

# CLINICAL IMPLICATIONS OF RHINOCEROS REPRODUCTIVE TRACT ANATOMY AND HISTOLOGY

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**Abstract:** Reproductive tracts or tissues from five male black rhinoceroses (*Diceros bicornis*), two male white rhinoceroses (*Ceratotherium simum*), two male one-horned Asian rhinoceroses (*Rhinoceros unicornis*), seven female black rhinoceroses, and six female white rhinoceroses from multiple institutions were examined to characterize their anatomy and histology. Some observations and measurements were obtained from in situ tracts of intact animals before or during necropsy. Formalin-fixed tissues were dissected and examined histologically. Retrospective reproductive data from each rhinoceros was obtained from the institutions of origin. Reproductive histology of these species was similar to that of other mammals. Male accessory gland structure varied among species, and the Asian rhinoceros epididymis was more loosely attached and had larger duct diameters than did the epididymides of the African species. Although histology was typically mammalian, rhinoceros reproductive morphology combined characteristics of several different mammals. Defining this unique morphology of rhinoceroses may help in understanding their reproductive physiology and will effect the development of appropriate reproductive techniques.

**Key words:** *Diceros bicornis*, *Ceratotherium simum*, *Rhinoceros unicornis*, reproduction, anatomy, histology.

## INTRODUCTION

All members of the family Rhinocerotidae are endangered. This situation has compelled managers of captive populations to attempt to induce genetically important, nonproductive individuals to reproduce,<sup>4</sup> yet rhinoceros reproductive dysfunction is poorly understood. Currently there is limited information on female rhinoceros reproductive physiology and endocrinology,<sup>5,7,10,12,13,19</sup> and even less documentation is available on these aspects in the male rhinoceros.<sup>3,11,16,20</sup> Resolution of reproductive difficulty has been further hampered by lack of information concerning surgical access to and proper orientation of the reproductive organs. Rhinoceros size and morphology have also impaired ultrasonographic and electroejaculation techniques and basic culture and biopsy techniques.<sup>1,22</sup> There are only four reports devoted to gross rhinoceros anatomy,<sup>8,20,22,24</sup> and only one of these covers histology.<sup>24</sup> Comparison of rhinoceros and domestic animal morphology can assist with development of reproductive techniques. We studied the reproductive morphology of male and female white rhinoceroses (*Ceratotherium simum*), male and female black rhi-

noceroses (*Diceros bicornis*), and male greater one-horned Asian rhinoceroses (*Rhinoceros unicornis*).

## MATERIALS AND METHODS

### Reproductive tracts

Reproductive tracts or tissues from five male black rhinoceroses, two male white rhinoceroses, two male one-horned Asian rhinoceroses, seven female black rhinoceroses, and six female white rhinoceroses were examined to characterize their anatomy and histology. The reproductive tracts of two male greater one-horned Asian rhinoceroses, two male and three female white rhinoceroses, and two male and three female black rhinoceroses were examined and removed at necropsy to observe tract orientation. The accessibility of the tract via the vagina, per rectum, and through the abdominal wall was digitally examined and measured in situ and in unfixed specimens of the female rhinoceroses. Observations of the morphology of the external genitalia of the male were made on living rhinoceroses before euthanasia. The other three male black rhinoceroses, four female black rhinoceroses, and three female white rhinoceroses were provided as intact or formalin-fixed partial tracts by veterinarians or pathologists of other institutions. Formalin-fixed tracts were measured before dissection and sample collection for histopathology. Measurements of one female black rhinoceros (SB 125) and one female white rhinoceros (SB 45) were not included because sections of only major organs were provided. One black male rhinoceros did not have testicles (SB 413), and only testicles were provided for two other black male rhinoceroses (SB 66 and SB 471).

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**Table 1.** Male rhinoceros reproductive history summaries.

Species	Studbook no.	Age at death (yr)	Proven breeder	No. offspring	Interval since last offspring <sup>a</sup>
Black	SB 413	mature	no	unknown	ND
	SB 56	33	yes	6	3 yr
	SB 66	27	no	unknown	
	SB 271	15	yes	5	1 yr
	SB 471	10	no	unknown	ND
White	SB 40	30+	yes	7	>1 yr
	SB 23	31	yes	0	ND
Asian	SB 14	30	yes	1	14 yr
	SB 19	32	yes	1	~7 yr

<sup>a</sup> ND = not done.

### Histology

To assess histologic features, representative reproductive tract samples were submitted for routine embedment and processing. Paraffin-embedded tissues were sectioned at 3–4  $\mu\text{m}$  and stained with hematoxylin and eosin. Based on clinical impressions of epididymal differences among rhinoceros species, the luminal diameter of five ducts per epididymis (caput and cauda) were measured with a calibrated reticle on a microscope. Epididymal duct diameters were measured from four black rhinoceroses, two white rhinoceroses, and two Asian rhinoceroses.

### Retrospective surveys

Questionnaires on the animals in the study were sent to the respective zoologic parks of origin requesting data on studbook number, age, cause of

death, and reproductive history of all subject rhinoceroses. Data requested for male tracts included fertility history, particularly the number of live offspring sired. Reproductive information requested on females included conception and parity history. Additional data were obtained from studbooks. Data were collected on nine males and 13 females (Tables 1, 2). The males examined were 15–33 yr old, and females were 4–32 yr old.

### Anatomical drawings

Oriental drawings were made from fresh in situ reproductive tracts, and structural drawings were made from formalin-fixed tissues.

## RESULTS

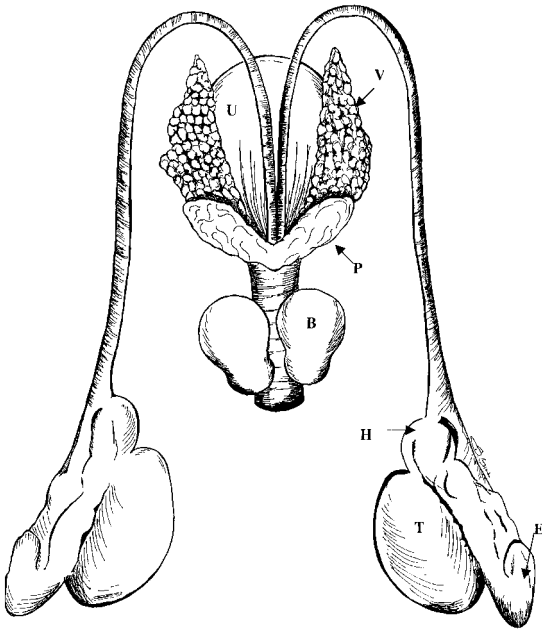
### Male gross and microscopic anatomy

Line drawings of black rhinoceros and Asian rhinoceros male reproductive tracts represent gross appearance and orientation (Figs. 1, 2, 8). Figure 8 represents the orientation of the reproductive tract of all three species. In this figure the sizes of the glands and testicle are proportional to each other based on formalin-fixed measurements of black rhinoceros organs (Table 3). The penis is represented in the relaxed in situ position (not proportional). Lengths and widths were measured for paired glands and for each lobe of the prostate. Circumferences of the penis and testes were measured at the widest point. Observations of the morphology of the erect penis and scrotum were made on one male of each species on living rhinoceroses before euthanasia.

