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Research Article

A cross-sectional study on serum testosterone and cortisol concentrations in pre-musth and non-musth Asian bull elephants (*Elephas maximus*) using radioimmunoassay (RIA) in Assam, India

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Abstract

Musth is a normal physiological phenomenon exhibited predominately by adult healthy dominant bull elephants. The condition is characterized by aggressive behavior due to increased reproductive activity in adult male elephants of both Asian and African species. Physiological, anatomical, and behavioral manifestations are commonly observed during annual musth due to the remarkable elevation of testosterone in the body. The present study investigates serum testosterone and cortisol concentrations in adult bull Asian elephants during pre-musth and non-musth conditions using Radioimmunoassay (RIA). Serum testosterone (67.68 ± 9.98 ng/mL) and cortisol (419.42±56.64 ng/mL) concentrations in elephants in pre-musth were significantly higher (p>0.01) than in normal elephants. The Pearson correlation between testosterone and cortisol in normal elephants was statistically insignificant (r=-0.067, p=0.791), while in elephants in the pre-musth period, it was insignificant but positively correlated (r=0.302, p=0.561). The regression model predicts that in the pre-musth condition, testosterone increases by approximately 0.05 (ng/mL) as cortisol increases by 1 ng/mL on average, whereas in normal adults, testosterone decreases by 0.001 (ng/mL). However, 9.11% of the variability in testosterone can be explained by the linear association between testosterone and cortisol in elephants during the pre-musth period, in contrast the same was only 0.45% in normal adults. This is the first interim investigation reported on the hormonal profile of elephants in relation to the pre-musth period from the state of Assam, India.

Keywords: Assam, Cortisol, Correlation, Musth, Testosterone

Highlights

- RIA enabled hormonal analysis during pre-musth in Asian bull elephants
- Elephants revealed higher testosterone and cortisol levels in pre-musth state
- Observational signs to identify pre-musth include behavioural changes, temporal gland secretion and urine dribbling
- First report on hormonal dynamics (testosterone and cortisol) in Asian bull elephants from Assam, India

INTRODUCTION

Musth is a normal physiological phenomenon exhibited by an adult, healthy, dominant bull elephant with prominent sexual connotations. This is a physical and behavioral manifestation observed due to a remarkable elevation of testosterone levels, which is characterized by significant aggression (Glaeser *et al*., 2022). In wild, musth plays a vital role in ensuring that only the best genes pass down the generations, thus ensuring better chances of survivability for the species (Sarma, 2001 and Sarma, 2003). Musth is a sign of healthy bull elephant, generally between 20-40 years of age, but may also be observed as early as 12 years and as late as 65 years of age (Brown *et al*., 2007). It is important to recognise the signs of musth, which can be broken down into definable stages viz, pre-musth, early musth, full musth and post-musth (Brown *et al*., 2020). Typical signs of musth includes erratic/ aggressive behaviour, frequent erection of the penis, dribbling of urine, hypertrophy of bulbourethral glands, dedicated tendency to search female elephants in oestrous and characteristic swelling and discharge from the temporal gland which gets more thick and sticky during various stages of musth (Brown *et al*.,

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2020; Glaeser *et al*., 2020 and Mahato, 2022). However, period and stages of musth vary considerably among bulls in terms of both duration and intensity. It is a periodic condition with higher activity of reproductive hormones like androgen in the plasma.In some cases, testosterone levels might elevate to 6-10 times than normal during musth. A musth elephant, whether wild or captive, is extremely dangerous to both humans and other elephants. Hence, the scientific investigation of musth elephants remains a strenuous and challenging task, particularly because of their violent behaviour towards humans and other elephants during that period. Elephants in musth often discharge a thick tar-like secretion called temporin from the temporal ducts on the sides of the head (Anonymous, 2020). Report reveal that temporin contains proteins, lipids (cholesterol), phenol and 4-methyl phenol (Rasmussen *et al*., 1984), cresols, farnesol, and its derivative (Glaeser *et al*., 2020). The bulls in peak musth period may often produce a characteristic low, pulsating rumbling sound known as "musth rumble" which can be heard by other elephants from a long distance (Poole,1987). In some parts of India, captive elephants in musth are traditionally tied to strong trees, with limited food and water until the period passes off. Study on the hormonal status of Asian elephants (*Elephas maximus*) are very scanty, particularly in regards to musth periods from the North-eastern part of the country.

