ANDROGEN MEDIATED BEHAVIOR OF SAMBAR DEER STAGS (*Cervus unicolor*) DURING RUT SEASON

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled "**ANDROGEN MEDIATED BEHAVIOR OF SAMBAR DEER STAGS *(Cervus unicolor)* DURING RUT SEASON**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,

13. 04. 10

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CERTIFICATE

Certified that this thesis, entitled **ANDROGEN MEDIATED BEHAVIOR OF SAMBAR DEER STAGS *(Cervus unicolor)* DURING RUT SEASON** is a record of research work done independently by V. Vishnu Savanth under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Among the Indian ungulates none has adapted itself to a wider variety of forest types and environmental conditions than the sambar (Schaller, 1967). It is the largest Indian deer and bears the grandest horns. They belong to the family Cervidae under the order Artiodactyla. The race *C. u. unicolor* is from Ceylon; the Malay race *C. u. equinus* extends from Assam eastwards and the Indian race *Cervus unicolor niger* is exclusively confined to India. Sambar deer in Hindi is called as Sambar or Samar; in Marathi Sambar; in Tamil Kudoo marn; in Malayalam Kullay marn; in Kannada Kudawe or Kuddama; in Burma Sut and in Oriya Sambar (Mohanty, 2005).

These deer are found in habitats ranging from tropical seasonal forests (tropical dry forests and seasonal moist evergreen forests), subtropical mixed forests to tropical rainforests. Their range covers a vast majority of territory that is classified as tropical rainforest, but their densities are probably very low there.

The Indian sambar inhabits much of southern Asia (as far north as the south-facing slopes of the Himalayan Mountains), mainland Southeast Asia (Burma, Thailand, Indochina, the Malay Peninsula), southern China (including Hainan Island), Taiwan, and the islands of Sumatra and Borneo in Indonesia. This deer has been seen congregating in large herds in protected areas such as national parks and reserves in India, Sri Lanka, and Thailand. The subspecies of Indian sambar in India and Sri Lanka are the largest of the genus with the largest antlers both in size and in body proportions. The South China sambar of Southern China and mainland Southeast Asia is probably second in terms of size with slightly smaller antlers than the Indian sambar. The Sumatran sambar, that inhabit the Malay Peninsula and Sumatra, and the Bornean sambar seem to have the smallest antlers in proportion to their body size. The Formosan sambar is the smallest *Cervus unicolor* with antler-body proportions more similar to the South China sambar.

The Indian sambar is a strongly built and large species of deer. Males are larger than the females. The coat is long, thick and dark brown in color with chestnut marks on the rump and underparts. The females are lighter in tone in comparison with the males. Old stags tend to become very dark in course of time. During hot weather, much of the hair is shed. Males have a thick mane of hair around the neck. Sambar stags are heavily built and are known to weigh up to 300 kgs and can grow to a height ranging from 135 - 150 cms at the shoulders. Sambars have the largest and the best developed facial glands. The large, rugged antlers are typically rusine, the brow tines being simple and the beams forked at the tip. Sambars of central India appear to be larger than those found in other regions. The male members of this species have antlers that can grow to a length of 90-95cms. These animals have a life expectancy ranging between 16 - 20 years.

Sambar deer are more solitary in their ways than other species of deer, mostly living alone, or in very small groups. They are mostly nocturnal. So they feed mainly at night and retire into dense forest at daybreak and do not usually come out till dusk. It is, therefore, very difficult to locate these animals during day time in dense forest. Their diet is mostly composed of grass, sprigs, fruits and bamboo buds. They can swim with the body submerged, only the face and antlers left exposed over the water surface. The sambar has extremely sharp senses of hearing and smell enabling them to easily detect predators. When alarmed they have a repetitive honking alarm call. When fleeing they raise their tails exposing the white underside. Sambars have a number of predators, mainly the tiger.

The rut takes place between October and November in most parts of India, even though there have been reports suggesting the occurrence of rut to be spread about seven months of the year (Schaller, 1967). The exact timing of the rut and birth of young depends on the location the deer lives in. Males establish territories, which they defend from rivals. They pair up with females who visit their territory. During the rut the males have a loud bellowing rutting call, which is quite distinctive. The gestation period is about eight months, after which normally a single unspotted calf is born.

In males the antler cycle is initiated by velvet antler formation. The antlers commence to grow in May and are in velvet during the rains and clear of velvet by November. In this stage thickly furred skin (velvet) will develop over the seal of the antler. This stage is found to be steaming up stage of males, in which they gain more body weight and do not show any breeding activities. When the antlers are in the velvet stage of their growth, the sambar move into more open habitats such as woodland glades and clearings so as to avoid any injury to the antler. This stage lasts for a few months and after the shedding of velvet it culminates in the breeding stage. Such males will have hardened antlers and will be emitting loud bellows while going after the receptive females of the herd. Each stag's harem is limited to a few hinds. After the rut, he deserts them and leads a solitary life till the return of the mating season. The young are born at the commencement of the monsoon in late May or early June. Young stags remain with the hinds. Each buck seems to have its own breeding cycle which may not be synchronized with the other bucks in the herd.

Stags show overt seasonal fluctuation in the testosterone level in synchronization with their reproductive cycle and their antler stages. Behavioral amendments are also seen accompanying the above mentioned changes (Pereira *et al.*, 2005).

The use of medical diagnostics on feces to determine an individual's internal physical condition is not a new science. However, only recently has the connection been made that feces contain hormones that have been eliminated from the circulation, which can provide insights into the internal physiology of an individual (Ziegler and Wittwer, 2005). Reproductive steroid hormone levels provide important information regarding the reproductive status of animals (Washburn *et al.*, 2004). Monitoring hormone profiles through the use of fecal steroid assays offers a noninvasive and potentially long-term means of assessing adrenocortical, testicular, and ovarian hormones in many vertebrate taxa. Because feces can be collected without capturing or interfering with animals, methods that use fecal steroid metabolites have become increasingly popular in both captive

and free-ranging studies. In combination with behavioral observations, researchers are now able to investigate many of the proximate physiological mechanisms that underlie animal behavior (Beehner and Whitten, 2004).

Behavioural studies are of great importance in increasing our understanding and appreciation of animals. In addition to providing knowledge about the complexity and density of behaviour, such studies also provide information crucial to improvements in the welfare of animals maintained in captivity. In this respect, development of ethogram and behavioural score which is associated with the androgen level can help a lot in interpreting complex behaviour of the males of this species (Roshin, 2005).

The sambar deer population is beyond manageable numbers in most of the Indian zoos. Since the animals maintained at the captivity are at no short of food at any time of the year, and that the threat of predators in zilch, the seasonality aspect of reproduction is far gone. They have now adapted to breed throughout the year, thus letting the population go out of control. This brings in space and economic constraints to maintain them in captivity. Published data regarding the breeding in deer, especially sambar deer, are hardly any. Therefore, this study was carried out to shed light on certain facets of sambar deer breeding so that strategies can be worked out to find out ways to curtail the exploding population. Some of the main objectives taken up for the study were to:

1) Identify the stags in order of hierarchy.
2) Observe and prepare a complete ethogram of sambar deer stags in rut season and correlate it with fecal testosterone levels.
3) Assess the morphological changes, breeding performance, preferences and frequencies of the alpha to lower order males.

Review of Literature

2. REVIEW OF LITERATURE

Deer are hoofed, ruminant mammals in the Cervidae family (order Artiodactyl) and are among the most graceful and attractive of animals. This family consists of 17 genera and about 53 species. Deer are native to all parts of the world except Antarctica, Australia, central and southern Africa and Madagascar, and have adapted to virtually every land habitat, from dry deserts to woodlands, prairies, marshes and Arctic regions. Deer are the only animals that grow antlers, which are composed of skin, nerves, blood vessels, fibrous tissue, cartilage and bone, and thus should not be confused with horns, which are a keratinized tissue that grow from their base under the control of underlying mesenchymal cells. Except for the reindeer (*Rangifer tarandus*), antlers develop only in male deer and in most species this occurs in the spring of the animal's second year of life (Price *et al.*, 2005).

2.1 SEASONALITY OF DEER BREEDING

Webster *et al.* (1991) reported that reproductive seasonality in red deer stags is due to the entrainment of an endogenous circannual rhythm by photoperiod and that this entrainment is mediated via melatonin secretion from the pineal gland.

Sambar deer in New Zealand as per Semiadi *et al.* (1994) were in velvet antler between January and April (mean = 125 days; SD = 22.6 days), and in hard antler between May and November (mean = 231 days; SD = 40.0 days), during which time rutting behavior was observed. He also claimed that the reproductive pattern of tropical deer is not strongly linked to day length.

The experiment conducted by Rolf and Fischer (1996) provided evidence that in fallow bucks the reproductive system is under strict photoperiodic control

and directly and/or indirectly dictates the course of the antler cycle via gonadal hormones.

Raman (1997) affirmed that chital density and group size measures show significant seasonal and monthly changes that are closely related to monthly rainfall and type of habitat.

Seasonality is an adaptive response of animals to their environment, which changes cyclically with seasons. Animals use annual changes in seasonal variables such as photoperiod, temperature, rainfall and food availability to regulate physiological functions such as reproduction, migration, coat change, hair or feather growth, voluntary food intake, milk production, behavior and diapauses (Garcia *et al.*, 2002).

In a study conducted by Willard and Randel (2002) on a herd of spotted deer, irrespective of seasonal fluctuations in testicular and epididymal sperm content, at no time were stags found to be azoospermic. Testis weight, total epididymal weight, total testis sperm content and total epididymal sperm content were greater in mature hard antlered than in velvet/no antlered stags. These data indicate that while seasonal changes in testis sperm content and morphology do occur and pattern antler cycles in the axis stag, these changes are less pronounced than in more temperate species of cervids.

Matsuura *et al.* (2004) professed that because of the difficulty of knowing exact conception dates in free- ranging deer, most studies use estimates derived from parturition dates of fetal age, based on the premise that gestation periods are consistent among females.

Acharjyo *et al.* (2005) studied the births of mouse deer in Indian zoos and suggested that this species has no fixed birth/breeding season in captivity.

Flores *et al.* (2005) observed that the duration of rut season lasted for 50.8 and 71.2 days respectively at two different red deer farms. He also stated that, on both farms, deer had kept their characteristic reproductive seasonality.

Bubenik (2006) averred that the antler cycle in male deer is closely associated with the photoperiodic regulation of its reproduction. A cascade of events involving several hormones such as melatonin, prolactin, luteinizing hormone and testosterone mediates the primary effect of the photoperiod. The pineal gland serves as a neuroendocrine transducer of circadian and circannual variation of photoperiod, producing melatonin in response to the onset of darkness.

Asher (2007) emphasized that entrainment of seasonal reproduction cycles of temperate cervid species is affected by endogenous recognition of photoperiodic changes, with a majority of species initiating mating activities during decreasing day length of late summer and autumn. He also reported that equatorial region cervids are completely aseasonal.

Haigh (2007) opined that all the physiological events of the reproductive cycle in wapiti and red deer are tied to the gestation length so that calf is born at a specific time to avoid inclement weather and to ensure plenty of high quality forage.

White tailed deer exhibit seasonal reproductive cycle as reported by Jacobson (2007).

Mulley (2007) and Morse and Miller (2009) reported that fallow deer had a seasonal breeding cycle and peak rut occurred in early to middle October.

In pampas deer populations inhabiting subtropical to temperate, births can occur all year round and the peak roughly coinciding with pasture abundance as claimed by Ungerfeld *et al.* (2008 a).

In Hangul deer, according to Bhat *et al.* (2009) rutting activity began by late September and extended up to the first week of November. The peak of the rut was from October 9 to 20 after which it faded out.

2.2 ANTLERS

2.2.1 Antler Characteristics

For most species of deer, males start the growth of antlers form a pedicel on each frontal bone in late spring or early summer. The velvet, a true skin which supplies the growing antler with nutrient material, is shed in the fall and normally takes only a day or so to peel off. Antlers remain hard until they are cast after the period of rut, which normally occurs in late fall or early winter (Riney, 1954).

Jaczewski *et al.* (2004) stated that in red deer stags, the full antler cycle lasted from 355 to 373 days. The soft and the hard antler phases lasted 142 - 161 and 205 - 223 days, respectively.

As per Price and Allen (2004) deer antlers are the only mammalian appendages capable of repeated rounds of regeneration; every year they are shed and regrow from a blastema into large branched structures of cartilage and bone that are used for fighting and display. In the case of some species they even represent the fastest rate of organ growth in the animal kingdom.

Colitti *et al.* (2005) reported that antlers elongate by a modified endochondral ossification process while intramembranous ossification takes place concurrently around the antler shaft.

Dimijian (2005) opined that deer antlers confer greater competitive ability on their owners.

Malo *et al.* (2005) emphasized that male red deer antlers could signal to other males not only their competitive ability at the behavioral level (fighting ability) but also at the physiological level (sperm competition).

Price *et al.* (2005) professed that each spring, deer shed antlers that were used for fighting and display during the previous mating season. Their loss is triggered by a fall in circulating testosterone levels, a hormonal change that is linked to an increase in day length. The subsequent re-growth of antlers during the spring and summer months is spectacular and represents one of the fastest rates of organogenesis in the animal kingdom. As androgen concentrations rise in late summer, longitudinal growth stops, the skin (velvet) covering the antler is lost and antlers are 'polished' in preparation for the mating season. He also reported that the antlers of a 200-kg adult red deer may weigh as much as 30 kg but take only 3 months to grow.

Bartos and Bahbouh (2006) concluded that in contrast to antler size, fluctuating assymetry is unlikely to play any significant role in sexual selection as an indicator of individual quality in red deer.

In their study with Iberian red deer, Gomez *et al.* (2006 a) found that high percentage of milk protein resulted in increased antler weight or length. Results also suggested that body growth during this early stage might affect antler size positively through growth precocity. Thus, males that reached a higher weight at weaning also developed antlers earlier. Antlers that developed earlier were also heavier and had a greater base perimeter.

Asher *et al.* (2007) avowed that in wapiti and red deer, during the period in which antlers are in velvet, stags do not normally engage in any sexual behavior.

During rut, stags become extremely aggressive toward one another. Antler rubbing/ thrashing serve to both mark territory and remove the dried velvet tissue on the surface of the hard antler. He also opined that rubbing of the neck is probably a form of scent marking.

In roe deer, antler size is an honest signal of male phenotypic quality and may be used as a cue for rival males and females to assess male fighting ability, sexual vigor and/or phenotypic quality (Vanpe, 2007).

The observations done by Ungerfeld *et al.* (2008 b) on pampas deer bucks illustrated that the brow and the trez tine were first observed 22.8 ± 0.6 and 45.9 ± 0.9 days after the first antler cast. Velvet shedding was observed 103.3 ± 2.1 days after the first antler cast. Both antlers casts were observed earlier in adult cycle bucks than in first antler bucks, although the interval between both casts was not different. The interval from the brow tine observation to the trez tine observation was shorter in first antler cycle bucks than in adults.

According to Morrow *et al.* (2009) antlers are appendages of the skull, composed of a solid, bony core and are unique in that they undergo an annual cycle of rapid growth in preparation for the breeding season (rut) and are cast after the rut, females generally do not grow antlers (reindeer are the only exception).

Ungerfeld *et al.* (2009) averred that female contact stimulated male pampas deer, increasing antler mass, size and darkness, as well as possibly hard antler period length.

2.2.2 Seasonality of Antler Growth

Moore (1938) described antler growth in deer as manifestation of the rut which follows a seasonal cycle controlled by the testicular hormones mainly.

Deer living near the equator, where seasonal day length changes are slight, tend to be asynchronous in both their antler and sexual cycles (Brown *et al.*, 1978).

Loudon and Curlewis (1988) in a herd of spotted deer found only little evidence of a clear seasonal synchrony in the antler cycle.

Jaczewski *et al.* (2004) asserted that annual antler cycles in the cervidae species of the temperate climate are closely associated with the length of day. The annual antler cycle consists of soft and hard phase. In red deer, the soft phase begins in February/March. It encompasses antler growth and mineralization and is ended by velvet shedding. Velvet shedding is followed by the hard phase of the antler cycle, which, in red deer starts around the end of July. At that time a period of increased reproductive activity begins. In this species, the peak of mating season takes place in September- October. The hard phase of the antler cycle lasts until March and is followed by antler casting.