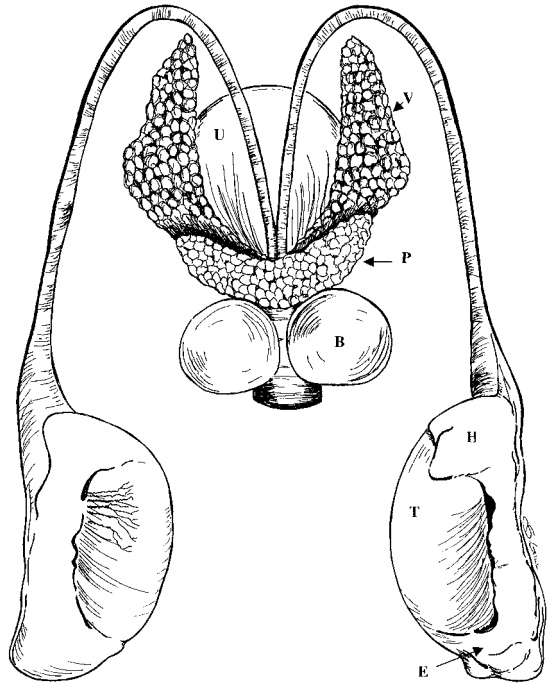
Testes were located in the dorsal aspect of the preputial fold. Although the horizontally positioned testes in these species were not visually apparent on the live animal, they could be located in situ in the whole animal by palpating the skin in a hori-

**Table 2.** Female rhinoceros reproductive history summaries.

Species	Studbook no.	Age at death (yr)	No. pregnancies	No. births
Black	SB 187	14	0	
	SB 125	31	4	3
	SB 412	4	unknown	
	SB 235	25	5	5
	SB 180	23	5	5
	SB 163	24	7	7
	SB 328	14	2	2
White	SB 178	23	0	
	SB 24	32	0	
	SB 32	27	0	
	SB 579	21	0	
	SB 45	27	0	
	SB 751	19	1	1



**Figure 1.** Line drawing of a male black rhinoceros reproductive tract, illustrating the relationship and morphology of the gonads and accessory sex glands. T = testis; H = head of epididymis; E = tail of epididymis; V = vesicular gland; P = prostate; B = bulbourethral gland; U = urinary bladder.



**Figure 2.** Line drawing of a male Asian rhinoceros reproductive tract, illustrating the relationship and morphology of the gonads and accessory sex glands. T = testis; H = head of epididymis; E = tail of epididymis; V = vesicular gland; P = prostate; B = bulbourethral gland; U = urinary bladder.

zontal line along the dorsal preputial fold from the inguinal area to the caudal border of the prepuce. Palpation was difficult because the hind leg was in the way. The testicles could be adjacent to the inguinal rings, or if more caudal, their distal aspect protruded between the rear legs. The testes were more obvious in Asian rhinoceroses than in African rhinoceroses. Testicular length and circumference

were similar in all species. Because of the small number of individuals examined, testicular dimensions were not analyzed statistically.

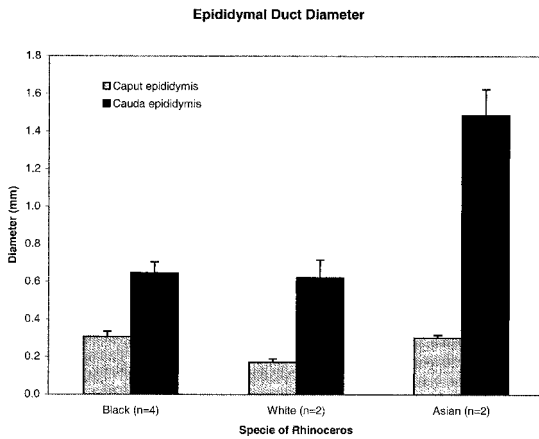
The testes had a thick connective tissue capsule. The parenchyma was partitioned by thick connective tissue septa that divided the seminiferous tu-

**Table 3.** Dimensions (cm) of male rhinoceros formalin-fixed reproductive tracts.

Species	Studbook no.	Testis		Vesicular gland		Prostate		Bulbourethral gland		Penis	
		Length	Circumference <sup>a</sup>	Length	Width	Length of lobe	Width of lobe	Length	Width	Length	Circumference <sup>a</sup>
Black	SB 413	ND <sup>b</sup>	ND	16	5	17	ND	6	5	28	21
	SB 56	11	15.5	15	4	14	ND	7	6	24	19
	SB 66	13	15	ND	ND	ND	ND	ND	ND	19	22
	SB 271	8.5	17	13	4	14	3	6	5.5	23	19
	SB 471	17.1	25.5	ND	ND	ND	ND	ND	ND	ND	ND
White	SB 40	14	17	14	4	10	ND	11	6	38	23
	SB 23	10	15.5	17	4	14	4	9	7	ND	ND
Asian	SB 14	15	20.4	16.5	6.5	ND	ND	11.5	7	33	22
	SB 19	19	33.0	19	7	16	ND	11	9	32	20

<sup>a</sup> At widest point.

<sup>b</sup> ND = not done.



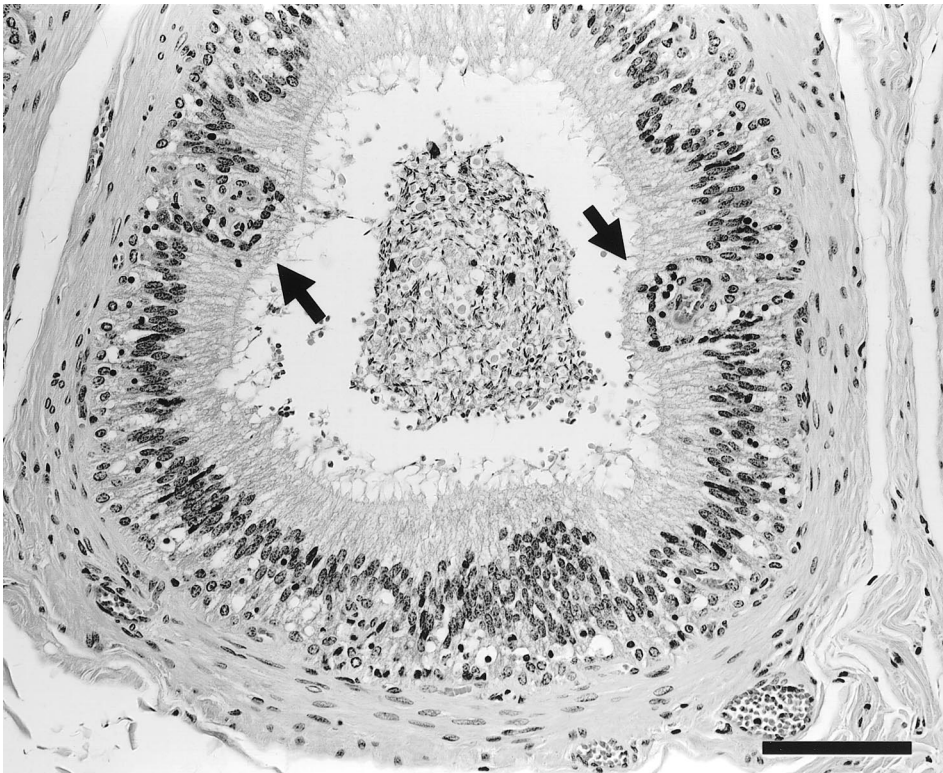
**Figure 3.** Mean ( $\pm$ SEM) epididymal luminal diameters from the caput (head) and cauda (tail) epididymis, demonstrating smaller diameter ducts in African species of rhinoceros.

bules into lobules. Testes contained a moderate amount of interstitial connective tissue, with small aggregates of interstitial cells (Leydig cells) interspersed around seminiferous tubules. The seminif-

erous tubules with active spermatogenesis were 180–200  $\mu$ m in diameter in all three species.

