

Gross Anatomy of the Reproductive Tract of Female Black (*Diceros bicornis michaeli*) and White Rhinoceros (*Ceratotherium simum simum*)

R.W. Godfrey, C.E. Pope, B.L. Dresser, and J.H. Olsen

Center for Reproduction of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, Cincinnati (R.W.G., C.E.P., B.L.D.); Busch Gardens, Tampa (J.H.O.)

Reproductive tracts were collected from three black rhinoceros and two white rhinoceros at necropsy. All females were nulliparous, except for one primiparous white rhinoceros. The animals ranged in age from 7 days to 28 years. All nulliparous animals had a constriction in the vaginal canal, which appeared to be a hymen. The primiparous female had only remnants of a hymen. The total length of the tract averaged 102 cm in three adult animals (≥ 21 years old). The distance from the vulva to the external cervical os averaged 40 cm in these animals. The endometrium of a 28-year-old nulliparous black rhinoceros and a 27-year-old nulliparous white rhinoceros exhibited signs of hyperplasia, whereas this condition was not present in the other animals. It is not known if this condition was related to the fertility of these animals. The cervix of the rhinoceros was firm, and the lumen followed a very tortuous path through eccentric rings of tissue. Visual examination of the ovaries revealed the presence of surface follicles on the ovaries in both species. The overall size of the reproductive tract, especially the vagina and uterine horns, and the extremely tortuous cervical lumen will present obstacles that must be overcome when developing artificial insemination and embryo transfer procedures for use in rhinoceros.

Key words: cervix, uterus, endometrium, Perrisodactyla

INTRODUCTION

Artificial insemination (AI) and embryo transfer (ET) procedures have been used successfully in both domestic and exotic animals. The use of AI and ET has allowed a greater utilization of genetic selection for various traits in domestic livestock. In the smaller population of captive rhinoceros, the importance of breeding

Received for publication August 13, 1990; accepted October 3, 1990.

Address reprint requests to Dr. R.W. Godfrey, Center for Reproduction of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220.

unrelated animals has become a crucial matter. By developing methods of AI and ET, it will be possible to achieve a higher level of genetic diversity in the population by breeding unrelated pairs of animals even though the male and female are not at the same location. The use of AI will allow a more widespread distribution of males, whereas ET will increase the amount of genetic influence provided by females of the captive population.

Before the techniques of AI and ET can be used successfully, it will be necessary to gain information about the anatomy of the female reproductive tract. The ability to deposit semen or embryos at the proper site within the female tract will be critical to the success of AI and ET. Another consideration involves developing methods to collect embryos or oocytes nonsurgically from the female. The objective of this report is to describe the gross anatomy of the reproductive tract of female black and white rhinoceros. Reproductive tracts from animals of different ages were utilized to evaluate anatomical changes related to sexual maturity.

MATERIALS AND METHODS

Reproductive tracts were obtained from three black rhinoceros (*Diceros bicornis michaeli*) and two white rhinoceros (*Ceratotherium simum simum*). The black rhinoceros were 7 days (No. 2058), 3 years (No. 367), and 28 years (No. 38) of age at the time of death. The white rhinoceros were 21 (No. 751) and 27 (No. 45) years old. White rhino 751 was primiparous (gave birth to a male calf 10 years prior to death), whereas the remaining animals were nulliparous.

Measurements taken of each tract consisted of the lengths of the various sections, i.e., vestibule, vagina, cervix, uterine body, and uterine horns. The vestibule was defined as the section of the tract from the vulva anteriorly to the site of the hymen. In all of the tracts evaluated, the hymen, or its remnant, was located immediately anterior to the urethral orifice, which was in the floor of the vestibule. The vagina was defined as the portion of the tract between the hymen and the external cervical os. The length of the cervix was measured as the straight line distance from the external os to the internal os. The uterine body was measured from the internal cervical os to the internal bifurcation of the uterine horns. The length of the uterine horns was measured from the internal bifurcation to the uterotubal junction. The diameter of the uterine horn was much greater than the oviduct in each animal, so it was readily visible where the two structures joined. The diameter of the uterine horns was measured, and the average of the right and left horn was determined. When ovaries were present, their length, width, and depth were recorded. The number and diameter of visible follicles were also recorded. In the dissection of one tract (No. 367) the vulva was not included; therefore, the total length is underestimated.