Pereira *et al.* (2005) declared that pampas deer stags exhibited a seasonal cycle that modulated sexual behavior and the antler cycle.

Bubenik (2006) averred that the antler cycle in male deer is closely associated with the photoperiodic regulation of its reproduction. In most boreal cervids antlers grow during the late spring and early summer, mineralize before the rut and are cast thereafter.

2.3 PHYSICAL CHANGES

2.3.1 Weight Gain

Asher *et al.* (1987) reported that fallow deer bucks exhibited pronounced liveweight gains over spring and summer months, to reach a peak mean weight and rapid liveweight losses over the rutting period with a minimum mean liveweight. Mean neck girth and serum testosterone levels increased during late

summer and peaked at before the onset of the rut. Thereafter both measures declined during winter and spring months.

Monfort *et al.* (1993) reported that antler length, body weight and chest girth were maximal during pre-rut in Eld's deer.

Vanpe (2007) confirmed that in roe deer, male body mass and antler size positively affect male breeding success.

2.3.2 Other Changes

In fallow deer the weights of the testes and epididymides reach a maximum in October and November at the time of the rut and fall to an appreciably lower value in the spring and summer at the time of minimal sexual activity as reported by Chapman and Chapman (1970) and Chaplin and White (1972).

Male secondary sexual characteristics, including swelling of the neck musculature, growth of the neck mane and development of the rutting odour in the urine, were all more advanced in the melatonin-treated red deer stags (Lincoln *et al.*, 1984).

Loudon and Curlewis (1988) declared that in a herd of spotted deer there was a fixed relationship between stage of the antler cycle and testis diameter; minimum testis diameter occurred 1-2 months after antler casting whereas maximum testis diameter occurred when stags were in hard antler. Changes in body weight, circumference of the neck and plasma testosterone concentrations largely paralleled those of testis diameter.

Asher *et al.* (1989) affirmed that adult entire fallow bucks demonstrate marked seasonal variation in LH and testosterone secretion. The dynamic interaction between these two hormones causes the profound changes in testis

size, neck girth and liveweight associated with the highly seasonal pattern of breeding in this species.

Gosch and Fischer (1989) reported that in fallow deer maximum testicular size occurs just before rut commences and minimum size was recorded when antlers started to regrow.

Monfort *et al.* (1993) observed maximal scrotal circumference and combined testes volume in mid-winter, whereas peak neck girth and behavioral aggression occurred three months later in the case of Eld's deer.

Blottner *et al.* (1996), Roelants *et al.* (2002) and Wagener *et al.* (2010) reported that adult roe deer males show seasonal cycles of testicular growth and involution. The exact timing of these cycles requires endocrine regulation and local testicular control by autocrine/ paracrine factors.

Georitz *et al.* (2003) found that most parts of the male reproductive tract showed distinct circannual changes in size and texture in roe deer stags. These changes were most pronounced in the testes, seminal vesicles and prostrate. All reproductive organs were highly developed during the rut only. The volume of ejaculates, total sperm number and percentage of motile and intact spermatozoa also showed a maximum during this period.

Gomez *et al.* (2006 b) claimed that in case of Iberian red deer neck circumference showed a time course reaching the highest values during the days of decreasing photoperiod.

Blake *et al.* (2007) observed that in reindeer, neck muscles thicken during the rut season.

Haigh (2007) stressed that the scrotal circumference increases markedly and peaks at about the same time of the onset of the rutting season in wapiti and red deer.

Jacobson (2007) avowed that as a seasonal breeder white tailed deer stags show annual changes in secondary sexual characteristics like increased body weight, swollen neck and development of large antlers.

2.4 ETHOLOGY

Lehner (1987) described focal animal sampling as a method which restricts data gathering for a sample period to one animal because of an interest in differences within and between individuals, sexes or ages. Also, focal-animal sampling can be employed when the behaviors occur too rapidly to record accurately data from several individuals. When necessary, sampling can be increased to focal pair (e.g., communication) or focal group (e.g., dominant-subordinate relationships). Any scale of measurement can be used in focal animal sampling.

Isvaran (2005) professed that behavioral variation in ungulate populations is an exciting area of research, which is likely to provide insights not only into the evolution of ungulate behavior but also, more generally, into the evolution of individual decision-making.

Vanpe (2007) opined that behavioral ecology is the study of the ecological and evolutionary basis for animal behavior and of the role of behavior in enabling an animal to adapt to its environment, enhancing its chances of survival and reproduction.

2.4.1 Behavioral Observations

Fighting behavior is sensitive to changes in the potential benefits of fighting: stags fight most frequently and most intensely where potential benefits are high and tend to avoid fighting with individuals they are unlikely to beat as observed by Clutton- Brock *et al.* (1979).

Ethogram was defined by Lehner (1987) as a set of terms and descriptions of the behaviors of an animal. It may be a comprehensive ethogram of all behaviors of a species or it may be for only one sex, age group or type of behavior.

In Eld's deer, Monfort *et al.* (1993) asserted that behavioral aggression scores were the lowest throughout the summer (July-September), but increased steadily thereafter until all males displayed peak and sustained aggressive behavior in spring.

Bowyer *et al.* (1994) hypothesized that the marking of trees by male moose late in rut attracts females not successfully bred during the peak rut.

Semiadi *et al.* (1994) avowed that rutting behavior in sambar stags was marked by wallowing, thrashing the ground with antlers, head-rubbing on posts and trees, and urinating on their head and antlers.

Adams *et al.* (2001) proposed that scent marking is an important component of rutting behavior in cervids and may be used for both male- male and male- female communication.

McElligott *et al.* (2002) in a study with male fallow deer found that reproducers had a greater chance of reproducing again in the following year than the non-reproducers.

Pelletier (2005) reported that all adult male bighorn rams spent less time feeding during the rut compared with the pre-rut.

Breeding behavior of male pampas deer during rut was characterized by predominately anogenital sniffing, flehmen, urine sniffing, chasing and mounting behavior as reported by Pereira *et al.* (2005).

As per Vannoni *et al.* (2005) in gregarious and polygynous deer, males compete intensely for females and show a highly developed vocal display during the breeding season.

Bhat *et al.* (2009) observed that the mature hangul stags, which may be found in small groups in summer, became intolerant of each other and separated during the rut. He also observed that the most conspicuous feature of rut was the reverberating resonant roaring calls by the stag.

2.4.2 Selecting the Dominant Male

Bowyer (1983) reported that differences in the mineral intake between dominant and subordinate individuals were related dominance hierarchies in elk.

Bartos (1980) observed that in red deer, the more dominant the stag, the earlier the antler casting occurred, but Forand *et al.* (1985) reported contradictorily that dominant white tailed bucks retained their antlers longer than the subordinates.

Krebs and Davies (1987) stated that the strongest individuals are despots, grabbing the best quality resources and forcing others into low quality areas or excluding them from the resource altogether.

Fraser and Broom (1997) avowed that reproductive success depends on fighting ability; the strongest stags are able to command the largest harems and enjoy the most copulations.

The results of the studies done by Komers *et al.* (1997), suggest that dominance rank is the most important factor in determining the level of reproductive behaviors exhibited.

Stewart *et al.* (2000) reported that body size and age are highly correlated with antler size, fighting ability and reproductive success in male cervids.

Li *et al.* (2001) affirmed that during early summer, Pere David's deer stags established their social rank by displaying, sparring, and fighting.

McElligott *et al.* (2001) asserted that body mass was related to prerut dominance rank which was in turn strongly related to rut dominance rank, and thus there was an indirect relationship between mating success and body mass.

Saseendran *et al.* (2003) confirmed that in a population of sambar deer, males were found to control the group led by alpha male. Alpha male was identified by its good physical appearance, sharp and long antlers, positioning at vantage points to take the major share of feed and its mates.

Price and Allen (2004) described antlers as undoubtedly one of nature's most dramatic and beautiful symbols of male strength and dominance.

Estep and Dewsbury (2005) reported that in species in which dominant hierarchies are formed either by males or by females, higher-order animals usually engage in more sexual behavior than lower order animals.

Blake *et al.* (2007) claimed that in reindeer, dominant males in free ranging system are generally between 6-10 years old with body size, antler size and antler branching correlated with dominance.

As per Mulley (2007), fallow deer bucks will fight vigorously during the pre rut to establish dominance.

Taillon and Cote (2007) quoted that achieving a high social rank may be advantageous for individuals at high population densities, because dominance status may determine the priority of access to limited resources and reduce individual loss of body mass. The establishment of dominance relationships between individuals involves variable levels of aggressiveness that can be influenced by resource availability.

Vanpe (2007) observed that in roe deer breeding chances tends to decline in relation to the loss of dominance.

Vanpe *et al.* (2008) opined that red deer follows dominance rank–based mating systems (e.g., harem holding, roving).

2.4.3 Harem Formation

As per Pemberton *et al.* (1992) both harem membership data and observations of mating and other estrous behavior can be used to identify males most likely to father a specific calf.

Observations made by Semiadi *et al.* (1994) indicated that although the dominant rutting sambar stag collected a harem, the dominant stag displayed a high degree of tolerance toward the presence of other stags in hard antler within the harem.

McElligott *et al.* (2001) reported that larger mature fallow bucks have advantages over other males when competing for matings.

Yoccoz *et al.* (2002) claimed that prime-aged males are most often the harem holders among red deer.

The study conducted by Bonenfant *et al.* (2004) on European red deer established that social segregation was the lowest during the rut (indicating aggregation).

In Chinese water deer, high levels of sniffing, parades and pursuits, concomitant of the highest concentrations of androgen, could allow the males to detect the furtive estrus in the females present in their territory. In adult males, the onset of androgen secretion, in October, was concomitant with the first manifestations of territoriality (Mauget *et al.*, 2007).

2.5 HORMONE ASSAY

2.5.1 Non- Invasive Techniques

Yamauchi *et al.* (1997) opined that the reproductive endocrine status of sika deer can be successfully monitored by means of fecal steroid analysis without disturbing the focused animals.

Yamauchi *et al.* (1999) asserted that blood sampling is sometimes extremely difficult in the wild species such as those of cervidae, because it often requires capturing and immobilizing of the target animal.

Monitoring gonadal status non- invasively through the analysis of fecal, salivary, or urinary steroid metabolites is an option. These approaches offer advantages in safety and ease of sample collection, and in general data are comparable to circulating hormone profiles (Brown, 2000).

Hamasaki *et al.* (2001) professed that in nontractable species such as cervids, blood sampling is impractical because it requires capture, immobilizations and/or restraint.

Beehner and Whitten (2004) emphasized that by extracting steroid metabolites from feces, researchers can track endocrine activity non- invasively in free- ranging animals. This method is particularly suited for behavioral research because it permits delays between sample collection and sample processing, thus allowing behavioral observations to continue.

Washburn *et al.* (2004) claimed that reproductive steroid hormone levels provide important information regarding the reproductive status of animals.

Pereira *et al.* (2005) averred that fecal steroid analysis is a practical and reliable non-invasive method for the evaluation of the endocrine status of free-ranging Pampas deer.

Ziegler and Wittwer (2005) avowed that fecal analysis techniques have allowed us to assess reproductive functions during seasonal changes in ovarian cycling, conception, pregnancy, and male fertility, and levels of glucocorticoids during reproductive functions and stress assessments. RIAs have been performed to quantify steroids since the 1960s. It is the most routine method available and is accurate and reproducible.

2.5.2 Testosterone Levels

Suzuki *et al.* (1992) found that testes size, seminiferous tubules and plasma testosterone concentrations showed conspicuous annual changes in Sika deer of Hokkaido, Japan.

As stated by Bubenik *et al.* (1996) in adult male pudu testosterone levels exhibited two, almost equal peaks; the first peak (2.8 ng/ml) was detected in March (rut) and the second one (2.7 ng/ml) in October (spring).

During his study on male white tailed deer Ditchkoff *et al.* (2001) observed that mean levels of serum testosterone increased during the breeding season until late October, approximately 1- 2 weeks before the peak of the rut.

Hamasaki *et al.* (2001) reported the highest levels of testosterone concentration for the most dominant sika deer stag compared with the subordinates.

As per Skinner and Harrington (2003) the mean testosterone concentration (ng/ml ± sd) in plasma from 16 territorial sika deer stags was 6.33 ± 0.57 (range 2.36 to 10.46), significantly higher ($p<0.001$) than in plasma from six nonterritorial stags, in which it was 1.17 ± 0.13.

2.5.3 Testosterone Vs Season

Brown *et al.* (1978) opined that in white tailed deer, androgen concentration show seasonal pattern with an annual low in May and a peak in late November.

Schams and Barth (1982) emphasized that in roe deer stags plasma values of testosterone began to increase during the second half of February and reached a plateau during April. The highest average levels of testosterone was observed before the start of the rutting season (mid-July to mid-August) and steadily declined thereafter.

Sempere *et al.* (1992) , Blottner *et al.* (1996) and Roelants *et al.* (2002) found that roe deer are seasonal breeders and the testosterone showed a maximum concentration during the rut.

Suttie *et al.* (1992) asserted that the rut or mating season in red deer stags is characterized by high mean testosterone concentrations.

The study conducted by Monfort *et al.* (1993) on Eld's deer showed that between mid-summer and mid-autumn, testosterone concentrations were low and increased about six fold above baseline values as winter approached.

Yamauchi *et al.* (1997) and Kameyama *et al.* (2002) suggested that testosterone secretion in adult male sika deer is mainly controlled by annual rhythm in the same manner as in other temperate cervids. They reported a peak in testosterone secretions during the months of September and October.

Hamasaki *et al.* (2001) observed higher levels of testosterone in male sika deer during the month of October.

Fecal testosterone concentrations peaked in December–January (summer), March (early autumn) and in August– September (winter–spring), with minimal values from April–July in male pampas deer (Pereira *et al.*, 2005).

Haigh (2007) declared that in wapiti and red deer, preceding the rut, testosterone concentrations, scrotal circumference and percentage of normal sperm increases sharply, reaching a peak just before the rut.

Umapathy *et al.* (2007) professed that in spotted deer, mean testicular volume, serum testosterone concentration, semen volume, sperm concentration, percentage of morphologically normal sperm, and percentage of motile sperm

were higher in hard antler deer (peaked from March to May) than in deer with velvet antlers or in deer in which the antler has been shed.

The seasonal onset of reproductive activity (rut) in stags is associated with rising circulating levels of gonadotropins and consequently testosterone secretion (Woodbury and Haigh, 2007).

2.5.4 Testosterone Vs Antler

Chapman and Chapman (1970) reported that in fallow deer, the antlers are cleaned of 'velvet' in August and September when testicular activity is increasing and are shed in the spring and summer when activity is at a minimum.

In adult roe deer, plasma androgen concentrations rose briefly in April and markedly in August which corresponded with the breeding period. The time of casting to maximum growth was about 60 days; mineralization of the antlers then lasted for 45 days only which was corresponding to the testicular activity (Sempere and Boissin, 1981).

Lincoln *et al.* (1982) , Yamauchi *et al.* (1997), Price and Allen (2004), Price *et al.* (2005), Gomez *et al.* (2006 b) and Ungerfeld *et al.* (2009) claimed that in most cervids, concentration of plasma testosterone was associated with the phases of antler development.

Suttie *et al.* (1984) asserted that pedicle initiation was associated with increasing plasma testosterone levels and antler development occurred when testosterone levels were low or decreasing. Cleaning of the velvet was associated with high levels of plasma testosterone. Antler casting occurred when plasma testosterone concentrations were low or undetectable.

According to Muir *et al.* (1988) and Bartos *et al.* (2009) in red deer stags, antler growth per day was primarily dependent on changes in testosterone concentration.

Chapman and Harris (1991) presented data that was equivocal as to the role of steroids in driving the antler cycle in Reeves' muntjac.

Suttie *et al.* (1992) provided a description of the ontogeny of the seasonal nadir in mean testosterone in red deer stags, which was believed to facilitate antler casting and antler regeneration.

Carrasco *et al.* (1997) assigned testicular atrophy as a possible etiology for deformed antlers and retained velvet in red deer.

Bartos *et al.* (2000) concluded that a plasma androgen concentration at least above a minimal threshold level is a necessary prerequisite for normal antler regrowth in fallow deer.

