Reproductive Monitoring in Captive Southern White Rhinoceros (*Ceratotherium simum simum*)

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Abstract

Reproduction was monitored in three Southern white rhinoceros females using faecal progestagen concentrations, changes in vulval swelling and observations of courtship behaviours. The average length of the oestrous cycle was 29.6±1.8 days (range 25-38 days, n=7). Vulval swellings were scored on a scale of 0 (no swelling) to 3 (very swollen). Level 3 swellings were associated with oestrus and/or mating in two females, subsequent ovulation and/or pregnancy was confirmed by elevated progestagen concentrations. One female exhibited Level 2 vulval swellings on several occasions and some signs of oestrus but failed to ovulate. Each female had an acyclic period characterized by low progestagen concentrations. In summary, for some individuals, vulval swellings may be a simple indicator of oestrus that could be used for breeding management decisions. Behaviour observation and confirmation of ovulation/conception using hormone analysis is recommended in the assessment of reproductive status in white rhinoceros.

1. Introduction

The Southern white rhinoceros (*Ceratotherium simum simum)* is the most abundant of the five extant rhinoceros species. Despite the reproductive success of wild populations, the captive white rhinoceros population is not self-sustaining [Swaisgood et al., 2006], captive-born (F_1) females have a reproductive rate as low as 8% despite exhibiting appropriate mating behaviours [Hermes et al., 2006; Swaisgood et al., 2006]. Numerous studies have established the effectiveness of serum, urinary or faecal progesterone concentrations and ultrasound to monitor ovulation, reproductive cyclicity and pregnancy in captive white rhinoceros [Hindle et al., 1992; Radcliffe et al., 1997; Schwarzenberger et al., 1998; Patton et al., 1999; Strike & Pickard, 2000; Brown et al., 2001; Hermes et al., 2006]. However, several of those studies report many females are acylic or have erratic reproductive cycles or reproductive pathologies [Hermes et al., 2004, 2006]. In cyclic females, short 30-35 day cycles and long 65-70 day cycles, or a combination of short and long cycles have been reported.

Changes in the size or swelling of the vulva had previously been noted by keeper staff at Hamilton Zoo and it was thought that these changes may be indicative of oestrus. In other species, fluctuations in vulvar tumescence can be used in determining the stage of the oestrous cycle [tayra, Poglayan-Newall et al., 1989; sow, Sterning et al., 1998; giant panda, Durrant et al., 2003]. While vaginal cytology is used routinely to determine optimum timing for mating or artificial insemination in domestic carnivores it was considered impractical for white rhinoceros because of the length of the genital tract.

The aim of this study was to monitor the reproductive status of three females using faecal progestagen concentrations and determine the predictive value of changes in vulval swellings in determining the onset of oestrus.

2. Methods

2.1. Animals

The rhinoceros herd at Hamilton Zoo consists of one adult breeding male, three females (5-11 years of age) and two sub-adult male offspring; the adult male and two adult females were wild born and transferred from Kruger National Park, South Africa to Hamilton in 1999 (Table 1). All animals share an outdoor enclosure, approximately 2000 square metres in size, during the day and are housed in individual yards at night.

Table 1: Description of Southern White rhinoceros at Hamilton Zoo.

Studbook #	Name	Sex	Year born	Origin
1356	Zambesi	M	Est. 1992	Wild-born Kruger
1357	Moesha	F	Est. 1994	Wild-born Kruger
1358	Caballe	F	Est. 1995	Wild-born Kruger
1353	Kito	F	Jun 2000	Captive – born Auckland Zoo to wild-born parents and conceived in Kruger. Transferred to Hamilton Nov 2004.
1409	Inkosi	M	Jan 2002	Hamilton Zoo: Sire 1356, Dam 1358
1502	Mtoto	M	Feb 2004	Hamilton Zoo: Sire 1356, Dam 1358

2.2 Vulval changes and behavioural observations

Data on vulval swellings was collected daily during the regular morning crush training session. Initially measurements of the swellings were taken but this was found to have too much variability between keepers. Instead a grading system (0-3) was developed, based on descriptions, to provide greater consistency. The presence of any discharge was also noted. Behavioural observations were collected on an opportunistic basis by the keepers and on days of suspected activity volunteer zoo hosts were asked to observe the rhinos for the day.

