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THE RELATIONSHIP BETWEEN VAGINAL ELECTRICAL IMPEDENCE AND HORMONE PROFILES DURING PREGNANCY AND PARTURITION OF A WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

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Abstract: In this study, an attempt was made to use vaginal electrical impedance to predict calving in a female white rhinoceros (*Ceratotherium simum simum*) and to determine the relationship between vaginal electrical impedance and hormonal profiles during pregnancy. The principle behind vaginal electrical impedance is that a change in the ionic balance of vaginal and cervical mucus occurs in response to changes in reproductive hormones. Three times weekly vaginal electrical impedance readings and fecal samples were collected from midgestation to calving (a 6-mo period). The extracted fecal samples were analyzed for immunoreactive estrogens, progestagens, and corticoids by RIA. Vaginal electrical impedance readings did not decrease before calving but remained consistent throughout the last 140 days of pregnancy. Fecal progestagens in the white rhinoceros decreased between day 17 and day 1 before calving, whereas estrogens increased between 4 and 2 mo before calving, with an additional increase occurring 1 mo before calving. Fecal corticoids increased 5 mo before calving, slowly declined, and increased again within 3 wk before calving. A decline in vaginal electrical impedance was noted 168 days before calving and remained at low levels for 4 wk. At the time of this decrease, the female became aggressive toward the male and began lactating. Fecal progestagens and estrogens did not change during this time; however, fecal corticoids increased as vaginal electrical impedance readings returned to normal along with her behavior and cessation of lactation. In summary, the use of vaginal electrical impedance could not predict parturition in the white rhinoceros. However, an anomaly occurred during pregnancy that was supported by vaginal electrical impedance readings, a change in female behavior, premature lactation, and a subsequent increase in fecal corticoids. The etiology of this physiological anomaly is unknown, yet it did not compromise pregnancy.

Key words: White rhinoceros (*Ceratotherium simum simum*), hormones, pregnancy, vaginal electrical impedance.

INTRODUCTION

Vaginal electrical impedance (VEI) has been used previously to detect estrus and to determine the timing of ovulation in cattle, horses, sheep, pigs, and buffalo (*Bubalus bubalis*).[6,7,10] The principle behind VEI measurements is that a change in the ionic balance of vaginal and cervical mucus occurs in response to changes in reproductive hormones such as estradiol and progesterone. Studies in cattle have shown that fluids within the vagina have high electrical resistance in the luteal phase and that this resistance decreases during the follicular phase.[8,11] In addition to endocrine changes that occur during the normal estrous cycle in cattle, placental shifts in steroid hormone production around the time of calving might also be detected by VEI. This has been reported previously in cattle, with a decline in VEI beginning around 14–15 days before parturition.[12,16]

In the black rhinoceros (*Diceros bicornis*), an increase in progestagens, as determined by fecal hormone analysis, occurs 15 mo before parturition, decreases during the last 2 wk of pregnancy, and reaches nadir values within 3 days after parturition.[17] In contrast, fecal estrogens remain low until 5 mo before calving, with a significant increase 1 mo before parturition.[5] In another study of a pregnant black rhinoceros, fecal progestagen concentrations decreased 2 wk before parturition, and an increase in estrogens was observed 2 mo before calving with elevated estrogen levels peaking the day of parturition.[3] In the white rhinoceros, fecal pregnane levels have been reported to rise until the seventh month of gestation and then remain at similar levels through parturition.[14] Nevertheless, questions remain regarding the hormonal relationships during gestation and parturition in the white rhinoceros, and assessments by other measures of reproductive function may be of value in further defining pregnancy and the timing of parturition in this species. Specifically, if a relationship exists between VEI

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and hormones in the rhinoceros, as determined in other species (e.g., cattle and horses). VEI might represent an additional tool to predict more closely the timing of calving and thus permit alternative housing or closer monitoring of delivery to assure calf survival and health.

The objectives of this study were to determine the relationship between VEI and hormonal profiles during pregnancy in a white rhinoceros (*Ceratotherium simum simum*) and to determine if VEI measurements can be used to predict the timing of parturition. Because previous studies have indicated that immunoreactive fecal steroid hormone analysis can accurately reflect serum steroid hormone concentrations in the rhinoceros,[3] fecal profiling of immunoreactive progestagens, estrogens, and corticoids were conducted to compare to VEI measures.

