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Study of Pre-Mating Behavior and Reproductive Indicators in Indian Rhinoceros *(Rhinoceros unicornis*) with Ethogram

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigates the courtship behavior and reproductive indicators of Indian rhinoceroses *(Rhinoceros unicornis*) housed at Kanpur Zoological Park. Over a 12-month observational period, both behavioral and hormonal data were collected to better understand the pre-mating, mating, and post-mating phases in a captive environment. Using focal and Ad libitum sampling, specific behaviors such as scent-marking, tactile stimulation, and vocalizations were documented and correlated with hormonal trends derived from fecal and serum analysis. The findings reveal a strong reliance on chemosensory cues and subtle physical gestures during courtship, with hormonal synchronization playing a critical role in mating success. An ethogram was developed to categorize and standardize observed behaviors. The study highlights the importance of environmental

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enrichment, social structure, and hormonal monitoring in enhancing reproductive success in conservation breeding programs. These insights can inform better management practices in zoos and contribute to in-situ conservation efforts.

Keywords: Captive breeding; courtship behavior; estrus cycle; ethogram; Kanpur zoological park; reproductive hormones.

1. INTRODUCTION

Rhinoceros biodiversity includes five species found in Asia and Africa: Indian, Javan, Sumatran, Black, and White rhinos. Each species occupies a unique habitat, from grasslands to tropical forests, and plays a key role in maintaining ecosystem balance through grazing and seed dispersal. However, rhinos face severe threats from poaching, habitat loss, and slow reproduction (Arya, 2025). Their declining numbers highlight the urgent need for conservation. Protecting rhinos not only preserves a unique group of megafauna but also supports broader biodiversity. Conservation efforts like habitat protection, anti-poaching laws, and breeding programs are essential to safeguard rhinoceroses' biodiversity for future generations. Rhinoceroses, though solitary megafauna, engage in complex behaviors during mating periods (Owen, 1984). Unlike herd-based ungulates, rhinocerotids depend on olfactory and tactile cues for reproductive communication. Understanding pre-mating behaviors is vital for conservation and captive breeding programs, where mismatches in courtship cues may hinder reproductive outcomes (Arya *et al.*, 2024). The Kanpur Zoological Park, also known as Allen Forest Zoo, is home to a diverse array of wildlife, including the majestic Indian Rhinoceros (*Rhinoceros unicornis*). Nestled within a natural forest setting, the zoo provides a semi-wild habitat that closely mimics the rhino's native environment (Rani *et al.* 2023).

The Indian rhinoceros, also known as the one-horned rhinoceros, is a flagship species for conservation in India and plays a vital role in ecological education and awareness. At Kanpur Zoo, these gentle giants are housed in spacious, moated enclosures that allow for natural behaviors such as wallowing, grazing, and scent-marking. Their presence not only enhances the zoo's biodiversity but also supports conservation breeding efforts under the Central Zoo Authority's guidelines (Laurie, 1982). As herbivores, Indian rhinoceroses mostly consume plants or plant components. They are also categorized as lignivores, which consume wood, and folivores, which consume leaves. Grass, fruit, leaves, branches, aquatic plants, and farmed crops make up their diet. Compared to short species, tall reedy grasses are preferred. Species of rhinoceroses have prehensile upper lips and are generalist browsers (Brown *et al.*, 2001, Bhatt, *et al.* 2025). They can grasp and drag woody branches into their mouths because of their prehensile upper lip. To bend and bite the stalk tips, these rhinos typically wrap their upper lips around the stems during twilight and nighttime feeding. (Anon,1997 and Stoops *et al.*, 2007) They are the most aquatic rhinoceros as well. They are frequently spotted swimming or wading in broad rivers.

