CHAPTER 71

Rhinoceros Theriogenology

Robert Hermes and Thomas Bernd Hildebrandt

Over the last few decades, the six rhinoceros species have become important icons in the saga of wildlife conservation.[12] Recent surveys have estimated the wild black *(Diceros bicornis)*, southern white *(Ceratotherium simum)*, northern white *(Cerathoterium cottoni)*, Indian *(Rhinoceros unicornis)*, Javan *(Rhinoceros sondaicus)*, and Sumatran rhinoceros *(Dicerorhinus Sumatrensis)* populations to be, at most, 3610, 11,330, 7, 2500, 70, and 300, respectively. Protected against habitat loss, poaching, and left undisturbed, rhinoceroses reproduce well in the wild. However, small and decreasing populations make successful captive management increasingly important.

From the first descriptions of the reproductive anatomy and estrous cycle to the present use of advanced assisted reproduction technologies, researchers and veterinarians have attempted to understand the function and dysfunction of the reproductive biology of these charismatic species.[10,22] This chapter briefly reviews current knowledge on rhinoceros theriogenology.

MALE REPRODUCTIVE ANATOMY AND CLINICAL ASPECTS

The external and internal genitalia of the male rhinoceros include the prepuce, prepucial fold, penis, accessory sex glands, duct deferens, epididymis, and testes (Fig. 71-1).[44-47,59]

The relaxed musculocavernous penis is fully covered by the prepucial fold. During urination and territorial marking, the penis exits the sheath, pointing caudally between the hind legs. The fully erect penis points cranially; it has characteristic horizontal flaps and a mushroom-shaped process glandis, both being specific to each species. The horizontal flaps and process glandis suggest that the rhinoceros is a cervical inseminator. During up to a 45- to 60-minute intromission, the flaps unfold in the female's vagina while the process glandis locks into the portio and cervical folds.[44,59]

The paired set of accessory sex glands consists of cigar-shaped, multilobulated, seminal glands, the prostate with two triangular lobes and an isthmus across the urethra, and compact, round, or elongated bulbourethral glands. An ampulla at the end of the deferens ductus has not been described for the rhinoceros. In adult white rhinoceros, it has been shown that the volume of the accessory sex glands correlates with semen quality.[45,47,59]

The testes and tightly attached epididymides are located in the dorsal aspect of the prepucial fold. Their position in the prepucial fold shifts from vertical, clearly visible and palpable, caudal to the relaxed penis to more horizontal and nonpalpable, adjacent to the inguinal rings.[45,47,59] Thick skin, dense testicular capsule and changing positions limit possible conclusions from testicular palpation about functional status or pathologic lesions. Clinical examination of the testes and accessory sex glands relies on transcutaneous and transrectal ultrasound, respectively. Whereas transrectal examination of the accessory sex glands requires a chute-trained animal or sedation, transcutaneous ultrasound of the testes can be achieved through the bars of the enclosure with very little training.

Semen Collection, Evaluation, and Preservation

The evaluation of breeding soundness to determine a bull's reproductive potential consists of two components, clinical examination of the reproductive tract and semen collection. Semen collection and evaluation in rhinoceros are aimed at the determination of causes for reproductive failure or for semen donation and

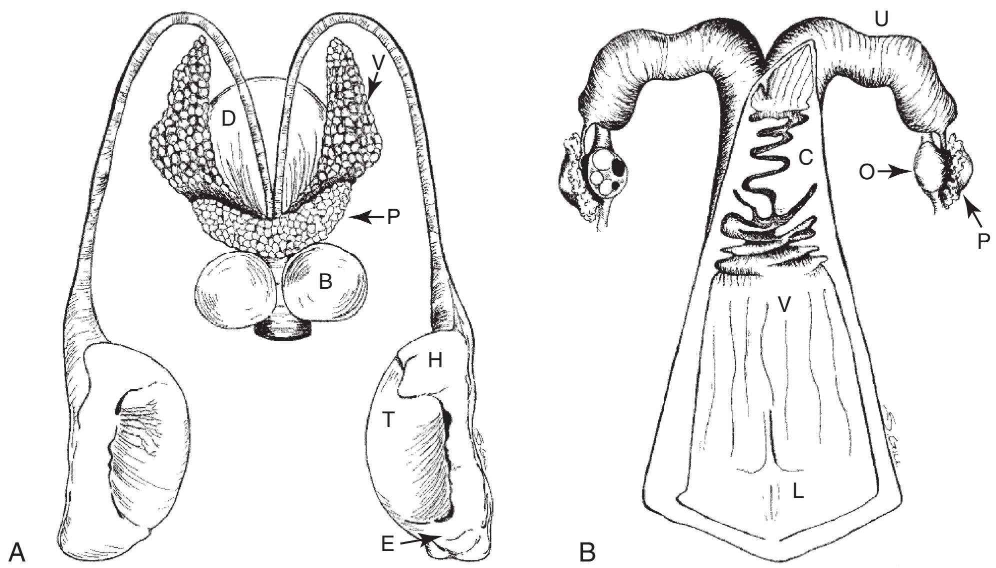

Figure 71-1

Male and female reproductive anatomy of the rhinoceros. **A**, Male reproductive organs from an Indian rhinoceros. **B**, Female reproductive organs from an African rhinoceros. *B*, Bulbourethral gland; *C*, cervix; *E*, tail of epididymis; *F*, fallopian tube; *H*, head of epididymis; *L*, vulva; *O*, ovary; *P*, prostate; *T*, testis; *D*, urinary bladder; *U*, uterine horn; *V*, vagina; *V*, vesicular gland. *(From Schaffer NE, Foley GL, Gill S, Pope CE: Clinical implications of rhinoceros reproductive tract anatomy and histology. J Zoo Wildl Med 32:31–46, 2001.)*

TABLE 71-1 Rhinoceros Semen Characteristics (Mean)

Species	Collection Method	Volume (mL)	Concentration (Sperm × 10^6/mL)	Motility (%)	Normal Morphology (%)	Post-Thaw Motility (%)
Black rhinoceros	Manual	62	15	—	—	—
	Electroejaculation	17-58	18-58	40-50	29	—
White rhinoceros	Manual	0.7	7	—	—	—
	Electroejaculation	30-80	165	71	69	20-78
Indian rhinoceros	Manual	4-17	1738	16	—	—
	Electroejaculation	92-160	124-377	48-72	34-39	50-55
Sumatran rhinoceros	Post coital	104	25	60	40	40-60
	Electroejaculation	21	1.5	55	8	—

preservation for assisted reproduction for males with known, good semen quality. Values generated might therefore differ greatly and their interpretation must be put carefully in context with the method of collection, the male's libido, presence or absence of mating or masturbation, territorial behavior, and diagnosed reproductive tract disease. Semen quality provides indications on the current breeding potential of a male. Poor semen quality does not exclude remaining chances of fertilization but is mostly associated with a lack of breeding activity.[17,36,41,44,53]

Semen Collection

Semen can be collected postcoitally, by manual stimulation, rectal massage, or electroejaculation, or postmortem (Table 71-1).[14,38] Postcoital collection, manual stimulation, and rectal massage give regular access to sperm for preservation and cryopreservation and later use in assisted reproduction without medical restraint. During postcoital semen collection, the ejaculatory fluid is captured draining from the vulva. Mating behavior, proper intromission, and ejaculation are prerequisites for this collection method, but are usually absent in males for which semen evaluation is warranted. Manual stimulation with or without light sedation has been successful in individual black, white, and Indian rhinoceroses. However, it is time-consuming, limited to well-trained individuals, and requires a restraint chute. It is characterized by inconsistent collection success and sometimes only a minute ejaculate volume 0.2 mL. Attempts to collect semen by the use of an artificial vagina have produced ejaculates but failed to produce sperm.