RESULTS

The dimensions of the sections of the rhino reproductive tracts are presented in Table 1. In the black rhinoceros, the proportion of the tract occupied by the vagina decreased as the age of the animals increased. The percentage of the total tract length taken up by the vagina in the 7-day-old female (40.6%) was twice that of the 28-year-old female (20.7%). The 3-year-old female was similar to the 7-day-old female in proportion of the tract occupied by the vagina (34.5%). The percent of the tract that

TABLE 1. Measurements of reproductive tracts from black and white rhinoceros

Studbook No.	Black rhinoceros			White rhinoceros	
	2058	367	38	751	45
Age	7 days	3 years	28 years	21 years	27 years
Length of					
Vestibule ^a					
cm	6.8	— ^d	15.5	19.6	14.3
% ^b	19.6	—	14.5	18.5	15.3
Vagina ^c					
cm	14.1	25.0	22.1	30.1	19.0
%	40.6	34.5	20.7	28.4	20.3
Cervix					
cm	2.8	10.5	17.0	13.0	12.0
%	8.1	14.5	15.9	12.3	12.9
Uterine body					
cm	.5	2.7	5.5	3.5	7.5
%	1.4	3.7	5.2	3.3	8.0
Uterine horns ^e					
cm	10.5	34.3	46.5	39.9	40.6
%	30.3	47.3	43.6	37.6	43.5
Total (cm)	34.7	72.5	106.6	106.1	93.4
Diameter of					
uterine horn ^e (cm)	0.5	1.5	2.0	3.1	4.0

^aVulva to distal surface hymen.

^bPercent of total tract length.

^cProximal surface of hymen to external cervical os.

^dVulva was missing from tract, so total length is reported without vestibule.

^eRepresents average of left and right horns.

was cervix or uterine horn increased with age. The cervical portion of the 7-day-old female tract (8.1%) was slightly greater than half that of the 3 (14.5%)- and 28 (15.9%)-year-old females. The proportion of the tract consisting of uterine horn increased from 30% in the 7-day-old female to greater than 40% in the 3- and 28-year-old females. A similar pattern was also evident in the proportion of the tract occupied by the uterine body, which increased from 1.4% in the 7-day-old female to approximately 4% in the older animals. The vestibule increased in absolute size with age but the proportion of the entire tract consisting of the vestibule did not appear different between the two animals (Nos. 2058 and 38). The absolute length of the vagina, cervix, uterine body, uterine horns, and total tract length increased with age in the three black rhinoceros.

The vestibule, vagina, and total tract length were greater in the younger (No. 751) than in the older (No. 45) white rhinoceros. The proportions of the tract taken up by the vestibule and vagina were also greater in the younger female, whereas there was no difference in the proportion occupied by the cervix. The uterus (uterine body and horns) made up a larger percentage of the total length of the tract in the older animal than in the younger one (51.5% vs. 40.9%, respectively).

The tract of the oldest (28-year-old) black rhinoceros was slightly longer than that of the oldest (27-year-old) white rhinoceros (106.6 vs. 93.4 cm, respectively). The biggest differences between the tracts were in the lengths of the cervix and

uterine horns. The oldest black rhinoceros (No. 38) had a 4–5 cm longer cervix and 6–7 cm longer uterine horns than both white rhinoceros (Table 1). The uterine body of the older white rhinoceros occupied a larger proportion of the entire tract than that of the oldest black rhinoceros (8.0% vs. 5.2%, respectively). The two white rhinoceros also had larger diameter uterine horns than the oldest black rhinoceros (3.1 and 4.0 vs. 2.0 cm, respectively).