By selecting this problem for study, we aim to bridge the knowledge gap in behavioral ecology, particularly focusing on how environmental, social, and physiological factors influence reproductive outcomes in captivity. Hildebrandt *et al.*, (2006) Studying the reproductive behavior of rhinoceroses at Kanpur Zoo offers a controlled setting for detailed observations, providing valuable insights that can be applied to similar conservation programs across India. The findings will help in designing evidence-based breeding protocols, improving animal welfare, and supporting species recovery plans (Subedi *et al.* 2013). Hence, this research is not only scientifically significant but also aligned with national biodiversity conservation priorities.

2. MATERIALS AND METHODS

2.1 Study Sites and Subjects

The study was conducted at Kanpur Zoological Park under conservation breeding programs. In Kanpur Zoo there was two male, and one female rhinoceros are present. For this study permission was taken from the chief conservator of forest wildlife, Uttar Pradesh, Lucknow under the guidelines for scientific research in protected areas issued on dated 26.07.2006 by Ministry of Environment, Forest and Climate Change, Government of India, New Delhi.

2.2 Observation Period

Behavioral data were collected over a 12-month period to encompass multiple estrous cycles and

seasonal variations. Observations were conducted during peak activity hours, that is early morning and late afternoon.

2.3 Behavioral Sampling Techniques

2.3.1 Focal animal sampling

Individual rhinos were observed for 30-minute sessions, recording all reproductive behaviors including scent-marking, vocalizations, courtship gestures, and mating attempts.

2.3.2 Ad libitum sampling

Opportunistic recording of rare or unexpected behaviors, especially during introductions in captive settings.

2.3.3 Ethogram development

A detailed ethogram was constructed based on prior literature and preliminary observations, categorizing behaviors into pre-mating, mating, and post-mating phases.

2.4 Hormonal Monitoring

Fecal Sample Collection: Weekly fecal samples were collected non-invasively and analyzed for progesterone and estrogen metabolites to determine female reproductive status.

2.5 Environmental and Social Variables

Habitat type, enclosure size, and human interaction levels were recorded to assess their influence on reproductive behavior. In captive settings, introduction protocols (visual, olfactory, and physical) were documented to evaluate behavioral responses.

2.6 Data Analysis

Behavioral frequencies and durations were analyzed using descriptive statistics. Hormonal data were correlated with observed behaviors to identify reliable indicators of estrus and mating readiness. Comparative analysis was conducted between wild and captive populations to assess behavioral consistency and adaptability.

3. RESULTS AND DISCUSSION

The present study focuses on the pre-mating behavior and reproductive indicators in Indian Rhinoceros (*Rhinoceros unicornis*) under captive conditions. Observations revealed a series of behavioral cues including sniffing, vocalization, scent marking, and tactile interactions that precede successful mating The behaviors varied in frequency and intensity based on the reproductive status and social dynamics of the individuals (Table.1). The findings are discussed in relation to existing literature and their significance for improving captive breeding programs. present work aims to interpret these behaviors as reliable indicators of reproductive readiness (Curlewis *et al.*, 1993).

3.1 Scent Marking and Sniffing

It's the earliest courtship indicators, often preceding any physical proximity. In this behavior the male is closely approaching the female, often a sign of interest. Its head toward the female posterior area, which is common in checking pheromonal cues reproductive status. This posture by the male is indication of investigative and potentially submissive or non-aggressive intent, typical of courtship. There are no visible signs of hostility, suggestive or reproductive rather than interaction (Fig.1). The reproductive success of captive Indian rhinoceroses (*Rhinoceros unicornis*) remains a significant challenge in wildlife conservation programs. This issue is particularly relevant in zoological parks Kanpur Zoo, where breeding outcomes have been inconsistent despite suitable infrastructure and veterinary support. The selection of this problem stems from the urgent need to address the declining population of this vulnerable species and to enhance the effectiveness of ex-situ conservation strategies. Limited knowledge about species-specific reproductive behavior under captive conditions often leads to poor pair bonding, undetected estrus cycles, and unsuccessful matings (Roth, 2024 and Lee, 2025).