Electroejaculation under general anaesthesia is the method of choice for obtaining a semen sample in untrained, captive, or wild rhinoceroses. It has been adapted and applied in all captive rhinoceros species and provides reliable sampling for semen evaluation, assessment of current breeding potential, and sperm preservation for assisted reproduction purposes.* Prior to electrostimulation, ultrasound helps determine the position of the accessory sex glands, facilitating accurate placement of the electrostimulation probe and avoiding urine contamination from accidental stimulation of the bladder or bladder neck. The electrostimulation probe is placed onto the pelvic part of the urethra and prostate. Electric current induces ejaculatory contraction of the accessory sex glands and pelvic urethra. Manual massage of the pelvic and penile urethra between stimulation sets funnels the emitted ejaculatory fluids into the thermally and light-insulated collection tube. Sperm-rich fractions are emitted at the beginning of the induced ejaculatory process, followed by less concentrated fractions of sperm and aspermatic seminal plasma.

The core of semen evaluation is based on the sperm-rich fractions of the ejaculate. Semen evaluation involves assessment of ejaculate volume, color and odor, and sperm motility, morphology, viability and concentration (see Table 71-1). In general, ejaculates can be obtained from bulls as young as 6 and up to 42 years of age. Sperm quality is not affected by age and can remain high until advanced age, despite the presence of increasing testicular fibrosis. Sperm production and androgen concentration are consistent throughout the year and do not show seasonal influences.[17] Recorded ejaculate volumes range from 0.1 to 200 mL and seem to be greatly influenced by collection method, probe operator, probe design, or stimulation protocol applied. Ejaculates collected postcoitally or by electroejaculation show the most consistent mean volumes, from 17 to 160 mL, presumably representing the naturally ejaculated volume range.

*References 17, 36, 41, 44, 45, and 53.

The preparation of epidydimal sperm after castration or postmortem represents a unique opportunity for a final male gamete rescue. The extraction of spermatozoa from the epidymis or vas deferens rescues the remaining stock of mature gametes. Extraction is best performed on site shortly after the donor's death to avoid autolysis or heterolysis of the tissue and temperature changes affecting sperm quality. Cooled transport (4° C) of the testis and epidymis in saline solution is an alternative to on-site preparation if the death occurs unexpected Cryopreserved sperm conserves the male's ability to produce offspring posthumously. The authors used sperm extracted and cryopreserved postmortem for artificial inseminations, which have resulted in pregnancy.

Semen Assessment and Preservation

Rhinoceros spermatozoa are similar in size to those of the stallion.[2] Sperm characteristics show huge ranges (see Table 71-1). Good- and poor-quality semen has no significant differences in terms of ejaculate volume, pH (8.5 to 9.0), or osmolarity (290 to 300 mOsm/kg).[53] Sperm motility, viability, and morphology therefore represent the most relevant indicators to rate current semen quality. In domestic species, sperm motility and morphology are positively correlated with pregnancy rate. In the rhinoceros, analogous to domestic species, the percentages of motile and morphologically normal sperm in sperm-rich fractions are used to rate a bull's semen quality. Semen quality is considered as a prognostic indicator of the sperm's fertilizing potential and thus of the bull's breeding potential. Sperm with motility of 75% is considered to have excellent fertilizing potential. Fertilizing potential of sperm with motility of less than 75% or less than 50% is considered intermediate or poor.[17,36] However, samples with 60% motility have proven sufficient for assisted reproduction and semen preservation.[25] Variations in semen quality among collections may occur and repeated evaluations are suggested to confirm poor semen quality and limited breeding potential of a bull. In white rhinoceroses, social structure and subordinate behavior have been identified as a cause of the high incidence of reduced semen quality in males without or long past breeding history. The subordination of males in multimale institutions has corresponded with low sperm quality. Socially subordinated males display decreased reproductive fitness in the presence of a territorial male. Altering the captive social structure by separating nonbreeding but territorial males, exclusive access of subordinate males to breeding females, or introduction of new challenging males might improve libido and reproductive parameters.

Rhinoceros spermatozoa are not sensitive to slow chilling. The motility of white rhinoceros sperm chilled to 4° C after suspension in equine, bovine, or custom-made extenders remains almost constant for 24 hours.[17] In another study, we noted that high-quality sperm from Indian and black white rhinoceroses show reduced motility values after only 72 hours of chilling. This low sensitivity of rhinoceros sperm to chilling facilitates sperm transport to distant locations for use in regional AI breeding programs.[20]

Cryopreservation of sperm has been shown in white, Sumatran, and Indian rhinoceroses and was successfully used for artificial insemination in white and Indian rhinoceroses.* Cryopreservation of male gametes permits the use of sperm from unrepresented, wild, or deceased males, thus improving the genetic diversity of a population. In general, cryopreservation of sperm induces cell damage, resulting in cell death or loss of function. Motility, viability, morphology, and acrosome integrity of the sperm are the main characteristics used to assess semen quality after thawing. Single- or double-stranded sperm DNA fragmentation has recently been added as a critical parameter for assessing post-thaw sperm integrity.[32] Fast fragmentation of the rhinoceros sperm nucleus in comparison with other eutherian species, including the stallion, suggests that semen processing and current freezing methods used will need further substantial improvement to increase cell and DNA integrity and longevity of rhinoceros sperm after cryopreservation.

Cryopreservation of Sperm

A critical precondition to cryopreserving rhinoceros sperm is a high-quality semen sample. Even in one ejaculate, only few sperm-rich fractions with highly motile sperm qualify for preservation. Standard or modified equine extenders or a skim milk–egg yolk or TEST–egg yolk medium are used to cryopreserve the sperm, with good success. Glycerol and dimethylsulfoxide (Me_2SO) in concentrations of 5% or 6.25% are used as cryoprotectants.[17,24,25] In a Sumatran rhinoceros, Me_2SO was identified as the superior cryoprotectant compared with glycerol. In large-scale studies in white rhinoceroses, Me_2SO, as an exclusively tested cryoprotectant, produced reproducible good results.[36] However, in a recent comparative study in Indian rhinoceros, no significant difference in post-thaw results was found between glycerol and Me_2SO.[53] Sperm is generally frozen in 0.5-mL straws in liquid nitrogen vapor. This freezing method provides good post-thaw results across species, and is relatively simple and easy to apply under field conditions. A new freezing technology, multithermal gradient directional freezing (Core Dynamics, Orangeburg, NY), facilitates freezing of large volumes (8 mL/vial).[16] Thanks to precise control over ice crystal formation, it is reported to result in less cell damage and higher gamete survival. In a comparative study, directional freezing has been superior to freezing over liquid nitrogen vapor in white rhinoceroses. However, high liquid nitrogen consumption and vulnerable electronics make this technology logistically more challenging.

*References 16, 17, 24, 25, 37, and 53.