Each tract examined had some type of constriction in the posterior section of the tract just anterior to the urethral orifice. This hymenal membrane exhibited varying degrees of patency in the animals evaluated. In the 7-day-old female, the hymen was almost complete with only two small (<1 mm) perforations visible. The older white rhinoceros female (No. 45) had a hymen which was palpable vaginally in situ. There were two holes in the membrane, which were each approximately 3 cm in diameter. The primiparous white rhinoceros (No. 751) had vestiges of a hymen present in the vaginal canal. The remnants of the hymen appeared to be in the same location as the intact membranes in the nulliparous animals. One section of the hymen in the primiparous white rhinoceros female appeared to run longitudinally in the vagina for approximately 10 cm, thus dividing that section of the vagina into two channels. This division stopped well short of the external cervical os, approximately 20 cm posteriorly. The tracts of the two remaining black rhinoceros (Nos. 367 and 38) also appeared to possess a hymen. The hymen had one hole (0.5 cm in diameter) in the 3-year-old female. The complete structure of the membrane in the 28-year-old female was not discernible, since the tract was cut open prior to preserving, but there was tissue present in the vaginal canal that had the appearance of a hymen. It did not look as if the membrane was intact, and most likely it had one or more holes in it.

The cervical canals of the mature black and white rhinoceros appeared to be very tortuous (Figs. 1–3). The cervix contained several (three or four) large folds of dense fibrous connective tissue with crypts between each fold of tissue. The lumen of the cervix of the primiparous white rhinoceros (No. 751; Fig. 1) possessed several right angle turns and blind pockets among the crypts, whereas the lumen did not appear to be as tortuous in the cervix of the other white rhinoceros (No. 45; Fig. 2). At the external os, a very prominent fornix was present (Fig. 1). The cervixes of the two white rhinoceros were wider than that of the oldest black rhinoceros (9.5 vs. 7.0 cm, respectively). The cervix of the oldest black rhinoceros (No. 38; Fig. 3) appeared similar to that of the older white rhinoceros, in both the number and the complexity of folds. The cervixes in the younger black rhino tracts (Nos. 367 and 2058) were less rigid than those of the older animals, but evidence of eccentric rings of tissue was evident. The lumen of the 7-day-old rhinoceros cervix was very small (<2 mm) and appeared almost closed.

Upon dissection, the endometrium of the older white rhinoceros (No. 45) appeared to be hyperplastic (Fig. 4). It was characterized by the presence of fluid-filled vesicles evenly distributed throughout both uterine horns. The fluid in the vesicles appeared clear. The number and size of vesicles were uniform throughout the entire length of each horn and was possibly a potential source of decreasing the size of the lumen of the uterine horns. A similar condition was present in the uterine horns of the 28-year-old black rhinoceros (No. 38), although the vesicles were not as large or as densely packed as in the white rhinoceros. The appearance of the vesicles in the black rhinoceros was also different. The vesicles were dark brown and did not seem to be as turgid, indicating a decreased fluid volume within the vesicles. There was no