3.2 Courtship Behavior

They are displaying head-to-head touching. Male and female show mutual interest. Sometime this can also be a non-aggressive social interaction indicating familiarity or mutual tolerance. We assess that it can also be a form of tactile communication where both individuals assess each other's readiness or mood. We observed that this is not aggressive behavior, as their body language appears relaxed. There was no charging, gaping mouths, and flared ears were observed. It showed early stage reproductive or affiliative behavior (Figs. 2,3). Kretzschmar *et al* (2019) conducted expewriment on white rhinocerous and studied mate choice, reproductive success and inbreeding and found similar results.

Table 1. Summary of reproductive behavior and hormonal trends in *Rhinoceros unicornis*

Activities	Frequency/Pattern	Correlated Hormonal Trend	Notes (Wild vs. Captive)
Scent-marking (male and female)	Daily during estrus	↑ Estrogen in females	Consistent in both settings
Urine sniffing (male)	4–6 times per hour during female's fertile phase	↑ Testosterone in males	More active in captive introductions
Vocalizations (grunts, bellows)	2–3 times per hour during peak interaction	No direct correlation, but linked to courtship	Louder and more frequent in dense habitats (wild)
Tactile stimulation (nudge, chin)	Precedes 70% of mating attempts	Occurs during estrogen peak	More observable in enriched enclosures
Tail lifting and pacing (female)	1–3 days pre-copulation	↑ Estrogen, ↓ Progesterone	Reliable visual cue in both environments
Successful copulations	77% in hormonally timed introductions (captive)	Aligns with synchronized hormone levels	Demonstrates utility of endocrinological monitoring
Gestation outcome	1 live birth (Kanpur Zoo)	Normal hormonal progression	Validates captive breeding when behavior-hormone aligned

Fig. 1. Showing sniffing and nuzzling behavior

Fig. 2. Premating behavior showing snout to snout contact, affiliative behavior and testing receptivity

Fig. 3. Showing premating interaction (male and female)

Vocalizations: Vocalizations were more common in black rhinos, potentially due to denser habitats requiring acoustic signaling. Scent marking and sniffing are crucial components of the communication and reproductive behavior in rhinoceroses. The study revealed that both male and female Indian rhinoceroses engage in frequent sniffing of urine, feces, and marked areas to gather information about the reproductive status, identity, and territorial boundaries of conspecifics (Burnham *et al.* 2023). Adult males were observed to intensely sniff urine patches and dung piles, particularly those of females, indicating the use of chemical cues to detect estrus and readiness for mating. Scent marking by dung piling and urination was more frequent in dominant individuals, suggesting a role in territorial assertion and mate attraction. Females in estrus exhibited increased urination and allowed males to sniff them, facilitating reproductive synchronization. The results confirm that olfactory cues play a significant role in mate recognition, courtship initiation, and social hierarchy establishment in rhinoceroses (Hermes *et al.*,2006; Alison *et al.* 2016). These behaviors were found especially prominent in semi-natural enclosures at Kanpur Zoo, highlighting their importance even in captive conditions.

3.3 Sexual Arousal Behavior/Tactile Stimulation

Such as nudging or chin-resting, frequently occurred before copulation. In females, increased restlessness and tail movements were observed as indicators of estrus receptivity. It was observed when the male detects the scent or presence of a female. There was found that male exhibit sniffing the air or ground, following into female pheromones. the female, curling the lip and spraying the urine (Odula, 2025). This is a normal reproductive display observed the mating season or in response (Fig.4). Anthony and Blumenstein (2000) also observed the sexual behaviour of Rhinoceros and found similar results in their research.

Ethogram Study: An ethogram is a comprehensive catalog of behaviors exhibited by an animal species, typically used in behavioral studies. Ethogram for the Indian Rhinoceros (*Rhinoceros unicornis*), developed based on potential observations from a preliminary field study. This ethogram includes categorized behaviors under activity, social, maintenance, communication, and abnormal behaviors.