Diseases of the Male Reproductive Tract

Penis

Mating or masturbation on foreign objects may cause trauma to the penis, resulting in superficial lesions, edema, or abrasion (Fig. 71-2). Superficial lesions are treated by and responsive to topical ointment. More severe abrasions might cause edema further, preventing retraction of the penis. With the penis permanently unprotected, edema increase and further secondary abrasions might occur to the free-hanging and swinging penis. Similar to the stallion, blood circulation of the glans is disturbed and aggressive therapy is necessary to prevent further abrasions, ulceration, and irreversible penile damage. Replacement of the penis into the sheath is the primary goal, best achieved by bandaging the penis to the abdominal wall. Blunt trauma of the erect penis can cause penile fracture, resulting in inability to obtain intromission thereafter.[21]

Accessory Sex Glands

Diseases of the accessory sex glands are rare. Prostate cysts have been documented in one white rhinoceros. Cystic formations in the prostate are presumably painful and could be interpreted as the reason for lack of libido.

Testis

Testicular fibrosis, atrophy, trauma, or neoplasia and epididymal cysts are disorders described in the male rhinoceros (Fig. 71-3).[17,30,31,53] Testicular fibrosis is a common finding in older males. In white and Indian rhinoceroses, testicular fibrosis starts forming at the age of 15 years.[17,53] Fibrotic areas, located in the interstitium, appear in the ultrasound as bright dots (approximately 0.1-0.3 mm) within the testicular parenchyma. The size and number of fibrotic spots are positively correlated with age and are regarded as signs of testicular aging, but with no influence on semen characteristics.

Figure 71-2

A, Swelling within the prepucial fold *(arrows)* diagnosed as a hematoma or seroma by ultrasound. **B**, Deep fissure and purulent infection in the upper inguinal area of a male after territorial fight. **C, D,** Increasing edema and prolapse of the penis. **E,** Complete body bandage to reposition the prolapsed penis into the sheath, reinitiating penile blood circulation.

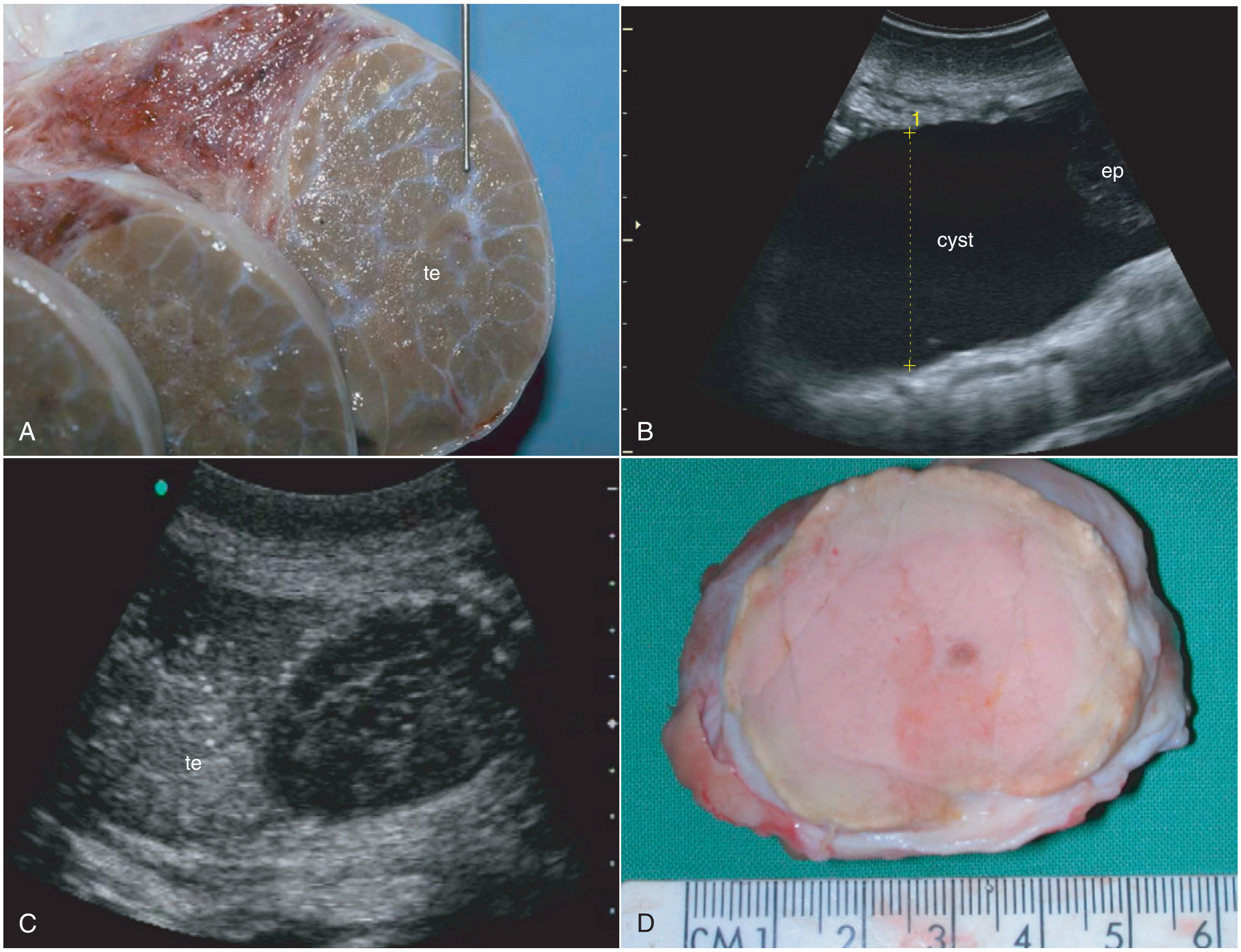

Figure 71-3

A, Interstitial fibrosis *(probe)* in the postmortem sliced testis *(te)* of an old Indian rhinoceros. **B,** Epidydimal cyst adjacent to the epidymis *(ep)* in a sterile white rhinoceros. **C, D,** Ultrasound image and postmortem preparation of a seminoma in the testis of a white rhinoceros.

Different from age related fibrosis, physical trauma, testicular neoplasia or epididydimal cysts may influence sperm production, causing subfertility or infertility.[30,31] During territorial fights or rough courtship, the opponent's horn forks into the inguinal area of the male, causing blunt trauma of the gonads (see Fig. 71-2). Hematoma or seroma of the testis may be visible as asymmetric swelling of the prepucial fold or by visualizing fluid cavities in and around the testis using ultrasound. Effects of hematoma or seroma on sperm production might be temporary. However, severe trauma might induce testicular necrosis and atrophy or permanent stenosis of the spermatic cord, resulting in infertility.

Testicular neoplasia is characterized as solid mass within the testicular parenchyma (see Fig. 71-3).[30,31] Diagnosed by ultrasound, it is further characterized by testicular biopsy. Seminoma has been described in black and white rhinoceroses. Hemicastration of the affected testis prevents further growth and possible metastasis and ensures the animal's breeding potential. A hemicastrated black rhinoceros resumed good-quality semen production after surgery, with proven fertility.

Epidydimal cysts as a defect of the gonaduct system have been detected in Sumatran and white rhinoceroses by the authors and Schaffer[43] (see Fig. 71-3). Filled with clear fluid, their dimensions can range from 1 to 10 cm. Ejaculates from effected males are consistently aspermatic or of low quality. The cause of these cysts is unclear. Previous trauma resulting in the formation of cysts might induce seroma and stenosis of the gonaduct system. Transcutaneous fine-needle aspiration of the cystic fluid can be attempted to resolve the cyst(s), resuming sperm passage and fertility.

