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Research paper

Prolactin in managed female white and black rhinoceroses: Associations with age, reproductive status, and season

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ABSTRACT

White rhinoceroses (*Ceratotherium simum*) and black rhinoceroses (*Diceros bicornis*) maintained under human care complement *in situ* conservation efforts by safeguarding genetic diversity and providing opportunities to study their biology. Prolactin (PRL), a pituitary hormone, could augment our understanding of rhino reproduction and physiology beyond what steroid hormone monitoring alone provides. The goals of this study were to characterize circulating PRL concentrations and evaluate associations with age, reproductive status, and season in adult, non-pregnant female white (n = 27) and black (n = 15) rhinoceroses. A previously validated PRL assay was utilized to measure serum samples collected monthly over a year. Reproductive status was determined by progesterone monitoring. Of the individuals with sufficient data; 15 white rhinos were classified as reproductively active and 6 as inactive. Additionally, 9 black rhinos were classified as reproductively active and none as inactive. In reproductively active cohorts, circulating PRL was lower in white (19.3 ± 3.4 ng/mL) compared to black rhinoceroses (40.0 ± 7.3 ng/mL; p = 0.0030). Variation in PRL concentrations was not associated with age in either white or black rhinoceroses (p = 0.3065; p = 0.7833, respectively). Within white rhinoceroses, reproductively active females had higher circulating PRL than inactive females (20.7 ± 4.8 ng/mL vs 7.6 ± 2.6 ng/mL, respectively, p = 0.0306) and PRL varied by season with lower concentrations in the winter compared to spring, summer, and fall (p = 0.0002). In black rhinoceroses, PRL did not vary significantly across seasons (p = 0.1033). These findings provide baseline data for non-pregnant white and black rhinoceroses and suggest that both reproductive status and season are associated with PRL variation in white rhinoceroses.

1. Introduction

White rhinoceros (*Ceratotherium simum*) and black rhinoceros (*Diceros bicornis*) populations are conservation priorities due to persistent threats from habitat loss and poaching [1,2]. Populations maintained under human care complement *in situ* conservation efforts by safeguarding genetic diversity and providing opportunities to study rhinoceros biology. Advances in endocrine monitoring, assisted reproductive technologies, and coordinated population management have greatly improved our ability to support the propagation of these species [3]. Analyses of progesterone and its metabolites in serum and feces have provided critical insight into estrous cycle lengths and pregnancy duration in rhinoceroses (henceforth referred to as rhinos) [4–8]. However, these steroid profiles largely represent ovarian activity; expanding endocrine assessments to include pituitary hormones may provide deeper insight into reproductive function/dysfunction, metabolism, and health.

One pituitary signal of particular interest is prolactin (PRL), a multifunctional protein hormone involved in over 300 physiological processes including its namesake function in lactation induction, as well as immune modulation and metabolic regulation [9]. Within the reproductive axis, PRL influences ovarian activity and seasonality [9,10]. Although PRL has not been extensively studied in rhinoceroses there are several papers reporting preliminary data that set a foundation for this work [5,11,12], and research in other species provides additional important context.

Many PRL-mediated responses appear to require concentrations within an optimal physiological range [13–15]. PRL deficiency has been associated with suppressed reproductive processes, including impaired implantation, inhibited mammary gland development, and reduced maternal behavior [9,16–20], while excessive PRL concentrations (hyperprolactinemia) have been associated with anovulation, amenorrhea, and galactorrhea across multiple species [9,21–24]. Evidence from African elephants (*Loxodonta africana*) further illustrates the importance

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of maintaining PRL within an appropriate physiological range. Approximately half of acyclic females in North American zoos exhibit elevated circulating PRL, whereas others display persistently low PRL concentrations, and both conditions are associated with ovarian inactivity and lack of reproductive success [25,26]. Together, these findings suggest a potential association between PRL dysregulation and impaired reproductive activity in large pachyderms.

Early work in free-ranging black rhinos revealed that PRL concentrations were largely undetectable when measured using a radioimmunoassay developed for human (*Homo sapiens*) prolactin [11]. Subsequent studies employing equine (*Equus ferus caballus*) PRL assays have detected measurable concentrations in rhino serum. In a Sumatran rhino (*Dicerorhinus sumatrensis*), circulating PRL increased at the end of gestation and into the early postpartum period [5]. More recent results from our laboratory using a different equine PRL assay validated for rhino serum suggested that PRL concentrations may differ across sex and reproductive states of managed black and white rhinos [12]. However, there are other factors that could impact such comparative PRL results. In many mammals, PRL secretion varies with photoperiod, even in species that do not exhibit a defined breeding season [9,27–29]. Additionally in humans and laboratory mice (*Mus musculus*), serum PRL declines with advancing age [30,31]. The primary objective of the present study was to utilize the validated equine PRL assay to evaluate circulating PRL in relation to reproductive status, as defined by progesterone profiles, in non-pregnant black and white rhinos. Additional analyses were performed to assess the potential effects of season or rhino's age on circulating PRL concentrations. Establishing the presence or absence of these associations is a necessary first step toward determining whether PRL monitoring alongside other hormone monitoring could inform management decisions for rhinos in human care.

