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RESEARCH COMMUNICATION

# REPRODUCTIVE PARAMETERS IN FREE-RANGING FEMALE BLACK RHINOCEROSES *(DICEROS BICORNIS)* IN ZIMBABWE

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ABSTRACT

KOCK, N., MORTON, D. & KOCK, M., 1991. Reproductive parameters in free-ranging female black rhinoceroses in Zimbabwe. *Onderstepoort Journal of Veterinary Research,* 58, 55–57 (1991)

Samples and data were collected from twenty-eight female black rhinoceroses *(Diceros bicornis)* during translocation efforts carried out by the Department of National Parks and Wildlife Management in Zimbabwe. Biological data were collected, cytological examination of vaginal smears was performed, and serum concentrations of follicle stimulating hormone, luteinizing hormone, progesterone, oestriol, and 17-β-oestradiol were determined by radio-immuno-assay. Prolactin levels were determined for 3 pregnant animals, 1 of which was sampled before and after parturition. Vaginal cytology was not found to be helpful for indicating the oestrous cycle stage for the black rhinoceros, but progesterone and 17-β-oestradiol levels were found to be useful indicators of pregnancy and possibly of oestrous cycle stage as well.

## INTRODUCTION

The black rhinoceros *(Diceros bicornis)* is critically threatened with extinction, the African population having dropped from a total of about 65 000 in 1970 to fewer than 4 000 at the present time (Cumming, 1987), a decline that has come about largely as the result of poaching. Although about 150 black rhinoceroses are kept in zoological gardens throughout the world, the species does not thrive in captivity, many succumbing to a haemolytic syndrome, the aetiology and pathogenesis of which remain obscure (Douglass, Plue & Cord, 1980; Miller & Boever, 1982, 1983; Miller, Chaplin, Paglia & Boever, 1986; Miller, 1987; Paglia, Valentine, Miller, Nakatani & Brockway, 1986; Paul, Du Toit, Lloyd & Mandisodza, 1988). Little regarding their reproductive cycles is known with certainty, the information available coming mainly from captive animals (Rowlands & Weir, 1984). Female black rhinoceroses reach sexual maturity between 60–121 months of age and often mate for the first time soon after. Gestation lasts approximately 15 months (Bothma, 1989). The length of the oestrous cycle is from 4 to 5 weeks, and oestrus lasts from 1 to 6 days, the only outward sign being a slight prominence of the vulva (Rowlands & Weir, 1984).

## METHODS AND MATERIALS

During 1988 and 1989, capture and relocation projects carried out by the Zimbabwe Department of National Parks and Wildlife Management (DNPWM) successfully translocated over 60 black rhinoceroses, 28 of which were female. Of these, 23 were classified as reproductively mature (>5 years old) and 5 as immature. Reproductive data and biological samples were collected from them, and follow-up information and samples were gathered as logistics permitted after transport and relocation.

At capture, the presence or absence of calves with the females was noted and the mammary glands were examined for lactation. The vulvar appearance was classified as normal without discharge, normal with discharge, swollen without discharge, or swollen with discharge. Sterile swabs, 15 cm in length, used to swab the vaginal vault, were immediately placed separately in sterile glass test tubes containing small amounts of normal saline solution. Smears were made from these swabs within 6 h of extraction and fixed in 10 % methanol. The smears were later stained with Leischman stain and evaluated for cellularity, major cell type, and predominant epithelial cell type. Cellularity was subjectively classified as high or sparse. The major cell type and predominant epithelial cell type were determined by examining 10 high/dry microscope fields (40 ×), and noting the most prevalent cell type and the predominant type of epithelial cell. In all cases the cell type was either neutrophil or epithelial cell. Vaginal epithelial cells were of 4 kinds, single or small groups of large, non-cornified cells, with abundant cytoplasm, rafts of smaller, non-cornified cells with scant to moderate amounts of cytoplasm, single small cells with eccentric nuclei (parabasal cells), and cornified epithelial cells.

Venous blood was collected and stored in insulated containers until serum could be harvested after centrifugation later in the day. The serum samples were placed in liquid nitrogen for storage until hormonal assays were performed. Radio-immuno-assay determinations of FSH, LH, progesterone, oestriol, 17-β-oestradiol, and prolactin were performed in duplicate using standard radio-immuno-assay kits designed for humans[1]. The results reported are mean values of duplicate samples, the differences within pairs being within acceptable ranges of coefficients of variation in all cases. Validation of the radio-immuno-assay results was done by spiking previously assayed samples with known increments of progesterone, 17-β-oestradiol, oestriol, and prolactin, by subsequent analysis. In all cases, the levels determined were in agreement with the increments added to the plasma samples, and all results were within the published coefficients of variation for the respective assay kits. It was not possible to validate the kits for LH or FSH, as pure samples of these hormones were not available.