2.3 Faecal sample collection and extraction

Faecal samples were collected three times per week from individual night yards during cleaning. Samples were taken from the middle of a dung ball, placed in labelled containers and frozen until processing. Samples were freeze-dried, sifted to remove vegetation, pulverised and 0.2g dry powder extracted in 5ml of 80% methanol:distilled water by shaking for 20 minutes. A portion of the supernatant was diluted 1:10 (1:100 to 1:1000 during pregnancy) in PBS buffer and frozen until progestagen analysis.

2.4 Progestagen enzymeimmunoassay

Faecal progestagen concentrations were quantified using an EIA method. The monoclonal antiserum CL425 [provided by Coralie Munro, University of California, Davis, CA] was produced against 4-pregnen-11-ol-3, 20-dione hemisuccinate:BSA. The antiserum cross-reacts with progesterone, 100%; 5α-pregnane-3β-ol-20-one, 96%; 5α-pregnane-3α-ol-20-one, 36%; 17β-hydroxyprogesterone, 15%; pregnenolone, 13%; 5β-pregnane-3α-ol-20-one, 7%; 5β-pregnane-3α,17α-diol, 20α-one, 5% [Brown et al., 1994]. Sensitivity of the assay is 5 pg/well. Binding inhibition curves of serially diluted pooled rhino faecal sample were parallel to the standard curve. The intra- and inter- assay coefficients of variations were <10% for control samples. All faecal data are expressed on a dry weight basis (i.e. nanograms progestagen per gram dry faeces).

2.5 Data analysis

Data are presented as mean ± SEM. Faecal progestagen concentrations during the luteal phase were calculated using an iterative process for each female [Graham et al., 2002] and concentrations were considered above baseline when they exceeded 2 standard deviations above the mean value for that animal. Ovulation was assumed to have occurred when progestagen concentration remained elevated for at least two consecutive samples. Oestrous cycle length was calculated as the interval between successive nadirs in progestagen concentration that included a luteal phase.

3. Results

3.1 Vulval swelling

A simple grading system (0-3) was developed, based on descriptions, to provide greater consistency between observers. A score of 0 represented 'no swelling', 1 represented 'moderately full', score of 2 was described as 'swollen' and a score of 3 as 'very swollen' and typical of oestrus (Fig. 1). The swellings are bubble-like in appearance and it is the fullness of the 'bubble' that is evaluated. Vulvar swelling varies within and among females: Female #1357 has just one 'bubble' while Female #1358 has three, Female #1353 did not exhibit the 'bubble-like' swellings of the mature females but on one occasion the lips of the vulva were noticeably tight and swollen.

Vulval swellings were identified on seven occasions, of which three (Score 3) corresponded to periods of mating and/or nadirs in progestagen (Fig. 2). The remaining four observations (all at Score 2) were at 30 day intervals in Female #1357 during an extended anovulatory period (Figure 2), the male showed some courtship behaviour on one of these days.

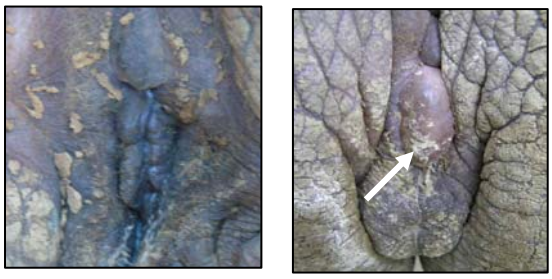

Figure. 1: Vulva scores in the white rhinoceros; left) score of 0 typical of anoestrus or luteal phase, right) score of 3 typical of oestrus, arrow indicating 'bubble-like' appearance.

3.2 Faecal progestagen profiles and behaviour observations

Individual profiles of faecal progestagen concentrations for the three females are presented in Figure 2. At the start of the study in May 2005 the two mature females (#1357, 1358) were anovulatory, Female #1357 (estimated age 11 yr) failed to ovulate throughout the 13 month study period. Female #1358 resumed ovarian activity and was mated on 27th September and 3-4th November, a 38 day oestrous cycle. Pregnancy was confirmed by an absence of oestrus activity and faecal progestagen concentrations (Fig. 3), expected parturition is March-April 2007. A winter anovulatory interval of 87 days was also identified in the young female (#1353, aged 5 years), this female resumed ovarian activity in October and five cycles ranging from 25-33 days (28.2±1.2 days; mean±SEM) were measured before the onset of another extended anovulatory interval in February 2006. The average of the 7 recorded cycles was 29.6±1.8 days.