MATERIALS AND METHODS

Validation of vaginal electrical impedance to predict parturition and measures of probe repeatability and precision

The objective of this initial preliminary experiment was to verify the use of VEI to predict calving in cattle (*Bos taurus*) as comparative supportive data for assessments made in a female white rhinoceros and to determine the nature of any change in VEI measurements that might be observed at parturition using the specific VEI probe employed in this study. Vaginal electrical impedance measurements were recorded on Holstein and Jersey cows ($n = 21$) from 105 days of gestation to calving. Vaginal electrical impedance measurements were acquired using a commercially available electrical conductivity probe (Ovatest meter; Animark Inc., Aurora, Colorado 80011, USA). Statistical analysis of cattle VEI values through parturition was performed using ANOVA specific for repeated measures.[18]

Before and between measurements, the Ovatest VEI probe was disinfected using Nolvasan (1% v/v water; Fort Dodge Animal Health, Overland Park, Kansas 66225 USA) after each cow. The VEI meter was checked for performance (i.e., repeatability of readings) at the beginning and end of each sampling period in clean water. Moreover, the Ovatest meter was calibrated to verify changes in the ionic content of a standard solution using sodium chloride (NaCl). Briefly, dilutions of NaCl (1 mM to 1 M) in water were quantified using the VEI probe in which three consecutive measurements were recorded for each dilution. Statistical analysis for these measurements was performed using the Student's t-test for mean separation, and coefficients of variation (CV%) were calculated to assess repeatability and precision of the Ovatest meter.[18]

Vaginal electrical impedance measurements and hormonal profiling in a white rhinoceros

A white rhinoceros ("Tombi"; ~32 yr of age) housed at the Memphis Zoo (Memphis, Tennessee 38112, USA) was thought to be pregnant with multiple breeding dates (mounting events) recorded but with an unknown conception date. Based on data for cattle and other species, we reasoned that VEI measurements may have application as a relatively noninvasive tool to predict parturition in the white rhinoceros. The female rhinoceros in this investigation was operant trained to come to a training barrier, which allowed the VEI probe to be inserted into the vagina and measurements acquired without the animal being restrained. Before each use the probe was disinfected using Nolvasan (1% v/v water), and the VEI meter was checked for performance (i.e., repeatability of readings) at the beginning and end of each sampling period in clean water. Vaginal electrical impedance measurements were collected in replicate three times weekly for 6 mo until calving; although occasionally the ambulatory animal would not permit intravaginal insertion of the probe at the training barrier for a measurement to be acquired. Fecal samples were collected three times weekly starting 6 mo before calving and then daily 4 mo before calving through 1 wk postcalving. Steroid hormones from the fecal samples were extracted in 5 mL of 80% methanol, placed in a shaker for 12–14 hr, and then centrifuged at 2,500 rpm for 15 min.[17] After the supernatant had been pipetted off, the fecal samples were analyzed for immunoreactive estrogens (estradiol-17β antibody[4]), progestagens (progesterone antibody[9]), and corticoids (cortisol antibody; Diagnostic Systems Laboratories, Inc., Webster, Texas 77598, USA) by RIA. The cross-reactivity of the progesterone antiserum with other steroids was as follows: progesterone, 100%; 5α-pregnane-3,20-dione, 6.0%; 11-deoxycorticosterone, 2.5%; 17α-hydroxyprogesterone, 1.2%; 5β-pregnane-3,20-dione, 0.8%; 11-deoxycortisol, 0.4%; 20α-dihydroprogesterone, 0.1%. The cross-reactivity of the cortisol antiserum with other steroids was as follows: cortisol, 100%; prednisolone, 45.6%; 11-desoxycortisol, 12.3%; corticosterone, 5.5%; prednisone, 2.7%; cortisone, 2.1%; 17α-hydroxyprogesterone, 1.0%; progesterone, 0.2%. The cross-reactivity of the estradiol antiserum with other steroids was as follows: estradiol-17β, 100%; estrone, 10%; and other steroids <0.1%. The intraassay coefficients of variation (CV%) for immunoreactive estrogens, pro-

Table 1. Selected excerpts from clinical records for selected days during pregnancy through parturition for a pregnant white rhinoceros ("Tombi") at the Memphis Zoo (Memphis, TN 38112 USA) as reported by Zoo Veterinarian, C. Tabaka, D.V.M.

13 August 2001 (Day −170; VEI 193, 213) For the first time today the female rhino ran off the male several times. This is typically a sign that birth is impending.