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TABLE 1 Summary of data and hormonal results measured from serum and plasma samples collected from female black rhinoceros at the time of capture in Zimbabwe

| Id. | Age | Calf | Lact | Vulva[1] | Estriol (ng/mℓ) | Estradiol -β-17 (pg/mℓ) | FSH (μIU/mℓ) | LH (μIU/mℓ) | Progesterone (ng/mℓ) |
|---|---|---|---|---|---|---|---|---|---|
| 8702 | Adult | Yes[2] | Yes | 2.3 | 1,22 | 31,46 | 1,94 | 1,46 | 0,46 |
| 8704 | Adult | No | No | 2.3 | 0,98 | 58,55 | 1,68 | 1,52 | 0,28 |
| 8808 | Adult | Yes | Yes | 2.3 | 1,76 | 48,00 | 2,12 | 1,96 | 0,74 |
| 8812 | Adult | Yes | No | 2.3 | 1,39 | 148,99 | 2,24 | 2,57 | 29,26 |
| 8814 | Adult | Yes | No | 1.3 | 1,87 | 218,00 | 1,79 | 2,61 | 23,38 |
| 8815 | Calf | — | — | 1 | 1,87 | 1,00 | 2,46 | 2,55 | 1,22 |
| 8816 | Subad | — | — | 1.3 | 1,37 | 59,65 | 2,51 | 2,54 | 0,70 |
| 8819 | Adult | No | No | 1 | 1,29 | 81,21 | 1,94 | 1,83 | 11,09 |
| 8822 | Adult | Yes[3] | Yes | 2.3 | 1,40 | 96,19 | 2,37 | 2,33 | 0,70 |
| 8823 | Adult | No | No | 2 | 1,44 | 93,23 | 2,30 | 2,51 | 2,91 |
| 8824 | Adult | No | Yes | 2.3 | 1,42 | 71,70 | 2,55 | 2,44 | 0,48 |
| 8825 | Adult | No | No | 2.3 | 1,40 | 73,28 | 2,31 | 2,68 | 11,56 |
| 8826 | Adult | Yes | No | 1.3 | 1,32 | 66,63 | 2,38 | 2,60 | 3,68 |
| 8828 | Adult | No | Yes | 2 | 1,32 | 107,74 | 2,14 | 2,91 | 0,65 |
| 8829 | Adult | No | No | 1 | 1,40 | 109,08 | 2,61 | 2,17 | 9,96 |
| 8833 | Adult | Yes | Yes | 1.3 | 1,42 | 73,15 | 2,21 | 2,33 | 0,77 |
| 8834 | Calf | — | — | 1 | 1,35 | 32,54 | 2,25 | 3,60 | 0,72 |
| 8835 | Adult | Yes | Yes | 1.3 | 1,69 | 138,00 | 1,95 | 2,47 | 1,70 |
| 8836 | Adult | No | No | 1 | 1,84 | 2,00 | 2,02 | 2,57 | 1,80 |
| 8839 | Adult | Yes | No | 1.3 | 1,91 | 22,00 | 2,25 | 2,44 | 14,09 |
| 8845 | Adult | Yes | Yes | 1.3 | 1,71 | 174,00 | 2,43 | 2,34 | 3,58 |
| 8846 | Calf | — | — | 1 | 1,83 | 1,00 | 1,95 | 2,20 | 1,39 |
| 8848 | Adult | No | No | 1 | 1,92 | 70,00 | 2,39 | 2,37 | 1,05 |
| 8849 | Adult | Yes | No | 1.3 | 1,68 | 90,00 | 2,45 | 2,97 | 10,12 |
| 8850 | Subad | — | — | 1 | 1,99 | 1,00 | 2,36 | 3,41 | 0,99 |
| 8882 | Adult[4] | No | No | — | 2,06 | 600,00 | — | — | 1406 |
| 8902 | Adult[4] | No | No | — | — | 30,00 | — | — | 6430 |
| 8904 | Adult[4] | No | No | — | — | 90,00 | — | — | 1170 |

[1] Appearance of vulva: 1 = normal, 2 = swollen, 1.3 = normal with mucoid discharge, 2.3 = swollen with mucoid discharge