Epididymal attachment to the testes varied among the different rhinoceros species. The epididymides in Asian rhinoceroses were tightly adhered to the testes (Fig. 2); those of the white rhinoceroses were loosely attached, and those of the black rhinoceroses were even more loosely attached (Fig. 1). The epididymal body was dorsal to the testes and was covered by a thick connective tissue capsule. The Asian rhinoceroses epididymal duct diameters were larger than those of the African rhinoceroses (Fig. 3). The epididymal duct was lined by a columnar epithelium that ranged from tall columnar in the caput and corpus to low columnar in the cauda epididymis. One Asian rhinoceros (SB 19), two white rhinoceroses (SB 23, SB 40), and one black rhinoceros (SB 271) had papillary projections of vascular tufts within the epididymal epithelium (Fig. 4). The significance of these tufts is unknown; however they were observed in at least one male from each of the three species examined.

Because of incomplete tracts, the ductus deferens lengths were not measured. There was no ampulla



**Figure 4.** Epididymal ducts from the caput epididymis of an Asian rhinoceros, demonstrating the invagination of the epithelium with small vascular tuft formations (arrows). Bar = 100  $\mu$ m.



**Figure 5.** Vesicular gland of a black rhinoceros. Club-shaped projections extend into the lumen for a short distance. Bar = 100  $\mu$ m.

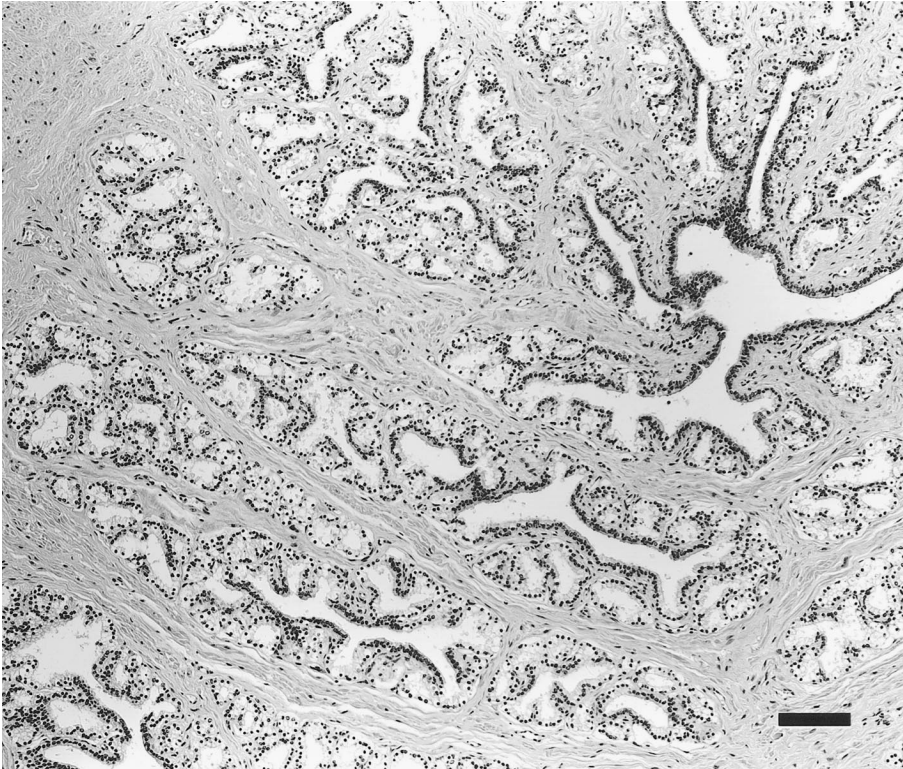
evident at the ductus deferens/urethral junction. Histologically, the ductus deferens consisted of an attenuated simple cuboidal epithelium with occasional glandlike extensions off the lumen and with a thick muscular coat.

The vesicular glands were paired, multilobed, and sacculated, extending cranially and dorsally. They were often partially covered dorsally by the prostate, and they were oblong and cigar-shaped if the sacculations were filled with vesicular secretions or soft collapsed sacs. Histologically, vesicular glands were multicystic with widely dilated lumina. They were divided by dense connective tissue bands, which formed the gross multilobed appearance. There was a central duct with highly infolded epithelium forming branching projections (Fig. 5). Columnar epithelial cells lined the glands.

The prostate in all three species had two triangular lobes joined by an isthmus across the urethra just caudal to the urinary bladder. The isthmus could be palpated per rectum in the intact animal. The prostate from Asian rhinoceroses had a more pronounced lobular surface pattern. In all three species, the pelvic urethra appeared to dilate as it extended caudally from the prostate. In one Asian rhinoceros (SB 19) and one white rhinoceros (SB 23),

a dilated section of pelvic urethra (5 cm long  $\times$  2 cm diameter and 8 cm long  $\times$  2 cm diameter, respectively) was observed when fluid was infused into the posterior urethra of the unfixed tract. The ducts of the vesicular and prostate glands and the ductus deferens emptied into the urethra from the colliculus seminalis. Prostatic parenchyma was divided into lobules by thick connective tissue septa, resulting in a multilobed gland (Fig. 6). Lobules contained dilated central ducts with alveolas-like extensions. The glands were lined by a tall columnar epithelium in the alveoli and a columnar to cuboidal epithelium in the centrally located ducts. Large ducts were lined with a pseudostratified columnar epithelium.

The prominent bulbourethral gland varied in size and morphology among species. The Asian rhinoceros and black rhinoceros bulbourethral glands were small and round compared with the white rhinoceros bulbourethral gland, which was more elongated and irregular. All of the bulbourethral glands were covered by a thick muscular layer. Histologically, they consisted of multiple ducts and channels lined by foamy cuboidal epithelium. Sloughed



**Figure 6.** Prostate of an Asian rhinoceros. In some prostate samples, concretions (corpora amylacea) located within the gland lumen were large enough to be grossly visible. Bar = 100  $\mu$ m.

cells were frequently observed within the lumina. (Fig. 7).

The tip of the penis was a double layer of flared annular skin folds, with the second layer creating a deep fornix. In the relaxed penis, the tip was curved caudally starting just distal to the lateral projections of the penis. This curvature of the penis results in caudal and sometimes horizontal urination and the partial curvature may be maintained until full erection. Two lateral projections lay between the tip and the middle of the penis. They were flaccid flaps on the relaxed penis, and their shape varied among species. In black rhinoceroses, the projections were connected to the penis in a dorsal to ventral slope from proximal to distal. When erect, these flaps protruded laterally as much as 20–25 cm in the living animal, becoming firm and slightly cupped. White and Asian rhinoceroses had longer, more streamlined and horizontal dorsolateral cups.