15 August 2001 (Day −168; VEI 20, 21) Readings have fallen through the floor. Unable to see if she has milk as her teats are extremely sensitive. She is having trouble breathing after mild exercise. 3:30 PM, Keeper was able to express milk from teats. Animal is highly uncomfortable. Shifting weight constantly. Urinating in unusual areas. Not standing still. Has run off male from patio once today.

18 August 2001 (Day −165) Unable to obtain reading today. She will not stand for it. She is too uncomfortable when the probe starts to enter her vaginal tract.

25 August 2001 (Day −158) Still unable to obtain reading. Her abdomen is more pendulous. She is extremely uncomfortable.

12 September 2001 (Day −140; VEI 174) Appears the recent readings as well as lactation were a false alarm. She is not lactating currently.

30 January 2002 (Day 0) Gave birth this morning. Newborn was seen nursing for the first time.

Day, days before calving; VEI, vaginal electrical impedance reading (relative units, RU).

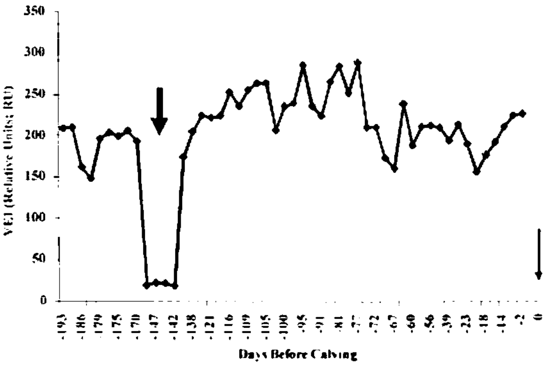

Figure 1. Vaginal electrical impedance readings (VEI) during pregnancy in a white rhinoceros. Unlike that observed in cattle, a decline in VEI before parturition was not observed: VEI remained constant throughout most of pregnancy (148–289 relative units; RU). However, VEI readings decreased 180 units 168 days before calving (large arrow) and remained at these low levels for 4 wk before returning to previous baseline levels, the etiology and physiologic relevance of which is not known. Small arrow indicates parturition (day 0).

gestagens, and corticoids were 5.8%, 10.44%, and 7.3%, respectively. In addition, clinical notes of the activity patterns (i.e., behavior) and observations of the female rhinoceros during pregnancy were compiled by the animal caretakers and zoo veterinarian and utilized for descriptive correlations to hormonal profiles and VEI readings (see Table 1).

RESULTS AND DISCUSSION

The results of the VEI probe calibration in NaCl indicated that as the concentrations of NaCl increased, the conductance measurements (i.e., electrical resistance) decreased ($P < 0.05$) from 694.7 ± 1.5 relative units (RU; no NaCl) to 24.7 ± 0.3 RU in the 1 M NaCl solution, with a CV% among measurements within concentrations of NaCl of 2.9 ± 0.46 %. In cattle, VEI measurements decreased ($P < 0.05$) from 105.0 ± 3.1 RU 104 days before calving to 73.7 ± 1.7 RU 2 days prior to calving. In the present study, a 31.7 RU (30%) decrease in VEI readings was recorded before calving, which suggested that VEI as detected with the Ovatest probe could be used as an indicator of parturition in the dairy cow. This further suggested that it may have application in other large herbivores as well, and therefore, it was applied to the white rhinoceros in this case study when the authors were presented with the task of trying to predict calving from an unknown conception date.

Unlike results obtained in cattle, in the white rhinoceros VEI readings before calving did not decrease but remained high (148–289 RU) throughout most of pregnancy through parturition (Fig. 1). Fecal progestagens (Fig. 2A) decreased 236.2 ng/g between day 17 and day 1 before calving, which is similar to the study conducted by Schwarzenberger et al.,[17] which reported a decline in fecal progestagens in the black rhinoceros during the last 2 wk of pregnancy. However, a previous report in the white rhinoceros observed that whereas fecal pregnane levels rose through the seventh month of gestation, they remained at these high levels thereafter with no appreciable decline through parturition.[14] Fecal estrogens (Fig. 2B) increased 15.4 pg/g between 4 and 2 mo before calving, with an additional increase of 13.5 pg/g occurring 1 mo before calving. Czekala and Callison[5] reported that salivary estrogens in the black rhinoceros remained near baseline until 5 mo before delivery, then underwent a gradual but significant increase until 1 mo before delivery. This is similar to the estrogen profile for the white rhinoceros profiled in the current study. Fecal corticoids (Fig. 2C) increased 5 mo before calving, slowly declined, and then increased again within 3 wk before calving. Fecal cortisol and cor-