2. Materials and methods

2.1. Animals

This study was conducted as part of a larger collaborative effort under the American Institute of Rhinoceros Science (AIRS) initiative and focused on samples voluntarily collected from non-pregnant female black (n = 15) and white (n = 27) rhinos managed across 25 institutions in the United States and eastern Canada (range 1–4 rhinos per institution; Table 1). Females that were determined to be pregnant at the time of sample collection (based on progesterone concentrations and/or parturition records), or that had received contraceptive treatment were excluded from the study. Information regarding other hormonal treatments or reproductive medications was not systematically collected across institutions and could not be accounted for in this study. Daily management and husbandry practices varied by facility but followed the general guidelines described in the Rhinoceros Husbandry Manual [32]. Ages ranged from 5.2 -49.6 years in white rhinos and 3.3–29.2 years in black rhinos (Table 1). Institutional Animal Care and Use Committee approval was obtained from the Center for Conservation & Research of Endangered Wildlife (CREW) of the Cincinnati Zoo & Botanical Garden (#22–173), and George Mason University (#1833567–1).

2.2. Sample collection

All animals were trained for voluntary blood collection according to each institution's established protocols. The specific timing of sample collection relative to feeding, exercise, or other husbandry activities was at the discretion of each institution and was not recorded. Institutions were asked to collect blood samples approximately once per month (± 3 days) for 12 months, using a standardized protocol provided by the AIRS team. Whole blood was collected into additive-free serum tubes without gel separator (provided by AIRS), allowed to clot at ambient temperature for 30–60 min or under refrigeration for up to 4 h, then centrifuged to separate serum from blood cells. The serum portion was transferred into cryovials and stored at −20°C until shipment. Samples were shipped overnight on dry ice to CREW for analysis. Upon arrival at CREW, samples were maintained at −20°C and thawed only immediately before analysis to minimize freeze/thaw cycles. Across individuals, 259 serum samples were analyzed from white rhinos and 170 from black rhinos, with 2–16 samples per individual for white rhinos and 6–15 samples per individual for black rhinos (Table 1).

2.3. Reproductive status determination

Reproductive status was assigned based on evidence of ovarian activity during the study period, as determined by longitudinal progesterone monitoring (Table 1). In addition to the monthly serum samples, participating institutions conducted more intensive sampling during two approximately 12-week windows as part of the broader AIRS reproductive assessment effort. Progesterone profiles were derived from fecal progesterone values (2–3 samples per week; obtained as described in Windle et al., 2026 [33] and/or serum progesterone values (1 sample per week; collected as described above). Each institution self-selected collection windows to align with local management seasonality, typically one winter and one summer 12-week period. Rhinos with progesterone concentrations that increased above baseline, were classified as reproductively active regardless of cycle regularity (n = 15 white rhinos; n = 9 black rhinos). Individuals with persistently low progesterone concentrations across all samples were considered reproductively inactive (n = 6 white rhinos; n = 0 black rhinos). Individuals without sufficient progesterone data were excluded from analyses involving reproductive status (not assigned [NA]; n = 6 white rhinos; n = 6 black rhinos).

2.4. Prolactin immunoassay

Prior to use, serum samples were vortexed to ensure thorough mixing and then centrifuged briefly to pellet any cellular debris. The supernatant was analyzed in duplicate with the equine PRL ELISA (CSB-EL018724HO, Cusabio, Houston, TX, USA) according to manufacturer's instructions. This assay was previously validated for use on rhino serum, including dilutional linearity, parallelism with the standard curve, and biological validation [12]. Black rhino serum samples were run neat. White rhino serum samples repeatedly produced high coefficients of variance (CV) between neat duplicates (>15%). Therefore, following the manufacturer's recommendation, white rhino serum samples were diluted 1:1 with phosphate-buffered saline (PBS) to improve assay precision. Despite this effort, a portion of the samples still had duplicate CV values greater than 15% and could not be included in the study (n = 26). The intra-assay precision was calculated by averaging the coefficient of variation across duplicate wells for all accepted samples; mean CV = 6.1% for white rhinos (n = 259 samples) and 5.4% for black rhinos (n = 170 samples). Inter-assay precision was monitored by including a serum sample, previously evaluated in an earlier study [12], on every plate; the inter-assay CV was 17% (n = 20 plates). If a value fell outside of the standard curve, the sample was diluted as needed and re-analyzed. Samples falling below the limit of the standard curve were assigned a value of 1.25 ng/mL, calculated as one-half the lower limit of detection (2.5 ng/mL) as recommended by Hornung and Reed (1990; [34]); n = 15 white rhino samples and n = 0 black rhino samples.

2.5. Statistical analyses

Analyses were conducted in R (ver4.6.1; R Foundation for Statistical Computing, Vienna, Austria) using a structured internal R workflow built upon publicly available packages. Linear mixed-effects models were fit using the *lme4* package (ver2.0.6), with Kenward-Roger-adjusted Type III F-tests implemented via *lmerTest* (ver3.2.1). Model diagnostics, including assessment of normality, homogeneity of variance, influential observations, and model fit, were conducted using

Table 1
Individual demographic, reproductive, and progesterone summary information for female rhinoceros included in this study.