[2] Calf approximately 1 month old

[3] Calf < 1 month old

[4] Known to be pregnant at capture

TABLE 2 Hormonal results measured from serial serum samples collected from three female black rhinoceroses, *Diceros bicornis*, that were known to be pregnant at the time of capture

| Id. | Calf | Lactating | Estriol (ng/mℓ) | Estradiol -β-17 (pg/mℓ) | FSH (μIU/mℓ) | LH (μIU/mℓ) | Progesterone (ng/mℓ) | Prolactin (ng/mℓ) |
|---|---|---|---|---|---|---|---|---|
| 8882[1] | No | No | 2,06 | 600,0 | — | — | 1406,00 | 2,1 |
| 8882[2] | Yes | Yes | 0,95 | 50,00 | — | — | 5,80 | 8,0 |
| 8902[3] | No | No | — | 30,00 | — | — | 6430,00 | ND |
| 8902[4] | No | No | — | 220,00 | — | — | 7770,00 | ND |
| 8904[5] | No | No | — | 90,00 | — | — | 1170,00 | ND |
| 8904[6] | No | No | — | 420,00 | — | — | 6850,00 | ND |

ND = Not detectable

[1] Sample taken 12 months into gestation

[2] Sample taken 1 week after parturition

[3] Sample taken about 13 months into gestation

[4] Sample taken about 14 months into gestation

[5] Sample taken about 13 months into gestation

[6] Sample taken about 14½ months into gestation

## RESULTS

Eleven of the 23 mature animals had calves, 2 of which were less than a month old, and 8 animals were lactating. Four had normal vulvas without discharge, 7 had normal vulvas with discharge, 2 had swollen vulvas without discharge, 7 had swollen vulvas with discharge, and the appearances of 3 animals were not recorded.

Neutrophils predominated in most of the vaginal smears (16/20), often accompanied by large, often single, non-cornified epithelial cells (16/20). Although parabasal and non-cornified epithelial cells were seen regularly in the smears, they did not predominate in any of the smears.

Table 1 is a summary of the field data and hormonal results. Marked variations were observed in progesterone and 17-β-oestradiol levels, while those of oestriol, FSH, and LH, varied little. Table 2 is a comparison of hormonal results from serial samples taken from 3 animals known to be pregnant at the time of capture. The stage of pregnancy at the time of sampling was determined following parturition.

## DISCUSSION

Without detailed information concerning ovarian cyclicity in the black rhinoceroses, it is difficult to use the hormonal values presented in this study to define the precise oestrous cycle stages. The results suggest a correlation between 17-β-oestradiol and reproductive cyclicity, evidenced by the marked variations in the values expected for a group of non-synchronized animals, and the levels rose with advancing pregnancy (Table 2). The variation in progesterone, as with 17-β-oestradiol, also suggests reproductive cyclicity. In humans, progesterone varies according to the stage of the reproductive

cycle and increases after ovulation (Guyton, 1976). It is likely that 17-β-oestradiol and progesterone can be used together to monitor reproductive cyclicity in the black rhinoceros, although more data with follow-up information are needed to correlate hormonal levels with precise oestrous stages. Elevated levels of 17-β-oestradiol with low levels of progesterone levels, as seen in Animals 8704 and 8823 (Table 1) may indicate pre-ovulatory phases, and elevated levels of both hormones, as in Animals 8812 and 8814 (Table 1), might indicate either post-ovulatory phases or early pregnancy. That both of these animals also had vaginal discharges and were captured with older calves that were no longer suckling lends credence to this speculation. Lactating animals had significantly lower levels of progesterone, while other hormonal values varied little. This agrees with hormonal changes in other species during lactation, where pituitary directed prolactin production suppresses the production of other gonadotrophic hormones (Guyton, 1976). Prolactin rose, as was expected, in serial samples from Animal 8882 taken at 12 months gestation and again shortly after parturition.

Oestriol, LH, and FSH varied little in the rhinoceroses, not even in animals known to be pregnant. Both LH and FSH are glycoprotein hormones that have steady secretory patterns with superimposed pulses. Serum levels in humans, therefore, vary greatly throughout the day, making analyses of results on single blood samples of questionable value anyway (Guyton, 1976). Since neither of these nor oestriol varied much between the rhinoceroses in this study, it is possible that the antibodies in the radio-immuno-assay kits were unable to bind the rhinoceros hormones to any extent. In the absence of antibodies specific for these hormones in the black rhinoceros, however, it is not possible to validate this suggestion.

