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Fecal Hormonal Dynamics in the Last Month of Gestation in a Captive Black One-Horn Rhinoceros (Rhinoceros unicornis): A Case Report

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ABSTRACT

One-horned black rhinoceros, also known as the Indian rhinoceros (Rhinoceros unicornis) is critically endangered due to poaching and habitat loss. The primary aim of this study was to investigate the levels of fecal progesterone and estrogen in the Indian great one-horned rhino during the last month of 472 days gestation and to observe the associated physical and behavioral changes. Notably, 48 hours before calving, estrogen levels increased by 234.56 ng/g, while progesterone levels decreased by 1034 ng/g. These hormonal changes provide critical insights into the physiological processes leading up to parturition, offering valuable information for the management of breeding programs for this critically endangered species in the wild and captivity.

Keywords: Rhinoceros, Gestation, Estrogen, Progesterone, Fecal metabolites

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INTRODUCTION

The Great One-Horned Rhinoceros (Rhinoceros unicornis), also known as the Indian rhinoceros, is a remarkable species that once roamed across the northern part of the Indian subcontinent. Today, due to habitat loss, poaching, and other anthropogenic pressures, their population is confined to a few protected areas in India and Nepal. As of recent estimates, the wild population is approximately 3,700 individuals, a testament to successful conservation efforts but still a number that underscores the need for continued vigilance and innovative conservation strategies (Talukdar et al., 2020). One such strategy is captive breeding, which is crucial in conserving the Great One-Horned Rhinoceros. Captive breeding programs serve as a genetic reservoir, ensuring that the species can be reintroduced into the wild if necessary and that genetic diversity is maintained. These programs also provide an opportunity to study the species in a controlled environment, yielding insights that can inform conservation efforts in the wild (Dinerstein, 2003). A significant challenge in captive breeding programs is predicting the calving time of female rhinoceroses. Accurate prediction is crucial for ensuring that the necessary preparations are made to provide the best care for both the mother and the calf. However, predicting calving time is fraught with difficulties due to the long gestation period of approximately 15 to 16 months and the subtlety of physical and behavioral changes that occur during this time (Schwarzenberger et al., 2000). One of the primary methods used to predict calving is the monitoring of hormonal changes, particularly estrogen and progesterone levels. These hormones play a critical role in pregnancy and can provide valuable information about the stage of gestation. However, traditional methods of hormone monitoring, which often involve blood sampling, can be invasive and stressful for the animals. This has led to the development of non-invasive techniques, such as fecal hormone analysis, which allow for the monitoring of reproductive status without causing undue stress (Garnier et al., 2002). Fecal hormone analysis has emerged as a powerful tool in the non-invasive monitoring of reproductive status in rhinoceroses. By analyzing fecal samples, researchers can track changes in estrogen and progesterone levels throughout the gestation period. This method has been shown to be effective in predicting the timing of calving and in diagnosing pregnancy (Hindle et al., 1992). The use of fecal samples is advantageous because it eliminates the need for physical restraint and sedation, which can be risky for both the animal and the handlers. Moreover, fecal sampling can be done frequently and easily, providing a continuous stream of data that can be used to monitor the health and reproductive status of the rhinoceros (Brown et al., 2001). In addition to hormonal monitoring, observing physical and behavioral changes can also provide clues about the impending birth. In the last month of gestation, female rhinoceroses may exhibit noticeable changes in their physical appearance. One such change is the development of the udder, which becomes more pronounced as the body prepares for lactation. This physical change is often accompanied by behavioral changes, such as increased restlessness or nesting

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behavior, which can signal that calving is imminent (Patton et al., 1999). The external body appearance of the rhinoceros can also provide important information. As the gestation period progresses, the abdomen may become more distended and the animal may exhibit changes in posture or gait. Careful observation of these changes, combined with hormonal data, can help caretakers predict the timing of calving with greater accuracy (Hermes et al., 2020). Stillbirth and perinatal death rates in captive rhinoceros populations range from 6-17% (Hermes et al., 2007). There is a notable scarcity of information regarding the physical and hormonal validation of captive one-horned Indian rhinoceroses during the last month of gestation. This report aims to fill that gap by providing detailed insights into the physical and hormonal changes observed in these rhinoceroses during this critical period. Understanding these changes is crucial for accurately predicting calving time and ensuring better preparation for both neonatal and maternal care.