Rhino ID	Inst ID	# Samples*	Species	Avg Age (years)	Avg PRL (ng/mL)	SD PRL	Repro Status**	Serum P4 range (ng/mL)	Fecal P4 range (ng/g)	Last Partuition (years)†
DB-2	Inst-1	13	White Rhino	26.92	6.14	1.27	Inactive	.03 −.13	8.79–136.47	-
DB-3	Inst-1	11	White Rhino	28.00	2.48	1.65	Inactive	.03 −.10	13.61–66.46	-
DB-4	Inst-1	12	White Rhino	26.92	1.89	1.19	Inactive	.03 −.11	10.18–84.50	-
DB-9	Inst-10	4	White Rhino	28.42	6.67	1.28	Active	.03–4.26	16.24–1634.34	12.4
DB-12	Inst-10	10	White Rhino	7.50	16.37	7.11	Active	.11–1.55	33.63–230.90	-
DB-16	Inst-14	11	Black Rhino	12.67	29.54	14.65	Active	.32–1.03	33.66–163.42	1.8
DB-17	Inst-15	11	Black Rhino	29.17	16.27	12.86	NA	-	-	4.5
DB-19	Inst-15	12	Black Rhino	19.5	27.23	20.83	NA	-	-	0.6
DB-26	Inst-18	12	White Rhino	23.58	21.12	17.86	Active	-	21.12–475.77	2.0
DB-27	Inst-18	11	White Rhino	26.42	9.59	3.16	Active	.17–1.75	10.20–633.77	1.1
DB-28	Inst-18	11	White Rhino	12.75	10.13	3.27	Active	-	18.13–550.99	1.0
DB-29	Inst-18	12	White Rhino	7.67	9.98	1.29	Active	.03 −.39	25.00–697.81	-
DB-32	Inst-19	12	Black Rhino	25.83	87.42	39.82	Active	.028–2.59	22.43–214.11	-
DB-33	Inst-19	13	Black Rhino	23	64.67	24.64	Active	.07–2.21	24.33–123.00	10.7
DB-36	Inst-19	12	White Rhino	23.25	23.53	9.71	Active	.14–1.42	21.89–200.00	-
DB-40	Inst-19	3	White Rhino	5.17	41.02	18.18	Active	-	61.76–20,000	-
DB-46	Inst-21	13	Black Rhino	25.08	25.30	16.56	Active	.15–1.36	21.67–265.10	6.1
DB-48	Inst-22	11	White Rhino	27.17	12.44	2.55	Inactive	.03 −.33	9.76–55.86	-
DB-55	Inst-24	11	Black Rhino	18.67	81.13	44.65	NA	-	-	-
DB-59	Inst-25	8	Black Rhino	17.5	34.26	28.86	Active	.82–4.86	-	3.7
DB-61	Inst-26	10	White Rhino	25.92	58.76	45.80	Active	.03–2.76	-	-
DB-65	Inst-26	12	White Rhino	13.58	24.80	12.31	Active	.03–2.91	-	-
DB-80	Inst-3	12	White Rhino	36.83	12.41	3.71	Active	-	10.80–320.02	25.9
DB-114	Inst-40	15	Black Rhino	14.92	64.18	31.59	Active	.41–3.60	48.13–288.35	2.7
DB-115	Inst-41	6	Black Rhino	22.5	76.81	15.40	Active	1.02–3.02	33.78–239.71	-
DB-121	Inst-43	7	White Rhino	5.17	10.41	0.70	Inactive	-	12.12–545.54	-
DB-130	Inst-47	10	White Rhino	7.42	48.64	35.19	Active	.03–2.71	13.42–816.18	-
DB-134	Inst-50	9	Black Rhino	24.42	37.44	22.27	Active	.20–2.19	27.34–293.63	1.3
DB-140	Inst-52	12	White Rhino	11.08	14.16	2.73	Inactive	-	14.98–35.75	-
DB-118	Inst-52	7	White Rhino	49.58	13.89	4.73	NA	-	-	-
DB-150	Inst-54	16	White Rhino	8.50	43.39	36.13	Active	.13–2.85	29.60–563.49	-
DB-155	Inst-55	8	White Rhino	10.25	13.56	12.32	NA	-	-	1.3
DB-156	Inst-55	10	White Rhino	11.42	27.39	10.88	NA	-	-	2.9
DB-157	Inst-55	10	White Rhino	11.42	18.30	5.84	NA	-	-	-
DB-160	Inst-56	8	White Rhino	7.25	35.83	29.69	Active	.09–2.26	16.77–300.14	-
DB-162	Inst-56	6	White Rhino	7.42	41.17	34.62	Active	.03–2.48	16.60–365.13	-

(*continued on next page*)

Table 1 (*continued*)

Rhino ID	Inst ID	# Samples*	Species	Avg Age (years)	Avg PRL (ng/mL)	SD PRL	Repro Status**	Serum P4 range (ng/mL)	Fecal P4 range (ng/g)	Last Partuition (years)†
DB-167	Inst-58	7	White Rhino	19.50	69.14	58.57	NA	-	-	-
DB-174	Inst-6	12	Black Rhino	5.75	29.14	25.49	NA	-	-	-
DB-175	Inst-6	12	Black Rhino	3.25	26.14	29.19	NA	-	-	-
DB-176	Inst-6	12	Black Rhino	11.92	23.32	5.92	NA	-	-	3.2
DB-180	Inst-7	2	White Rhino	21.92	24.25	3.36	NA	-	-	-
DB-184	Inst-8	13	Black Rhino	11.83	46.18	31.92	Active	-	29.88–243.64	-

Abbreviations used: ID, identifier; Inst, institution; NA, not assigned; PRL, prolactin; P4, progesterone; Repro, reproductive; SD, standard deviation.
*__Indicates the number of samples__ included in analysis __from each__ rhinoceros; the final number analyzed may differ to sample exclusions as described in Methods.
**Reproductive status was determined from progesterone profiles. Individuals whose progesterone concentrations showed any evidence of elevation above baseline, regardless of cycle regularity, were classified as active. Individuals whose progesterone concentrations remained persistently low across all available monitoring were classified as inactive. Individuals with insufficient progesterone data were not assigned (NA).
† Indicates the number of years between the most recent calving event and the sample collection period. Nulliparous individuals are indicated with a dash.

performance (ver0.18.2) and *car* (ver3.1.5) packages.