Several studies have been conducted to understand the role of circulating androgens, glucocorticoids, and other hormonal metabolites during the annual musth cycle outside India. However, no such studies have been reported from the northeastern part of India. The purpose of this study was to investigate the serum testosterone and cortisol concentrations in pre-musth and non-musth adult captive Asian bull elephants in Assam.

MATERIALS AND METHODS

Study area and sample collection: A total of 24 departmental captive bull elephants aged between 16 to 69 years (Mean±SE age =38.875±0.79 years) maintained at three different facilities of Assam, India, were included in the study. Blood samples were collected (without sedation) through venipuncture of the ear vein using 26 gauge needles following appropriate protocols and prior approval (No BT/ PR16778/NER/95/340/2017) of the Forest Department, Govt. of Assam. The elephant handlers (mahout) conveyed that some of the elephants were showing altered behavioural that includes occasional reduce responsiveness and obedience towards the command made by them along with physiological manifestations like frequent protrusion of penis, urination, touching near temporal fossa and swelling under the tail (Hypertrophied bulbo-urethral gland). Further, detailed anamnesis and book records also revealed that the elephants exhibiting these behavioural signs have been entering their annual musth cycle periodically. Six out of 24 bull elephants exhibiting pre-musth signs were included in the study based on the criteria as described by Glaeser *et al.,* 2022 (Table 2). The changes in behavioural and physiological signs were recorded and used to differentiate pre-musth elephants (entering into their annual musth cycle) from the normal adult elephants. Behavioural signs (like restlessness, lack of interest in feeding, mild to moderate aggression, erratic behaviour, frequent erection of the penis along with swelling of the bulbourethral gland and occasional trumpeting), temporal gland secretion, urine dribbling (occasional/frequent) and a loss of body condition were considered as qualitative indicators of pre-musth state in this study. The samples were collected in clot activator vial to separate the serum and kept in ice pack for transportation to the laboratory and stored at -80°C until further use. These samples were analysed within 48-72 hours of collection to maintain its integrity and to reduce biological variability. Comparative cross-sectional and co-relational descriptive study on serum testosterone and cortisol concentrations were conducted in elephant during pre-musth (n=6) and in normal adult bull elephants (n=18) from Kaziranga National Park, Orang National Park and Pabitora wildlife sanctuary, Assam, India.

Radioimmunoassay (RIA): The facilities available at Nuclear Research Laboratory under the Department of Animal Physiology, College of Veterinary Science, Khanapara, Guwahati, Assam, India were utilized to process the samples. The serum samples were subjected to radioimmunoassay (RIA) which is considered as highly sensitive and precise in-vitro assay technique that can measure slightest amount of specific antigen in biological fluid. Serum concentration of testosterone and cortisol were analysed using commercially available RIA kits procured from Immunotech, Company of Beckman Coulter, Czech Republic following manufacture's instructions and as per published literature (Smitha *et al*., 2011). The intra and inter assay coefficient of variations were calculated at less than 15.8% and 16.9% in testosterone and 6.0% and 10.4% for cortical respectively. Serum

estosterone and cortisol concentrations of 24 adult male elephants are the domain of study. The concentrations were expressed in ng/mL. (Catogoue ID for RIA kit for cortisol (REF-IM1841) and testosterone (REF-IM1119).

Statistical analysis: Statistical analysis was performed using SAS 9.3 software. The data were subjected to descriptive statistical analysis including correlation and regression. It was further tested by student 't' test for inference and interpretation (Snedecor & Cochran, 1967).

RESULTS

In the present study, adult Asian bull elephants in the pre-musth period showed a typical symptom which includes increased restlessness, lack of interest in feeding, mild to moderate aggression, erratic behaviour, frequent erection of the penis along with swelling of the bulbourethral gland and occasional trumpeting. Discharge from swollen temporal glands, continuous urine dribbling, and a loss of body condition were also recorded. The distribution of serum measurement of testosterone and cortisol level is depicted in Table1 and Fig. 1. Concentrations of testosterone in the serum of elephants during pre-musth (67.68±9.98 ng/mL) were significantly (p <.001) higher than that of normal elephants (0.65±0.18 ng/mL). Similarly, cortisol concentrations were also found to be significantly higher (p <.001) in elephants during pre-musth (419.42±56.64 ng/mL) than that of normal elephants (173.99±13.03 ng/mL).