MATERIALS AND METHODS

Study Area

The sampling was conducted in Sanjay Gandhi Biological Park commonly known as Patna Zoo. To conduct this work written approval from The Chief Wildlife Warden (CWLW) Bihar was obtained (Wildlife L. No. 338; Dated: 03/07/2020).

Observational Measurements

A captive female Rhino (RANI) age 19 years, entered estrus in the early morning of 06/03/2019. In the evening, transferred to a compatible male enclosure and mating occurred between 6.35 to 7.30 pm on the same day. Continuous observation was conducted, with no further signs of estrus detected. During last months of gestation (days 442 to 472), behavioral and physical changes were monitored and fecal samples were collected at weekly intervals for three weeks and then on daily basis until calving to estimate the fecal progestogens and estrogen levels. Gestation length was calculated using the days of observed mating (Day 0) and parturition.

Fecal progesterone and estrogen metabolites

Fresh feces were collected within one hour of defecation from individual animals, placed in a metal trough, mixed thoroughly to create a homogenized sample, and approximately 200 g was collected in a polybag with a zip. The samples were spread on aluminum foil and placed in a hot-air oven at 60 °C until completely dry. After drying, the feces were pulverized and sieved. Approximately 20 g of fine fecal powder was transferred to a labeled l falcon tube to identify the individual animal samples. The tube was then sealed airtight and preserved at -20 °C until further processing in the laboratory. Fecal progestogen and estrogens metabolites were extracted using the previously described protocol (Kumar et al., 2014; 2021). Fecal progesterone and estrogen enzyme immunoassays (EIAs) were performed as described previously (Kumar et al., 2014, 2021, 2022; Umapathy et al., 2015).

RESULTS AND DISCUSSION

The primary aim of this study was to investigate physical and non-invasive hormonal changes, specifically estrogen and progesterone fecal metabolite levels, in the last month of gestation in captive greater One Horn female rhinoceroses. The hormonal patterns observed in this case report provide valuable insights for the management of captive breeding programs, allowing for better prediction of calving and improved care for pregnant rhinoceroses.

Physically, the greater one-horned rhinoceros appeared substantial, with her abdomen becoming more pronounced by day 462 (11 days before calving), her udder and teats had enlarged and were visible from a distance between the inguinal folds, regardless of the viewing angle (Fig.1a, b & c). By day 465 (eight days before calving), she mostly preferred to stay in the area where she had anticipated delivering her calf. This was her fourth parity, and the male calf was born in the early morning (16/06/2020) after completing a gestation period of 472 days (Fig.1d). The labor process, from onset to completion, lasted approximately 40 minutes. . Throughout the last month of gestation, estrogen levels gradually increased, with a significant spike to 234.56 ng/g observed 48 hours before calving (Table: 1; Fig. 2). At the same time, progesterone levels remained relatively stable until the final days, when a marked decrease to 1034 ng/g was recorded 48 hours before parturition. These hormonal shifts reflect the physiological adjustments necessary for the onset of labor in the greater one-horned rhinoceros.

Fig. 1: Showing udder (a) and teat (b) visible from a distance from inguinal fold c) the abdomen became bulky d) Live male calf delivered on day 472 of gestation.

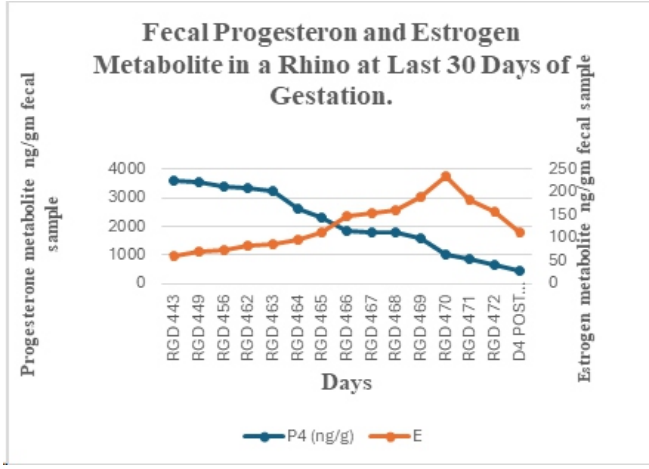

Fig. 2: Line diagram Progesterone levels remained relatively stable and a marked decrease was recorded 48 hours before parturition. Estrogen levels gradually increased, with a significant spike observed 48 hours before calving.