For all models examined, individual rhino nested within institution was included as the random effect to account for repeated measures and potential institution-level variation. PRL concentrations were log-transformed prior to analysis to improve conformity with model assumptions of normally distributed residuals and homogeneity of variance. Model-adjusted means and standard errors, as well as pairwise comparisons, were generated using the *emmeans* package (ver 1.10.6) on the log-transformed values. Pairwise comparisons were conducted using Fisher's protected LSD, where mean separations were only interpreted when the overall F-test was significant at $p \leq 0.05$. Results are reported as F statistics with Kenward-Roger-adjusted numerator and denominator degrees of freedom (e.g., $F_{1,21.3} = 11.2$) alongside exact p-values. For data presentation, model-adjusted means were back-transformed to the original scale by exponentiation, and standard errors were approximated using the delta method.

Initial analysis compared PRL concentrations between species (fixed effect); because all black rhinos were categorized as reproductively active, this comparison was limited to reproductively active females from both species. Given the observed species difference and the possibility of differential antibody cross-reactivity, all subsequent analyses were conducted within species. For white rhinos, three separate models were evaluated with reproductive status (active vs inactive), meteorological season (winter, spring, summer, fall), or age (continuous) as a fixed effect. Reproductive status could not be evaluated in black rhinos as no individuals were reproductively inactive; therefore, only meteorological season and age were modeled individually as fixed effects. Season was defined using conventional North American definitions: Winter (December 1 – February 28), Spring (March 1 – May 31), Summer (June 1 – August 31), and Fall (September 1 – November 30).

3. Results

Circulating concentrations of PRL were lower in reproductively active white rhinos (19.3 ± 3.4 ng/mL) compared to reproductively active black rhinos (40.0 ± 7.3 ng/mL; $F_{1,21.3} = 11.2$, $p = 0.0030$; Fig. 1). Species comparisons were conducted on reproductively active females only, as all black rhinos that could be assessed for reproductive status were considered active. Given the observed species difference in PRL concentrations and the possibility that assay antibody affinity may differ between species, all remaining analyses were conducted separately for black and white rhinos.

In white rhinos, PRL concentrations were not associated with increasing age (slope = −0.01, $F_{1,26.62} = 1.09$, $p = 0.3065$). However, reproductively active females had higher circulating concentrations of PRL than inactive females (20.7 ± 4.8 ng/mL vs 7.6 ± 2.6 ng/mL, respectively, $F_{1,11.3} = 6.1$, $p = 0.0306$; Fig. 2). PRL also varied by season with concentrations lower in winter compared to other seasons (winter:12.5 ± 2.5 ng/mL, spring:18.0 ± 3.5 ng/mL, summer:17.1 ± 3.4 ng/mL, fall:18.6 ± 3.6 ng/mL; $F_{3233.2} = 6.9$, $p = 0.0002$; Fig. 3A).

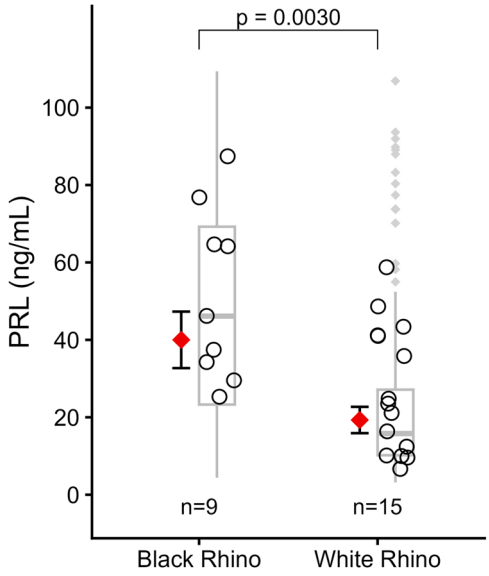


Fig. 1. Circulating prolactin (PRL) concentrations in reproductively active black and white non-pregnant female rhinos. Red diamonds indicate back-transformed, model-adjusted means with standard error bars; means differed between species ($F_{1,21.3} = 11.2$). Individual points represent the overall mean PRL concentration for each rhino (n = number of rhinos per species). Boxplots depict the distribution of all individual samples with outliers shown as diamonds.

Reproductive status association with PRL concentrations could not be evaluated in black rhinos because no individuals were classified as reproductively inactive. In black rhinos, circulating PRL concentrations were not affected by age (slope = −0.005. $F_{1,8.36} = 0.08$, $p = 0.7833$). Additionally, PRL variation across the four seasons approached but did not reach the pre-established significance threshold ($F_{3153.2} = 2.1$, $p = 0.1033$). Mean PRL concentrations by season were: spring 30.4 ± 5.5 ng/mL, summer 33.6 ± 5.8 ng/mL, fall 42.0 ± 7.5 ng/mL, and winter 36.7 ± 6.5 ng/mL (Fig. 3B).