Pereira *et al.* (2005) found that there were significant correlations between fecal testosterone and antler phases and also claimed that antler casting and regrowth occurred under low testosterone concentrations, whereas velvet shedding was associated with high concentrations of testosterone.

Blake *et al.* (2007) reported that in reindeer, increasing levels of testosterone cause the antlers to harden and the velvet to split and shed.

Woodbury and Haigh (2007) avowed that antler growth cycles are closely related to sexual cycles in stags and are directly attributable to variations in seasonal photoperiod influencing gonadal steroidogenic activity. They reported that testosterone levels peak immediately before rut and it is the rapid decline in its level that causes antler casting. They also found that antler growth occurs at a low testosterone concentration and is seen increasing when the antler growth nears completion. Velvet shedding and antler hardening is a consequence of high testosterone levels as per their reports.

2.5.5 Testosterone Vs Behavior

Endocrine control results in stags being most sexually active and aggressive at the peak of the sexual cycle in October (the rut), with dead bony antlers which can function as weapons in inter-male rivalry (Lincoln *et al.*, 1984).

Bubenik *et al.* (1999) claimed that the testosterone levels were higher in dominant male pudu than in the subordinate ones.

Li *et al.* (2001) declared that in Pere David's deer there were statistically significant correlations between some male reproductive behaviors, such as anogenital sniffing, urine sniffing, urine spraying, wallowing, bellowing, antler adorning, antler swags mud, chasing, herding hinds, chin resting, mounting and copulating, with the fecal testosterone concentrations. These results suggested that seasonal reproductive behaviors in stags are strongly associated with circulating testosterone.

As per Pelletier *et al.* (2003), a study done on bighorn rams showed that fecal testosterone was directly correlated with social rank.

Skinner and Harrington (2003) reported that in a group of sika deer the activities directly associated with mating were significantly different ($p < 0.05$) between the territorial and non territorial groups (flehmen: $t = 3.495$, df: 3; mating calls: $t = 3.725$, df: 3; matings: $t = 3.992$, df: 3).

Asa (2005) opined that in general, androgens are responsible for reproductive behavior, ranging from aggression related to mate or territory defense to scent marking, courtship and copulation. Social factors can also cause an increase in hormone levels. Mating activity stimulates acute increase in testosterone.

Pereira *et al.* (2005) stated that there were significant correlations between fecal testosterone and reproductive behavior.

Roshin (2005) claimed that there exists a positive correlation between the breeding score and fecal testosterone in spotted deer in velvet stage, velvet shedding stage and rutting stage. But no correlation was seen during antler casting stage and antler growth stage.

Wildt (2005) stated that in the case of cervids the seasonal elevations in testosterone have been associated with increase in sexual activity, aggressiveness and testicular size. These factors were maximally coincident with the female cyclicity.

Blake *et al.* (2007) asserted that in reindeer, increasing levels of testosterone during the rut influences an increase of aggressive behavior towards other males, along with herding and chasing of females. The stags also show vocalization, scent marking, establishing dominance and urinating on its hind feet.

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out at the State Museum and Zoo, Thrissur, Kerala, India for a period of four months from 11th June to 11th October, 2009. The zoo was chosen for the execution of the study because of the large number of sambar deer maintained there. There were a total of 70 sambar deer in the enclosure, during the commencement of the study, of which 22 were males including 16 adult stags.

3.1. SELECTION OF THE SEASON OF STUDY

The birth registers maintained at the zoo were scanned prior to the study to find out the season during which maximum delivery was recorded. The timing of breeding activity was determined by deducting the length of gestation period from the date of delivery. As per the birth registers of past nineteen years, November-February appeared to be the period during which maximum number of delivery occurred, therefore the peak breeding season was found to be during March- June. But the recent years average shows that the breeding season tends to be a bit later than the past nineteen years average. There were also observations suggesting that breeding activity usually occurs during the cooler climates especially during the rainy season, hence the study was done integrating both the factors and was carried out from June to October, 2009.

3.2. IDENTIFICATION OF MALE POPULATON

A preliminary observation was carried out to rank the stags in the herd on a dominance hierarchy table. The characters that were taken into account were the ability of a stag to occupy vantage positions during the feeding time, lead a larger harem, capacity to aggressively dictate other members of the herd and carry out most of the breeding activities in the herd. The dominant stags appeared to be

physically more massive that the subordinate animals had more magnificent antlers and were slightly darker in shade.

Based on the above mentioned characteristics, top three stags in the rut/ hard antler stage, on a chronological order of dominance namely H_1, H_2 and H_3 were selected. Three more superior stags in late stages of velvet growth, which were expected to come into rut/ hard antler stage during the observation period were also selected and were named V_1, V_2 and V_3 as per descending order of dominance. Hence, a total of six animals were selected for the study.

3.3. BEHAVIOURAL OBSERVATION

The normal activities of the deer during the rut and non rut periods were observed before the study methodology was devised. An ethogram developed by Roshin, 2005 who did a similar work on spotted deer stags, was adapted and was modified slightly to accommodate the behavioral differences between the two species. The observations were carried out from 0600 to 1800 hours to go with the normal activity time schedule of the deer as well as the zoo. The twelve hours study period was divided into twelve segments of one hour duration each and each animal was allotted two such segments every week. The stag and the segment in which the observation is to be taken were selected randomly. The animals were observed from outside the enclosure not more than five meters away and each and every activity of the deer during that particular one hour period was recorded in the ethogram.

Therefore a total of 318 hours of observation was involved in the study spanning over four months and each animal received about 53 hours of observation.

3.3.1. Recording Method

3.3.1.1. Focal Animal Sampling /Focal Sampling

The behaviors of the stags were recorded by focal animal sampling method. Lehner (1987) described focal animal sampling as a method which restricts data gathering for a sample period to one animal because of an interest in differences within and between individuals, sexes or ages. Also, focal-animal sampling can be employed when the behaviors occur too rapidly to record data accurately from several individuals. When necessary, sampling can be increased to focal pair (e.g., communication) or focal group (e.g., dominant-subordinate relationships). Any scale of measurement can be used in focal animal sampling.

Focal animal sampling means, observing one individual for a specified period of time and recording all the instances of its behavior usually for several different categories of behavior (Martin and Bateson, 1993).

3.3.1.2. Ethogram

Lehner (1987) defined ethogram as a set of terms and descriptions of the behaviors of an animal. It may be a comprehensive ethogram of all behaviors of a species or it may be for only one sex, age group or type of behavior.

The ethogram used for this study adapted and modified from Roshin, 2005 is presented below.

Sl.No.	Behaviour	Description
1.	Feeding and drinking	Involves the individual gathering of food/water with mouth.

2.	Rumination	Individual engages in rumination of food.
3.	Rubbing/grooming	Individual licks its body or rubs against tree/wall
4.	Head held high	Head with antlers are held high.
5.	Vocalization	Bellowing, mate calls.
6.	Territory marking	Chasing away the other males from certain area by the superior males of the herd.
7.	Fighting	Fighting among the superior males, superior and inferior males etc. by clashing the antlers.
8.	Urine spraying	Spraying urine onto its own face, neck and antler.
9.	Resting	Individual will not show any specific activity and will be lying down.
10.	Sniffing	Sniffing the lower abdomen, vulva or urine of the females.
11.	Chasing the female	Males going after the receptive female of the herd.
12.	Mounting	Mounting over the female in attempt to mate.
13.	Service	Thrust and ejaculation.
14.	Flehmen	The flattened surface of the tip of the nostril is brought to the mouth and the nostril tip is placed in contact with the roof of the muzzle.

3.4. PHYSICAL AND BEHAVIOURAL CHANGES

Various changes seen on the physical condition of the stags were noted down. The observations were mainly resorted to the shifting antler stages, size of the stag, changes in the coat color, neck and testicular circumference. Behavioral observation, if any, additional to that in the ethogram, were also looked for.

3.5. HORMONE LEVEL ESTIMATION

3.5.1. Fecal Sample- Collection and storage

The fecal pellets were collected on a weekly basis from all six stags for a period of four months. The samples were colleted within half an hour of voidance and were labeled and kept in polyethylene covers at -20 ° C until extraction for RIA.

3.5.2. Extraction for RIA

Frozen fecal samples were dried in a conventional oven. Each sample was powdered and mixed thoroughly. A sub sample weighing 0.2 g was mixed with 5 ml of 90 per cent ethanol in a test tube and vortexed briefly. The tubes were boiled in a water bath (90° C) for 20 minutes, adding ethyl alcohol to avoid it from boiling dry. The extract was brought up to pre boil levels with 90 per cent ethanol and centrifuged at 1500 rpm for 20 minutes. The extracts were poured off into another storage vial. To the remaining fecal powder 90 per cent ethanol was added again and vortexed for 30 seconds and centrifuged at 1500 rpm for 15 minutes. The first and second extracts were combined and dried down and reconstituted in one ml of methanol and vortexed for a brief period. The methanol extracts were stored at -20 ° C until RIA analysis (Brown et al., 2002).

3.5.3. RIA for Fecal Testosterone

Radioimmunoassay was done to determine the testosterone concentration in the fecal samples. The RIA kit used for the purpose was ^{125}I labeled Testosterone (Direct) kit (M/s Immunotech, France).

3.5.4. Procedure for Estimation of Testosterone

The radioimmunoassay of testosterone is a competition type of assay. Samples or calibrators were incubated with ^{125}I labeled testosterone in antibody coated tubes. After incubation, the liquid content of the tube was aspirated and the bound radioactivity was determined in a gamma counter. A standard curve was prepared and unknown values were obtained from the curve by interpolation.

Step 1 Additions *	**Step 2** Incubation	**Step 3** Counting
To antibody coated tubes, add successively: 50 µL of calibrator, control or sample and 500 µL of tracer Mix	 Cover the tubes Incubate 3 hours at 37 ° C in water bath	 Aspirate carefully the content of tubes (except the 2 tubes for total cpm) Count bound cpm and total cpm for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm (cpm – count per minute)

Results were obtained from the standard curve by interpolation. The curve serves for the determination of testosterone concentrations in samples measured at the same time as the calibrator.

3.6. HAREM FORMATION

Each one of the six stags was observed for the size of the harem they formed. The details regarding the members of the harem like the sex, age group and antler stage were also recorded.

3.7. CORRELATION BETWEEN ANDROGEN LEVEL, ANTLER STAGE AND BEHAVIOUR

Statistical correlation was drawn between the various antler stages and the concentration of fecal testosterone observed during those periods. Correlations were also made regarding the influence of androgen on the behavior of the stags. The differences seen in the behavior of the stags were also correlated to the shifting antler stages.

3.8. STATISTICAL ANALYSIS

The statistical analysis was done as described by Snedecor and Cochran (1994).

4. RESULTS

4.1 SEASONALITY OF DEER BREEDING

The birth records maintained at the zoo indicated that late stages of the rainy season and the whole dry season are the periods during which maximum births take place. End of dry season to beginning of rainy season was found to be the season which witnessed maximum number of breeding activity. The data are presented in Tables 1 and 2 and Figures 1 and 2.

4.2 THE DOMINANT MALE

The stags were selected based on the preliminary study wherein body and antler size, positioning at the feeding time, ability to control territories and possession of mates were the criteria. The six study animals selected were named as H_1, H_2, H_3, V_1, V_2 and V_3 in dominance order of hierarchy, presented in Plates 1 to 6. H_1, H_2 and H_3 were in hard antler stage and V_1, V_2 and V_3 were in velvet growth stage at the beginning of the study.

4.3 ANTLERS

4.3.1 Antler Characteristics

Observation of the stags before, during and after the four month study period revealed that the sambar deer antlers were in velvet growth for a duration of seven to eight months. The complete shedding of velvet took about 5-6 days. The stags were seen rubbing their antlers on the trees and the fencing in order to get the velvet off. Most of the trees in the enclosure were devoid of bark up to certain height mainly because of this activity. The hard antlers decorated the stags for the whole rut season which lasted for about four to five months. This was followed by the antler casting stage wherein, the stags lost their antlers either in a

Table 1. Month wise births recorded.

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	TOTAL
JAN																1				1
FEB			1					1		1						2				5
MAR			1		1	1		1	2	1	1					1		2		11
APR	3		1	1				1	1	4	5	2		1	3				1	23
MAY		1				1	1					1	2	2	3	1	2			14
JUN			3									1			1	1	6	5		17
JULY															2			2		4
AUG							1							4	3	2		1		11
SEP			2														1			3
OCT					1								1					1		3
NOV				4												2	1			7
DEC			1			1										1				3
TOTAL	3	1	9	5	2	3	2	3	3	6	6	4	3	7	12	11	10	11	1	102

Table 2. Month wise breeding estimated.

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	TOTAL
JAN		1				1	1					1	2	2	3	1	2			14
FEB			3									1			1	1	6	5		17
MAR															2			2		4
APR							1							4	3	2		1		11
MAY			2														1			3
JUN					1								1					1		3
JULY				4												2	1			7
AUG			1			1										1				3
SEP																1				1
OCT			1					1		1						2				5
NOV			1		1	1		1	2	1	1					1		2		11
DEC	3		1	1				1	1	4	5	2		1	3				1	23
TOTAL	3	1	9	5	2	3	2	3	3	6	6	4	3	7	12	11	10	11	1	102

Fig. 1. Month wise births recorded

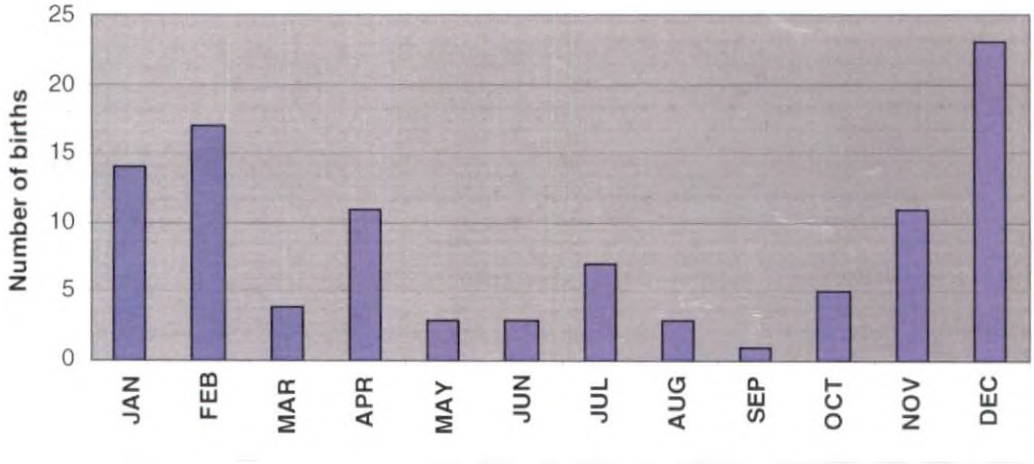


Fig. 2. Month wise breeding estimated

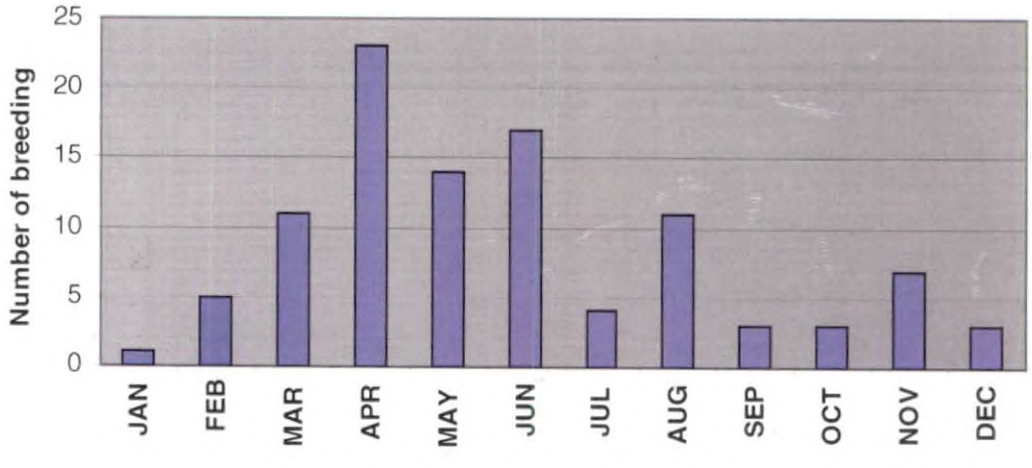


Plate 1. Stag H_1

Plate 2. Stag H_2

Plate 3. Stag H_3

Plate 4. Stag V_1

Plate 5. Stag V_2

Plate 6. Stag V_3

fight or by hitting against tree or fence. The stags lost both the antlers on the same day or there was even a gap of 2-3 days for the second one to fall. This stage did not last long, as antler growth is a rapid continuous cycle, and the initiation of the next set of antler set in without much delay. Various stages of antler cycle are shown in Plates 7 to 18.