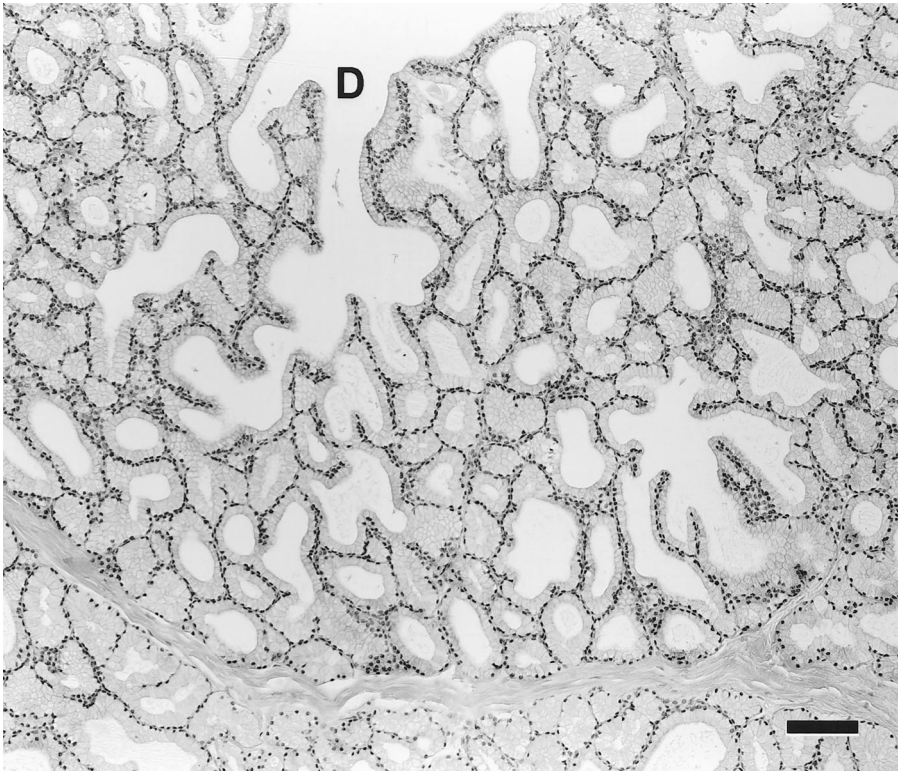
#### **Female gross and microscopic anatomy**

Female black and white rhinoceros reproductive tract morphology was similar. Line drawings of the African female rhinoceros illustrate the gross appearance and orientation (Figs. 9, 10). In Figure 9,

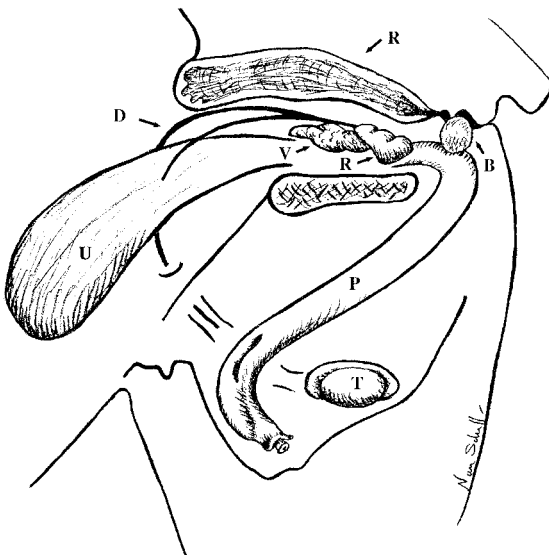
the various parts of the reproductive tract are proportionally represented based on measurements (Table 4) of unfixed tissues of the black rhinoceros. Figure 10 provides additional detail of internal structures (not proportional). Table 4 summarizes measurements made on fixed and unfixed organs. In situ length represents the straight line vulva–ovary distance before the tract was removed from the abdominal cavity. The mean ( $\pm$ SD) length was  $86.7 \pm 13$  cm for three black rhinoceroses and  $92.0 \pm 7$  cm for three white rhinoceroses.

In female white and black rhinoceroses examined at necropsy, the in situ reproductive tract coursed cranially towards the kidneys. The uterus and ovaries lay in the lumbar area and hung loosely from the uterine ligament, which attached to the dorsal abdominal wall lateral and caudal to the kidneys. The distance of the ovaries from the kidneys was  $14.8 \pm 4.3$  cm in three nulliparous female white rhinoceroses and one black rhinoceros as compared with 35 cm in a multiparous black female. The ovaries were suspended just cranial to the ends of the uterine horns and could be in close association with the colon.

Access to the reproductive tract was examined in

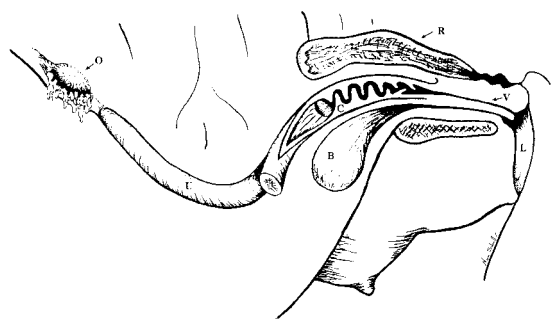


**Figure 7.** Bulbourethral gland of an Asian rhinoceros, consisting of tall foamy columnar cells lining alveolar spaces with a central duct (D). Bar = 100  $\mu$ m.



**Figure 8.** Abdominal orientation of the male rhinoceros reproductive tract. P = penis; T = testis; D = ductus deferens; B = bulbourethral gland; A = prostate; V = vesicular gland; U = urinary bladder; R = rectum.

five laterally recumbent females at necropsy through an incision between the last rib and the point of the hip. The distance between the tuber coxa and last rib was  $7 \pm 1.5$  cm for two white rhinoceroses and  $15 \pm 3$  cm for two black rhinoceroses. Visualization of the ovaries was inhibited by their cranial position and by the position of the intestines. The ovaries were located by reaching



**Figure 9.** Abdominal orientation of the African female rhinoceros reproductive tract. O = ovary; U = uterine horn; C = cervix; V = vagina; L = vulva; B = urinary bladder; R = rectum.



**Figure 10.** Line drawing of the African female rhinoceros reproductive tract, illustrating the relationship and morphology of the ovaries and tubular tract. O = ovary; U = uterine horn; C = cervix; V = vagina; L = vulva.

caudally toward the cervix to palpate the uterine horn to a point cranial to the incision (25–38 cm from the point of the hip). In all animals, the uterine horn, but not the ovaries, could be exteriorized. In the two multiparous black rhinoceroses, the ovary could be retracted sufficiently for visualization just cranial to the incision.

Ovaries were flat and ovoid when inactive and round when they contained developing follicles or corpora lutea and/or cysts. Ovarian measurements varied with the presence of these structures; however, the ovaries examined were primarily inactive. The ovaries of one mature black and a white rhinoceros were within ranges previously published for six white rhinoceroses (volume =  $67.3 \pm 59 \text{ cm}^3$ ) and 10 black rhinoceroses ( $96.5 \pm 59 \text{ cm}^3$ ).<sup>8</sup> No large preovulatory follicles were found. In one white rhinoceros (SB 32), a yellow corpus luteum ( $2 \times 3.2 \times 1.6 \text{ cm}$ ) protruded from the surface of the ovary, and a thick-walled cystic, tan corpus luteum ( $1.3 \times 1.9 \text{ cm}$  with 0.4-cm central cyst) was present in another (SB 579). A hemorrhagic corpus luteum ( $3.0 \times 3.5 \text{ cm}$ ) and two or three follicles >1 cm were present in one black rhinoceros (SB 412).