TABLE 71-2 Female Reproductive Anatomy and Estrous Cycle

	LENGTH OR DIAMETER							
Rhinoceros Species	Vestibule (cm)	Vagina (cm)	Cervix (cm)	Uterine Body (cm)	Uterine Horns (cm)	Ovary (cm)	Ovulatory Follicle (cm)	Estrous Cycle (days)
Black rhinoceros	15	22-25	11-17	3-6	34-80	5-12	5.0	21-27
White rhinoceros	14-20	19-30	12-23	2-8	40-64	6-9	3.2-3.5	31-35 or 66-70
Indian rhinoceros	27	20	30	10	33	5	10-12	43-48
Sumatran rhinoceros	—	—	6-7	4	30-34	2-8	2-2.5	21-25

FEMALE REPRODUCTIVE ANATOMY AND CLINICAL ASPECTS

The female reproductive organs in the rhinoceros consist of the vulva, clitoris, vestibule, vagina, cervix, uterus bicornis, oviduct, and ovaries. The total length of the genital tract varies among species, from approximately 50 cm in the Sumatran to well over 100 cm in the white rhinoceros (Table 71-2). The hymenal membrane and cervix are uniquely shaped anatomic structures in the rhinoceros (see Fig. 71-1).[19,46,47,59]

The external genitalia are composed of vertical, symmetrical, outer and inner vulval labia and a prominent clitoris on the ventral commissure. The outer labia protrude caudoventrally and, in the white and greater one-horned rhinoceroses, are sometimes the only part of the external genitals visible from a distance. Changes of the external genitalia associated with estrous are small and subtle in all species. In most species, estrous is therefore noted only if the female vocalizes (Indian and Sumatran rhinoceroses) or if the male indicates estrous by his interest.

The vestibule and vagina in nulliparous females are separated by a hymenal membrane cranial to the urethral orifice. The patency of the hymen in young females is limited to small perforations of 0.1 to 0.5 cm. The hymen ruptures during first mating, with only vestiges of the membrane remaining thereafter. In females in which mating is rare or intromission is not observed, the hymenal status is often unknown. Reproductive failure in these females is generally associated with a persistent hymen. The presence or absence of the hymenal membrane in nulliparous females can be determined by vestibulovaginal palpation. In a study group of mature white rhinoceroses with reproductive failure, the incidence of a persistent hymen was 76%.[19] Specifically, in older females, the hymen becomes more fibrous and, despite dilated hymenal orifices (3 cm), is more difficult to rupture. Blunt surgical rupture of the hymen is an option to resolve this mechanical breeding barrier for mating in older females.

The cervix consists of three to five very tight interdigitated folds of fibrous connective tissue. The cervical cannel is tortuous, with 90-degree turns and blind pockets. The length and tightness of the cervical folds allow catheter passage only during estrous. The cervix is well definable as a tight cylindrical structure during rectal palpation.

The uterus bicornis consists of a short body and long uterine horns. The horns first run alongside as a pseudo-uterine body before parting laterally at the bifurcatio uteri. In the white rhinoceros, the total length of the uterine horns can exceed 50 cm.

The size of the ovaries and their functional structures vary greatly among species (see Table 71-2). Unlike the horse, the closest related domestic species of the rhinoceros, follicles rupture on the surface of the ovary. Both the uterus and ovary are not accessible for conclusive palpation.

Because palpation is of limited value in the rhinoceros, clinical examination of the reproductive tract is commonly performed by transrectal ultrasound.[19,34,40,54] Ultrasound allows an efficient and accurate determination of the reproductive status, function, and diagnosis of reproductive pathology. Portable commercial systems equipped with 2- to 8-MHz ultrasound probes are sufficient for diagnostic imaging. In black, Indian, and Sumatran rhinoceroses, the genital tract is easily accessible for the examiner. In the white rhinoceros, the length of the genital tract might exceed the examiner's arm reach and custom-made extensions of the ultrasound probe might be necessary to access the cranial part of the reproductive tract.[19] In chute-trained animals, transrectal examinations can be performed on a daily basis. In animals not trained, standing sedation is adequate for reproductive assessment.

Estrous Cycle

In the wild, rhinoceroses are described as polyestric nonseasonal breeders.[7,23] The estrous cycle length in the different rhinoceros species is diverse. Estrogen and progesterone profiles measured in the feces, urine, and serum using various hormone assays have characterized the estrous cycle length in four of the six rhinoceros species (see Table 71-2).[14,38] Individual differences in cycle length occur in all captive species, being most pronounced in the Indian rhinoceros, with recorded extremes ranging from 36 to 86 days. Although the estrous cycle in the Sumatran, black, and Indian rhinoceros shows only individual variations, two different estrous cycle lengths were reported in the white rhinoceros.[5,28,51] The shorter 30- to 35-day cycle is believed to be fertile. The longer cycle, when observed in females without a mating history, is noted as the first sign of reproductive aging and reduced fertility. However, the authors also observed long cycles in relation to embryonic resorption after artificial insemination.

Ovulation in the rhinoceros, like in most mammals, is induced by a single, preovulatory, luteinizing hormone (LH) surge that occurs at the end of the follicular phase reported for the Indian and Sumatran species.[39,54] The Sumatran rhinoceros, different from the other species, is additionally described as an induced ovulator. In this species the LH surge and ovulation are triggered by mechanical stimulation of the vagina and cervix when the female is mated. Similar to the diversity of estrous cycle length, the dimension of the ovulatory follicle deviates considerably among species (see Table 71-2). The Graafian follicle in the Indian rhinoceros, with an average diameter of 120 mm, is the largest reported in a mammal.[20,34,35,40] Luteinization and formation of hemorrhagic follicles, which exceed the dimensions of the species-specific Graafian follicle by 10 to 30 mm, are known to occur in all four captive rhinoceros species. Except for the Sumatran rhinoceros as induced ovulator, these anovulatory structures are regarded as nonphysiologic. The incidence of hemorrhagic follicles in captive black, white, and Indian rhinoceroses and the possible effect on reproduction is of great concern.

Anestrous

Anestrous is a common finding in white rhinoceroses and is considered as a primary cause for the low reproductive rate in captivity. Anestrous also occurs in individual black, Sumatran, and Indian rhinoceroses. In the white rhinoceros, 50% of females have been determined to be in anestrous.[28,51] Endocrine data, ultrasound, and postmortem findings have revealed that anestrous is associated with different status of ovarian activity. Young females in anestrous exhibit high ovarian activity and regular follicular waves. However, ovulatory-sized follicles do not ovulate but become atretic or hemorrhagic.[19] This phenomenon has been reported to occur in other species as well.[34,35,39,54] Over decades, this high follicular output is believed to lead to oocyte depletion. In middle-aged females, follicular development, and therefore ovarian activity, becomes irregular and then ceases, resulting in premature senescence.[18] Transporting young females to other facilities or the introduction of new males has shown to initiate or reinitiate regular estrous cycle activity in some animals.[49] These scarce data suggest that the optimal animal husbandry and behavioral needs that lead to regular estrous cycle activity are yet unknown.