In our study the udder and teat became more prominent eight days before calving and visible from a distance from either on left or right inguinal fold. Our finding is consistent with the findings of Hermes et al. (2020) in white rhinoceros where udder development was started about

Table 1: Fecal Progesterone and Estrogen Metabolite in last months of gestation period in a Greater One Horne Black Rhinoceros (Rani Gestation Period 472 Days)

Days	RGD 443	RGD 449	RGD 456	RGD 462	RGD 463	RGD 464	RGD 465	RGD 466
Progesterone Metabolite ng/gm fecal sample	3612.62	3557.66	3421.40	3335.81	3218.46	2620.08	2293.19	1825.18
Estrogen ng/gm fecal sample	60.32	69.73	72.56	81.71	87.96	97.41	113.48	147.71

Days	RGD 467	RGD 468	RGD 469	RGD 470	RGD 471	RGD 472	D4 POST CALV
Progesterone Metabolite ng/gm fecal sample	1790.16	1774.44	1593.83	1035.56	878.58	673.70	446.26
Estrogen ng/gm fecal sample	155.28	160.81	191.14	234.56	184.83	156.31	113.76

3 weeks prior to calving. In the present study, the gestation length recorded was 472 days and the male calf was delivered in the summer season. The average values of gestation length vary between different Rhino species. The average gestation length reported for black (n-43), white (n-54), and Greater One Horn rhinoceroses (n-23) were 461, 504, and 480 days, respectively. Gestation in captive rhinoceros is, on average, one week shorter when the birth occurs in summer as opposed to winter and sex of foetus did not affect the gestation length as reported (Stoops et al., 2016; Schwarzenberger and Hermes, 2023).

During the last month of gestation, estrogen levels showed a gradual increase, culminating in a significant spike to 234.56 ng/g observed 48 hours before calving. Meanwhile, progesterone levels remained relatively stable until the final days, when they markedly decreased to 1034 ng/g 48 hours before parturition. These hormonal changes suggest the physiological preparation for labor. The observed increase in estrogen levels shortly before calving is consistent with its role in promoting cervical ripening and enhancing uterine contractility, essential processes for successful parturition. The decline in progesterone levels aligns with the withdrawal of progesterone support, a common precursor to labor in many mammalian species. These findings are consistent with previous studies on rhinoceros reproductive physiology, such as those by Schwarzenberger et al. (1996) and Patton et al. (1999), which emphasize the importance of hormonal monitoring in managing reproductive health and predicting parturition. The pattern of progesterone decline is similar to that observed in white rhinoceros, horses, and other wildlife species, supporting its reliability as a predictor. The decline in serum progesterone concentration is not affected by a female's age, parity, or visible behavioral changes, making it a more universal predictor. Our findings align with the study by Hermes et al. (2020), which also reported a significant drop in serum progesterone levels one week before, with a more pronounced decrease 48 hours before parturition in white rhinoceros. The pre-partum E elevation is similar to other species such as the cow and the sheep (Catchpole, 1969). Kock et al. (1991) reported that serum estradiol concentrations were higher in black rhinoceros females later in pregnancy than in early pregnant or nonpregnant animals (Berkeley et al., 1997). Contrary to some studies that suggest behavioral changes as indicators of impending parturition, our research indicates that behavioral changes along with hormonal changes are more reliable predictors.

The findings of this study provide valuable insights into the reproductive physiology of Greater One Horn rhinoceroses and can be applied to enhance the accuracy of predicting parturition in captive breeding programs, thereby improving neonatal care and survival rates. The relatively small sample size may limit the generalizability of the findings to all Greater One Horn rhinoceroses or other rhinoceros species populations. Further researches with larger sample sizes and across different rhinoceros's populations are recommended to validate these findings and enhance our understanding of rhinoceros reproductive physiology.

CONCLUSION

In conclusion, this study demonstrates significant behavioral, physical and hormonal changes during the last month of gestation in captive Greater One Horn rhinoceroses, with increased estrogen and decreased progesterone levels 48 hours before calving indicating imminent parturition. These findings have direct applications in captive breeding programs, allowing for targeted interventions to ensure successful parturition and neonatal care. Implementing routine hormonal monitoring could markedly enhance reproductive success rates and contribute to the conservation of this species."

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