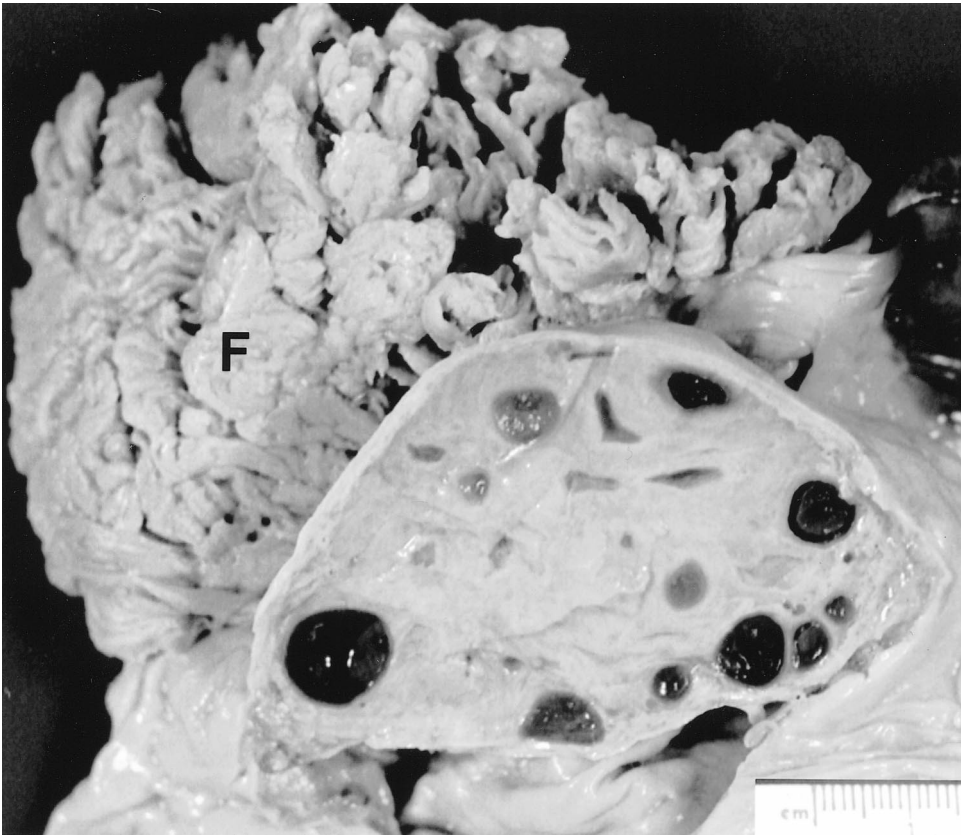
**Table 4.** Dimensions (cm) of female rhinoceros fixed and unfixed reproductive tracts.

Species	Studbook no.	Tissues <sup>a</sup>	Vulva–cervix distance	Cervix length	Length of uterine body (internal/external)	Length of uterine horns (right/left)	Ovaries		Total length (in situ/total)
							Right	Left	
Black	SB 187	fixed	ND <sup>b</sup>	13	2.5/7.0	40.0/44.0	8.0 × 4.0 × 2.0	7.0 × 4.3 × 2.0	ND
	SB 180	unfixed	40.0	12.0	2.0/10.0	53.0/49.0	12.0 × 5.0 × 3.3	10.0 × 4.0 × 2.3	75.0/113.0
	SB 412	unfixed	55.0	15.2	4.5/15.5	60.0/60.0	7.5 × 7.5 × 2.5	7.5 × 7.5 × 3.2	100/130
	SB 235	unfixed	45.0	12.0	4.0/16.0	80.0/72.0	12.0 × 5.0 × 1.2	10.0 × 4.0 × 2.2	85.0/137
	SB 163	fixed	ND	7.0	4.0/9.0	27.0/28.0	8.0 × 4.0 × 2.0	8.1 × 4.0 × 2.5	ND
	SB 328	fixed	ND	10.0	3.0/9.0	25.0/21.0	4.5 × 3.0 × 2.0	5.0 × 3.5 × 2.5	ND
White	SB 178	fixed	32	23.0	ND/13.0	48.5	ND	ND	116.5
	SB 24	unfixed	53.4	23.0	2.0/7.5	63.5/48.2	8.2 × 9.0 × 5.1	5.7 × 3.8 × 2.0	100.0/135.0
	SB 32	fixed	ND	19.0	4.0/12.5	43.5	5.5 × 7.0 × 3.0	6.0 × 3.0 × 2.0	ND
	SB 579	unfixed	46.0	13.0	7.0/16	42.0/46.0	8.5 × 4.0 × 4.0	8.0 × 6.0 × 2.5	86.0/104.0

<sup>a</sup> Measurements of unfixed tissues were collected at necropsy. All other measurements were derived from formalin-fixed tissues.

<sup>b</sup> ND = not done.





**Figure 11.** Cut section of ovary and fimbria (F) from a black rhinoceros. Small follicles are scattered in the ovarian cortex.

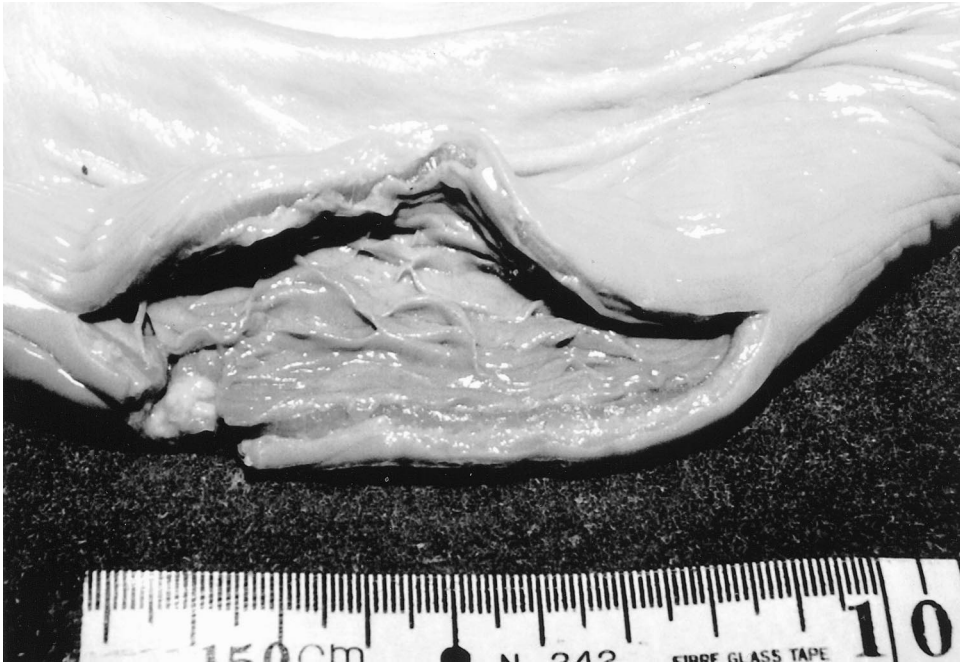
Structures resembling corpora fibrosa of cattle were present in three ovaries.

The ovaries lay within the ovarian bursa in a serosal invagination of the medial surface of the uterine ligament. The hilus attached to the lateral side of the ovary, and the opening of the bursa extended the length of the medial side of the ovary. The position of the ovary within the bursa varied with ovarian activity. The fimbriae were prominent, extending around the infundibulum and protruding out the bursa. They could be seen from the lateral side (Fig. 11). The infundibulum made up the medial border of the bursa, and the ovary formed the lateral border. The uterine tube was within the bursa on the lateral and ventral surface. Small (0.2–1 cm diameter) fimbrial cysts of the uterine tubes were observed in one white rhinoceros (SB 178) and one black rhinoceros (SB 125).

