency to approach the buck.

Conclusion. It can be concluded that it was possible to assess the noninvasive estrous cycle of sambar deer in Sri Lanka by visual estrus manifestations and fecal progesterone extraction and Radioimmunoassay. There was no seasonal effect on hind-buck sexual behaviors during female natural estrus in them in-situ habitat. One of the important characteristics of Sri Lanka's sambar deer that we found from this experiment is that they can breed throughout the year, or in other words, they are polyestrous. A seasonal factor did not affect the manifestations of the estrus in hinds other than the loss of appetite. A natural tendency of hinds in approaching the buck is a sexual receptivity measure that follows estrus.

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