4.3.2 Seasonality of Antler Growth

Even though the deer species throughout the world is known for its seasonality of antler and breeding cycles, most of the tropical deer do not exhibit that. The sambar deer herd selected for the study had been observed to breed throughout the year and also stags at various stages of the antler cycle were seen at any point of the year.

4.4 PHYSICAL CHANGES

4.4.1 Weight Gain

The stags were observed during various stages of antler cycle and there were marked variations noticed in terms of body condition as well as the size. The stags seemed to be in peak size and maintained the best body condition during the later velvet stages. The good body condition was maintained at the initial phases of the rut season but, during the later stages of rut/ hard antler phase, the stags were seen in a worn out condition indicative of the hardships undergone during the rut for maintenance of the harem as well as the dominance quotient. The worst body condition was observed in those stags whose antlers were freshly cast; the stags appeared skinny, emaciated, lesser coat thickness and some of them carrying the gore wounds sustained during the tussles for territory, hinds and rations. The whole of antler re-growth stage was found to be a recuperating stage during which the stag prepared itself to face the next rut.

Plate 7. Antler cast stage

Plate 8. Velvet growth - 1

Plate 9. Velvet growth – 2

Plate 10. Velvet growth - 3

Plate 11. Velvet growth – 4

Plate 12. Velvet growth - 5

Plate 13. Velvet growth – 6

Plate 14. Velvet shedding -1

Plate 15. Velvet shedding – 2

Plate 16. Hard antler

Plate 17. One antler cast

Plate 18. Both antlers cast

4.4.2 Other Changes

The scrotum showed significant enlargement in the stags approaching the rut season. The stags in the hard antler stage had much larger testes that those in antler cast or velvet growth stages. The coat color was slightly darker in shade in hard antler stags in comparison with those in other stages. The neck musculature was blown up in size during the rut season.

4.5 ETHOLOGY

4.5.1 Behavioral Observations

The behavioral observations for the selected six stags were recorded for a period of 16 weeks with two hours of observation on each stag every week. The observations were documented on the ethogram and were assigned breeding scores as per the score chart adapted and modified from Roshin, 2005.

Score chart for behavioral data collection

Sl. No.	Behavior (Diurnal)/ week	Scoring		
		5	3	1
1	Feeding and drinking	1-4	5-8	9-12
2	Rumination	1-3	4-6	7-9
3	Rubbing or grooming	21-30	11-20	1-10
4	Bellowing	5-6	3-4	1-2
5	Territory marking	4-5	2-3	1
6	Fighting	8-11	4-7	1-3
7	Resting	1-2	3-4	5-7
8	Sniffing	21-30	11-20	1-10
9	Flehmen	9-12	5-8	1-4
10	Chasing females	9-12	5-8	1-4

11	Mounting	6-12	3-5	1-2
12	Service	5-7	2-4	1
13	Other solitary	9-12	5-8	1-4
14	Urine spraying	5-6	3-4	1-2
15	Head held high	11-15	6-10	1-5

Some of the behavioral observations like feeding, resting, rubbing antler on tree, holding the head high, fighting, territory marking, urine spraying, chasing, sniffing and Flehmens' reaction are shown in Plates 19 to 28.

Stag H_1 maintained a behavioral score of 42 ± 11.73 during the hard antler phase whereas during the velvet phase it slipped to 9.66 ± 2.25 (Table 4, Fig. 3).

The behavioral score of stag H_2 was 42.85 ± 12.58 during the hard antler stage and it reduced to 12.6 ± 2.3 during the velvet period (Table 6, Fig. 4).

Stag H_3 had a behavioral score of 44 ± 14 during the rut season; it became 12.6 ± 3.13 during the velvet stage (Table 8, Fig. 5).

The stag V_1 maintained a behavioral score of 23.16 ± 12.84 during the velvet stage, but as it entered the rut season, its score shot up to 51.81 ± 5.54 (Table 10, Fig. 6).

V_2 had a behavioral score of 24.85 ± 13.83during the velvet phase, as it shifted to hard antler stage, the score increased to 51.9 ± 5.38 (Table 12, Fig. 7).

The behavioral score of V_3 was 30.33 ± 14.76 during the velvet antler stage, the shift to the rut took up the score to 53.75 ± 4.83 (Table 14, Fig. 8).

Plate 19. Feeding

Plate 20. Resting

Plate 21. Rubbing antler on tree

Plate 22. Head held high

Plate 23. Fighting

Plate 24. Territory marking

Plate 25. Urine spraying

Plate 26. Chasing

Plate 27. Sniffing

Plate 28. Flehmens reaction

Table 3. Frequency of behaviors observed – H_1 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	9	2	8	1	10	22	4	5	10	4	27	3	2	2
18/06/09-25/06/09	9		4		12	23	3	5	9	3	23	5	2	3
25/06/09-02/07/09	8		5		11	20	2	4	7	3	26	7	2	3
02/07/09-09/07/09	6		3		10	18	2	3	9	3	21	7	3	4
09/07/09-16/07/09	8		3		9	13	3	3	8	2	22	6	4	5
16/07/09-23/07/09	2		1		5	10	2	4	5	2	18	5	5	3
23/07/09-30/07/09	0		0		2	2	0	0	1	0	9	10	7	3
30/07/09-06/08/09	0		0		2	8	0	1	1	0	9	10	6	9
06/08/09-13/08/09	1		0		1	3	0	0	2	0	8	12	7	9
13/08/09-20/08/09	1		0		**2**	7	1	0	3	1	7	11	6	8
20/08/09-27/08/09	0		0		2	6	1	0	2	1	9	9	6	7
27/08/09-03/09/09	2		1		2	0	1	0	1	2	5	12	6	7
03/09/09-10/09/09	0		0		2	9	0	0	3	1	9	10	5	6
10/09/09-17/09/09	1		2		1	3	1	1	4	2	11	11	4	7
17/09/09-24/09/09	2		0		3	6	1	1	4	1	4	12	3	6
24/09/09-01/10/09	2		1		3	7	1	1	2	3	7	11	6	6

Table 4. Behavioral scores - H_1

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	5		5		5	5	5	5	3	5	5	5	5	5	**58**
18/06/09-25/06/09	5		3		5	5	3	5	3	3	5	3	5	5	**50**
25/06/09-02/07/09	3		3		5	3	3	3	3	3	5	3	5	5	**44**
02/07/09-09/07/09	3		1		5	3	3	3	3	3	5	3	3	5	**40**
09/07/09-16/07/09	3		1		5	3	3	3	3	1	5	3	3	3	**36**
16/07/09-23/07/09	1		1		3	1	3	3	1	1	3	3	1	3	**24**
23/07/09-30/07/09	0		0		1	1	0	0	1	0	1	1	1	1	**7**
30/07/09-06/08/09	0		0		1	1	0	1	1	0	1	1	1	1	**8**
06/08/09-13/08/09	1		0		1	1	0	0	1	0	1	1	1	1	**8**
13/08/09-20/08/09	1		0		1	1	1	0	1	1	1	1	1	3	**12**
20/08/09-27/08/09	0		0		1	1	1	0	1	1	1	1	1	3	**11**
27/08/09-03/09/09	1		1		1	0	1	0	1	1	1	1	1	3	**12**
03/09/09-10/09/09	0		0		1	1	0	0	1	1	1	1	1	3	**10**
10/09/09-17/09/09	1		1		1	1	1	1	1	1	1	1	3	3	**16**
17/09/09-24/09/09	1		0		1	1	1	1	1	1	1	1	3	3	**15**
24/09/09-01/10/09	1		1		1	1	1	1	1	3	1	1	1	3	**16**

Table 5. Frequency of behaviors observed – H_2 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	9		8		12	27	4	5	11	5	26	3	2	3
18/06/09-25/06/09	6		7		12	27	4	5	9	5	25	7	2	2
25/06/09-02/07/09	8		4		9	22	3	4	9	4	23	5	2	2
02/07/09-09/07/09	6		5		11	24	2	3	8	4	27	5	1	1
09/07/09-16/07/09	6		6		9	27	2	4	10	3	20	5	2	5
16/07/09-23/07/09	5		5		6	13	1	2	4	4	24	6	3	4
23/07/09-30/07/09	3		2		3	13	1	2	7	1	20	10	4	6
30/07/09-06/08/09	0		0		0	12	1	2	5	0	10	12	7	7
06/08/09-13/08/09	0		1		2	4	0	1	2	0	7	11	5	6
13/08/09-20/08/09	2		1		1	6	1	2	2	0	10	11	5	6
20/08/09-27/08/09	2		1		2	6	2	1	3	0	7	9	7	7
27/08/09-03/09/09	1		1		3	7	2	1	4	1	8	8	6	7
03/09/09-10/09/09	3		2		3	9	1	2	4	2	9	9	3	8
10/09/09-17/09/09	3		1		2	10	0	2	2	1	9	10	2	8
17/09/09-24/09/09	2		2		2	14	2	1	1	1	7	12	6	7
24/09/09-01/10/09	3		0		3	11	2	0	5	4	11	11	4	8

Table 6. Behavioral scores - H_2

WEEK	Flehmens reaction	*Mounting*	Fighting	Service	Chasing	Sniffing	Territory *marking*	Urine spraying	*Head held* high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	5		5		5	5	5	5	5	5	5	5	5	5	**60**
18/06/09-25/06/09	3		3		5	5	5	5	3	5	5	3	5	5	**52**
25/06/09-02/07/09	3		3		5	5	3	3	3	3	5	3	5	5	**46**
02/07/09-09/07/09	3		3		5	5	3	3	3	3	5	3	5	5	**46**
09/07/09-16/07/09	3		3		5	5	3	3	3	3	3	3	5	3	**42**
16/07/09-23/07/09	3		3		3	3	1	1	1	3	5	3	3	3	**32**
23/07/09-30/07/09	1		1		1	3	1	1	3	1	3	1	3	3	**22**
30/07/09-06/08/09	0		0		0	3	1	1	1	0	1	1	1	1	**10**
06/08/09-13/08/09	0		1		1	1	0	1	1	0	1	1	1	3	**11**
13/08/09-20/08/09	1		1		1	1	1	1	1	0	1	1	1	3	**13**
20/08/09-27/08/09	1		1		1	1	3	1	1	0	1	1	1	1	**13**
27/08/09-03/09/09	1		1		1	1	3	1	1	1	1	3	1	1	**16**
03/09/09-10/09/09	1		1		1	1	1	1	1	1	1	1	3	1	**14**
10/09/09-17/09/09	1		1		1	1	0	1	1	1	1	1	5	1	**15**
17/09/09-24/09/09	1		1		1	3	3	1	1	1	1	1	1	1	**16**
24/09/09-01/10/09	1		0		1	3	3	0	1	3	3	1	3	1	**20**

Table 7. Frequency of behaviors observed – H_3 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	9		8		10	25	4	5	14	4	21	3	1	3
18/06/09-25/06/09	12		11		12	26	4	6	14	5	27	3	3	2
25/06/09-02/07/09	9		9		12	21	3	5	12	3	20	4	2	4
02/07/09-09/07/09	9		10		9	21	2	4	11	4	19	5	3	3
09/07/09-16/07/09	10		7		8	20	3	4	9	2	13	5	3	5
16/07/09-23/07/09	7		4		6	15	2	3	10	3	11	8	4	6
23/07/09-30/07/09	5		3		4	12	1	2	7	2	7	9	4	7
30/07/09-06/08/09	4		0		0	10	0	1	5	0	8	10	5	6
06/08/09-13/08/09	4		1		2	7	0	1	4	0	8	9	7	9
13/08/09-20/08/09	3		1		2	7	0	1	3	1	6	9	6	9
20/08/09-27/08/09	4		1		1	4	1	1	3	2	7	8	7	6
27/08/09-03/09/09	5		2		2	2	2	2	3	2	9	9	5	7
03/09/09-10/09/09	4		1		3	6	1	1	4	1	11	11	7	8
10/09/09-17/09/09	7		1		1	0	1	1	5	1	9	10	6	7
17/09/09-24/09/09	6		2		4	2	2	3	2	0	10	12	4	9
24/09/09-01/10/09	6		3		1	5	3	0	4	1	8	11	5	8

Table 8. Behavioral scores - H_3

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
18/06/09-25/06/09	5		5		5	5	5	5	5	5	5	5	3	5	**58**
25/06/09-02/07/09	5		5		5	5	3	5	5	3	3	5	5	3	**52**
02/07/09-09/07/09	5		5		5	5	3	3	5	3	3	3	3	5	**48**
09/07/09-16/07/09	5		3		3	3	3	3	3	1	3	3	3	3	**36**
16/07/09-23/07/09	3		3		3	3	3	3	3	3	3	3	3	3	**36**
23/07/09-30/07/09	3		1		1	3	1	1	3	1	1	1	3	1	**20**
30/07/09-06/08/09	1		0		0	1	0	1	1	0	1	1	1	3	**10**
06/08/09-13/08/09	1		1		1	1	0	1	1	0	1	1	1	1	**10**
13/08/09-20/08/09	1		1		1	1	0	1	1	1	1	1	1	1	**11**
20/08/09-27/08/09	1		1		1	1	1	1	1	1	1	3	1	3	**16**
27/08/09-03/09/09	3		1		1	1	3	1	1	1	1	1	1	1	**16**
03/09/09-10/09/09	1		1		1	1	1	1	1	1	3	1	1	1	**14**
10/09/09-17/09/09	3		1		1	0	1	1	1	1	1	1	1	1	**13**
17/09/09-24/09/09	3		1		1	1	3	3	1	0	1	1	3	1	**19**
24/09/09-01/10/09	3		1		1	1	3	0	1	1	1	1	1	1	**15**

Table 9. Frequency of behaviors observed – V_1 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	3		2		4	9	1	2	4	1	9	11	7	9
18/06/09-25/06/09	4		1		3	9	1	2	3	1	10	11	6	8
25/06/09-02/07/09	3		1		3	8	3	1	3	1	10	9	7	6
02/07/09-09/07/09	5		3		4	7	2	3	4	0	9	8	4	7
09/07/09-16/07/09	8		7		7	14	3	3	7	3	7	8	4	3
16/07/09-23/07/09	9		8		9	19	3	3	8	3	15	5	3	4
23/07/09-30/07/09	12		10		11	28	4	5	13	4	26	2	1	2
30/07/09-06/08/09	11		11		11	25	5	6	15	4	25	1	1	1
06/08/09-13/08/09	12		11		12	20	5	6	14	3	23	1	1	1
13/08/09-20/08/09	8		10		9	27	5	6	15	3	29	1	2	1
20/08/09-27/08/09	8		8		10	22	4	5	13	4	30	2	2	3
27/08/09-03/09/09	7		7		11	21	3	4	14	3	21	3	3	3
03/09/09-10/09/09	8		9		11	20	4	4	11	2	20	2	4	4
10/09/09-17/09/09	9		8		10	23	4	3	10	3	17	4	2	5
17/09/09-24/09/09	9		7		8	24	3	5	12	4	27	6	2	1
24/09/09-01/10/09	10		11		9	20	5	2	14	4	22	4	1	2