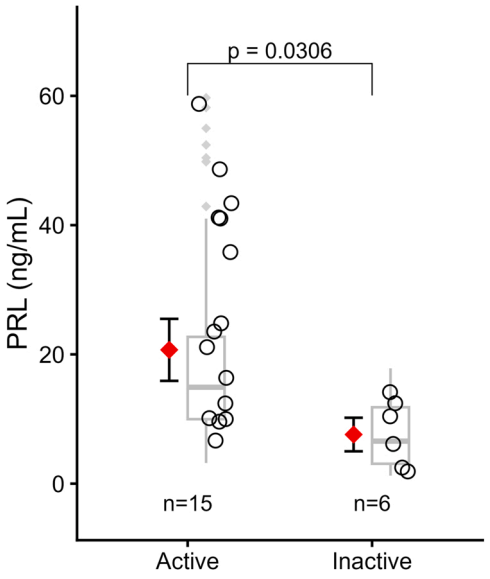


Fig. 2. Serum prolactin (PRL) concentrations in non-pregnant female white rhinos by reproductive status. Red diamonds indicate back-transformed, model-adjusted means with standard error bars; means differed between reproductively active and inactive females ($F_{1,11.3} = 6.1$). Individual points represent the overall mean PRL concentration for each rhino (n = rhinos per reproductive status). Boxplots depict the distribution of all individual samples, with outliers shown as diamonds.

4. Discussion

This work significantly expands on our earlier study of rhino PRL [12] by employing the same validated assay to characterize PRL concentrations in non-pregnant black and white rhinos and evaluate how PRL varies with reproductive status, age, and season. Circulating PRL concentrations differed between the two rhino species, with higher concentrations observed in black rhinos than in white rhinos. Similar species differences have been reported in other large mammals, including Asian elephants (*Elephas maximus*) and African elephants [35], reinforcing that physiological baselines may differ substantially between closely related species [36]. However, even when amino acid sequences are highly conserved between species [12], post-translational modifications such as glycosylation can alter epitope presentation and assay behavior [37]. Thus, we cannot rule out the possibility that the observed species difference reflects differential antibody recognition rather than a true difference in circulating concentrations. These findings highlight the importance of evaluating each rhino species independently when assessing endocrine function.

In white rhinos, reproductively inactive females exhibited lower PRL concentrations than reproductively active individuals, which contrasts with early studies in elephants that reported hyperprolactinemia was often associated with reproductive acyclicity [35]. More recent published work indicates that reproductive dysfunction in elephants can happen in females with both high and low PRL [26,38,39]. Our data demonstrate a range in PRL concentrations among individual rhinos that may be expected with natural biological variation, but there are no existing rhino PRL reference values for comparison. Regardless, no individual's mean PRL concentration fell outside two standard deviations of the population mean, and although the range in concentrations was smaller for reproductively inactive females, there was overlap with concentrations measured in reproductively active individuals. Therefore, study findings provide no evidence of abnormal PRL concentrations in white rhinos regardless of their reproductive status.

Factors potentially contributing to the lower mean PRL concentrations found in reproductively inactive white rhinos were considered. It was noted that all six females were nulliparous. In African elephants, females that have produced at least one calf are more likely to exhibit normal ovarian cycles and prolactin profiles [26] suggesting that reproductive history may be a relevant factor associated with PRL function and regulation in large pachyderms. However, there were also nulliparous female white rhinos in the reproductively active group of this study, and they were producing higher PRL than most individuals in their cohort (Table 1). In fact, the highest mean PRL was recorded for a nulliparous, reproductively active individual (58.76 ng/mL; Table 1). Furthermore, for rhinos in human care, parity is contingent with