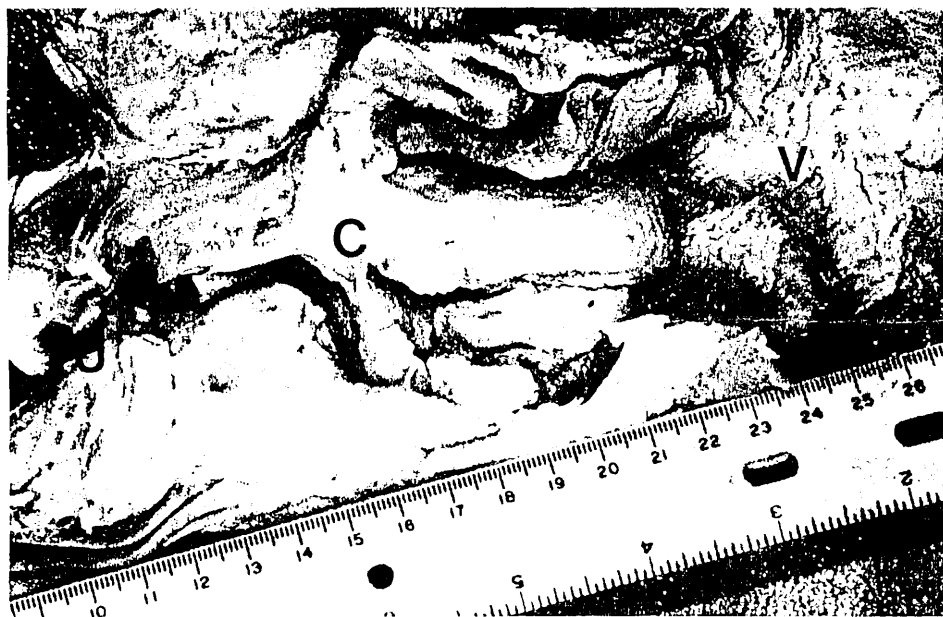


Fig. 1. Cervix (C) of a 21-year-old primiparous white rhinoceros (No. 751), showing the tortuous cervical lumen. The cervix is oriented with the vagina (V) to the right and the uterus (U) to the left. The photo was taken after the tract was fixed in 10% buffered formalin.

indication of endometrial hyperplasia in any of the remaining tracts examined. The endometrium of the primiparous white rhinoceros (No. 751) was characterized by the presence of longitudinal folds of tissue throughout the entire length of the horn. These folds were thin and did not contain any fluid. Upon dissection, the endometrium had a ruffled appearance due to these folds of tissue. There was also a difference in the diameter of the left and right uterine horns of the primiparous white rhinoceros (3.5 vs. 2.7 cm, respectively). It is assumed that the larger horn may have been the one where the calf was carried during the earlier gestation.

The oviducts were thin, convoluted tubes and terminated in large, fimbriated tissue adjacent to the ovary in the adult black and white rhinoceros. The fimbria of one white rhinoceros (No. 45) appeared dark red in color and was spongy to the touch at the time of necropsy (Fig. 5). This tissue appeared to be engorged with blood at the time of necropsy. The fimbria of the black rhinoceros (No. 38) was also very prominent, but did not appear to be as spongy or blood engorged as that of the white rhinoceros. This may be due to the fact that the black rhinoceros tract was placed in formalin prior to shipment to our laboratory for evaluation.

The ovaries of the adult black and white rhinoceros (Nos. 38 and 751) were similar in appearance but not in size (Table 2). The black rhinoceros had larger ovaries than the white rhinoceros based on linear dimensions as well as calculated volume. The 3-year-old black rhinoceros had ovaries that were similar in size to those of the 21-year-old white rhinoceros. The ovaries of the 7-day-old black rhinoceros were significantly smaller than any of the other ovaries. Only the three older animals (Nos. 38, 45, and 751) had any visible follicles on their ovaries. The follicles were