Estrous Induction

The hormonal induction of ovulation to overcome anestrous has been attempted in three different species, testing over 15 different induction protocols.[9,27] Various combinations of synthetic progestin, sometimes preceded by prostaglandin F_{2a} (PGF_{2a}) and followed by different combinations of follicle-stimulating hormone (FSH), pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), and gonadotropin-releasing hormone (GnRH), have failed to induce ovulation. Results have suggested a low species-specific receptor affinity of the primarily used synthetic progestin, altrenogest (Regumate). The use of another synthetic progestin, chlormadinone acetate combined with hCG, achieved better receptor affinity and induced estrous and ovulation in white rhinoceroses.[51] Chlormadinone acetate (30 mg/day) for 45 days and hCG (10,000 IU), injected on day 55 of treatment, induce ovulation and luteal activity. The authors observed that the effectiveness of a modified protocol, using synthetic GnRH analogue implants instead of hCG to induce ovulation, is 81%. However, initiation of a regular estrous cycle activity using this protocol has not been achieved. Another successful protocol to induce ovulation in anestrous females with regular follicle development is the use of a synthetic GnRH analogue.[16,20] Folliculogenesis is monitored by ultrasound and, when a preovulatory sized follicle is identified, GnRH analogue implants are used to induce ovulation within 48 hours.

GESTATION AND BIRTH

The gestation period of rhinoceroses is the second longest in land mammals after the elephant, ranging from 15 to 18 months. Pregnancy can be diagnosed by elevated progesterone concentrations 3 to 5 months after conception or by ultrasound 2 to 4 weeks after conception.[16] The embryonic vesicle is detectable by ultrasound as early as 15 days postovulation.[30] In the larger species, white and Indian rhinoceroses, transrectal imaging of embryonic and fetal structures is limited to the first trimester. Dimensions of the amnion and allantois during the second and third trimesters become too large for transrectal visualization of the fetus. Pregnancy diagnosis based only on the differentiation of the allantois and amnion against the female's bladder during this period might be challenging for the inexperienced examiner and may result in a false-negative pregnancy diagnosis. In contrast, we have noted that transcutaneous ultrasound in the lower flank and inguinal areas during the third trimester provides reliable images of fetal parts and fetal life signs, such as movement of the extremities or fetal blood flow.

Early embryonic loss has been reported to occur in captivity and in the wild in black, white, and Sumatran species.* Early pregnancy loss may be associated with uterine inflammation or pyometra in individual cases but is clinically uneventful. Luteal insufficiency has been a suspected cause in animals with a history of embryonic resorption in captivity. One black and one Sumatran rhinoceros supplemented with synthetic progestin subsequently carried their pregnancies to term. However, whether luteal deficiency is occurring and the synthetic progestin used actually has any supplementing effect, based on its low receptor affinity, is controversial. When progesterone profiles during a normal and supplemented pregnancy were compared in one Sumatran rhinoceros, no difference was detected.[38] These results question the existence of luteal deficiency in rhinoceros.

Hand Rearing

Hand rearing has been successful using bovine whole-milk formulations. However, considering the low solid (8.3% to 8.8%), protein (1.2% to 1.5%), and very low fat (0.2% to 0.6%) but high sugar (6.5% to 6.9%) content of rhinoceros milk, bovine skim milk or equine foal milk exchanger are probably more appropriate replacements.[7,11,13,56] Colostrum should be provided, if at all possible, within 24 hours of birth. To prepare for hand rearing, colostrum or maternal serum as colostrum replacement should be collected antepartum. It is recommended that substitutions of vitamins, gamma globulin and paraimmunity inducers are added to the milk. During the first 3 days, 10% of body weight is fed; thereafter, it is 15% to 20% of body weight/day. Total amounts should be divided into 7 to 10 feedings/day. The total amount of milk increases to 20 to 35 liters/day. Milk is slowly substituted with boiled rice, hay, soft fruits, and vegetables, starting at 2 weeks. Weaning can begin as early as 6 months, but should be complete at 12 to 15 months. Recommended food items introduced for weaning can include horse feeds or high-fiber ungulate pellets, together with high-quality hay and browse. For more detailed information on formulas and feeding schedules, husbandry guidelines are an excellent source of information.

Maternal inexperience is responsible for postpartum maternal aggression, which can cause fatal injuries to the newborn and often make hand rearing imperative. Measures such as a mixed exhibit with small mammals during pregnancy, oxytocin application to enhance lactation, or short-term sedation of the female to facilitate first suckling have been attempted to avoid rejection of the newborn and need for hand rearing.[52]

Birth

Physiologic labor and expulsion of the fetus in the different rhinoceros species are brief, ranging between 1 and 2 to 3 hours. Birth weight of the calf ranges from 24 to 70 kg, depending on the species. The calf stands within the first hour after birth and suckles after 2 to 3 hours. A longer interval until first suckling of up to 14 hours might occur in inexperienced mothers but seems not to harm the calf. The placenta is expelled within 6 to 7 hours. It is not uncommon for the female to eat the placenta.

Dystocia

Dystocia and stillbirth occur in all captive rhinoceros species, with the highest stillbirth rate in the Indian rhinoceros.[48] Since 1971, a total of 226 Indian rhinoceros calves have been born; 24% of them were stillborn or did not survive the first 3 months. Early detachment of the placenta, malposition, or fetal malformation (e.g., cyclopia, cerebral aplasia) are incidental causes reported for stillbirth. Three cases of craniofacial

*References 4, 8, 28, 34, 39, and 40.

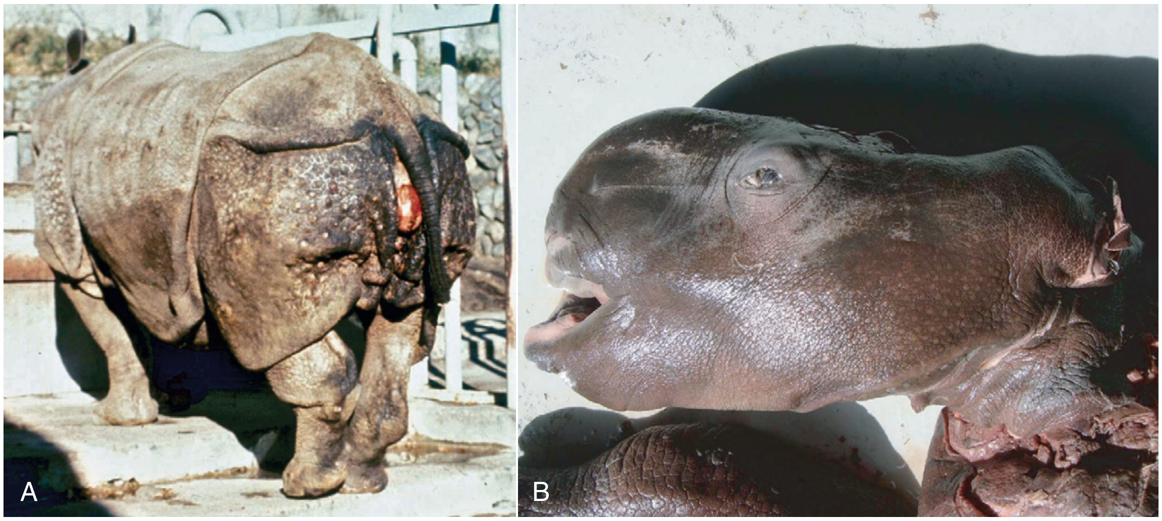

Figure 71-4

A, Vaginal leiomyoma in an Indian rhinoceros protruding from the vulva. **B,** Dissected head of a stillborn Indian rhinoceros with cerebral aplasia after fetotomy.

malformation in Indian rhinoceroses have recently suggested[26] that undetected neural tube defects may be associated with more stillbirths than previously considered. The causes for the high rate of stillbirth and neonate mortality in Indian rhinoceroses are yet unknown.