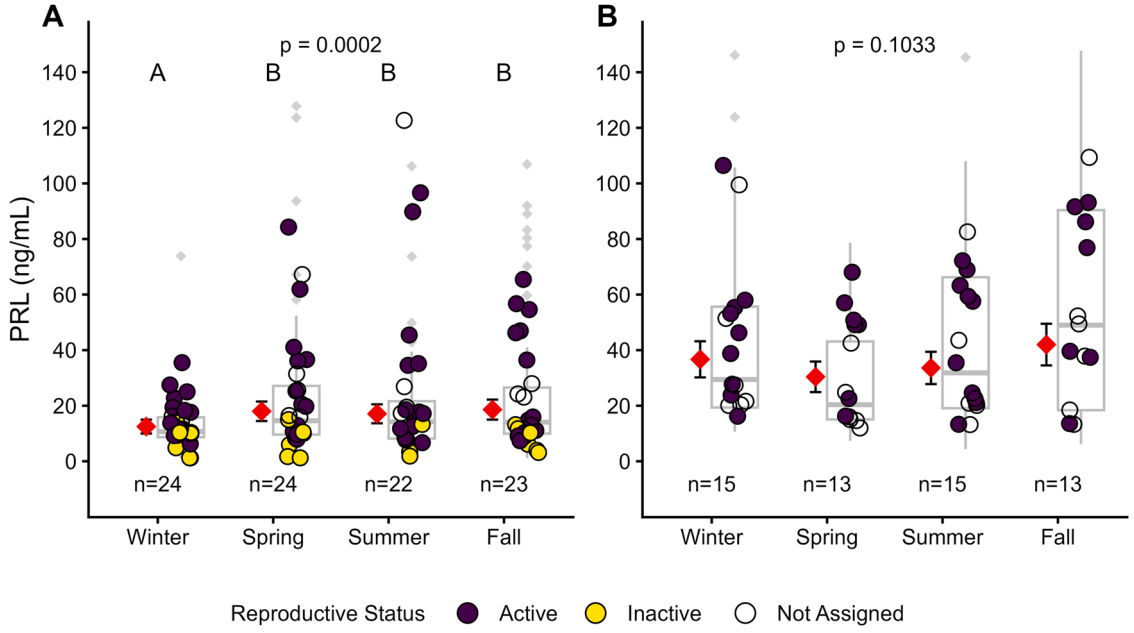


Fig. 3. Seasonal variation in serum prolactin (PRL) concentrations in white (A) and black (B) non-pregnant female rhinos. Red diamonds indicate back-transformed, model-adjusted means with standard error bars. Letters in panel A denote mean separation results ($F_{3233.2} = 6.9$), groups sharing the same letter did not differ. Individual points represent the mean PRL concentration for each rhino, colored by reproductive status (active, inactive, not assigned). Boxplots depict the distribution of all individual samples, with outliers shown as diamonds. Sample sizes (n) indicate the number of rhinos contributing observations per season.

management strategies.

Age was another consideration since four of the six reproductively inactive white rhinos were > 26 years old, and lower PRL concentrations have been reported in older women and mice [30,31]. Furthermore, increasing age is negatively associated with normal prolactin status in elephants [26]. However, three of the older rhinos (>23 years of age) in the reproductively active cohort had individual mean PRL concentrations greater than the inactive group average (21.1–58.7 ng/mL vs. 5.7 ng/mL, respectively), including one female almost 26 years of age who had the highest mean PRL of all the white rhinos in the study (Table 1).

Ultimately, it appeared that lower PRL concentrations accompany, or reflect, reduced reproductive function in white rhinos. Experimental and clinical studies have demonstrated that insufficient PRL signaling can impair reproductive processes, including ovarian function and successful lactation [9,16,17]. However, we are not proposing that is happening in rhinos since this study was not designed to test cause and effect. A more likely explanation could be related to PRL's close association with progesterone that was first demonstrated in a previous case study of a pregnant white rhino [12]. Although very preliminary, that pilot study also reported PRL concentrations in serum for a small number of rhinos in different reproductive states that suggested PRL fluctuated with reproductive activity. The highest PRL concentrations were associated with pregnancy, followed by sexually mature and/or luteal females, with lower concentrations measured in serum of sexually immature females or males [12]. Data in this study further support the close association of PRL and progesterone seen in the pilot study. Reproductively active white rhinos exhibited higher mean circulating PRL concentrations (Fig. 2), a wider range in values (Table 1), and greater standard deviations within individuals than reproductively inactive rhinos, suggesting that PRL varied with ovarian activity. A similar, and even more pronounced variation in PRL concentrations was noted in black rhinos, though a comparison to reproductively inactive black rhinos was not possible.

If PRL concentrations do mimic progesterone patterns, the lack of reproductive activity, ovulation, and luteal function in reproductively inactive females would be consistent with the persistently low PRL concentrations observed in this study. In contrast, more variable and higher mean PRL concentrations would occur in reproductively active rhinos. Cyclic patterns of PRL connected to ovarian activity have been reported in several species including mice, horses, and African elephants [30,31,36]. However, proof of this close connection between progesterone and PRL in rhinos will require a sampling frequency greater than once monthly to reveal short-term fluctuations across estrous cycles or other reproductive transitions.

In addition to reproductive state, PRL secretion in many mammals is influenced by photoperiod, with concentrations typically increasing during periods of longer day length and declining during intervals of shorter day lengths [10,24,28]. Although neither black nor white rhinos are considered seasonal breeders, both natural history and management conditions may expose animals to seasonal environmental influences [40]. In the wild, breeding activity is often associated with periods of increased forage availability [29]. Rhinos maintained under human care typically do not experience substantial nutritional changes throughout the year, but are exposed to seasonal differences in housing, light exposure, and activity patterns [41]. The lower PRL concentrations observed during winter in white rhinos may therefore reflect responses to seasonal changes in light exposure experienced by animals housed in North America. In contrast, seasonal variation in black rhinos was not statistically supported, possibly due to the more limited statistical power available for this species. Although seasonal influences on PRL may differ between rhino species, the overall impact of the season appeared minor and could have been confounded by variation in latitudes, local light environments, or institution-specific management practices. The limited number of non-pregnant black rhinos available for analysis prohibited further evaluation of these factors.

Although this study substantially increases our knowledge of rhino PRL and builds on the preliminary information presented in the initial pilot study [12], there are limitations. Despite the relatively large number of rhinos included, there were a limited number of reproductively inactive individuals in the analysis. Consequently, the lower PRL concentrations observed in inactive white rhinos should be viewed as an indication that PRL varies with reproductive state, rather than definitive evidence. Additionally white rhino serum exhibited high CVs when run neat necessitating dilution and indicating a potential matrix interference not experienced in black rhino serum. Also, the timing of sample collection relative to daily husbandry activities was not standardized across institutions, which may contribute to within-individual variation in measured PRL concentrations [9]. Furthermore, it is not known if black rhino PRL would vary similarly between reproductive states since no females in the study were considered reproductively inactive. Finally, PRL concentrations were evaluated using monthly serum samples collected over a single year, which provides only a snapshot of endocrine dynamics in a long-lived species.

5. Conclusion

There was no clear evidence of abnormal PRL concentrations within the study populations. However, in this study a few differences were noted. Black rhinos had higher PRL than white rhinos. PRL concentrations were higher in reproductively active white rhino females compared to reproductively inactive females, indicating an association with reproductive state. Seasonal influences on PRL appeared to vary between species, and were minimal. Continued longitudinal monitoring at greater frequencies across reproductive states, including pregnancy and cyclic activity, will be important for further clarifying how PRL dynamics could serve as a complementary tool for reproductive management of rhinos in human care.