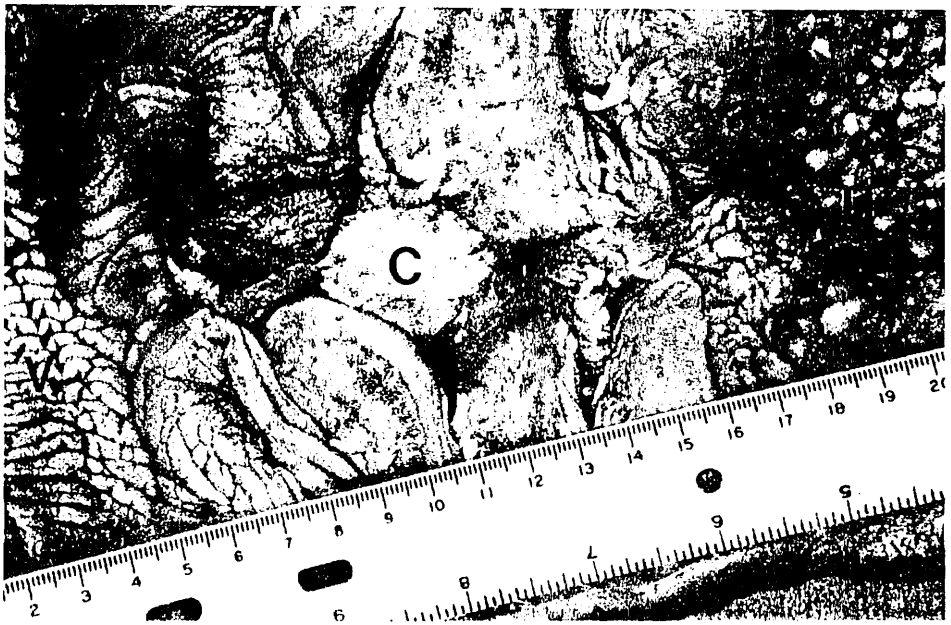


Fig. 2. Cervix (C) of a 27-year-old nulliparous white rhinoceros (No. 45). The cervix consists of several folds of rigid tissue. The cervix is oriented with the vagina (V) to the left and the uterus (U) to the right. The photo was taken after the tract was fixed in 10% buffered formalin.

not measured on the ovaries of one white rhinoceros (No. 45), but there were five antral follicles found, three of which were visible on the ovarian surface. Five oocytes were harvested from the ovaries, and the results of *in vitro* culture procedures are reported elsewhere [Godfrey et al., 1990]. The ovaries from females 38 and 751 were dissected, and 15 and 14 oocytes were recovered, respectively. The oocytes did not appear to be mature, and *in vitro* culture procedures were not attempted. There were no follicles visible on the ovaries from the 7-day-old and 3-year-old females, and no oocytes were recovered. No corpora albicantia or corpora hemorrhagica were found on any of the ovaries examined.

DISCUSSION

The uterine anatomy of the rhinoceros appears to match best the description of the long bicornuate as given by Mossman [1989]. The relatively long uterine horns, in relation to the entire length of the tract, and the small uterine body are similar to the general appearance of canine and porcine uteri. The fact that the horns are long will be important when developing ET procedures. The volume of media needed to flush the uterine horns will have to be large to flush the entire horn adequately. It is not known from the present data if the uterus undergoes changes in tone or size in multiparous rhinoceros. It did appear that the uterine horns may become thicker, as indicated by the diameters of the uterine horns of the primiparous female, with an increase in parity. This is not known with certainty, because it was not possible to ascertain which horn carried the pregnancy in this animal. The position of the tract