Segments of uterine tubes were available from two white rhinoceroses (SB 178, SB 579) and two black rhinoceroses (SB 125, SB 412). Histologically, the uterine tubes were lined by a tall columnar

epithelium that was highly infolded. There was a variable amount of layered muscle around the mucosa.

The uterus of both species examined consisted of a short body and long tubular horns. The horns were uniformly  $2 \pm 0.4$  cm in diameter in the black rhinoceroses and  $4 \pm 1.0$  cm in the white rhinoceroses. The fusion of the muscular layers of the uterine horns adjacent to the uterine body gave the external impression of a longer body. The external unfixed length of the uterine body was on average longer in three white rhinoceroses ( $16.2 \pm 9$  cm) than in three black rhinoceroses ( $13.8 \pm 3$  cm), but internal lengths were 3.5 cm in all individuals of both species. The average of six unfixed uterine horn lengths of black rhinoceroses was not different than the average of six unfixed lengths from white rhinoceroses ( $62.3 \pm 12$  cm and  $53.3 \pm 9$  cm, respectively), but fixed uterine horn lengths were shorter than unfixed lengths of both species. There was a prominent dorsal intercornual ligament at the uterine bifurcation that could not be palpated per



**Figure 12.** Cut section of uterine horn of a white rhinoceros demonstrating the longitudinal folds of the endometrium.

rectum in these individuals. The uterine horns hung in a loose convex curve in the mesometrium and were retractable; however, the heavy tract and thick rectal wall made retraction difficult. The endometrium in eight normal reproductively quiescent white rhinoceroses and black rhinoceroses consisted of thin longitudinal folds that projected 0.5–2 cm into the lumen (Fig. 12). The endometrium was lined by tall columnar epithelium with marked infolding, and abundant endometrial glands were uniformly distributed over the uterine mucosa (Fig. 13). Three animals (SB 32, SB 412, SB 579) had luteal tissue in ovarian sections, and their endometrial surface epithelium was lined by a foamy tall columnar epithelium, similar to endometrial epithelium seen in other species under progesterone influence. The myometrium was always prominent.

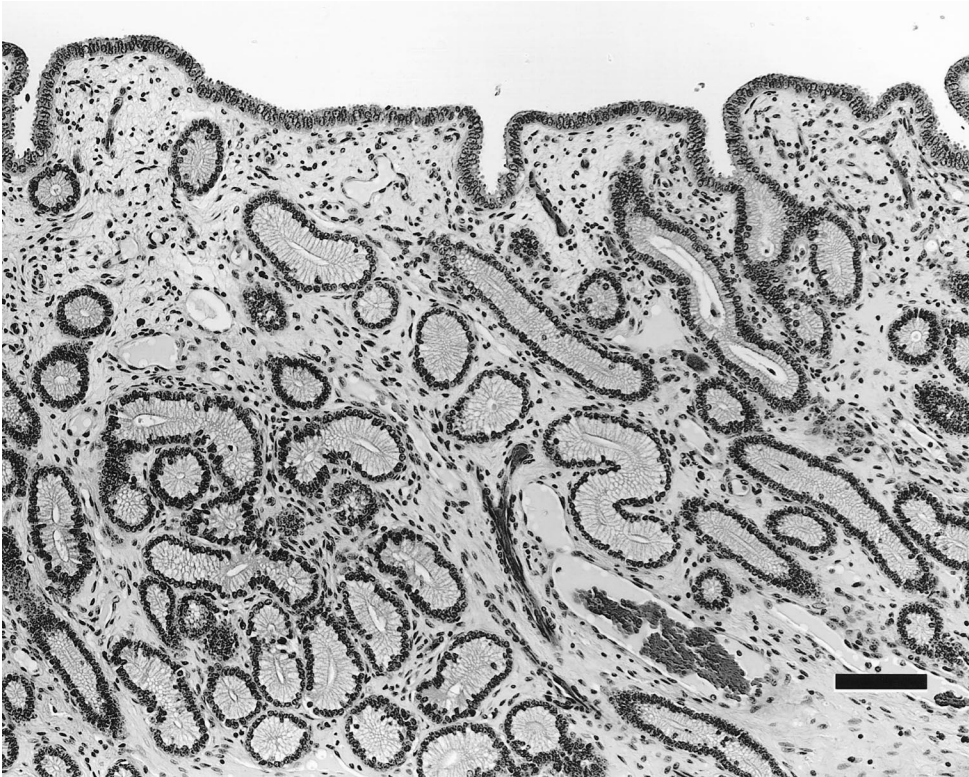
The rhinoceros cervix had a complicated system of thick, fibrous, circular and semicircular folds. The folds projected caudally and tended to be annular at either end of the cervix. Because of vaginal folds and irregularities of the cervical folds, the external os was often poorly defined. In one white rhinoceros (SB 751), a cervical fold extended from the external os and become bulbous (Fig. 14). At the midcervix, the folds interdigitated, effectively closing the canal. In only one black rhinoceros (SB 412) was the lumen patent. In other animals, the

lumen alternated between the crests of these folds, often at 180° angles. The cervical lumen was round, ovoid, or reniform, and the surface often had longitudinal rugae. The cervix was located cranial to the brim of the pelvis. The unfixed cervix was longer in white rhinoceroses than in black rhinoceroses ( $18.6 \pm 5$  cm and  $11.5 \pm 3$  cm, respectively), contradicting a previous report.<sup>8</sup>

The longitudinal wrinkling of the caudal vagina became transverse in the cranial vagina, creating folds around and sometimes obliterating the external os. In the anterior vagina, the numbers of folds varied widely. Unlike the thick fibrous cervical folds, the vaginal folds tended to be thin, with sharp crests. Visually, it was difficult to distinguish where vaginal folds ended and cervical folds began.

Of the four vaginal samples examined histologically, all were lined by a thick keratinized squamous epithelium with a thick underlying layer of muscle (Fig. 15). Inflammatory cells were common in the lamina propria and consisted of scattered neutrophils and macrophages. Samples from the caudal aspect of the vagina had extensive rete peg formation and lymphocytic infiltrates.

The vulva lips were larger at the ventral border, and the clitoris was located in a fossa at the ventral commissure. The hymen, located at the opening to the vaginal vault, was just dorsocranial to the ridge



**Figure 13.** Uterine mucosa of a white rhinoceros, with the regular arrangement of coiled glands lined by a tall columnar epithelium. Bar = 100  $\mu$ m.