CRediT authorship contribution statement

Elizabeth M. Donelan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Louisa A. Rispoli:** Writing – review & editing, Supervision, Investigation. **Parker M. Pennington:** Writing – review & editing, Supervision, Conceptualization. **Terri L. Roth:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethics declaration

This study was conducted in accordance with the following guidelines for animal welfare and/or reporting: Rhino Husbandry Manual 2014. This study was approved by the IACUC.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] International Rhino Foundation. 2025 state of the rhino. (2025) Sept.
[2] Rhino Research Council. 2025 rhino research masterplan. (2025).
[3] T.L. Roth, That was then, this is now – over two decades of progress in rhinoceros reproductive science and technology, Theriogenology Wild 4 (2024), https://doi.org/10.1016/j.therwi.2023.100065.
[4] F. Schwarzenberger, W. Rietschel, J. Vahala, D. Holeckova, P. Thomas, J. Maltzan, et al., Fecal progesterone, estrogen, and androgen metabolites for noninvasive monitoring of reproductive function in the female Indian rhinoceros, *Rhinoceros unicornis*, Gen. Comp. Endocrinol. 119 (2000) 300–307, https://doi.org/10.1006/gcen.2000.7523.
[5] T.L. Roth, H.L. Bateman, J.L. Kroll, B.G. Steinetz, P.R. Reinhart, Endocrine and ultrasonographic characterization of a successful pregnancy in a Sumatran rhinoceros (*Dicerorhinus sumatrensis*) supplemented with a synthetic progestin, Zoo. Biol. 23 (2004) 219–238, https://doi.org/10.1002/zoo.10131.
[6] E.V. Berkeley, J.F. Kirkpatrick, N.E. Schaffer, W.M. Bryant, W.R. Threlfall, Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (*Diceros bicornis*), Zoo. Biol. 16 (1997) 121–132.
[7] J.L. Brown, A.C. Bellem, M. Fouraker, D.E. Wildt, T.L. Roth, Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring, Zoo. Biol. 20 (2001) 463–486, https://doi.org/10.1002/zoo.10028.
[8] M.L. Patton, R.R. Swaisgood, N.M. Czekala, A.M. White, G.A. Fetter, J. P. Montagne, et al., Reproductive cycle length and pregnancy in the southern white rhinoceros (*Ceratotherium simum simum*) as determined by fecal pregnane analysis and observations of mating behavior, Zoo. Biol. 18 (1999) 111–127, https://doi.org/10.1002/(SICI)1098-2361(1999)18:2.
[9] M.E. Freeman, A. Lerant, G. Nagy, Prolactin: Structure, function, and regulation of secretion, Physiol. Rev. 80 (2000) 1524–1585.
[10] C. Stewart, C.J. Marshall, Seasonality of prolactin in birds and mammals, J. Exp. Zool. Pt A 337 (2022), https://doi.org/10.1002/jez.2634.
[11] N. Kock, D. Morton, M. Kock, Reproductive parameters in free-ranging female black rhinoceroses (*Diceros bicornis*) in Zimbabwe, Onderstepoort J. Vet. Res. 58 (1991) 55–57.
[12] Donelan Roth, Reilly Rispoli, Prolactin enzyme-linked immunosorbent assay for rhinoceroses – another tool for assessing reproductive function and dysfunction in this taxon, Theriogenology Wild 2 (2023), https://doi.org/10.1016/j.therwi.2023.100035.
[13] L. Diaz, M. Diaz-Munoz, L. Gonzalez, S. Lira-Albarran, F. Larrea, I. Mendez, Prolactin in the immune system, in: G.M. Nagy, B.E. Toth (Eds.), Prolactin, InTech, 2013, https://doi.org/10.5772/53538.
[14] C.J. Marshall, A. Blake, C. Stewart, T.A. Liddle, I. Denizli, F. Cuthill, et al., Prolactin mediates long-term, seasonal rheostatic regulation of body mass in female mammals, Endocrinology 165 (2024), https://doi.org/10.1210/endocr/bqae020.
[15] R. Pirchio, C. Graziadio, A. Colao, R. Pivonello, R.S. Auriemma, Metabolic effects of prolactin, Front. Endocrinol. 13 (2022), https://doi.org/10.3389/fendo.2022.1015520.
[16] T.E.R.G. Huijsmans, A. Van Soom, K. Smits, J. Wauters, T.B. Hildebrandt, Review: the role of prolactin in the maternal investment-survival balance, Theriogenology Wild 5 (2024), 2773-093X.
[17] A. Bachelot, N. Binart, Reproductive role of prolactin, Reproduction 133 (2007), https://doi.org/10.1530/rep-06-0299.
[18] V.J. Wiebe, J.P. Howard, Pharmacologic advances in canine and feline reproduction, Top. Companion Anim. Med. 24 (2009).
[19] N.D. Horseman, W. Zhao, E. Montecino-Rodriguez, M. Tanaka, K. Nakashima, S. J. Engle, et al., Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene, EMBO J. 16 (1997) 6926–6935.
[20] C.A. Marks, L. Jalkanen, R. Savolainen, R.V. Short, Control of red fox (*Vulpes vulpes*) fertility with cabergoline: Dose response and timing of intervention, Reproduction 122 (2001) 147–154.