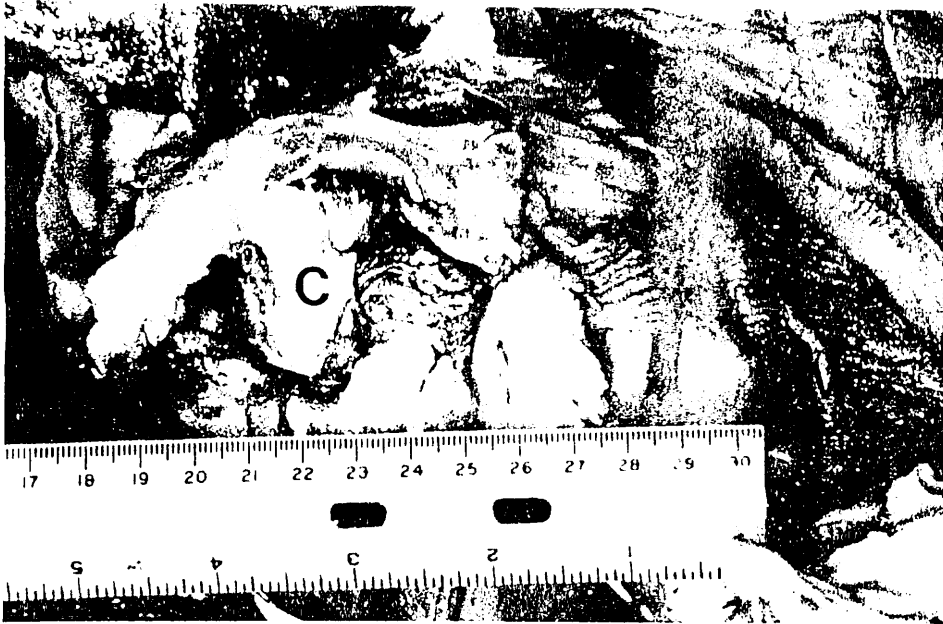


Fig. 3. Cervix (C) of a 28-year-old nulliparous black rhinoceros (No. 38). The cervix is oriented with the vagina (V) to the right and the uterus (U) to the left. The photo was taken after the tract had been fixed in 10% buffered formalin.

within the pelvic cavity may also vary with parity and pregnancy. The gravid uterus of a mature black rhinoceros during late gestation is pulled over the rim of the pelvis and drops into the abdominal cavity (Pope, personal communication, 1989). The length and flexibility of the vagina may also add to the difficulties in rectally palpating the uterus of a gravid or multiparous female.

The significance of the hymenal membrane in rhinoceros tracts is unclear. In the younger animals, the membrane appeared intact; in the older animals, the hymen was incomplete. A similar structure has been reported in the urogenital canal of African and Asian elephants (*Loxodonta africana* and *Elephas maximus*) by Balke et al. [1988a,b]. In the elephant, the presence of a hymen could be used as an indicator of parity, since the membrane was intact until the female gave birth to her first calf. It is possible that this can also be used as a measure of parity in rhinoceros. Since the hymen was not ruptured by intercourse, a pregnant elephant with an intact hymen was classified as primigravid [Balke et al., 1988b]. In the present set of data, the two older females had not produced a calf while in captivity, and the presence of the hymen would seem to indicate that they had not produced a calf in the wild either. If the presence of a hymen can be determined with a rectally guided ultrasound probe or vaginal palpation, some information regarding an individual animal's reproductive history can be obtained. The use of ultrasound may also be a potential tool to determine if there is any uterine hyperplasia and possibly the degree of hyperplasia, since the vesicles in the uterus were filled with fluid, and this fluid may appear echogenic on an ultrasound image.

A structure that may be difficult to manipulate during AI and ET procedures is

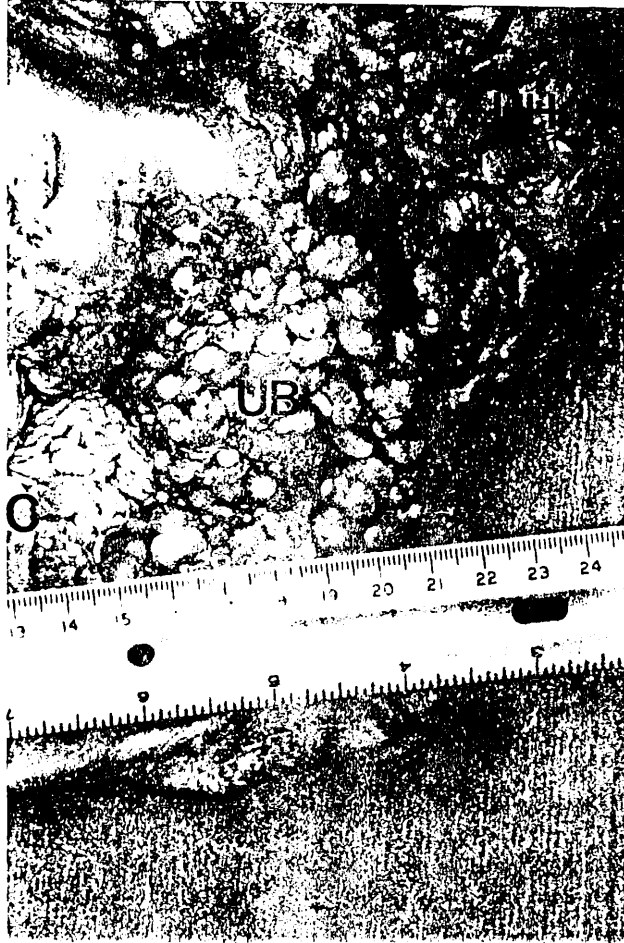


Fig. 4. The uterus of a 27-year-old nulliparous white rhinoceros (No. 45) exhibiting endometrial hyperplasia. The fluid filled vesicles were evenly distributed throughout the entire uterus. C, cervix; UB, uterine body, UH, uterine horn.