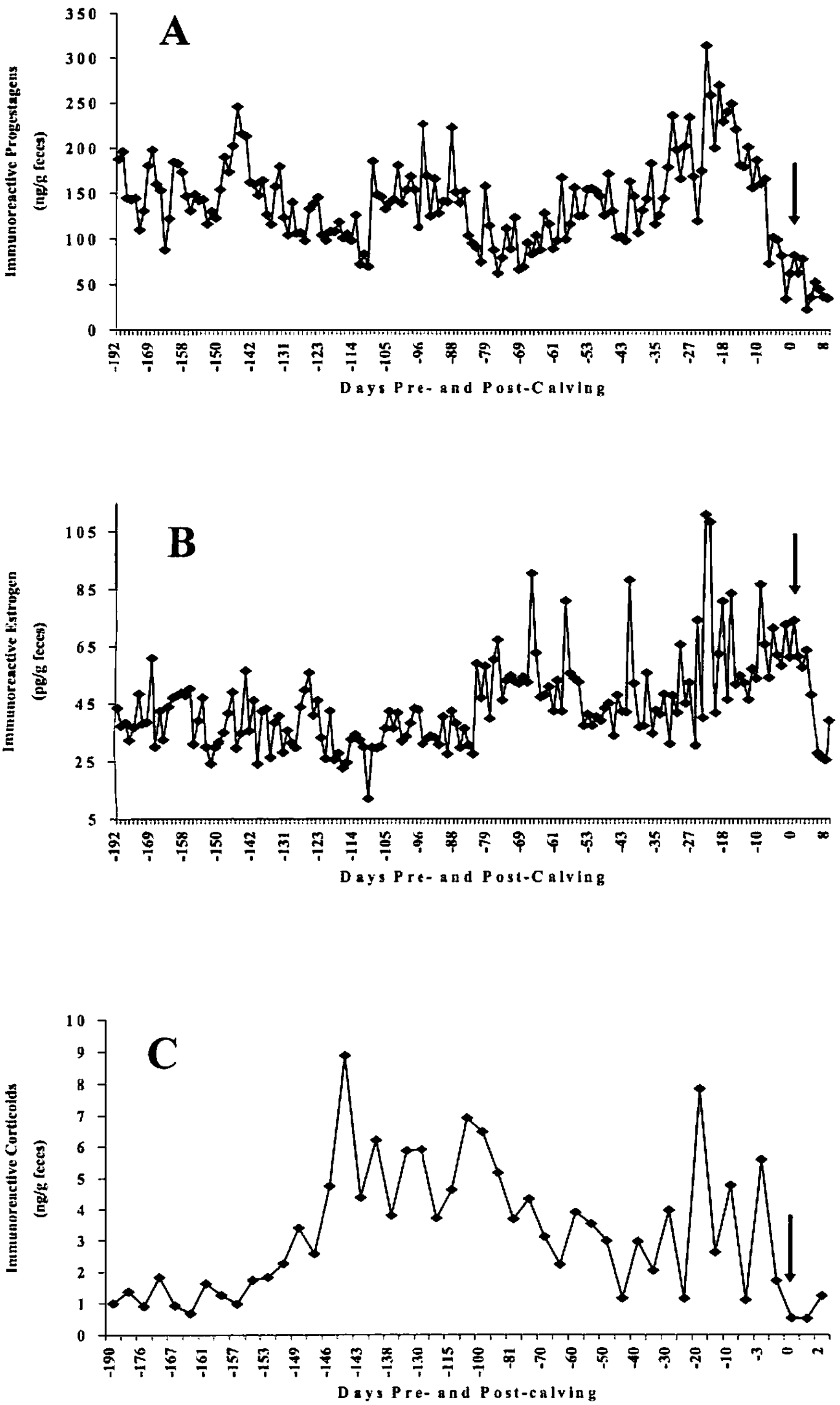

Figure 2. **A.** Immunoreactive fecal progestagens during pregnancy and through parturition (day 0) in a white rhinoceros. Fecal progestagens decreased 236.2 ng/g between day 17 and day 1 before calving. During the episode of VEI decline (168 days before calving; see Fig. 1), fecal progestagens did not change appreciably. **B.** Immunoreactive fecal estrogens during pregnancy through parturition (day 0) in a white rhinoceros. Fecal estrogens increased 15.4 pg/g between 4 and 2 mo before calving, with an additional increase of 13.5 pg/g occurring 1 mo before calving. During the episode of VEI decline (168 days before calving; see Fig. 1), fecal estrogens did not change appreciably. **C.** Immunoreactive fecal corticoids during pregnancy through parturition (day 0) in a white rhinoceros. Fecal corticoids increased 5 mo before calving, stabilized for the next 3 mo, and increased again within 3 wk before calving. Although fecal corticoids did not change during the VEI decline (168 days before calving; see Fig. 1), corticoid levels increased as the VEI readings returned to preepisode baseline levels. Arrows in A–C indicate parturition.

ticosterone have been measured previously in the white rhinoceros using high-performance liquid chromatography for assessments of stress.[19] In livestock species an increase in the secretion of cortisol occurs 2–3 days before parturition,[1] and prolonged doses of glucocorticoid will induce parturition in the mare.[2] The fecal corticoid profile of the white rhinoceros in this study suggests that, as in other species, elevations in cortisol late in gestation may be characteristic as well, though values were in fact relatively low on the day of calving.

Unexpectedly during this study a 180-RU decline in VEI readings occurred 168 days before calving (Fig. 1). The clinical records by the attending veterinarians and caretakers at this time (Table 1) also reported a change in the behavior of the female rhinoceros that paralleled the decline in VEI. Of note, the female became very agitated and exhibited premature lactation with milk able to be expressed manually from the teats, usually signs of impending parturition. However, neither fecal progestagens nor estrogens changed appreciably during this period. Although fetal corticoids did not change during the VEI decline, corticoid levels did increase as the VEI readings returned to previous baseline levels (148–289 RU) around 140 days before calving, with this episode lasting approximately 28 days. This decline in VEI and the behavior changes in this rhinoceros could have been an indication that the pregnancy may have been compromised in some way but was able to recover, although this cannot be substantiated and may in fact be a normal occurrence.