Table 1. Descriptive statistics of testosterone (ng/mL) and cortisol level (ng/mL) of pre-musth and non-musth elephants and their significance test

Attribute	Statistic(s)	Pre-musth (n=6)	Normal (n=18)	t (22)	p-value	Effect Size
Testosterone	Range	26.45-83.60	0.06-3.26			
(ng/mL)	Mean ±SE	67.68±9.98	0.65±0.18	12.18**	<.001	2.11
(1.84-5.35)	95%CI	42.02-93.33	0.27-1.02			
Cortisol	Range	307.00-692.00	104.50-260.90			
(ng/mL)	Mean ±SE	419.42±56.64	173.99±13.03	6.34**	<.001	1.82
(20-150)	95%CI	273.82-565.02	146.49-201.48			

**Significant at p(<.001)

Table 2. Results of Dichotomous outcome test regarding changes in behavioural signs exhibited by the elephants under study

Criteria / Animal	Temporal gland secretion	Urine dribbling	Erratic behaviour
Elephant 1	Present	Absent	Present
Elephant 2	Present	Present	Present
Elephant 3	Present	Present	Present
Elephant 4	Present	Absent	Present
Elephant 5	Present	Present	Present
Elephant 6	Present	Absent	Present

Note: The behavioural signs associated with pre-musth in male elephants are often subjective and should not be relied upon as the sole indicator for diagnosing the condition. Such behavioural changes should be further validated through biochemical and serological evaluations to reach a definitive conclusion.

DISCUSSION

Elephants exhibiting typical behavioural, physical, and physiological changes in the present study are in agreement with the findings recorded by Lincoln and

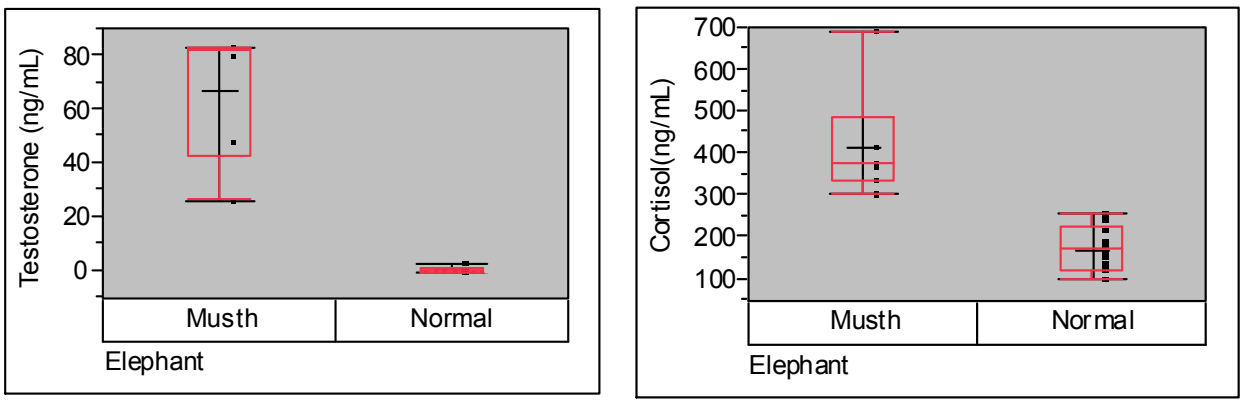


Fig. 1. Testosterone (ng/mL) and cortisol (ng/mL) variability chart

Ratnasoriya (1996), Sarma (2003) and Sukumar (2003). These findings expressed by the bull elephants vary considerably in terms of intensity. Behavioural changes and the elevation of testosterone noted in the serum of pre-musth elephants (n=6) might be due to the dramatic increased in the level of circulating androgen in plasma upon reaching sexual maturity. Previous studies have reported that the mean normal serum testosterone concentration in captive Asian elephants may vary from 1-10 ng/mL in pre-musth, while it may reach up to 20-50 ng/mL during musth (Ganswindt *et al*., 2005(a); Ganswindt *et al*., 2005(b) and Yon *et al*., 2008). In contrast to this, no elevation of testosterone during the musth period was reported in one of the studies which can be explained by difference in study pattern and type of biological sample used for estimation (Carmen, 2012).