the cervix. The cervix of the rhinoceros has eccentric rings similar to that of domestic bovine and ovine species [Sorenson, 1979]. The ability to pass a catheter through the lumen of the cervix may be difficult at times other than estrus, since the cervix of a female in estrus is less rigid and the lumen is less constricted. This may explain why the cervix of the older white rhinoceros appeared less tortuous. This animal had been given exogenous hormones to stimulate ovulation and may have been close to the time of estrus when the tract was obtained at necropsy [Godfrey et al., 1990]. The eccentric rings of tissue may also present a barrier since a catheter can be lodged in one of the crypts, preventing further penetration into the uterus. The physical size of the cervix may also be a difficulty. To insert a catheter through the cervix, it will be necessary to move the cervix with one hand in the rectum. If the technician is unable to grasp the cervix firmly and manipulate it, the passage of the catheter through the cervix may be impaired.



Fig. 5. The right uterine horn (UH), ovary (O), and fimbria (F) of a 27-year-old nulliparous white rhinoceros (No. 45). The fimbria appeared to be engorged with blood at the time of necropsy. Bar = 1 cm.

TABLE 2. Ovarian measurements from black and white rhinoceros taken at necropsy

	Black rhinoceros			White rhinoceros	
	2058	367	38	751	45
Studbook No.	2058	367	38	751	45
Age	7 days	3 years	28 years	21 years	27 years
Ovarian dimensions					
Length (cm)	3.0	6.2	8.5	6.0	NC ^c
Width (cm)	1.3	3.7	4.7	3.8	NC
Depth (cm)	.5	1.2	2.8	1.3	NC
Volume (cc) ^a	1.9	27.5	111.9	29.6	—
No. of follicles ^b	0	0	2	3	5
Diameter of					
Largest follicle (mm)	—	—	15	14	NC

^aCalculated as width × length × depth.

^bIncludes follicles visible on the surface of the ovary and in the interstitium after dissection.

^cData not collected.

An unexpected observation was the presence of endometrial hyperplasia in the two older rhinoceros (Nos. 45 and 38). The white rhinoceros (No. 45) had a more pronounced degree of hyperplasia than the black rhinoceros (No. 38). This may have been due to the age of the animals, the stages of the estrous cycle, or the hormonal treatment imposed on the white rhinoceros [Godfrey et al., 1990]. The hyperplasia was present in the entire uterus and was uniformly distributed. There was no indication of hyperplasia in the younger animals (7 days and 3 years old). Diehl et al.

[1986] reported a similar condition in an inbred strain of miniature swine. They reported that the hyperplasia was age dependent as opposed to being influenced by exogenous progestin or gonadotropin administration. Ramsay et al. [1989] found endometrial hyperplasia in a 46-year-old Asian elephant (*Elephas maximus*). The animal had been cycling normally for the previous 2 years prior and had ovulated as recently as 2 months prior to death. There was no speculation on the cause of the hyperplasia or its relationship to endocrine profiles. Based on the data being presented, it appears that endometrial hyperplasia in the rhinoceros is age related. It may not be proper to exclude totally hormonal influences on this condition, since the degree of hyperplasia was greater in the white rhinoceros that was being administered exogenous hormones (progestins and gonadotropins). The presence of endometrial hyperplasia may be related to the fertility of the animals involved. Abnormal growth of the endometrium may interfere with proper implantation and embryonic development in the uterus. Prior to this stage, endometrial hyperplasia may act as a physical barrier to conception. By occluding the uterine lumen, the hyperplastic tissue may prevent spermatozoa from reaching the upper sections of the tract (oviducts) and fertilizing oocytes. There may also be a negative effect on the ability to flush the uterus with media for ET due to occlusion of the uterine lumen.