Dystocia can remain unnoticed when the stillborn fetus is delivered without complications. Lack of labor progress 4 to 6 hours after rupture of the fetal membranes is an indication for dystocia and calls for veterinary intervention. The use of oxytocin, 100 IU, has been successful in resolving retention as a minimally invasive approach. Breech presentation or flexed extremities may require correction of fetal position and manual extraction under general anesthesia.[11,26] Cesarean section as a surgical approach for calf delivery is not an option in rhinoceroses because of their thick integument, enormous intestinal weight, and difficult wound management in unconditioned animals. Although laparoscopic accessibility of the abdomen has been demonstrated in the rhinoceros for uterine biopsy, a large incision, as is necessary to extract a 24- to 70-kg fetus, poses a great risk of postsurgical complications.[33] Fetotomy may become necessary when attempts at manual extraction fail.[48] Dissected into five parts, a dead and malformed fetus was extracted during two anesthesias from one female via the birth canal over two consecutive days (Fig. 71-4). All females with dystocia from which calves were extracted recovered fully. One female was reported to come into estrous 31 days after fetotomy and to fall pregnant when natural breeding was resumed.

REPRODUCTIVE PATHOLOGY

Noninfectious reproductive disorders pose one of the main obstacles for the reproduction of rhinoceroses in captivity. Until recently, the diagnosis of reproductive disorders was limited to postmortem examination and a few clinical signs, such as bloody vaginal discharge or massive tumor growth visible from the outside (see Fig. 71-4). The increased use of ultrasound has facilitated in vivo diagnosis of reproductive disorders and evaluation of reproductive potential in rhinoceroses.[15] In general, reproductive pathologies in female rhinoceroses involve ovarian, uterine, cervical, and vaginal tumors, endometrial and ovarian cysts, endometrial hyperplasia, and mucohydrometra (Figs. 71-5 to 71-7). Transrectal ultrasound-guided fine-needle aspiration is a new surgical approach to dissolve cystic formations.[15] However, there is a limited choice of treatments for this array of reproductive disorders. Neoplasia is the predominant disorder found in Asian species whereas uterine cystic hyperplasia is the prevailing finding in African species.[19] Cystic or neoplastic formations can be a hidden source of discomfort, preventing intromission, mating, and/or semen transport. The incidence of reproductive disorders is greater in nulliparous females and is positively correlated with age.[17,18] Reproductive disorders are regarded as age-related consequence of long nonreproductive periods. Diagnosed reproductive disorders might affect reproductive success and careful consideration has to be made regarding the remaining breeding potential of the female. The leiomyoma is

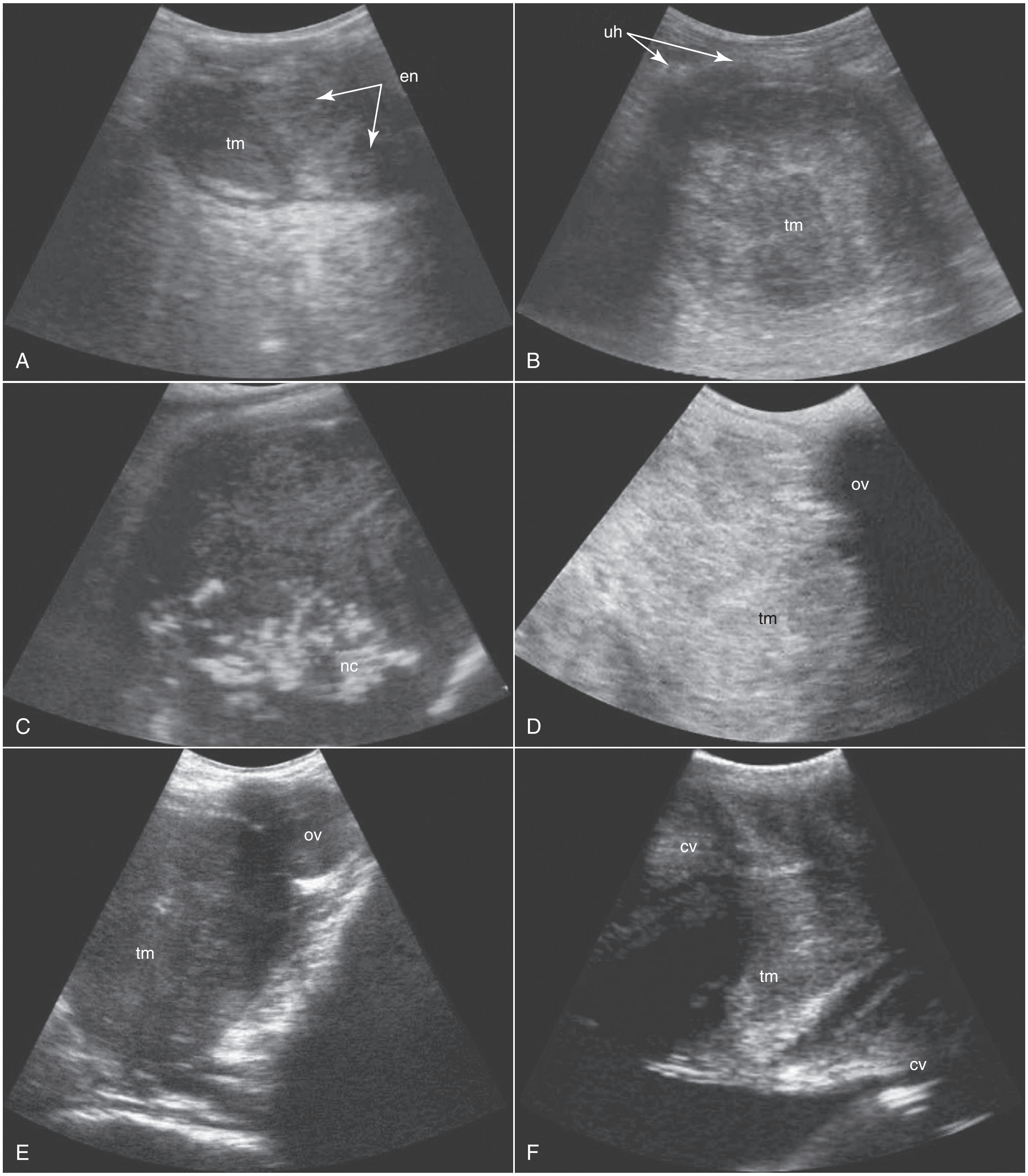

Figure 71-5

Ultrasound images of uterine **(A-C)**, ovarian **(D, E)**, and cervical **(F)** tumors *(tm)* in white rhinoceroses. **A,** Tumor is lower in echogenicity compared with the endometrium *(en)*. **B,** Tumor compressing the uterine horn *(uh)*. **C,** Tumor with central areas of necrosis *(nc)*. **D,** Paraovarian tumor in a cycling female. **E,** Large paraovarian tumor. **F,** Longitudinal view of a polyp located between the cervical folds *(cv)*. Scale bars = 1 cm.