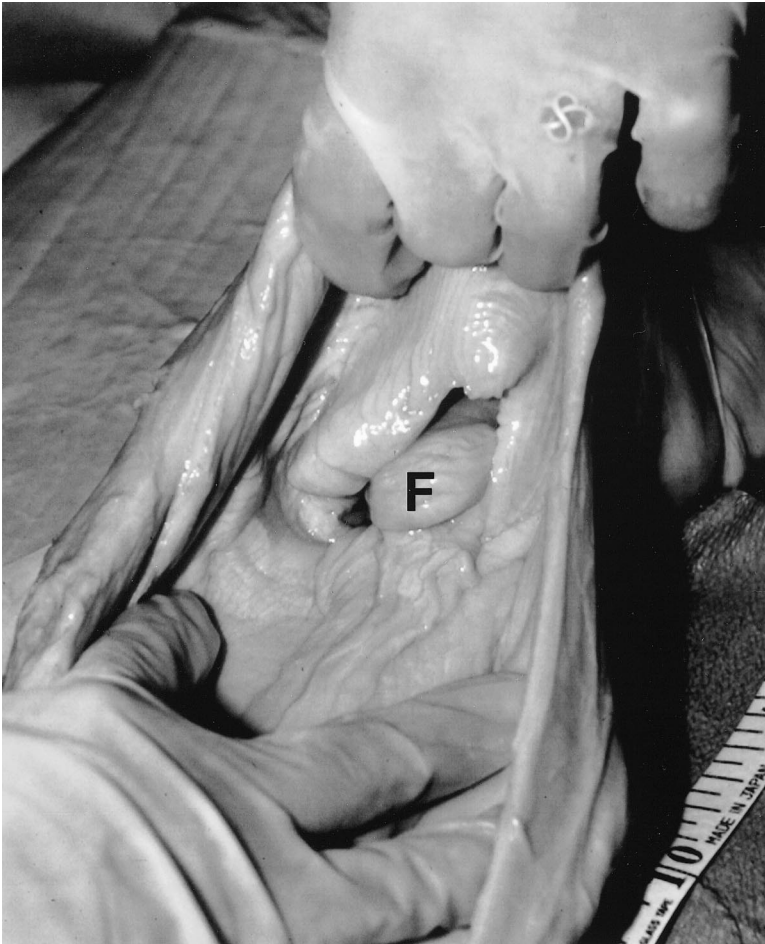
of the urethral diverticulum and blocked entry into the vagina. The hymen in three nonbred animals consisted of a transverse mucosal covering with one or two small 0.5–3-cm diameter holes separated by a midline vertical septum. The tissue of the hymen was reinforced by this septum, which extended cranially into the vagina, creating two channels in the caudal vagina. The hymen persisted as flaps of skin in five bred animals. One white rhinoceros (SB 24) had an intact hymen stretched on one side. The external urethral orifice was at the cranial extent of the diverticulum, 15–25 cm from the external surface of the vulva. There was no sub-urethral diverticulum.

### Survey results

Survey and studbook data were collected for nine males (Table 1), five of which had successfully produced live young. The white and Asian rhinoceroses died acutely, and the black rhinoceroses had prolonged periods of illness. One white rhinoceros male (SB 23) was breeding with a female but failed to produce offspring. Reproductive soundness of the pair was not assessed. Two others (SB 413 and

SB 471) died shortly after arrival from the wild. Of the successful breeders, two had produced one offspring, and three had produced multiple offspring (five, six, or seven individuals). The interval since siring their last offspring and time of death ranged from 14 yr (SB 14) to shortly before death (SB 40).

For the 13 females, reproductive information was obtained from surveys for seven and from studbooks for six others. Black rhinoceroses had more incidences of successful pregnancy ( $n = 5$ ) than did white rhinoceroses ( $n = 1$ ); however, all of the rhinoceroses but two were quiescent at time of necropsy. Five of six mature black rhinoceros females had produced multiple offspring, but none had given birth within the previous year, and no reports were available on estrus. Only one (SB 412), which died acutely, showed evidence of cycling. One white rhinoceros female (SB 178) bred to a fertile male did not become pregnant. Another (SB 751) carried a single fetus to term. According to records, all of the white rhinoceros females were housed with fertile males, but none had demonstrated breeding within the previous 5 yr, and none had recent histories of estrus. Although all of them died



**Figure 14.** External cervical os of a white rhinoceros with a cervical fold (F) protruding.

acutely, only one showed evidence of cycling. None of the males or females died from reproductive complications.

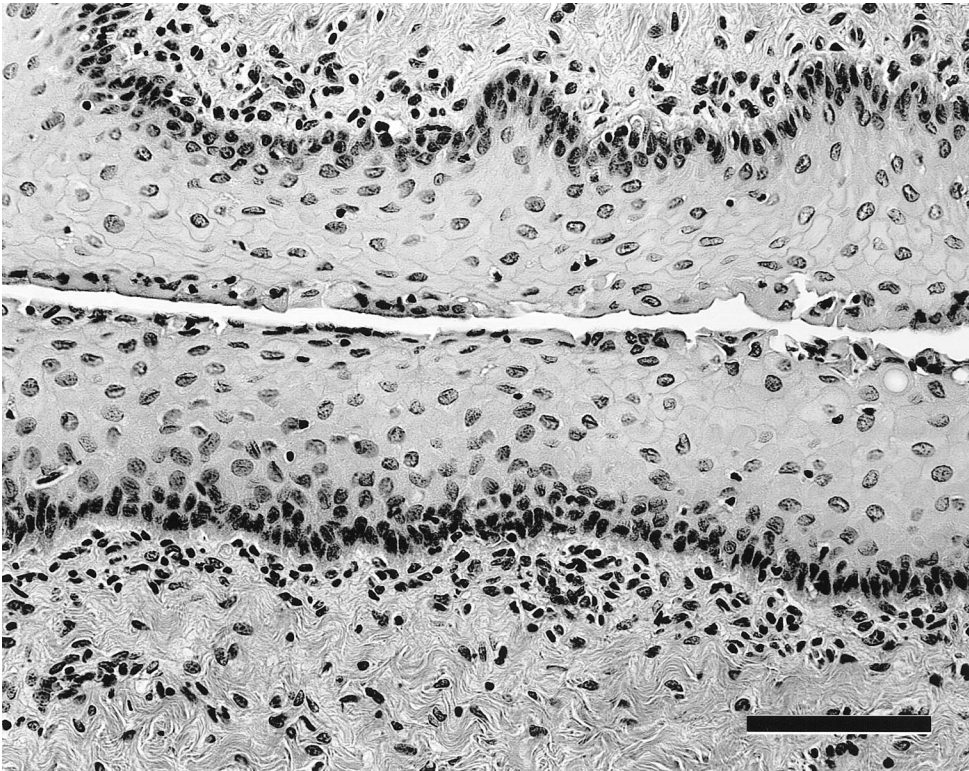
### DISCUSSION

The basic histology in this study was typically mammalian, although rhinoceros anatomy was unique, combining the characteristics of several domestic species.<sup>6</sup> To assess rhinoceros fertility, domestic animal reproductive techniques must be modified to accommodate this unique morphology.

Lateral penile flaps are distinctive to the rhinoceros and tapir. They are usually inside the vagina during intromission and significantly increase the diameter of the penis when erect. Unlike the other rhinoceros species, the size and cranial projection of the flaps of the Sumatran rhinoceros can inhibit intromission (Zainal, per comm.). The diameters of the flaps in this study were larger than the diameters

of domestic animal artificial vaginas (AV). Confinement of these flaps within domestic AVs has inhibited erection.<sup>21</sup> New AV designs for the rhinoceros must be developed that allow extension of these flaps.