The ovaries of both species of rhinoceros studied appeared similar. Follicles were observed on the surface of the ovaries in each species. Unlike horses, which are also members of the order Perissodactyla, the rhinoceros appeared to ovulate on the surface of the ovary at several sites. It has been reported that in domestic horses the preovulatory follicle migrates to a specific area of the ovary, called the *ovulation fossa*, prior to ovulation [Sorenson, 1979]. There also appeared to be only a single ovulation at each estrus, since only one large follicle was found on the ovaries of the 28-year-old black rhinoceros. It does appear possible to stimulate follicular growth with exogenous gonadotropins in the white rhinoceros [Godfrey et al., 1990], although ovulation was not achieved.

CONCLUSIONS

The reproductive tract of an adult female rhinoceros presents several obstacles to assisted reproductive technologies in these species. In nulliparous animals the hymen may prevent technicians from inserting a catheter very far into the vaginal canal. This will vary with the degree of patency of the hymen in an animal. The presence or absence of a hymen can possibly be used to determine if wild-caught animals have had a calf previously. The tortuous cervical lumen will make it difficult to pass a catheter through the cervix for AI or nonsurgical ET procedures. The size of the entire tract will make it difficult to manipulate the tract per rectum, and the length will make rectal palpation of the ovaries difficult.

ACKNOWLEDGMENTS

We thank the curatorial and veterinary staff at Busch Gardens, Caldwell Zoo, Kings Dominion Wild Animal Park, Miami Metro Zoo, and St. Louis Zoo for providing the reproductive tracts for this project. Thanks are also extended to Dr. Sylvie Gosselin of Merrell Dow Research Institute for histological evaluation of tissue samples, Dr. Nan Schaffer of the Lincoln Park Zoo for assisting in the recovery

of a reproductive tract, and Vickie Stidham for manuscript preparation. This project was supported in part by a grant from the Institute of Museum Services (IC-70171-87).

REFERENCES

- Balke, J.M.E.; Barker, I.K.; Hackenberger, M.K.; McManamon, R.; Boever, W.J. Reproductive anatomy of three nulliparous female Asian elephants: The development of artificial breeding techniques. *ZOO BIOLOGY* 7:99-113, 1988a.
- Balke, J.M.E.; Boever, W.J.; Ellersieck, M.R.; Seal, U.S.; Smith, D.A. Anatomy of the reproductive tract of the female African elephant (*Loxodonta africana*) with reference to development of techniques for artificial breeding. *JOURNAL OF REPRODUCTION AND FERTILITY* 84: 485-492, 1988b.
- Diehl, J.R.; Stuart, L.D.; Goodrowe, K.L.; Wildt, D.E. Effects of altrenogest and exogenous gonadotropins on ovarian function and embryo recovery in swine leukocyte antigen inbred miniature swine as influenced by cystic endometrial hyperplasia. *BIOLOGY OF REPRODUCTION* 35: 1261-1268, 1986.
- Godfrey, R.W.; Pope, C.E.; Dresser, B.L.; Bavis-ter, B.D.; Andrews, J.C.; Olsen, J.H. An attempt to superovulate a southern white rhinoceros (*Ceratotherium simum simum*). *THERIOGENOLOGY* 33:231, 1990.
- Mossman, H.W. Comparative anatomy. Pp. 19-34 in *BIOLOGY OF THE UTERUS*. R.M. Wynn and W.P. Jollie, ed., New York, Plenum Medical Book Company, 1989.
- Ramsay, E.C.; Leach, M.W.; Czekala, N. Post-mortem reproductive findings in a female Asian elephant. P. 55 in *PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF ZOO VETERINARIANS*, Greensboro, N.C., AAZV, 1989.
- Sorenson, A.M., Jr. Macroscopic female functional anatomy. Pp. 180-202 in *ANIMAL REPRODUCTION PRINCIPLES AND PRACTICES*. New York, McGraw-Hill Book Company, 1979.