In the mare, premature udder development and lactation (galactorrhea) early or midgestation can be associated with abnormalities of the placenta. This has been suggested to be an indication of impending abortion, in utero death of one twin fetus, placental separation, or bacterial/fungal-induced placentitis.[13] In this case study of a pregnant white rhinoceros exhibiting premature lactation 5 mo before calving, lactation ceased once the event subsided (i.e., as evidenced by a return of VEI and female behavior to normal). Similar spontaneous disappearance of premature mammary development has been known to occur in the mare as well when premature mammary development occurs in midgestation.[13] The decline in VEI in response to the changing ionic balance of vaginal and cervical mucus would suggest that a change in reproductive hormones had occurred, which typically precedes changes in VEI during the normal estrous cycle and pregnancy in other species.[6,7,12,17] Nevertheless no characteristic changes in fecal estrogens or progestagens as measured with the steroid hormone assays and respective antibodies used in this study were noted. Our calibration of the Ovatest probe using dilutions of NaCl to test conductivity showed that an increase in NaCl in solution decreased VEI (694.7 ± 1.5 RU in no NaCl to 24.7 ± 0.3 RU in 1M NaCl). This would suggest that a change in the ionic balance of the vaginal mucosa did occur in the female rhinoceros during this time, yet the etiology of this physiologic change that paralleled alterations in animal behavior and premature lactation is unknown. One can only speculate as to its cause, significance, and relationship to pregnancy in the rhinoceros. Although early pregnancy loss (by day 28 postbreeding) has been documented previously in the white rhinoceros,[15] an anomaly like the one that occurred in this report, which obviously resulted in a short-term yet noncompromising impact on pregnancy, has not been documented and characterized in relation to hormone profiles and VEI measures in this species. Further investigation of pregnancy and VEI changes in other female rhinoceros are needed to substantiate whether this is a unique individual animal- (or pregnancy-) specific observation or a characteristic of pregnancy for this species. Although the etiology and significance of the observed anomaly remains unclear, it did not compromise pregnancy, and a live birth resulted 140 days later. VEI did not appear useful for predicting impending parturition in the white rhinoceros, but our observations in this case report suggest that additional investigations are needed to understand further the physiological changes occurring in the rhinoceros during pregnancy and parturition.

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