Three pregnant animals, 8882, 8902, 8904, had elevated 17-β-oestradiol and progesterone levels, which rose as pregnancy progressed in Animals 8902 and 8904, and dropped dramatically after parturition in Animal 8882 (Table 2). Prolactin levels were either low or not detectable in these animals before parturition and rose in Animal 8882 sampled after parturition. This pattern is similar to that seen in the horse during pregnancy, another perissodactylate, or odd-toed ungulate like the rhinoceros (Fowler & Nelson, 1986), where progesterone and oestrogen rise soon after conception, increase during pregnancy, and decline after foaling (Roberts, 1986).

Correlations between hormonal levels and vulvar appearances in the black rhinoceroses in this study were somewhat uncertain because of small sample sizes in some of the categories. However, 17-β-oestradiol appeared to be elevated when the vulva was either swollen, with discharge, or both, but not when classified as normal without discharge. This is as was expected, for the vaginal mucosa, being influences by oestrogens, becomes moist, hyperaemic, and glistening when oestrogens peak during oestrus and pale and dry during dioestrus, when oestrogens are low (Noakes, 1979).

Vaginal mucosa undergoes cornification under the influence of oestrogen which provides protection during copulation and regresses under the influence of progestins (Noakes, 1979). Cytological examination of vaginal smears has proved rewarding in determining the stage of oestrus in the dog and the cat (Coles, 1986), but has not been helpful in most other domestic species. Differences in the vaginal smears of the black rhinoceroses were difficult to assess, and it is likely that, as in most domestic species, vaginal cytology may not be useful in predicting oestrus cycle stages in this species.

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## REFERENCES

BOTHMA, J. DU P. (ed). 1989. Game ranch management. 1st Edn. 120–121. Pretoria, South Africa: J. L. van Schaik.

COLES, E. H., 1986. Veterinary clinical pathology. 4th Edn. 260–262. Philadelphia: WB Saunders Company.

CUMMING, D., 1987. Small population management of black rhinos. *Pachyderm* 9, 12–19.

DOUGLASS, E. M., PLUE, K. E. & CORD, C. E., 1980. Hemolytic anemia suggestive of leptospirosis in the black rhinoceros. *Journal of the American Veterinary Medical Association* 177, 921–923.

FOWLER, M. E. & NELSON, L., 1986. Rhinocerotidae. *In:* FOWLER, M. E. (ed.) Zoo and wild animal medicine. 2nd Edn 934–938. London: WB Saunders Co.

GUYTON, A. C., 1976. Textbook of medical physiology. 5th Edn. London: WB Saunders Co.

MILLER, R. E., 1987. Haemolytic anemia in the black rhino. *Pachyderm* 9, 26–28.

MILLER, R. E. & BOEVER, W. J., 1982. Fatal haemolytic anemia in the black rhinoceros: Case report and a survey. *Journal of the American Veterinary Medical Association*, 181, 1228–1231.

MILLER, R. E. & BOEVER, W. J., 1983. Hemolytic anemia in the black rhinoceros *(Diceros bicornis). Proceedings of the Annual Meeting of the American Association of Zoo Veterinarians*, 51–53.

MILLER, R. E., CHAPLIN, H., PAGLIA, D. E. & BOEVER, W. J., 1986. Hemolytic anemia in the black rhinoceros—an update. *Proceedings of the Annual Meeting of the American Association of Zoo Veterinarians*, 7–8.

NOAKES, D. E., 1979. The normal breeding animal. *In:* LAING, J. A. (ed.). Fertility and infertility in domestic animals. 3rd Ed. 5-35. London: Bailliere Tindall.

PAGLIA, D. E., VALENTINE, W. N., MILLER, R. E., NAKATANI, M. & BROCKWAY, R. A., 1986. Acute intravascular hemolysis in the black rhinoceros: Erythrocyte enzymes and metabolic intermediates. *American Journal of Veterinary Research*, 47, 1321–1325.

PAUL, B., DU TOIT, R., LLOYD, S. & MANDISODZA, A., 1988. Haematological studies on wild black rhinoceros *Diceros bicornis)*—evidence of an unstable haemoglobin. *Journal of Zoology, London* 214, 399–405.

ROBERTS, S. J., 1980. Gestation and pregnancy diagnosis in the mare. *In:* MORROW, D. A. (ed.). Current therapy in theriogenology. 670–678. Philadelphia: WB Saunders Company.

ROWLANDS, I. W. & WEIR, B. J. 1984. Mammals: Non-primate eutherians. *In:* LANNING, G. E. (ed.). Marshall's physiology of reproduction, Vol. 1. 455–658. London: Churchill Livingstone.