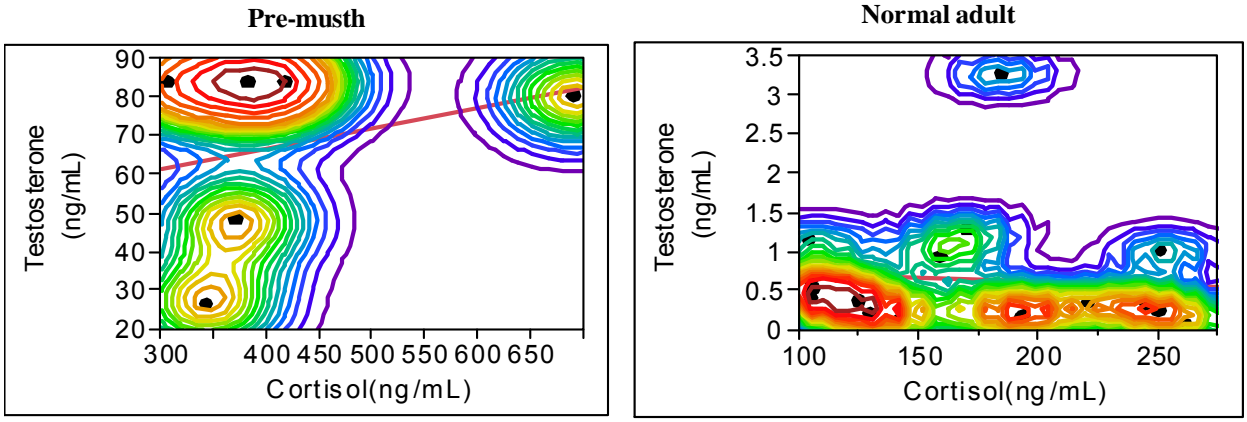
Similarly, in our study, an elevation of cortisol level was recorded during pre-musth stage corresponding to elevated levels of testosterone. This might be due to the fact that pre-musth or musth is considered as a stressful event, where the elephants stay in anger, shows increased restlessness, reduced intake of feed and water leading to decline in body condition. Hence, it is reasonable to expect increase in circulating glucocorticoid concentrations in response to musth episodes (Thomas *et al*., 1999; Whirledge and Cidlowski, 2010 and Carmen, 2012).

The regression model predicts that during pre-musth, testosterone level increases by approximately 0.05 (ng/mL) for every 1 ng/mL increase in cortisol, whereas the same decreaseby 0.001 (ng/mL) in case of normal adults (Fig. 2). Further, 9.11% of the variability in testosterone can be explained by the linear association between testosterone and cortisol in pre-musth whereas the same was only 0.45% in case of normal adults.

This study provided an important insight into the linkage between testosterone and cortisol. However, it is also essential to note certain limitations in our study due to the smaller sample size and factor ambiguity. Furthermore, longitudinal study would have provided a better understanding of hormonal variation over time. Therefore, the present study requires further refinement in these areas and exploration to yield more comprehensive findings. The purpose of the present cross-sectional study was to understand the status of hormones and behavioural signs depicted in the pre-musth condition. This knowledge would help in predicting and recognizing impending musth, allowing planning and formulating strategies well in advance to mitigate man-animal conflict. Also, to our knowledge, this is the first instance of investigation reported from this part of the country and would encourage other academician and wildlife veterinarians to devise more studies on these aspects.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: GM, KKS: Involved in investigation, conceptualization and methodology; SAA: Engaged in sample collection, data curation and preparation of original draft; AKS: Performed statistical analysis; TD, DD: Involved in editing and final proofreading.



Testosterone (ng/mL) = 45.37 + 0.05 × Cortisol (ng/mL)
R Sq=9.11%

Testosterone (ng/mL) = 0.80 - 0.001× Cortisol (ng/mL)
R Sq=0.45%

Fig. 2. Bivariate scatter plot and linear regression of testosterone (ng/mL) on cortisol (ng/mL) in pre-musth (n=6) and normal elephant (n=18).

Data availability statement: The data mentioned in the article will be available with the corresponding author.

Ethical permission: The present study was approved (No BT/PR16778/NER/95/340/2017) by the Department of Forest (Office of Chief Conservator of Forests, Wildlife & Chief Wildlife Warden, Assam), Government of Assam, to screen the rescued wild departmental captive elephants of Assam.

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