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REPRODUCTIVE BIOLOGY OF FEMALE AFRICAN RHINOCEROS

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The rapid decline of wild rhinoceros populations has led to an intense interest in captive propagation of rhinoceros species. Historically, captive rhinoceros have not been very prolific although there have been occasional pairs and groups of animals which have been quite successful reproductively. In order to evaluate non-reproductive animals and to initiate the development of assisted reproductive techniques, such as artificial insemination and embryo transfer, a number of recent studies have been undertaken on the reproductive biology of the female rhinoceros.

Initial endocrine work on the greater one-horned (Indian) rhinoceros, *Rhinoceros unicornis*, indicated that the oestrus cycle and pregnancy could be monitored readily via urinary steroid hormone analyses[8 9]. Studies of female black and white rhinoceros revealed these species' reproductive physiology to be quite different than the one-horned rhinoceros[7 13]. Subsequent studies of captive black and white rhinoceros have confirmed this difference and provided basic information about their oestrus cycle and pregnancy[4 5]. This paper summarizes the reproductive data derived from studies of captive female black and white rhinoceros.

WHITE RHINOCEROS *Ceratotherium simum*

Studies of the reproductive tract of the female white rhinoceros reveal the presence of a hymen, just cranial to the urethral opening, in nulliparous females[1 15]. The vagina is long and smooth, with occasional longitudinal folds of skin. The cervix is firm and the lumen of the cervix is tortuous. The internal and external os of the cervix are not readily identifiable due to copious folds of skin[15].

The white rhinoceros has a long bicornuate uterus. The body of the uterus is relatively short and divides into the two horns, which remain attached at the muscular layer for 20-30cm before separating[15]. The uterine horns then course separately 20-30 cm to the oviducts. The texture of the endometrium can vary considerably, presumably with different stages of the oestrus cycle[1]. The oviducts are thin and convoluted with large fimbria. Inactive ovaries are flat and ovoid, becoming more spherical with follicular activity. Overall adult tract length is approximately 100 cm[1].

Age at sexual maturity in captivity is reported as approximately 5-7 years for the female[2]. Reports of captive gestations range from ">15 months" to approximately 20 months[2]. No reports suggest a reproductive seasonality in captivity.

Initial urinary hormone metabolite studies suggest that neither direct estrone conjugate nor a pregnanediol-3-glucuronide (PdG) analysis were useful for monitoring the oestrus cycle of the white rhinoceros[7]. Subsequent work confirmed PdG is not found in post-oestrus urine samples[5]. Analyses of urine and faeces following injection of radio-labelled estradiol-17ß and progesterone indicated slightly more than half of the metabolized hormones were excreted in the faeces[4]. The vast majority of the radio-activity in the faeces was associated with unconjugated steroids while in the urine, amounts of conjugated and unconjugated hormone metabolites were roughly equal. Estradiol-17ß was the major estrogen metabolite excreted. Progesterone was the major unconjugated progestin found in faeces and 4-pregnanediol-20α-ol-3-one was the principal conjugated progesterone metabolite in urine[4].

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Serial analyses of urine samples utilizing a radio-immuno-assay (RIA) for estradiol-17ß demonstrated the northern white rhinoceros (*Ceratotherium simum cottoni*) to have an inter-estradiol peak interval of 24-25 days and the southern subspecies (*Ceratotherium s. simum*) to have an interpeak interval of 32 days[5]. Analyses of urine using an enzyme immuno-assay (EIA) for 20α-dihydroprogesterone (20α-DHP) showed peak concentrations occurred 8-12 days following breeding activity.

There have been few studies of steroid hormone excretion during pregnancy of the white rhinoceros. Elevated concentrations of PdG during pregnancy were found in a study of an individual white rhinoceros[6]. The PdG concentrations dropped sharply after parturition. Examination of a number of non-pregnant white rhinoceros urine samples demonstrated low to non-measurable amounts of PdG and C-19/C-21 progesterone metabolites, similar to concentrations observed in samples from non-pregnant black rhinoceros[14].

Two studies have evaluated white rhinoceros gonadotropins. A white rhinoceros pituitary gland evaluated for presence and activity of gonadotropins demonstrated luteinizing hormone activity[11]. No follicle stimulating hormone activity was identified in this luteinizing hormone-like compound. Analyses of non-pregnant white rhinoceros urine using two assays for equine chorionic gonadotropin (eCG) failed to detect any eCG-like activity[14].

BLACK RHINOCEROS *Diceros bicornis*

The reproductive tract of the female black rhinoceros is anatomically very similar to that of the white rhinoceros[1,15]. Early efforts at monitoring the oestrus cycle via vaginal cytology, vaginal discharge, and behaviour indicated that cytology was not useful for identifying oestrus[12]. A slight correlation was noted between vaginal discharge and motor activity with sexual receptivity, but attempts to artificially inseminate a female based on these observations were unsuccessful.

Captive black rhinoceros mature at a mean age of 5,25 years, with a range of 3-10 years[16]. Gestation in captivity ranges from 438-480 days[13]. While there is some clumping of births during the spring and fall, there is no clear seasonality to captive black rhinoceros reproduction[2] (Ramsay, unpublished data).