Table 10. Behavioral scores - V_1

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	1		1		1	1	1	1	1	1	1	1	1	1	**12**
18/06/09-25/06/09	1		1		1	1	1	1	1	1	1	1	1	1	**12**
25/06/09-02/07/09	1		1		1	1	3	1	1	1	1	1	1	3	**16**
02/07/09-09/07/09	3		1		1	1	3	3	1	0	1	3	3	1	**21**
09/07/09-16/07/09	3		3		3	3	3	3	3	3	1	3	3	5	**36**
16/07/09-23/07/09	5		5		5	3	3	3	3	3	3	3	3	3	**42**
23/07/09-30/07/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
30/07/09-06/08/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
06/08/09-13/08/09	5		5		5	3	5	5	5	3	5	5	5	5	**56**
13/08/09-20/08/09	3		5		5	5	5	5	5	3	5	5	5	5	**56**
20/08/09-27/08/09	3		5		5	5	5	5	5	3	5	5	5	5	**56**
27/08/09-03/09/09	3		3		5	5	3	3	5	3	5	5	3	5	**48**
03/09/09-10/09/09	3		5		5	3	5	3	5	1	3	5	3	3	**44**
10/09/09-17/09/09	5		5		5	5	5	3	3	3	3	5	5	3	**50**
17/09/09-24/09/09	5		3		3	5	3	5	5	3	5	3	5	5	**50**
24/09/09-01/10/09	5		5		5	3	5	1	5	3	5	5	5	5	**52**

Table 11. Frequency of behaviors observed – V_2 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	2		2		2	10	2	2	5	0	6	8	5	7
18/06/09-25/06/09	1		1		4	10	1	2	4	0	10	12	5	7
25/06/09-02/07/09	2		3		4	9	1	1	1	1	18	8	6	8
02/07/09-09/07/09	2		2		3	8	3	3	4	3	11	10	5	9
09/07/09-16/07/09	4		1		7	15	1	4	9	3	9	11	4	6
16/07/09-23/07/09	8		7		10	18	3	4	10	2	18	5	3	5
23/07/09-30/07/09	10		8		12	20	5	5	8	3	23	5	2	6
30/07/09-06/08/09	12		8		12	29	5	6	12	4	29	1	2	1
06/08/09-13/08/09	12		11		12	24	5	4	12	3	24	1	1	2
13/08/09-20/08/09	12		10		10	20	5	6	14	4	24	1	1	1
20/08/09-27/08/09	9		10		9	21	4	5	14	4	23	3	1	3
27/08/09-03/09/09	9		8		11	27	3	5	12	3	27	4	2	2
03/09/09-10/09/09	8		9		10	22	4	4	13	2	20	5	3	1
10/09/09-17/09/09	9		7		10	20	5	3	11	1	29	4	4	3
17/09/09-24/09/09	10		8		12	19	4	2	10	3	22	5	2	4
24/09/09-01/10/09	9		11		9	25	3	5	10	0	30	5	3	4

Table 12. Behavioral scores - V_2

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	1		1		1	1	3	1	1	0	1	3	1	1	**15**
18/06/09-25/06/09	1		1		1	1	1	1	1	0	1	1	1	1	**11**
25/06/09-02/07/09	1		1		1	1	1	1	1	1	3	3	1	1	**16**
02/07/09-09/07/09	1		1		1	1	3	3	1	3	3	1	1	1	**20**
09/07/09-16/07/09	1		1		3	3	1	3	3	3	1	1	3	3	**26**
16/07/09-23/07/09	3		3		5	3	3	3	3	1	3	3	3	3	**36**
23/07/09-30/07/09	5		5		5	3	5	5	3	3	5	3	5	3	**50**
30/07/09-06/08/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
06/08/09-13/08/09	5		5		5	5	5	3	5	3	5	5	5	5	**56**
13/08/09-20/08/09	5		5		5	3	5	5	5	3	5	5	5	5	**56**
20/08/09-27/08/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
27/08/09-03/09/09	5		5		5	5	3	5	5	3	5	5	5	5	**56**
03/09/09-10/09/09	3		5		5	5	5	3	5	1	3	3	3	5	**46**
10/09/09-17/09/09	5		3		5	3	5	3	5	1	5	5	3	5	**48**
17/09/09-24/09/09	5		5		5	3	5	1	3	3	5	3	5	3	**46**
24/09/09-01/10/09	5		5		5	5	3	5	3	0	5	3	3	3	**45**

Table 13. Frequency of behaviors observed – V_3 (Two hours observation each week)

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating
11/06/09-18/06/09	1		3		1	7	1	2	4	0	7	9	5	8
18/06/09-25/06/09	1		2		2	7	2	1	3	1	8	7	6	9
25/06/09-02/07/09	2		3		4	10	1	2	4	1	9	9	6	7
02/07/09-09/07/09	3		1		7	12	2	4	4	1	8	12	7	6
09/07/09-16/07/09	1		4		6	14	4	3	6	2	11	8	6	6
16/07/09-23/07/09	7		5		8	17	3	3	7	3	17	5	4	4
23/07/09-30/07/09	8		6		9	20	4	4	9	2	19	7	4	5
30/07/09-06/08/09	9		9		12	19	5	3	7	3	18	4	4	3
06/08/09-13/08/09	11		11		9	20	4	2	13	3	26	4	2	1
13/08/09-20/08/09	12		11		11	30	5	6	14	4	28	2	1	1
20/08/09-27/08/09	12		10		12	23	5	6	13	4	27	2	1	1
27/08/09-03/09/09	11		11		11	27	4	5	14	5	29	1	1	3
03/09/09-10/09/09	10		9		9	21	5	4	12	3	21	2	2	2
10/09/09-17/09/09	7		7		10	22	3	5	11	2	27	1	1	2
17/09/09-24/09/09	8		8		8	27	4	6	10	2	26	4	3	3
24/09/09-01/10/09	9		10		11	19	5	4	13	1	20	6	2	1

Table 14. Behavioral scores - V_3

WEEK	Flehmens reaction	Mounting	Fighting	Service	Chasing	Sniffing	Territory marking	Urine spraying	Head held high	Bellow	Grooming	Feed/drinking	Resting	Ruminating	TOTAL BEHAVIORAL SCORE
11/06/09-18/06/09	1		1		1	1	1	1	1	0	1	1	1	3	**13**
18/06/09-25/06/09	1		1		1	1	3	1	1	1	1	3	1	1	**16**
25/06/09-02/07/09	1		1		1	1	1	1	1	1	1	1	1	3	**14**
02/07/09-09/07/09	1		1		3	3	3	3	1	1	1	1	1	3	**22**
09/07/09-16/07/09	1		3		3	3	5	3	3	1	3	3	1	3	**32**
16/07/09-23/07/09	3		3		3	3	3	3	3	3	3	3	3	5	**38**
23/07/09-30/07/09	3		3		5	3	5	3	3	1	3	3	3	3	**38**
30/07/09-06/08/09	5		5		5	3	5	3	3	3	3	5	3	5	**48**
06/08/09-13/08/09	5		5		5	3	5	1	5	3	5	5	5	5	**52**
13/08/09-20/08/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
20/08/09-27/08/09	5		5		5	5	5	5	5	3	5	5	5	5	**58**
27/08/09-03/09/09	5		5		5	5	5	5	5	5	5	5	5	5	**60**
03/09/09-10/09/09	5		5		5	5	5	3	5	3	5	5	5	5	**56**
10/09/09-17/09/09	3		3		5	5	3	5	5	1	5	5	5	5	**50**
17/09/09-24/09/09	3		5		3	5	5	5	3	1	5	5	3	5	**48**
24/09/09-01/10/09	5		5		5	3	5	3	5	1	3	3	5	5	**48**

Fig. 3. Behavioral scores of H_1

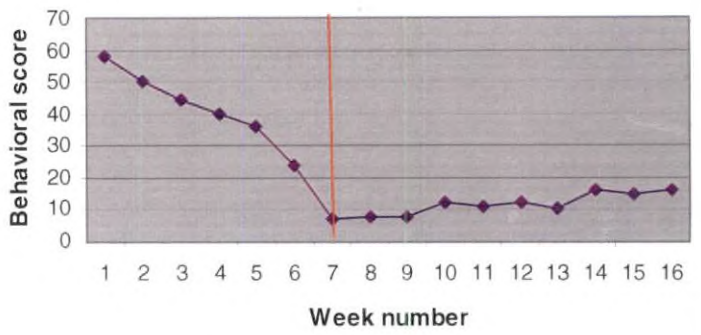


Fig. 4. Behavioral scores of H_2

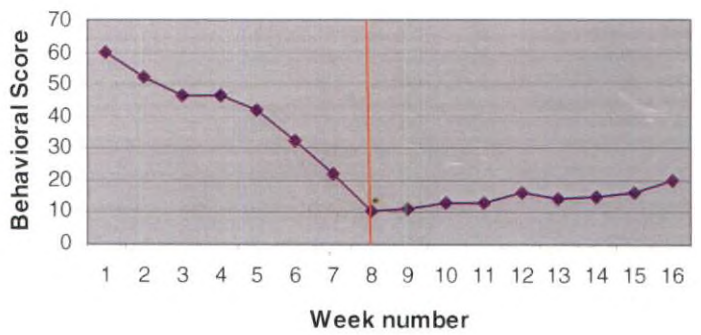
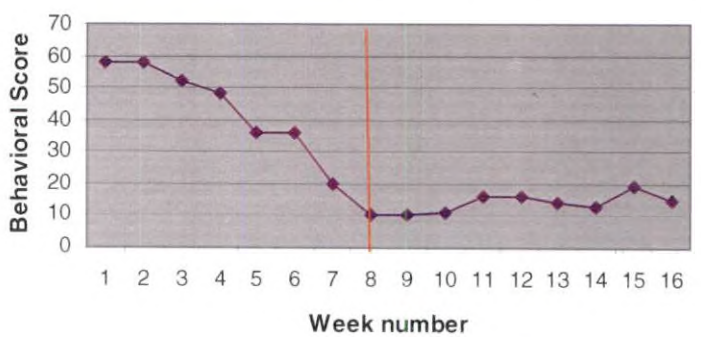


Fig. 5. Behavioral scores of H_3



(| - Week of antler casting)

Fig. 6. Behavioral scores of V_1

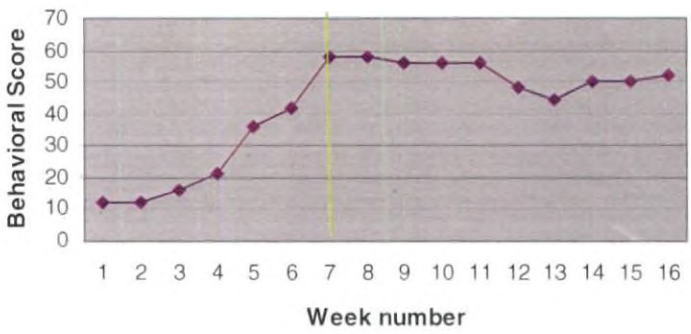


Fig. 7. Behavioral scores of V_2

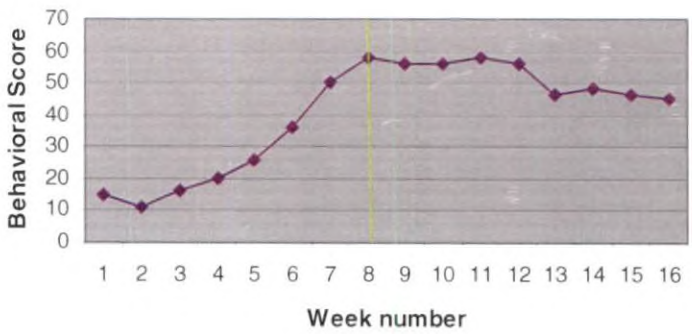
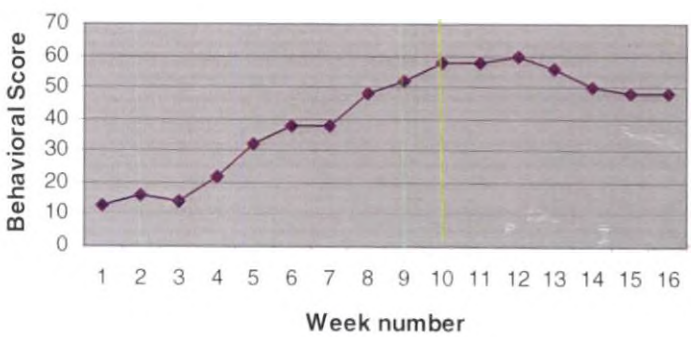


Fig. 8. Behavioral scores of V_3



(| - Week of velvet shedding)

Fig. 9. Comparison of behavioral scores of H_1, H_2 and H_3

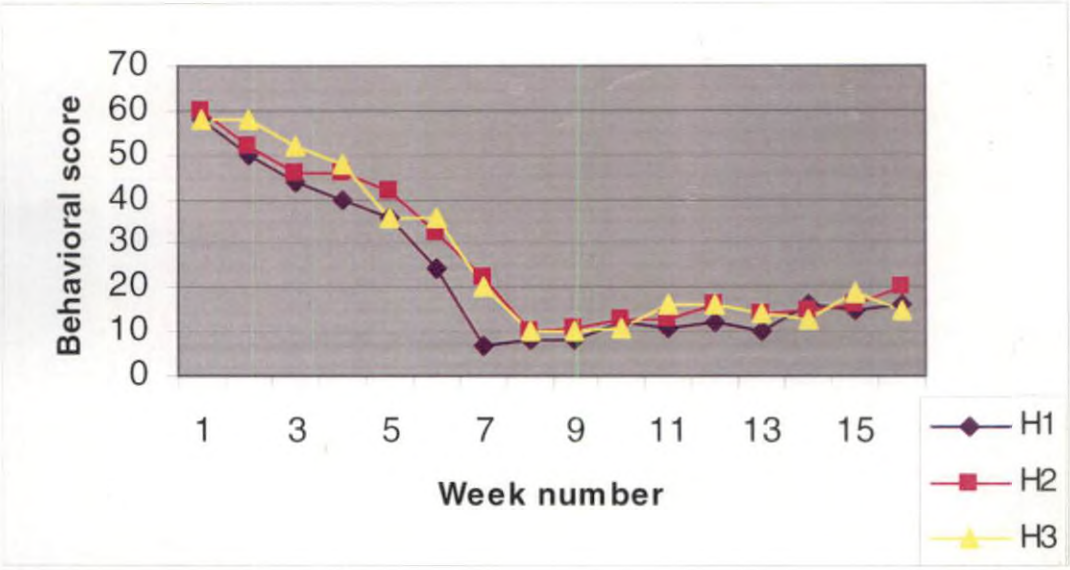
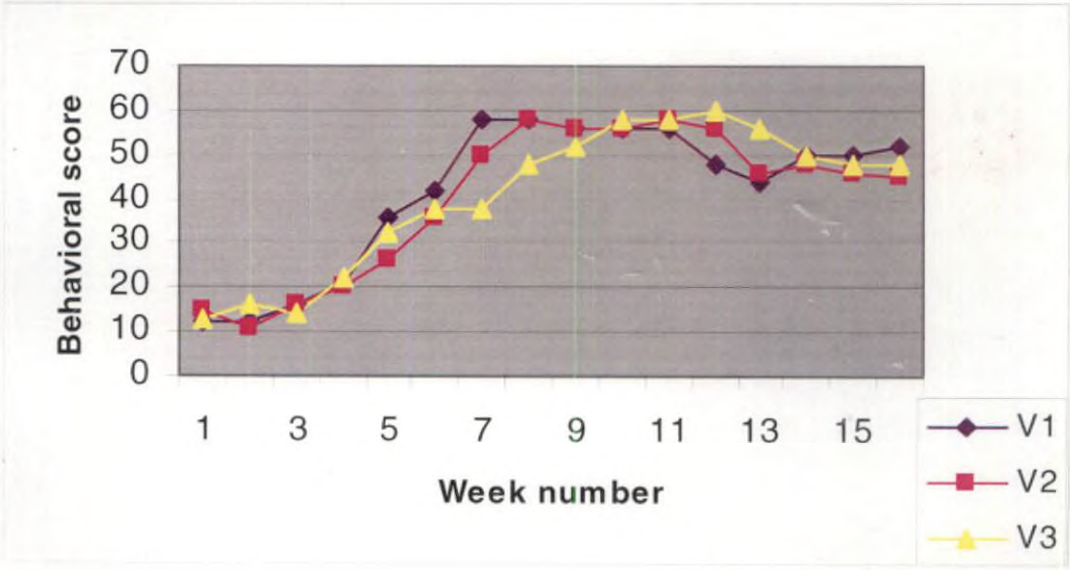


Fig. 10. Comparison of behavioral scores of V_1, V_2 and V_3



Figures 9 and 10 show comparison of behavioral scores of the hard antlered and the velvet antlered groups respectively.