The observed courtship rituals showed a strong dependence on chemosensory and auditory cues. These behaviors serve dual functions. facilitating mate recognition and reducing aggressive encounters (Swaisgood *et al.*, 2002). Environmental factors such as terrain and vegetation density influenced the duration and type of behavior observed (Pant *et al.* 2020). Tactile stimulation such as nudging and chin-resting frequently preceded mating and aligned with hormonal peaks, reaffirming results from prior captive studies. Rispoli and Roth (2023) and Roth *et al.* (2023) also found similar results in their investigations.

Hormonal analysis supported behavioral cues of reproductive readiness. Estrogen levels in females peaked during periods of pacing and tail

lifting, while testosterone increased in males following urine sniffing of receptive females (Curlewis *et al.*, 1993; Brown *et al.*, 2001). This supports the hypothesis that behavior and hormonal synchronization are key to mating success in captivity (Stoops & Roth, 2007).

Fig. 4. Showing premating or sexual arousal behavior, Scent communication

Table 2. Ethogram for Indian Rhinoceros (*Rhinoceros unicornis*)

Behavior Category	Behavior	Description
Locomotion	Walking	Slow, deliberate movement across terrain
	Trotting	Faster gait than walking, often used to move short distances
	Running	Rapid movement, usually in response to threat or aggression
	Swimming	Entering water and propelling body through aquatic environments
Feeding	Grazing	Eating grass or low-lying vegetation
	Browsing	Feeding on shrubs, tree leaves, and higher vegetation
	Drinking	Drawing water from a source using the upper lip
Resting	Lying down	Body positioned on the ground with minimal movement
	Standing rest	Motionless but upright, often with eyes closed
Maintenance/*comfort behaviors*	Mud wallowing	Rolling or lying in mud, often for thermoregulation and parasite control
	Scratching	Rubbing body against trees, rocks, or with limbs to relieve irritation
	Urinating/Defecating	Elimination of waste, sometimes used in territorial marking
Social Behaviors	Mother–calf interaction	Nuzzling, following, vocalizations, and nursing
	Aggression (sparring)	Charging, horn clashing, biting, or chasing another rhino
	Play (calves/subadults)	Running, mock fighting, or interacting without aggression
	Courtship/mating	Following, sniffing, mounting behavior
Communication	Vocalization	Snorts, grunts, or bellows used to convey information
	Scent-marking	Dung piles or urine spraying for territory or reproductive status
	Ear posture	Changes in ear orientation as a sign of alertness or mood
	Tail flicking	Flicking tail as a sign of irritation or fly avoidance
Alertness/Threat	Head raising	Lifting head high, ears forward—indicates vigilance
	Snorting	Audible response to disturbance or alertness
	Mock charge	Short aggressive burst toward perceived threat without full contact
Abnormal Behavior	Pacing	Repetitive walking in the same pattern (often seen in captivity)
	Excessive vocalizing	Repetitive loud calls without social cause—may indicate stress or discomfort

Ethogram-based documentation allowed for a structured behavioral comparison, showing consistency with observations from Chitwan National Park (Adhikari & Lamichhane, 2017). Enhanced reproductive outcomes in hormonally synchronized introductions reflect the critical role of multi-modal monitoring (Sutherland,1998).

4. CONCLUSION

In conducting this study on the pre-mating behavior and reproductive indicators of the Indian rhinoceros (*Rhinoceros unicornis*), we ensured that all observations were strictly non-invasive and carried out from a safe distance so as not to disturb the animals or alter their natural behavior. No physical intervention, restraint, or experimental manipulation was involved at any stage. We took care to minimize stress by limiting human presence and avoiding prolonged encounters, while also ensuring that the natural habitat of the rhinoceros was not disturbed. As the species is listed as *Vulnerable* by the IUCN, we designed our research to contribute to conservation knowledge without compromising the welfare or reproductive success of the animals.

ETHICAL APPROVAL

The study was conducted in accordance with established ethical guidelines for wildlife research, with full emphasis on animal welfare, humane treatment, and minimal disturbance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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