Testicular biopsy has not been used to assess male rhinoceros fertility. Rhinoceros testicles, like those of stallions, are positioned horizontally. Although equine biopsy techniques might be adapted for use in rhinoceroses, the variable and indistinct location of rhinoceros testicles within the scrotal sac would make stabilization a challenge.<sup>25</sup> If such stabilization is possible, significant quantities of semen might be recoverable percutaneously from the epididymis of living Asian rhinoceroses. Percutaneous epididymal aspiration is used for human fertility analysis and/or gamete recovery.<sup>14</sup> The relatively large caliber of the epididymal ducts of the Asian facilitated the needle aspiration harvest of



**Figure 15.** Vaginal epithelium of a black rhinoceros, with a small number of lymphocytes and plasma cells subjacent to the submucosa. Bar = 100  $\mu$ m.

postmortem epididymal semen.<sup>24</sup> This large duct size may facilitate percutaneous antemortem collection of sperm from Asian rhinoceroses.

Rhinoceros accessory glands are accessible per rectum and have characteristics of a variety of other domestic species. The vesicular gland sacculations are similar to that of the bull, the triangular, bilobed prostate is characteristic of the stallion, and the glands lack an ampulla as in the boar. These characteristics have been evident in ultrasonographs of the rhinoceros<sup>20</sup> and are similar to those seen in images of domestic animals<sup>26,27</sup> Because palpation of the covertly located testicles proved difficult, testicular ultrasonography may also be inhibited.

The dilatation of the posterior urethra of the rhinoceros is similar to an area of semen accumulation during ejaculation (seminal emission) in other species. Monitoring the flow of semen in this area has resulted in successful collection of semen from the rhinoceros.<sup>22</sup> Additional studies of semen flow dynamics in this area may improve methods for rhinoceros semen collection.

Aspects of the female rhinoceros reproductive tract are similar to other species. The female intact hymen may indicate nulliparity in both elephants

and rhinoceroses;<sup>8</sup> however, it may also indicate absence of successful copulation in the rhinoceros. The elephant penis may not enter the hymen,<sup>15</sup> but the rhinoceros penis extends well beyond the hymen during copulation. Full penetration eliminates the hymen in the rhinoceros. Intromission may have been incomplete in the one white rhinoceros in this study that was suspected of breeding but had an intact hymen stretched on only one side. As in another study,<sup>8</sup> only two holes were evident in the rhinoceros hymen as opposed to two to three holes and blind pouches in the elephant.<sup>15</sup> The rhinoceros uterus resembles that of a sow, with a short body and long tubular horns. Its thin longitudinal endometrial folds resemble those of the mare, and no ruminant-like caruncular development was evident. Like the mare and sow, rhinoceroses have diffuse placentation;<sup>5</sup> however, the rhinoceros does not appear to possess the equine ovulatory fossa. Histologically, the ovarian tissues were similar to tissues of the cow.<sup>24</sup> As in the cow, but unlike in the mare, surface corpora lutea and follicles can be prominent in the rhinoceros.

Few structures occurred on the rhinoceros ovaries examined. The 3.0-  $\times$  3.5-cm corpora luteum

in one black rhinoceros (SB 412) resembled a 3.0-cm image that was reported on ultrasonography.<sup>1</sup> The corpora lutea from white rhinoceroses in this study were smaller than those reported elsewhere,<sup>17</sup> possibly because of regression or dysfunction. The lack of significant ovarian activity in the animals in the present study may be partially due to either the advanced ages or the illnesses of the animals examined. Both wild and captive rhinoceros females can reproduce in their third decade of life.<sup>23</sup>

Analysis of reproductive problems depends on access to the reproductive tract; however, the large size, length, thickness of the rectal wall, and abdominal position makes most of the of the female rhinoceros reproductive tract inaccessible. We were unable to manipulate per rectum the anterior uterus and ovaries in all but the smallest of individuals. To use ultrasonography to examine the ovaries, operators have had to lengthen their reach with extensions.<sup>1,17,20</sup> These extensions have ranged in length from 100 to 122 cm for white rhinoceroses to 70 cm for black rhinoceroses and white rhinoceroses. In situ vulva–ovary distances were all <100 cm, suggesting extensions allowing the operator to reach at least this distance may be useful for ultrasonography of both species.

The female reproductive tract location within the abdomen varies widely because of variation in size of tracts and in length of the uterine ligament. In this study, unfixed total reproductive tract length exceeded that in prior reports,<sup>8</sup> possibly either because of individual variation or because the tissue in the earlier report may have shrunk due to preservation. In addition, our in situ ovary–vulva distances in white and black rhinoceroses (maximum of 100 cm) were shorter than total lengths. This laxity of the female reproductive tract facilitates retraction of the tract into the pelvic canal or displacement toward the lateral abdominal wall,<sup>18</sup> but it makes locating the tract difficult. Prolonged practice is therefore required to locate the female rhinoceros reproductive tract by ultrasonography.

An intact hymen and the length and complexity of the rhinoceros cervix will inhibit entry into the uterine lumen for diagnosis or treatment. To penetrate the intact hymen, a clinician must cut both the vaginal transverse barrier and vertical septum. Unlike the simple cervix of the mare and elephant, the rhinoceros cervix has a complicated series of folds. Several cranial vaginal folds that are contiguous with the cervical folds can obscure the external os. The vaginal and cervical folds are of palpably different thickness, which may help to identify the cervical os when trying to digitally locate the external os through the vagina. In this study, the num-

ber of cervical folds was the same as in the domestic cow and sow, but folds were deeper and tended to interdigitate. Flexible catheters will probably be required in the rhinoceros for penetrating the cervix to the uterine lumen. The cervical folds of elephants and mares can be traversed with a flexible endoscope, but this technique has not been reported in the rhinoceros.<sup>9,28</sup> Clinicians should also note that no suburethral diverticulum exists in the rhinoceros, as it does in the domestic cow. The urethral orifice should therefore be directly catheterized at the cranial recess of the large urethral diverticulum.

The lumen of the rhinoceros cervix may become patent during estrus, as it does in other species. The cervix was patent in one female (SB 412) that appeared to be under late estrus influence. In addition, the cervix of a live black rhinoceros in estrus was catheterized. In contrast, the cervix of a nonproductive white rhinoceros could not be dilated with estrus-inducing drugs.<sup>18</sup> Even though these findings suggest that the patency of the rhinoceros cervix increases under the influence of estrogen, other avenues for examination will need to be developed.

The paralumbar approach is another option to access the rhinoceros reproductive tract. Although the uterus of the rhinoceros is in the vicinity of the lumbar area, the ovaries are more cranial in the abdomen. Accessing them may be more difficult because of the caudal extent of the ribs and the nonpliable nature of the skin, particularly that of the white rhinoceros. Multiparous female ovarian ligaments may stretch sufficiently to allow visualization through a lumbar fossa approach. However, based on our experience, the entire reproductive tract is not readily manipulated.

The entire tract of the mare has been visualized with laparoscopy.<sup>2</sup> Although this procedure was used recently to access a white rhinoceros uterus via the flank, the ovaries were not visible.<sup>18</sup> Our study suggests that the 54-cm scope used in the procedure should have been sufficient to reach the ovaries if the nonretractable skin and difficulty encountered in locating the tract had not inhibited the operator. Laparoscopy may be easier in black rhinoceroses because their lumbar fossas are larger and the distance to their ovaries is shorter.

The large size and inaccessible location of the rhinoceros reproductive tract inhibit collection of samples or data in vivo, yet many reproductive procedures depend on access to these structures. The information presented here should assist the development of techniques for accessing the rhinoceros reproductive tract. Anatomic data are still needed

for the two lesser Asian rhinoceros species and also for the female greater Asian rhinoceros.

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