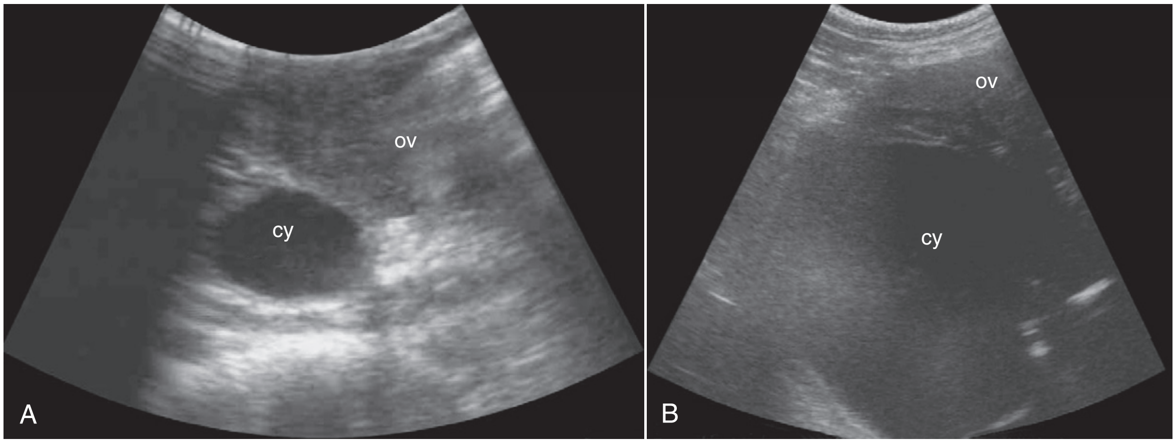

Figure 71-6

Ultrasonographic images of paraovarian cysts in white rhinoceroses. **A,** Small paraovarian cysts *(cy)* close to the ovary *(ov)* in a noncycling southern white rhinoceros. **B,** Paraovarian cyst. The small inactive ovary *(ov)* appears atrophic because of the mechanical pressure of the large cystic structure.

the most common uterine tumor across species, but endometrial adenoma and adenocarcinoma have also been documented to occur in white rhinoceroses.[58] The predominant location of neoplasia within the genital tract is species-specific. Although tumors occur mainly in the uterus in the white rhinoceros, the cervix and vagina are the most affected organs in the Indian rhinoceros. Excessive tumors may become necrotic or cause internal or external blood loss. In old females, regular bloody discharge from the vagina is a common clinical sign associated with genital tumours. Extensive chronic blood loss may even lead to anemia. In geriatric females, downregulation of ovarian activity using long-acting GnRH analogue formulations or GnRH vaccine might be an approach to stop further hormone-dependent tumor growth and blood loss. However, great care should be taken when managing old anestrous or artificially downregulated females in a herd together with a mature breeding bull. Old females with naturally ceased ovarian activity or artificially downregulated (GnRH vaccine) are disposed of any sexual signals and may be perceived as territorial rival to the breeding bull. Breeding bulls have repeatedly attacked or killed older females presumably in an attempt to eliminate a potential 'rival'.

Reproductive disorders, along with ovarian exhaustion, might render a female irreversibly infertile early during life. This phenomenon has been termed *asymmetrical reproductive aging*.[18] A reproducing female white rhinoceros in captivity may produce up to nine calves and, in between, exhibit approximately 90 estrous cycles during her reproductive life. Observations from the wild have confirmed that pregnancy and lactation represent the predominant endocrine status. With as few as 90 cycles, estrous is a relatively rare event during a female's reproductive life. These numbers are considerably lower than the up to 310 estrous cycles in captive, nonreproducing female rhinoceroses. By the age of 16 years, when first reproductive lesions are detected, nonreproducing females have already displayed the 90–estrous cycle allotment. The reproductive organs of nonreproducing female rhinoceros are exposed to prolonged periods of sex steroid fluctuations from continuous ovarian cycle activity. The central effects of this asymmetrical reproductive aging process include the progressive development of genital pathology, with subsequent subfertility or infertility and, presumably, the utilization of follicular stock at a higher rate. Because the incidence of reproductive disorders in parous females is significantly lower, achievement of pregnancy in young animals can be regarded as a prophylactic measure against reproductive disorders.[19]

Infectious diseases of the reproductive tract are rarely described. Endometritis caused by *Pseudomonas* or hemolytic streptococci has only been reported in several Indian rhinoceroses.[11,55] Vaginal purulent discharge was noted in these cases as the only clinical sign. Treatment consisted of vaginal lavage and systemic antibiotic treatment with penicillin and streptomycin.