[21] A. Majumdar, N. Mangal, Hyperprolactinemia, J. Hum. Reprod. Sci. 6 (2013), https://doi.org/10.4103/0974-1208.121400.
[22] O. Serri, C.L. Chik, E. Ur, S. Ezzat, Diagnosis and management of hyperprolactinemia, Can. Med. Assoc. J. 169 (2003).
[23] C. Sonigo, J. Bouilly, N. Carré, V. Tolle, A. Caraty, J. Tello, et al., Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration, J. Clin. Investig. 122 (2012), https://doi.org/10.1172/JCI63937.
[24] D.L. Thompson, E.L. Oberhaus, Prolactin in the horse: Historical perspective, actions and reactions, and its role in reproduction, J. Equine Vet. Sci. 35 (2015) 343, https://doi.org/10.2527/jas1986.633854x.
[25] N.A. Prado, S.W. Paris, J.L. Brown, Prolonged ovarian acyclicity is associated with a higher likelihood of developing hyperprolactinemia in zoo female African elephants, Zoo. Biol. 38 (2018) 180, https://doi.org/10.1002/zoo.21464.
[26] N.A. Prado, K. Carlstead, E.J. Malloy, S. Paris, N. Wielebnowski, L.L. Rockwood, et al., Ovarian cyclicity and prolactin status of African elephants (*Loxodonta africana*) in North American zoos may be influenced by life experience and individual temperament, Horm. Behav. 125 (2020), https://doi.org/10.1016/j.yhbeh.2020.104804.
[27] B.A. Schulte, J.A. Parsons, U.S. Seal, D.J. Plotka, L.J. Verme, J.J. Ozoga, Heterologous radioimmunoassay for deer prolactin, General. Comp. Endocrinol. 40 (1980) 59–68.
[28] G.E. Dahl, B.A. Buchanan, Tucker, Photoperiodic effects on dairy cattle: a review, J. Dairy. Sci. 83 (2000) 885–893, https://doi.org/10.3168/jds.S0022-0302(00)74952-6.
[29] N. Owen-Smith, J.O. Ogutu, Controls over reproductive phenology among ungulates: allometry and tropical-temperate contrasts, Ecography 36 (2013) 256–263, https://doi.org/10.1111/j.1600-0587.2012.00156.x.
[30] M. Vekemans, C. Robyn, Influence of age on serum prolactin levels in women and men, BMJ 4 (1975) 738, https://doi.org/10.1136/bmj.4.5999.738.
[31] T.A. Parkening, T.J. Collins, E.R. Smith, Plasma and pituitary concentrations of LH, FSH and prolactin in aged female C57BL/6 mice, J. Reprod. Fertil. 58 (1980) 377–386.
[32] L. Metrione, A. Eyers (Eds.), Rhino husbandry manual, International Rhino Foundation, Forth Worth, TX, 2014.
[33] S. Windle, D. Beetem, C. Cseplo, E. Donelan, K. Krupp, C. Consago, et al., Bull access influences fecal progesterone metabolites in managed female white rhinoceros (Ceratotherium simum): Insights from hormonal and behavioral monitoring at the wilds, Theriogenology Wild 9 (2026) 06–30, https://doi.org/10.1016/j.therwi.2026.100166.
[34] R.W. Hornung, L.D. Reed, Estimation of average concentration in the presence of nondetectable values, Appl. Occup. Environ. Hyg. 5 (1990), https://doi.org/10.1080/1047322x.1990.10389587.
[35] J.L. Brown, S.L. Walker, T. Moeller, Comparative endocrinology of cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants, General. Comp. Endocrinol. 136 (2004) 360, https://doi.org/10.1016/j.ygcen.2004.01.013.
[36] J.L. Brown, S. Paris, N.A. Prado-Oviedo, C.L. Meehan, J.N. Hogan, K.A. Morfeld, et al., Reproductive health assessment of female elephants in North American zoos and association of husbandry practices with reproductive dysfunction in African elephants (*Loxodonta africana*), PLoS. ONE 11 (2016), https://doi.org/10.1371/journal.pone.0145673.
[37] L.S. Haro, D.W. Lee, R.N.P. Singh, G. Bee, E. Markoff, U.J. Lewis, Glycosylated human prolactin: Alterations in glycosylation pattern modify affinity for lactogen receptor and values in prolactin radioimmunoassay, J. Clin. Endocrinol. Metab. 71 (1990) 379–383, https://doi.org/10.1210/jcem-71-2-379.
[38] N.A. Prado, M. Krcmarik, S. Paris, L. Rockwood, L. Brown, JL. Abnormal prolactin secretion is associated with changes in metabolic health biomarkers in acyclic female African elephants (*Loxodonta africana*), Theriogenology Wild 3 (2023), https://doi.org/10.1016/j.therwi.2023.100062.
[39] J.L. Brown, J. Lehnhardt, Secretory patterns of serum prolactin in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants during different reproductive states: Comparison with concentrations in a noncycling African elephant, Zoo. Biol. 16 (1997) 149–159.
[40] T.L. Roth, A review of the reproductive physiology of rhinoceros species in captivity, Int. Zoo. Yearb. (2006) 130–143.
[41] K. Radeke-Auer, A. Wittwer, J. Aust, M. Roller, D.W.H. Müller, F. Von Houwald, et al., Reproductive non-seasonality in rhinoceroses: A review of the in-situ literature and birth records of ex-situ institutions, J. Zoo. Aquar. Res. 10 (2022), https://doi.org/10.19227/jzar.v10i1.628.