4.5.2 Harem Formation

The stag H_1 possessed the largest harem followed by H_2 and H_3 with a membership of 17, 13 and 07 individuals respectively. But this continued only till they retained the antlers, the casting of antlers by these males and the entry of the velvet stags into rut season lead to a large dropout in the harem membership of the former stags and they preferred to join the harems of V_1, V_2 and V_3. Among these stags, V_1 collected the largest harem, followed by V_3, and V_2 not lagging far behind. The mentioned stags enjoyed a membership of 19, 14 and 13 individuals respectively. The harem sizes are depicted in Fig. 11 and 12.

4.6 HORMONE ASSAY

4.6.1 Testosterone Levels

The stag H_1 maintained a testosterone level of 14.66 ± 2.30 ng g^{-1} of dry feces for the six weeks that it remained in hard antler. A sudden decline in the hormone level to 4.6 ng g^{-1} of dry feces during the seventh week triggered the antler fall. The testosterone level thereafter maintained a low profile of 7.85 ± 2.32 ng g^{-1} of dry feces for the remaining ten weeks of observation (Fig. 13).

The testosterone level of stag H_2 was 14.07 ± 0.54 ng g^{-1} of dry feces during the hard antler phase and its decline by the eight week led to the antler casting. The testosterone level then was at a level of 9.12 ± 2.40 ng g^{-1} of dry feces for nine weeks (Fig. 14).

H_3 stag had testosterone levels of 14.85 ± 1.17 ng g^{-1} of dry feces for seven weeks before casting the antler. The casting was followed by testosterone levels of

Fig. 11. Harem size of H_1, H_2 and H_3

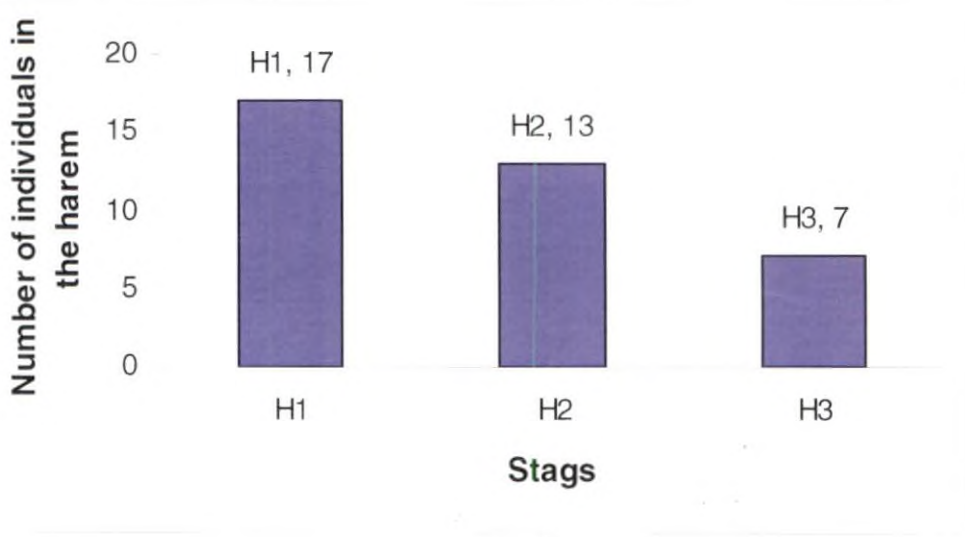
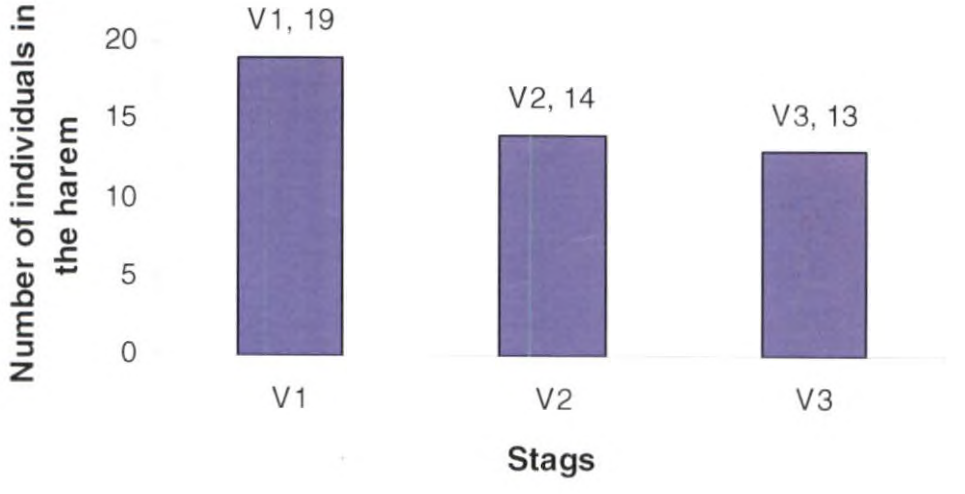


Fig. 12. Harem size of V_1, V_2 and V_3



9.56 ± 1.94 ng g^{-1} of dry feces for the rest of the study period of nine weeks (Fig. 15).

Stag in velvet stage initially, V_1, had testosterone levels of 13.52 ± 1.82 ng g^{-1} of dry feces, a sudden increase in the hormone levels by the seventh week caused the velvet to peel off welcoming the stag to the rut season. The stag from thereon maintained a hormone level of 18.65 ± 1.20 ng g^{-1} of dry feces (Fig. 16).

V_2 maintained testosterone levels of 12.45 ± 0.91 ng g^{-1} of dry feces for seven weeks. This was followed by the velvet shedding and the testosterone level was then maintained at 18.77 ± 1.03 ng g^{-1} of dry feces (Fig. 17).

The stag V_3 had the hormone levels at 12.32 ± 1.18 ng g^{-1} of dry feces before casting the velvet at the tenth week. The testosterone level after the velvet casting was higher at 18.72 ± 0.79 ng g^{-1} of dry feces (Fig. 18).

Table 15 compiles the testosterone levels of the selected stags over the study period. Fig. 19 and 20 compares the testosterone level gradations of the selected stags during hard antler stage and velvet growth stage.

4.6.2 Testosterone Vs Antler stage and behavior

The fecal testosterone levels of the six stags estimated by RIA revealed that testosterone exerts a strong grip on the antler cycle, as well as the breeding score of the stags. The stags in hard antler stage or rut season were found to have a significantly higher testosterone concentration in comparison with the velvet growth stage stags. The study also reveals that, it is the sudden dip in the testosterone concentration which causes the antler casting. Velvet shedding was preceded by an increase in the testosterone levels emphasizing its role. The increasing behavioral scores were also accompanied by higher levels of testosterone.

Table 15. Testosterone levels of the selected stags over the whole study period. (ng g^{-1} of dry feces)

	H1	H2	H3	V1	V2	V3
11/06/09-18/06/09	16.91	14.50	14.44	11.91	11.36	10.74
18/06/09-25/06/09	15.98		16.79	12.53	11.89	11.47
25/06/09-02/07/09	14.87	14.30	14.93	13.48	12.47	13.12
02/07/09-09/07/09	14.75	14.48	14.76	14.08	13.41	13.42
09/07/09-16/07/09	13.83	13.46	13.14	13.01		
16/07/09-23/07/09	8.56	13.08		18.47	13.97	13.74
23/07/09-30/07/09	4.60	7.57	9.51	19.92	18.21	14.33
30/07/09-06/08/09	6.09	4.63	6.37	18.70	19.95	
06/08/09-13/08/09	7.43	8.60	9.50	19.98	19.16	18.73
13/08/09-20/08/09	9.98	10.37	11.32	18.41		19.80
20/08/09-27/08/09	10.15	10.95		17.06	17.90	19.37
27/08/09-03/09/09	10.91	11.00	10.46	16.72		17.68
03/09/09-10/09/09	10.67	10.59	11.49		17.20	18.37
10/09/09-17/09/09		11.09	11.99	16.69	17.09	18.71
17/09/09-24/09/09	11.55		12.19		19.09	17.78
24/09/09-01/10/09	11.12	11.77	11.54	17.12	18.76	17.87

Week of antler casting

Week of velvet shedding

Fig. 13. Testosterone concentration of H_1 (ng g^{-1} of dry feces)

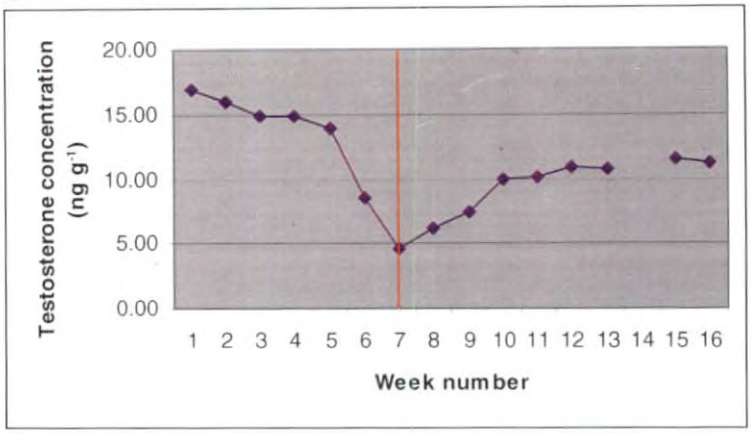


Fig. 14. Testosterone concentration of H_2 (ng g^{-1} of dry feces)

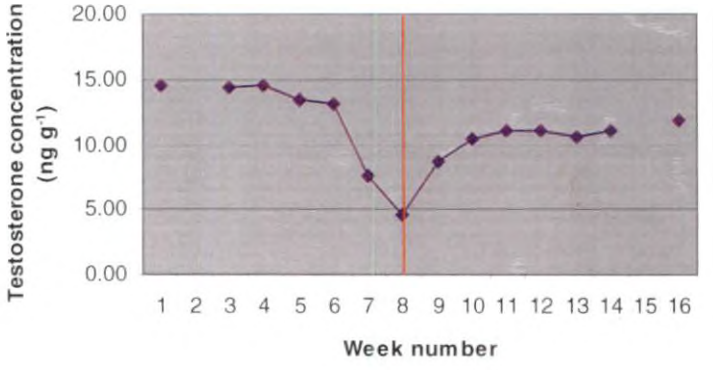
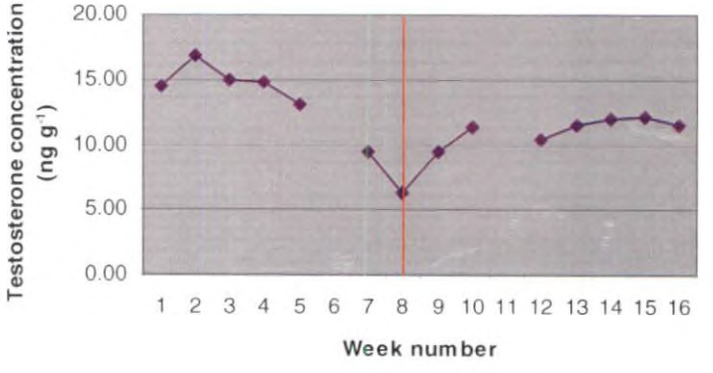


Fig. 15. Testosterone concentration of H_3 (ng g^{-1} of dry feces)



(| - Week of antler casting)

Fig. 16. Testosterone concentration of V_1 (ng g^{-1} of dry feces)

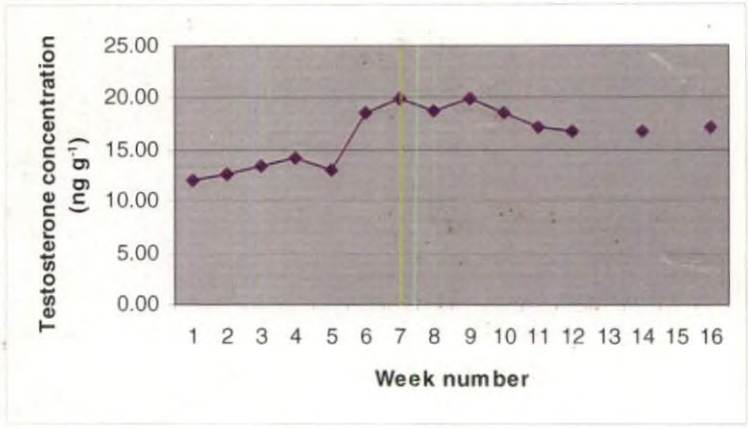


Fig. 17. Testosterone concentration of V_2 (ng g^{-1} of dry feces)

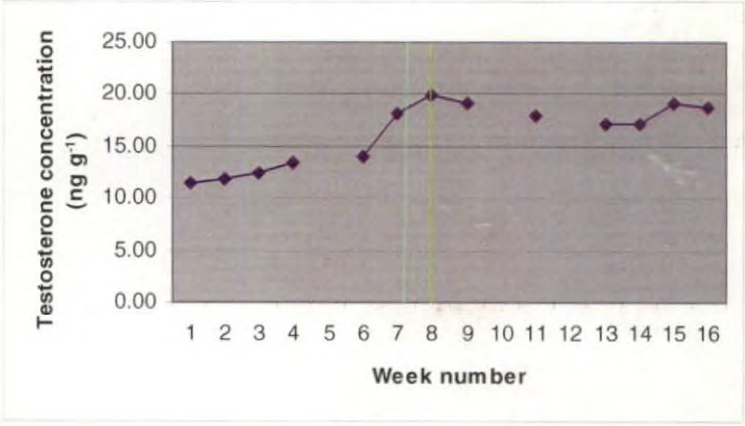
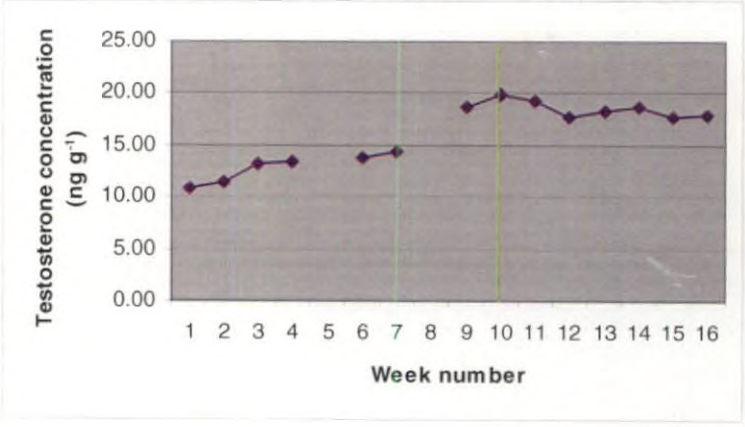


Fig. 18. Testosterone concentration of V_3 (ng g^{-1} of dry feces)



(| - Week of velvet shedding)

Fig. 19. Comparison of testosterone levels during hard antler and velvet stage – H_1, H_2 and H_3

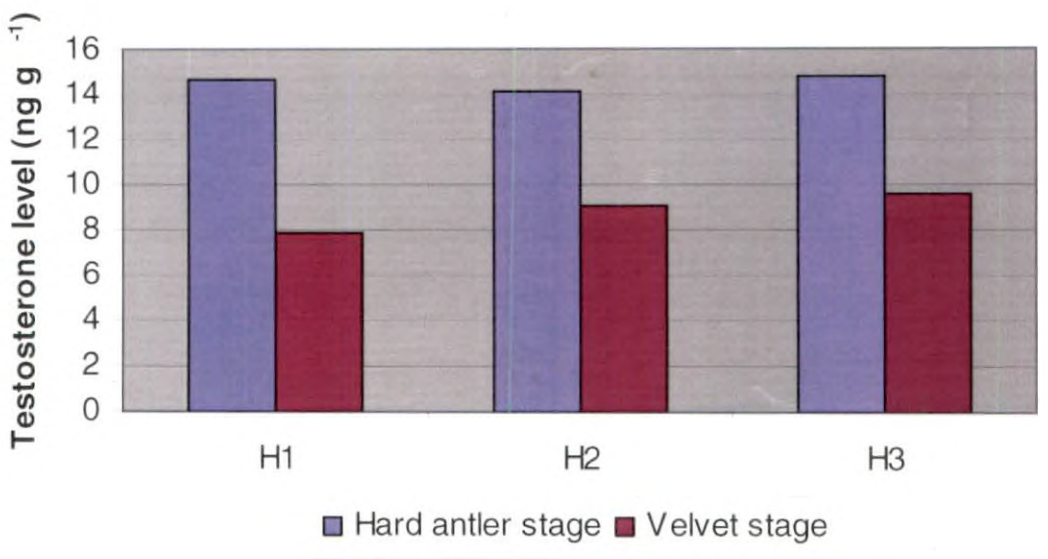


Fig. 20. Comparison of testosterone levels during hard antler and velvet stage – V_1, V_2 and V_3