Radio-labelled hormone infusion studies have not been performed in the black rhinoceros. Analyses of serial urine samples by an estrone conjugate RIA, an estradiol RIA, and a PdG RIA indicated these assays were not useful for monitoring the black rhinoceros oestrus cycle[13]. High performance liquid chromatography indicated conjugated estrone to be the principal estrogen metabolite excreted by the black rhinoceros[5]. The failure of the estrone conjugate RIA used by Ramsay *et al.*[13] to detect cyclic patterns, is unclear but may be related to the nature of the assay and its antibody. Analyses of serial urine, following hydrolysis, for total estrogens using a RIA indicates the black rhinoceros oestrus cycle has a 3-4 day follicular phase and a length of 21-22 days. Urinary 2α-DHP patterns indicate luteal phases of approximately 18 days[5]. Pregnanediol-3-glucuronide as not found in post-oestrus urine samples from black rhinoceros[5,13]).

Two studies of pregnant black rhinoceros suggest urinary PdG can be used to monitor the second half of gestation[6,13]. Concentrations measured in individuals varied widely[13] and subsequent EIA studies of urinary PdG and other C-19/C-21 progesterone metabolites suggest analyses for these metabolites are approximately 75% accurate[14]. Gas chromatography and mass spectrophotometry indicate pregnanetriols, not pregnanediols, are the most commonly excreted progesterone metabolite during pregnancy in the black rhinoceros[3]. Current studies are in progress to evaluate saliva analysis for progesterone metabolites as a method of pregnancy diagnosis[10] in this species (N. Czekala, San Diego Zoological Society, personal communication).

Analysis of a black rhinoceros pituitary gland for gonadotropins indicated the presence of lutenizing hormone activity[11]. In contrast to the white rhinoceros, the luteinizing hormone-like compound also possessed follicle stimulating hormone activity. No eCG-like activity was identified in pregnant or non-pregnant black rhinoceros urine samples using RIA or dipstick/enzyme technique[14].

DISCUSSION

Hormone data from the two African rhinoceros species indicate these species' reproductive physiologies differ remarkably from that of the greater one-horned rhinoceros. In the one-horned rhino, analysis of estrone conjugates, either by direct assay or following hydrolysis and chromatography of samples, clearly indicates ovarian follicular activity, while urinary PdG accurately reflects luteal activity[8,9]. The oestrus cycle of the greater one-horned rhinoceros is considerably longer (mean = 48 days, range = 39-64 days) than the oestrus cycles of the African species[5].

Sexual maturity occurs at a similar age in the three rhinoceros species examined. The greater one-horned rhinoceros' gestation in captivity has been consistently reported in the 462-489 day range[2]. This range is much tighter than those reported for African species, but probably reflects the closer scrutiny this rarer, Asian species receives in zoo collections. In contrast to African rhinoceros, pregnancy can be definitively identified in the greater one-horned rhinoceros by rising concentrations of urinary PdG, with a pronounced drop in PdG concentrations occurring following parturition[6,8]. The one gestation documented for the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is >498 days and among the longest recorded for the rhinoceros family (Zainal Zahari, Zoo Melaka, Malaysia, personal communication).

While much has been learned about the reproductive biology of female African rhinoceros, much remains to be studied. Although the data accumulated to date has been largely derived from urinary hormone metabolite analyses, efforts to acquire additional reproductive information via serum or plasma hormone analyses should not be ignored. Preliminary analyses of serum progesterone in African rhinoceros indicate it is possible to diagnose pregnancy from a single sample (J. van Heerden, Veterinary Faculty, Medunsa, Republic of South Africa, personal communication).

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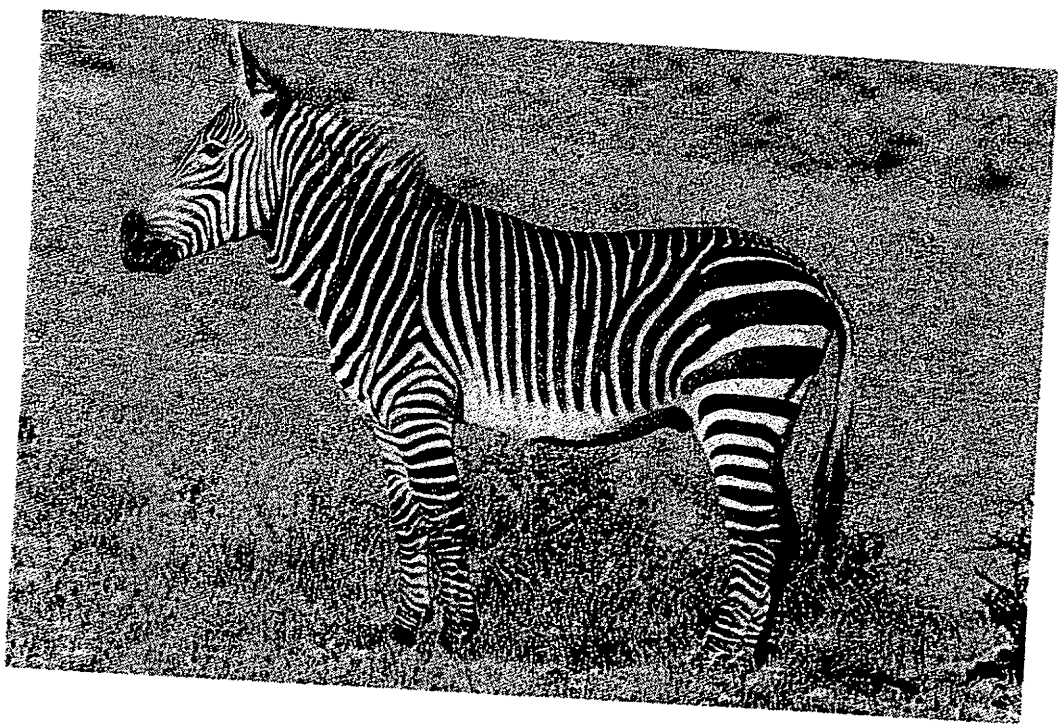

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