Faecal progestagen concentrations during the interluteal and anovulatory phases were 145.4±21.0 ng/g and 535.2±152.5 ng/g during the luteal phase. Faecal progestagen concentrations remained elevated throughout gestation and were higher (17,382±1298 ng/g; $P<0.01$) than those observed during non-pregnant luteal phases (Fig. 3). Behavioural observations found that the male initiated consort behaviour (follows female and always in close proximity – 10m) 7-8 days before mating. Chin rest (male rests his chin on the rump of the female) was observed on the day before and day of mating. Mating was preceded by mounting without penetration numerous times, mating in the conception cycle occurred over 2 days and the mating on 4th November lasted approximately 20 minutes.

Figures 2 & 3 here but unable to embed them into this template, they are provided as separate word file named Carter, Louise ARAZPA 2007 Fig 2&3.

Figure. 2: Faecal progestagen concentrations in three female Southern white rhinoceros. Arrows denote observed courtship or mating behaviour, asterisks represent Level 2 (#1353, 1357) or 3 (#1358) vulval swelling.

Figure. 3: Faecal progestagen concentrations in a pregnant white rhinoceros (mating November 2005). Note change in y axis scale.

4. Discussion

Measuring progesterone metabolites in faecal extracts proved a reliable method for monitoring oestrous cycles, anovulatory periods and pregnancy in three captive Southern white rhinoceros. The average length of the oestrous cycles reported here was 29.6±1.8 days (range 25-38 days, n=7) which confirms previous reports of 30-35 days [Hindle et al., 1992; Radcliffe et al., 1997; Patton et al., 1999; Brown et al., 2001].

We did not find evidence of the 65-70 day cycle reported by others but confirm the extended acyclic or 'flat line' pattern [Schwarzenberger et al., 1998; Patton et al., 1999; Brown et al., 2001]. Interestingly, all females resumed ovulation, including Female #1357 (data not shown) after varying intervals. Female #1353 had a second extended acyclic period beginning in February 2006 that lasted 222 days which may suggest a seasonal effect for this young captive-born female.

The pattern of extended periods of anovulation or erratic cycles for Female #1357 fit the pattern of premature senescence described by Hermes et al. [2004, 2006] or reproductive suppression [Hermes et al., 2006]. This female had previously exhibited regular oestrous cycles of 28-35 days throughout 2000-2002 [Morrow, unpub.] and keepers regularly observed mating and distinct vulval swelling during oestrus. Female #1357 also had vulval swellings typically indicative of oestrus on four occasions at 30 day intervals during the present study and were possibly due to follicular waves without ovulation and the formation of hemorrhagic follicles [Radcliffe et al., 1997; Hermes et al., 2006]. Vulval swelling varied between individuals but was only ever observed when progestagen concentrations were low, on two occasions swellings coincided with mating. Ultrasound examination would confirm the suspected relationship between vulval swelling and high oestrogen secretion by the pre-ovulatory follicle. Monitoring follicular activity using faecal oestrogen or oestrogen metabolites has not been successful in white rhinoceros [reviewed by Roth, 2006].

In summary, for some individual females, vulval swellings may be a simple yet worthwhile addition to behaviour observations in signaling the onset of oestrus and assisting in breeding management decisions. Behaviour observation and confirmation of ovulation/conception using hormone analysis is recommended in the assessment of reproductive status in white rhinoceros.

5. Acknowledgments

We thank all past and present Hamilton Zoo rhino keepers for conscientious collection of faecal samples and observations; we also acknowledge the volunteer Zoo Hosts for assistance with behavioural observations and Coralie Munro for the preparation of the progesterone antibody and HRP. The University of Waikato Biological Sciences Department provided travel and conference funding (LHC).