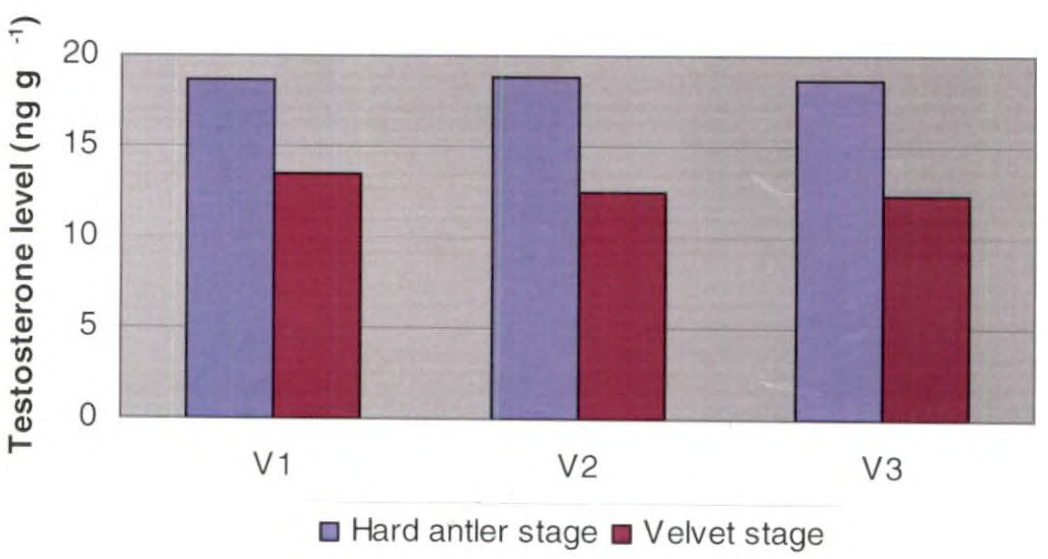


Fig. 21. Comparison of behavioral score and testosterone level – H_1

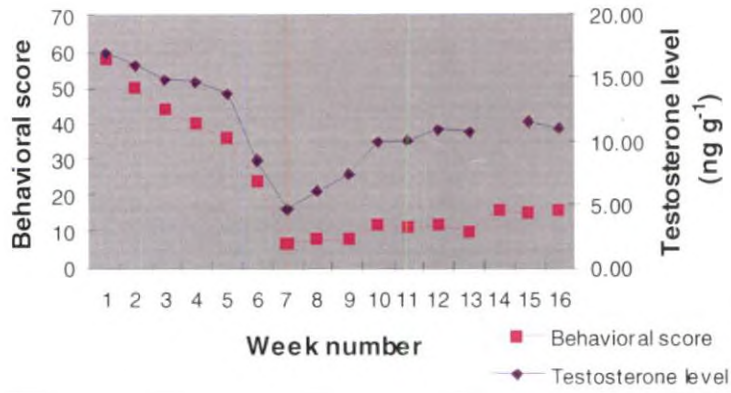


Fig. 22. Comparison of behavioral score and testosterone level – H_2

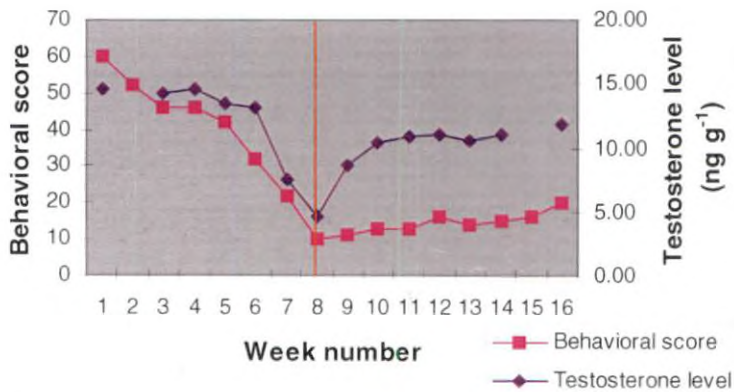
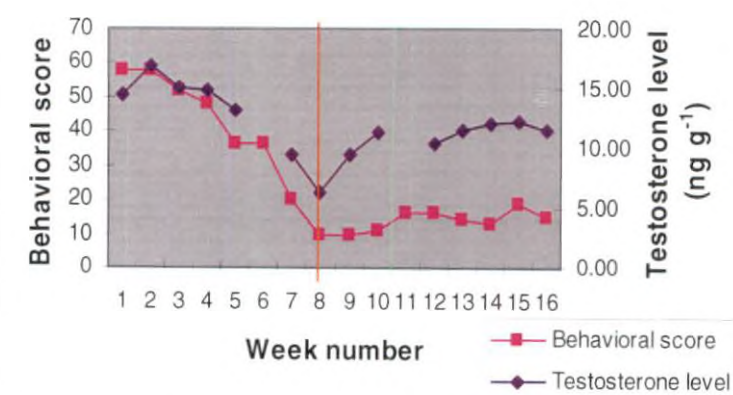


Fig. 23. Comparison of behavioral score and testosterone level – H_3



(| - Week of antler casting)

Fig. 24. Comparison of behavioral score and testosterone level – V_1

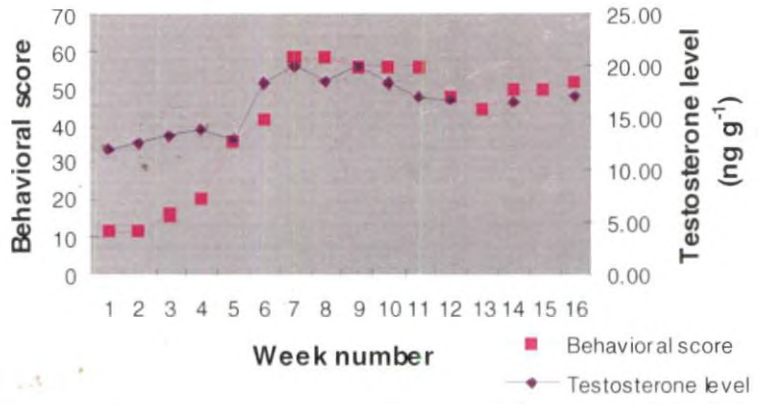


Fig. 25. Comparison of behavioral score and testosterone level – V_2

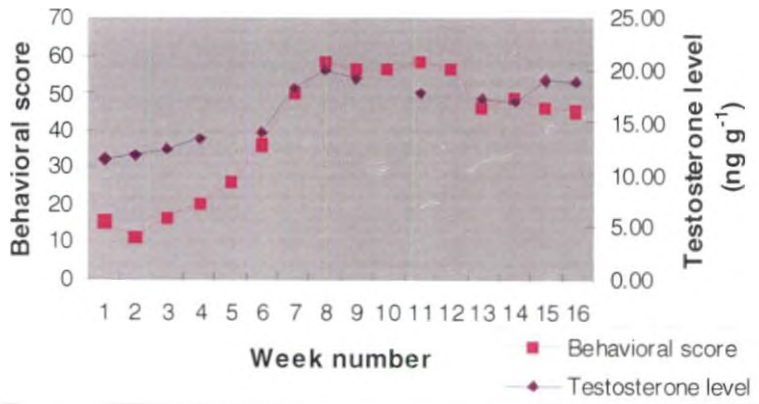
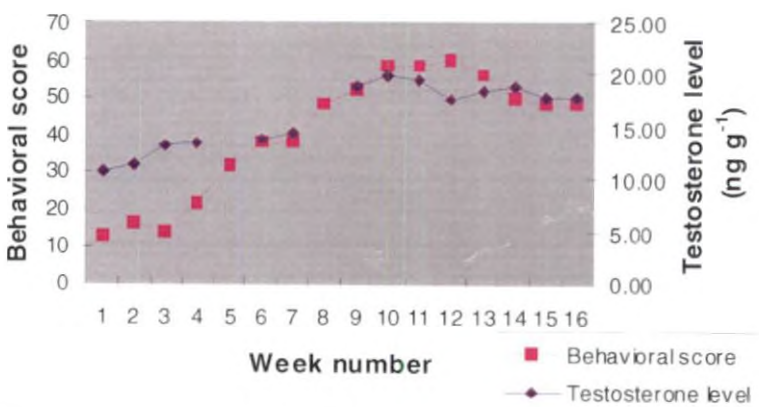


Fig. 26. Comparison of behavioral score and testosterone level – V_3



(- Week of velvet shedding)

4.6.3 Correlation between testosterone level and behavioral score

Statistical analysis to correlate the testosterone levels of all the stags during both hard antler and velvet stage to the behavioral scores showed a significant and positive correlation ($\rho = 0.875$, $p < 0.01$). Statistical analysis of the testosterone level and behavioral score during the hard antler stage alone also showed positive and significant correlation ($\rho = 0.791$, $p < 0.01$). The correlation between the testosterone level and the behavioral score was significant and positive during the velvet stage as well ($\rho = 0.805$, $p < 0.01$). Figures 21 to 26 compares the behavioral score and testosterone levels of the selected six stags.

Discussion

5. DISCUSSION

5.1 SEASONALITY OF DEER BREEDING

The breeding related activities of the sambar deer herd used for the study did not show any seasonality; there were males in rut season throughout the year. There was a slightly higher number of births occurring during the end of dry season and beginning of rainy season, but not significant enough to consider this as the breeding season of the species. The research by Willard and Randall (2002) on a herd of tropical spotted deer had similar reports.

The observations made by Acharjyo *et al.* (2005) in mouse deer in Indian zoos suggested that this species has no fixed birth/breeding season in captivity. Asher (2007) reported that equatorial region cervids are completely aseasonal.

In pampas deer populations inhabiting subtropical to temperate, Ungerfeld *et al.* (2008) reported that births can occur all year round, the peak roughly coinciding with pasture abundance.

5.2 THE DOMINANT MALE

Six top stags were selected from a total of 22 males. The characteristics taken into account for the selection were body and antler size, positioning at the feeding time, ability to control territories and possession of mates. The selected animals were named as H_1, H_2, H_3, V_1, V_2 and V_3 in dominance order of hierarchy. H_1, H_2 and H_3 were in hard antler stage and V_1, V_2 and V_3 were in velvet growth stage at the beginning of the study. The attributes used for the selection were in accordance with Saseendran *et al.* (2003) who claimed that in a population of sambar deer, males were found to control the group led by alpha male. Alpha male was identified by its good physical appearance, sharp and long antlers, positioning at vantage points to take the major share of feed and its mates.

Krebs and Davies (1987) stated that the strongest individuals are despots, grabbing the best quality resources and forcing others into low quality areas or excluding them from the resource altogether.

Fraser and Broom (1997), Komers *et al.* (1997), Stewart *et al.* (2000), Estep and Dewsbury (2005), Vanpe (2007) and Vanpe *et al* (2008) had the same opinion that the dominant stags are involved in more breeding activities in the herd.

5.3 ANTLERS

5.3.1 Antler Characteristics

The stags were observed to be in velvet growth for a duration of seven to eight months. The complete shedding of velvet took about 5-6 days. As per Riney (1954), for most species of deer, males start the growth of antlers form a pedicel on each frontal bone in late spring or early summer. The velvet, a true skin which supplies the growing antler with nutrient material, is shed in the fall and normally takes only a day or so to peel off. Antlers remain hard until they are cast after the period of rut, which normally occurs in late fall or early winter. Price *et al.* (2005) reported that in late summer, longitudinal growth stops, the skin (velvet) covering the antler is lost and antlers are 'polished' in preparation for the mating season. Asher *et al.* (2007) opined that antler rubbing/ thrashing serve to remove the dried velvet tissue on the surface of the hard antler.

The hard antlers decorated the stags for the whole rut season which lasted for about four to five months. This is followed by the antler casting stage wherein, the stag may lose its antler either in a fight or by hitting against tree or fence. The stag may lose both the antlers on the same day or there may even be a gap of 2-3 days for the second one to fall. Price *et al.* (2005) stated that in each spring, deer

shed antlers that were used for fighting and display during the previous mating season. Morrow *et al.* (2009) reported that antlers are cast after the rut.

5.3.2 Seasonality of Antler Growth

The sambar deer herd selected for the study had been observed to breed throughout the year and also stags at various stages of the antler cycle could be seen at any point of the year. Brown *et al.*, (1978) suggested that deer living near the equator, where seasonal day length changes are slight, tend to be asynchronous in both their antler and sexual cycles. The findings of only little evidence of a clear seasonal synchrony in the antler cycle in a herd of spotted deer by Loudon and Curlewis (1988) are also supportive of the present findings. This observation is also supported by Asher (2007) who reported that equatorial region cervids are completely aseasonal.

5.4 PHYSICAL CHANGES

5.4.1 Weight Gain

In this study, the good body condition was maintained at the initial phases of the rut season but, during the later stages of rut/ hard antler phase, the stags were seen in a worn out condition indicative of the hardships undergone during the rut for maintenance of the harem as well as the dominance quotient. Asher *et al.* (1987) reported that fallow deer bucks exhibited pronounced liveweight gains over spring and summer months, to reach a peak mean weight and rapid liveweight losses over the rutting period with a minimum mean liveweight. Monfort *et al.* (1993) reported that antler length, body weight and chest girth were maximal during pre-rut in Eld's deer. The above report was in complete agreement with the finding of the present study.

The worst body condition was observed in those stags whose antlers were freshly cast; the stags appeared skinny, emaciated, lesser coat thickness and some of them carried the gore wounds sustained during the tussles for territory, hinds and rations. The sambar stags seemed to be in peak size and maintained the best body condition during the later velvet stages. The whole of antler re growth stage was found to be a recuperating stage during which the stag prepared itself to face the next rut. McElligott *et al.* (2001) asserted that body mass was related to pre-rut dominance rank which was in turn strongly related to rut dominance rank, and thus there was an indirect relationship between mating success and body mass.

5.4.2 Other Changes

The scrotum showed significant enlargement in the stags approaching the rut season. The stags in the hard antler stage had much larger testes than those in antler cast or velvet growth stages. Monfort *et al.* (1993) observed maximal scrotal circumference and combined testes volume in mid-winter. Georitz *et al.* (2003) also supported the findings with the claim that all reproductive organs were highly developed during the rut only. This finding was supported by Haigh (2007) who stressed that the scrotal circumference increased markedly and peaked at about the same time of the onset of the rutting season in wapiti and red deer.

The coat color was slightly darker in shade in hard antler stags in comparison with those in other stages. The neck musculature was blown up in size during the rut season. Gomez *et al.* (2006 b) also claimed that in case of Iberian red deer, neck circumference showed a time course reaching the highest values during the days of decreasing photoperiod. Blake *et al.* (2007) observed that in reindeer, neck muscles thicken during the rut season.

5.5 ETHOLOGY

5.5.1 Behavioral Observations

The behavioral scores were very high during the hard antler stage for all the six stags in comparison with their own scores during the velvet antler period. Since sambar deer are nocturnal in habit, mounting and service could not be observed more than once, but other activities allied to breeding like chasing of females, sniffing and flehmen's reaction were observed many times. Territorial behavior, holding the head high, fighting, spraying urine upon its own body and face were also observed quite a few times. The results of the studies done by Komers *et al.* (1997), suggested that dominance rank is the most important factor in determining the level of reproductive behaviors exhibited. Skinner and Harrington (2003) had supportive observations that in a group of sika deer the activities directly associated with mating were significantly different between the territorial and non territorial groups. Pereira *et al.* (2005) observed that breeding behavior of male pampas deer during rut was characterized by predominately ano-genital sniffing, flehmen, urine sniffing, chasing and mounting behavior which were similar to the findings in the case of sambar deer stag in this study. As per Mulley (2007), fallow deer bucks will fight vigorously during the pre rut to establish dominance.

5.5.2 Harem Formation

The stag H_1 possessed the largest harem followed by H_2 and H_3 with a membership of 17, 13 and 07 individuals respectively. But this continued only till they retained the antlers, the casting of antlers by these males and the coming to rut of the till then velvet stags lead to a large dropout in the harem membership of the former stags and they preferred to join the harems of V_1, V_2 and V_3. Among the these stags, V_1 collected the largest harem, followed by V_3, and V_2 not lagging far behind. The mentioned stags enjoyed a membership of 19, 14 and 13

individuals respectively. Observations made by Semiadi *et al.* (1994) indicated that although the dominant rutting sambar stag collected a harem, the dominant stag displayed a high degree of tolerance toward the presence of other stags in hard antler within the harem. Fraser and Broom (1997) avowed that the strongest stags are able to command the largest harems and enjoy the most copulation which was supportive of the results of the present study. McElligott *et al.* (2001) reported that larger mature fallow bucks have advantages over other males when competing for mating. Yoccoz *et al.* (2002) claimed that prime-aged males are most often the harem holders among red deer.

5.6 HORMONE ASSAY

5.6.1 Testosterone Levels

In the present study, the testosterone levels of all the six stags were much higher during the rut season/ hard antler stage when compared to their own values during the velvet antler stage. During his study on male white tailed deer Ditchkoff *et al.* (2001) observed that mean levels of serum testosterone increased during the breeding season until approximately 1- 2 weeks before the peak of the rut. Washburn *et al.* (2004) claimed that reproductive steroid hormone levels provide important information regarding the reproductive status of animals. Pereira *et al.* (2005) averred that fecal steroid analysis is a practical and reliable non-invasive method for the evaluation of the endocrine status of free-ranging pampas deer.

The stags had the highest testosterone levels just before the velvet shedding and the hormone level reached the least values during the antler casting stages. Schams and Barth (1982) emphasized that in roe deer stags the highest average levels of testosterone were observed before the start of the rutting season and steadily declined thereafter which was completely supportive of the results of the present study. Sempere *et al.* (1992) , Blottner *et al.* (1996) and Roelants *et al.*

(2002) found that in roe deer the testosterone showed the highest concentration during the rut. Hamasaki *et al.* (2001) reported the highest levels of testosterone concentration for the most dominant sika deer stag compared to the subordinates.

5.6.2 Testosterone Vs Antler stage and Behavior

The fecal testosterone levels of the six stags estimated by RIA revealed that testosterone exerts a strong grip on the antler cycle, as well as the breeding score of the stags. Chapman and Chapman (1970) reported that in fallow deer, the antlers are cleaned of 'velvet' in August and September when testicular activity is increasing and are shed in the spring and summer when activity is at a minimum. Suttie *et al.* (1984) asserted that pedicle initiation was associated with increasing plasma testosterone levels and antler development occurred when testosterone levels were low or decreasing. Cleaning of the velvet was associated with high levels of plasma testosterone. Antler casting occurred when plasma testosterone concentrations were low or undetectable.

The stags in hard antler stage or rut season were found to have a significantly higher testosterone concentration in comparison with the velvet growth stage stags. The study also reveals that, it is the sudden dip in the testosterone concentration which causes the antler casting. Velvet shedding was preceded by an increase in the testosterone levels emphasizing its role. Lincoln *et al.* (1982), Yamauchi *et al.* (1997), Price and Allen (2004), Price *et al.* (2005), Gomez *et al.* (2006 b) and Ungerfeld *et al.* (2009) claimed that in most cervids, concentration of plasma testosterone was associated with the phases of antler development. The increasing behavioral scores were also accompanied by higher levels of testosterone. Pereira *et al.* (2005) also stated that there were significant correlations between fecal testosterone and reproductive behavior.

5.6.3 Correlation between testosterone level and behavioral score

Statistical analysis to correlate the testosterone levels of all the stags during both hard antler and velvet stage to the behavioral scores showed a significant and positive correlation ($\rho = 0.875$, $p < 0.01$). As per Pelletier *et al.* (2003), a study done on bighorn rams showed that fecal testosterone was directly correlated with social rank. Asa (2005) opined that in general, androgens are responsible for reproductive behavior, ranging from aggression related to mate or territory defence to scent marking, courtship and copulation. Wildt (2005) also had supportive views that in the case of cervids the seasonal elevations in testosterone have been associated with increase in sexual activity, aggressiveness and testicular size.

Statistical analysis of the testosterone level and behavioral score during the hard antler stage alone also showed positive and significant correlation ($\rho = 0.791$, $p < 0.01$). Li *et al.* (2001) declared that in Pere David's deer there were statistically significant correlations between some male reproductive behaviors, such as anogenital sniffing, urine sniffing, urine spraying, wallowing, bellowing, antler adorning, antler swags mud, chasing, herding hinds, chin resting, mounting and copulating, with the fecal testosterone concentrations. These results suggested that seasonal reproductive behaviors in stags are strongly associated with circulating testosterone.

In the present study, the correlation between the testosterone level and the behavioral score was significant and positive during the velvet stage as well ($\rho = 0.805$, $p < 0.01$). Roshin (2005) claimed that there exists a positive correlation between the breeding score and fecal testosterone in spotted deer in velvet stage, velvet shedding stage and rutting stage.

Summary

6. SUMMARY

The population of sambar deer is beyond manageable numbers in most of the Indian zoos. Published data regarding the breeding in deer, especially sambar deer, are hardly any. Therefore, this study was carried out to shed light on certain facets of sambar deer breeding so that strategies can be worked out to find out ways to curtail the exploding population. Some of the main objectives taken up for the study were to identify the stags in order of hierarchy, observe and prepare a complete ethogram of sambar deer stags in rut season and correlate it with fecal testosterone levels and to assess the morphological changes, breeding performance, preferences and frequencies of the alpha to lower order males.

The study was carried out at the State Museum and Zoo, Thrissur, Kerala, India for a period of four months from 11th June to 11th October, 2009. The zoo was chosen for the execution of the study because of the large number of sambar deer maintained there. There were a total of 70 sambar deer in the enclosure, during the commencement of the study, of which 22 were males including 16 adult stags.

A preliminary observation was carried out to rank the stags in the herd on a dominance hierarchy table. The characters that were taken into account were the body and antler size, ability of a stag to occupy vantage positions during the feeding time, lead a larger harem, capacity to aggressively dictate other members of the herd and carry out most of the breeding activities in the herd.

Based on the above mentioned characteristics, top three stags in the rut/ hard antler stage, on a chronological order of dominance namely H_1, H_2 and H_3 were selected. Three more superior stags in late stages of velvet growth, which were expected to come into rut/ hard antler stage during the observation period were also selected and were named V_1, V_2 and V_3 as per descending order of dominance. Hence, a total of six animals were selected for the study.

The observations were carried out from 0600 to 1800 hours to go with the normal activity time schedule of the deer as well as the zoo. The twelve hour study period was divided into twelve segments of one hour duration each and each animal was allotted two such segments every week. The stag and the segment in which the observation is to be taken were selected randomly. The animals were observed and each and every activity of the deer during that particular one hour period was recorded in the ethogram. Therefore a total of 318 hours of observation was involved in the study spanning over four months and each animal received about 53 hours of observation.

Fecal pellets were collected on a weekly basis from all six stags for a period of four months. The samples were collected within half an hour of voidance and were labeled and kept in polyethylene covers at -20 ° C until extraction for RIA.

Observation of the stags before, during and after the four month study period revealed that the sambar deer antlers were in velvet growth for a duration of seven to eight months. The complete shedding of velvet took about 5-6 days. The hard antlers decorated the stags for the whole rut season which lasts for about four to five months. This is followed by the antler casting stage wherein, the stag may lose its antler either in a fight or by hitting against tree or fence. The stag may lose both the antlers on the same day or there may even be a gap of 2-3 days for the second one to fall. This stage does not last long, as antler growth is a rapid continuous cycle, and the initiation of the next set of antler would set in without much delay.

The stags seemed to be in peak size and maintained the best body condition during the later velvet stages. The good body condition was maintained at the initial phases of the rut season but, during the later stages of rut/ hard antler phase, the stags were seen in a worn out state. The worst body condition was observed in those stags whose antlers were freshly cast; the stags appeared skinny,

emaciated, lesser coat thickness and some of them carrying the gore wounds sustained during the tussles for territory, hinds and rations. The whole of antler re growth stage was found to be a recuperating stage during which the stag prepared itself to face the next rut.

The scrotum showed significant enlargement in the stags approaching the rut season. The coat color was slightly darker in shade in hard antler stags in comparison to those in other stages. The neck musculature was blown up in size during the rut season.

Stag H_1 maintained a behavioral score of 42±11.73 during the hard antler phase whereas during the velvet phase it slipped to 9.66±2.25. The behavioral score of stag H_2 was 42.85±12.58 during the hard antler stage and it reduced to 12.6±2.3 during the velvet period. Stag H_3 had a behavioral score of 44±14 during the rut season; it became 12.6±3.13 during the velvet stage. The behavioral score of stag V_1 was 23.16±12.84 during the velvet stage, but as it entered the rut season, its score shot up to 51.81±5.54. Stag V_2 had a behavioral score of 24.85±13.83 during the velvet phase, as it shifted to hard antler stage, the score increased to 51.9±5.38. The behavioral score of V_3 was 30.33±14.76 during the velvet antler stage, the shift to the rut took up the score to 53.75±4.83.

The stag H_1 possessed the largest harem followed by H_2 and H_3 with a membership of 17, 13 and 07 individuals respectively till they retained the hard antlers. As V_1, V_2 and V_3 entered the rut season, V_1 collected the largest harem, followed by V_3, and V_2 not lagging far behind. The mentioned stags enjoyed a membership of 19, 14 and 13 individuals respectively.

The stag H_1 maintained a testosterone level of 14.66±2.30 ng g^{-1} of dry feces for the six weeks that it remained in hard antler. A sudden decline in the hormone level to 4.6 ng g^{-1} of dry feces during the seventh week triggered the antler fall. The testosterone level thereafter maintained a low profile of 7.85±

2.32 ng g^{-1} of dry feces for the remaining ten weeks of observation. The testosterone level of stag H_2 was 14.07±0.54 ng g^{-1} of dry feces during the hard antler phase and its decline by the eight week led to the antler casting. The testosterone level then was at a level of 9.12± 2.40 ng g^{-1} of dry feces for nine weeks. Stag H_3 had testosterone levels of 14.85±1.17 ng g^{-1} of dry feces for seven weeks before casting the antler. The casting was followed by testosterone levels of 9.56±1.94 ng g^{-1} of dry feces for the rest of the study period of nine weeks.

Stag V_1, which was in the velvet stage initially, had testosterone levels of 13.52±1.82 ng g^{-1} of dry feces, a sudden increase in the hormone levels by the seventh week caused the velvet to peel off welcoming the stag to the rut season. The stag from thereon maintained a hormone level of 18.65±1.20 ng g^{-1} of dry feces. V_2 maintained testosterone levels of 12.45±0.91 ng g^{-1} of dry feces for seven weeks. This was followed by the velvet shedding and the testosterone level was then maintained at 18.77±1.03 ng g^{-1} of dry feces. The stag V_3 had the hormone levels at 12.32±1.18 ng g^{-1} of dry feces before casting the velvet at the tenth week. The testosterone level after the velvet casting was higher at 18.72±0.79 ng g^{-1} of dry feces.

The fecal testosterone levels of the six stags estimated by RIA revealed that testosterone exerts a strong grip on the antler cycle, as well as the breeding score of the stags. The stags in hard antler stage or rut season were found to have a significantly higher testosterone concentration in comparison to the velvet growth stage stags. The study also reveals that, it is the sudden dip in the testosterone concentration which causes the antler casting. Velvet shedding was preceded by an increase in the testosterone levels emphasizing its role. The increasing behavioral scores were also accompanied by higher levels of testosterone.

Statistical analysis to correlate the testosterone levels of all the stags during both hard antler and velvet stage to the behavioral scores showed a

significant and positive correlation ($\rho = 0.875$, $p < 0.01$). Statistical analysis of the testosterone level and behavioral score during the hard antler stage alone also showed positive and significant correlation ($\rho = 0.791$, $p < 0.01$). The correlation between the testosterone level and the behavioral score was significant and positive during the velvet stage as well ($\rho = 0.805$, $p < 0.01$).

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ANDROGEN MEDIATED BEHAVIOR OF SAMBAR DEER STAGS (*Cervus unicolor*) DURING RUT SEASON

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ABSTRACT

The study was carried out at the State Museum and Zoo, Thrissur, Kerala, India for a period of four months from 11th June to 11th October, 2009. There were a total of 70 sambar deer in the enclosure, during the commencement of the study, of which 22 were males including 16 adult stags. A total of 318 hours of observation was involved in the study spanning over four months and each animal received about 53 hours of observation.

Top three stags in the rut stage, on a chronological order of dominance namely H_1, H_2 and H_3 were selected. Three more superior stags in late stages of velvet growth were selected and were named V_1, V_2 and V_3 as per descending order of dominance. Hence, a total of six animals were selected for the study.

Behavioral scores were allotted on the basis of the observations recorded on the ethogram and with the help of a standard score chart. Stag H_1 maintained a behavioral score of 42±11.73 during the hard antler phase whereas during the velvet phase it slipped to 9.66±2.25. The behavioral score of stag H_2 was 42.85±12.58 during the hard antler stage and it reduced to 12.6±2.3 during the velvet period. Stag H_3 had a behavioral score of 44±14 during the rut season; it became 12.6±3.13 during the velvet stage. The behavioral score of stag V_1 was 23.16±12.84 during the velvet stage, but as it entered the rut season, its score shot up to 51.81±5.54. Stag V_2 had a behavioral score of 24.85±13.83 during the velvet phase, as it shifted to hard antler stage, the score increased to 51.9±5.38. The behavioral score of V_3 was 30.33±14.76 during the velvet antler stage, the shift to the rut took up the score to 53.75±4.83.

Fecal testosterone level was measured by RIA on a weekly basis from all six stags. The stag H_1 possessed the largest harem followed by H_2 and H_3 with a membership of 17, 13 and 07 individuals respectively till they retained the hard antlers. As V_1, V_2 and V_3 entered the rut season, V_1 collected the largest harem with 19, followed by V_3 with 14, and V_2 not lagging far behind with 13 members.

The stag H_1 maintained a testosterone level of 14.66±2.30 ng g^{-1} of dry feces when it was in rut stage. The testosterone level after the antler casting in the seventh week maintained a low profile of 7.85± 2.32 ng g^{-1} of dry. The testosterone level of stag H_2 was 14.07±0.54 ng g^{-1} of dry feces during the hard antler phase and its decline by the eight week led to the antler casting. The testosterone level then was at a level of 9.12± 2.40 ng g^{-1} of dry feces. Stag H_3 had testosterone levels of 14.85±1.17 ng g^{-1} of dry feces before casting the antler. The casting was followed by testosterone levels of 9.56±1.94 ng g^{-1} of dry feces.

Stag V_1 had testosterone levels of 13.52±1.82 ng g^{-1} of dry feces in the velvet stage. After velvet shedding, the stag had hormone levels of 18.65±1.20 ng g^{-1} of dry feces. V_2 maintained testosterone levels of 12.45±0.91 ng g^{-1} of dry feces during the velvet stage. Velvet shedding was accompanied by an increase in testosterone level to 18.77±1.03 ng g^{-1} of dry feces. The stag V_3 had the hormone levels at 12.32±1.18 ng g^{-1} of dry feces before casting the velvet. The testosterone level after the velvet casting was 18.72±0.79 ng g^{-1} of dry feces.

The stags in the rut season were found to have a significantly higher testosterone concentration in comparison with the velvet growth stage stags. The study also reveals that, it is the sudden dip in the testosterone concentration which causes the antler casting. Velvet shedding was preceded by an increase in the testosterone levels emphasizing its role. The increasing behavioral scores were also accompanied by higher levels of testosterone.

Statistical analysis to correlate the testosterone levels of all the stags during both hard antler and velvet stage to the behavioral scores showed a significant and positive correlation ($\rho = 0.875$, $p < 0.01$). Statistical analysis of the testosterone level and behavioral score during the hard antler stage alone also showed positive and significant correlation ($\rho = 0.791$, $p < 0.01$). The correlation between the testosterone level and the behavioral score was significant and positive during the velvet stage as well ($\rho = 0.805$, $p < 0.01$).