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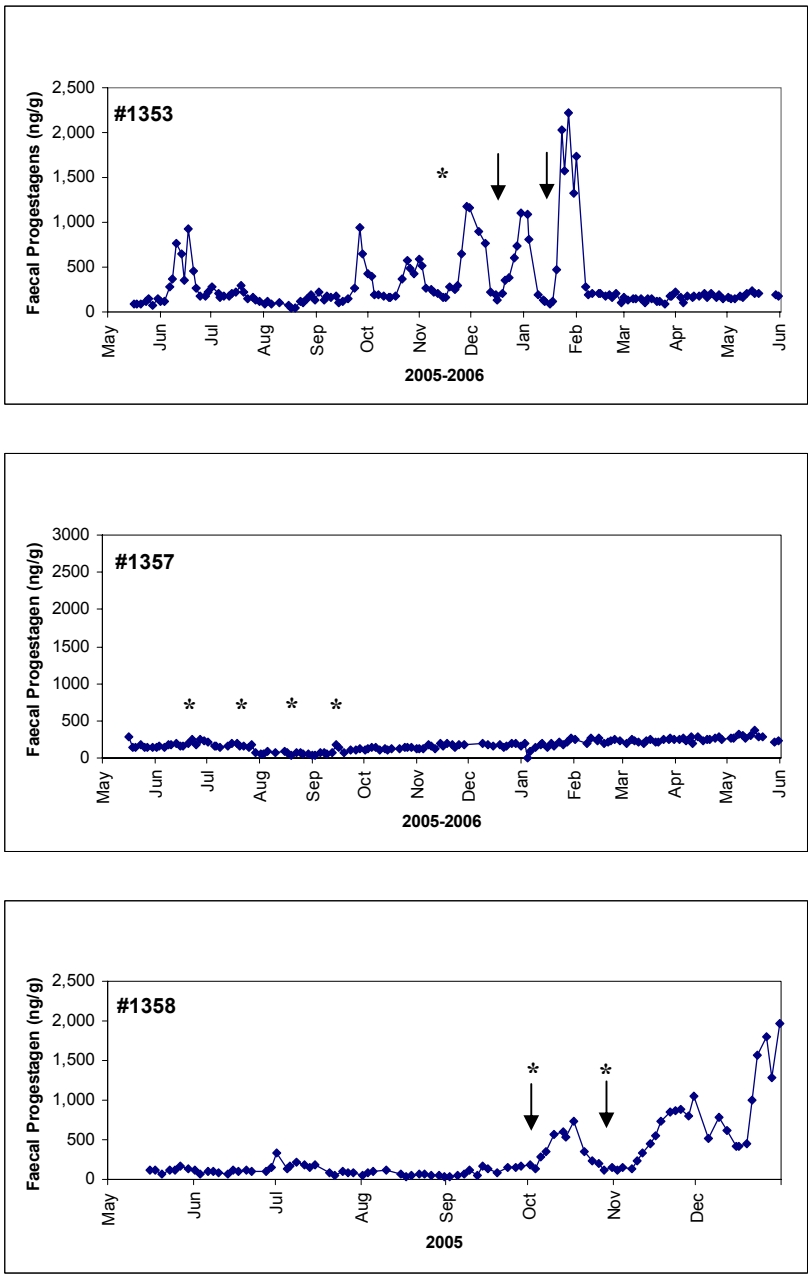

Figure. 2: Faecal progestagen concentrations in three female White rhinoceros. Arrows denote observed courtship or mating behaviour, asterisks represent Level 2 (#1353, 1357) or 3 (#1358) vulval swelling.

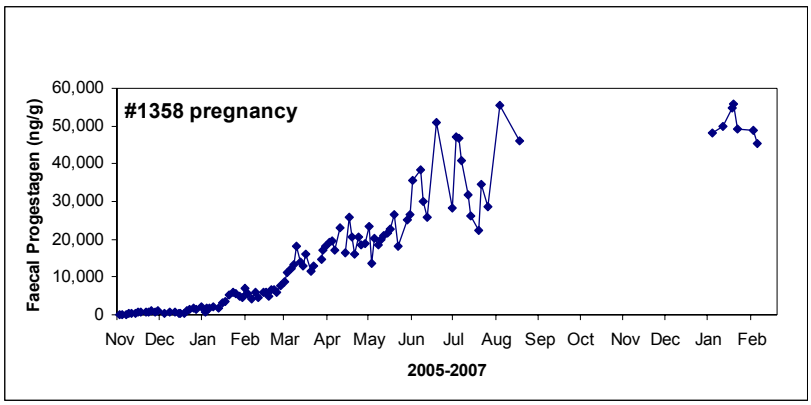

Figure. 3: Faecal progestagen concentrations in a pregnant White rhinoceros from mating in November 2005. Note change in scale of y axis.