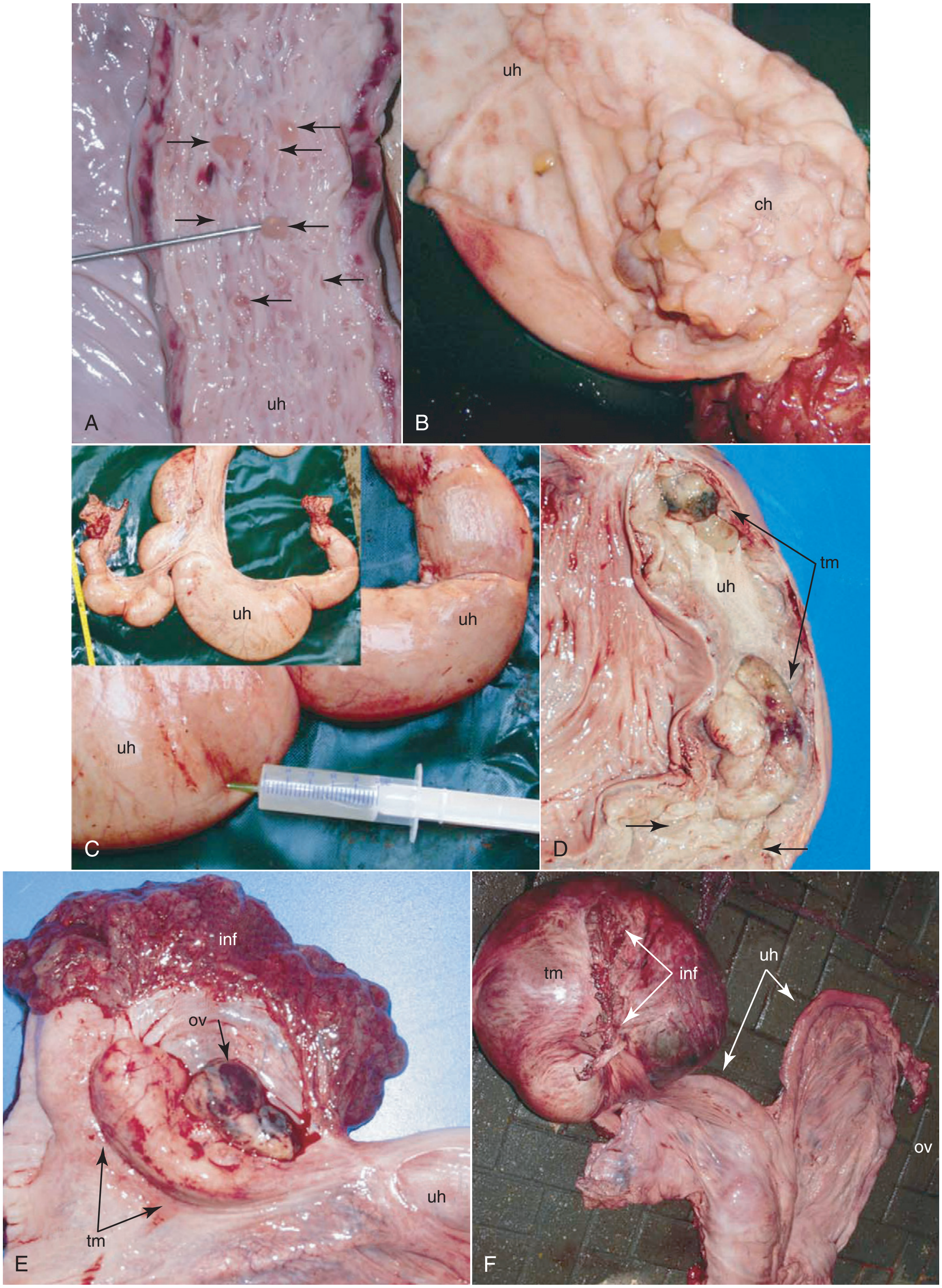

Figure 71-7

Postmortem preparations of genital pathology from white rhinoceroses. **A,** Multiple endometrial cysts (arrows) in the uterine horn. **B,** Multifocal cystic endometrial hyperplasia at the end of the uterine horn *(uh)* blocking the oviductal orifice. **C,** Hydromucometra. The uterine body and horns *(uh)* are filled with fluid. **D,** Cystic endometrial hyperplasia *(arrows)* and intraluminal adenoma *(tm)* in the uterine horn *(uh)*. **E,** Leiomyoma *(tm)* in the mesovarium close to the ovary *(ov)*, opposite the infundibulum *(inf)*. **F,** Paraovarian leiomyoma *(tm)*.

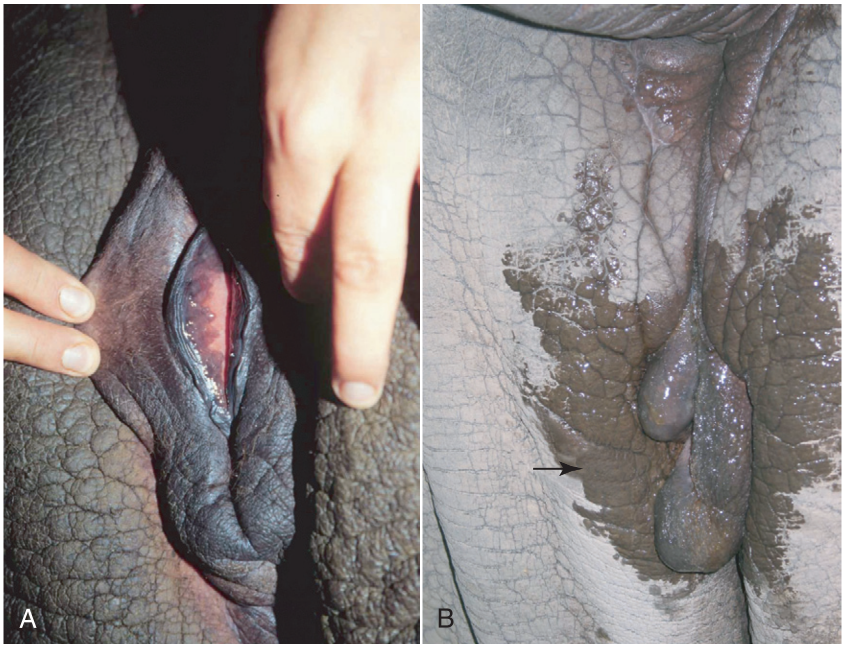

Figure 71-8

A, Symmetrical outer and inner vaginal labia in a white rhinoceros. **B,** Vaginal fissure of the outer labia *(arrow)* as the source for chronic vaginal discharge and reluctance to breed.

$PGF_{2\alpha}$ to enhance discharge should be used very carefully and in combination with a preceding cervical application of PGE to open the tightly closed cervix, facilitating passage prior to uterine contraction. Abortion and unnoticed fissures of the vulval labia, with purulent discharge from rough courtship or fights between rivals, should be considered as differential diagnosis to endometritis in animals with purulent vaginal discharge (Fig. 71-8).

ARTIFICIAL INSEMINATION

Artificial insemination (AI) has been successful using fresh and cryopreserved semen in white and Indian rhinoceroses.[16,20,37] So far, seven rhinoceroses have been conceived by AI worldwide. Yet only five of those were born alive. When considering the limited breeding success of both white rhinoceros species in captivity, skewed birth sex ratios in the Indian and black rhinoceros, and the disastrous situation of some rhinoceros species in the wild, AI might become a useful tool to overcome these crises.[1,60]

The estrous cycle in anestrous AI candidates has been induced using chlormadinone acetate plus GnRH analogue. The presence of a preovulatory follicle and accurate timing of ovulation were determined by preceding ultrasound examinations.[16,20] In cycling or postpartum females, GnRH analogue was used to hasten ovulation and to coincide with the insemination. The anatomic challenge for AI, the firm tortuous cervix, is overcome using a rhinoceros-specific AI catheter. Frequent use of AI in the future might help in establishing self-sustaining captive populations of threatened rhinoceros species and enhancing genetic diversity when using unrepresented captive or even wild semen donors.

ADVANCED ASSISTED REPRODUCTION TECHNOLOGIES

Sperm Sexing

In domestic species and a few wildlife species, using gender-biased sperm samples has been described as a tool to influence the gender of the offspring. AI in rhinoceros will facilitate the development and implementation of this technology in the future. The feasibility of sorting sperm by gender in black, white, and Indian rhinoceroses has been demonstrated by sorting the spermatozoa into X and Y chromosome–bearing populations based on their relative DNA differences.[2,3] A sorting purity of 94% for X chromosome–bearing spermatozoa was achieved. However, the slow sorting rate of 300 to 700 cells indicates that improvements could still be made before gender-sorted sperm can be used in future

AI programs. Using gender-sorted sperm might help in boosting critically small captive rhinoceros populations by producing more female offspring using X chromosome–bearing spermatozoa or adjusting skewed birth gender ratios, such as in the Indian or black rhinoceros.[1,60]

Gamete Rescue

In vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo transfer are well-established techniques for the production of embryos and as a solution to infertility in humans and domestic species. Although gamete rescue from male rhinoceroses is technically solved by different means of semen collection in vivo and postmortem epidydimal sperm extraction, the rescue of female gametes poses a bigger challenge. In the infertile rhinoceros, oocyte collection represents an option for female gamete preservation. Until recently, the collection of oocytes from deceased females has been the only source for these gametes. Successful ICSI of in vitro matured oocytes in a white rhinoceros collected postmortem was the first attempt at female gamete rescue and in vitro embryo production.[6] The repeated harvest of oocytes in live donors is a recent development. By transrectal, ultrasound-guided follicular aspiration, oocytes were collected from anesthetized live black and white rhinoceroses.[15,29] The ovaries of infertile females were superstimulated with a GnRH analogue to induce the development of a large number of follicles suitable for aspiration. Oocytes were collected by puncture of the follicles through the rectal wall under ultrasound guidance, using a long, flexible aspiration needle. The production of an embryo after IVF marked the first step toward banking and transfer of embryos produced in vitro from live oocyte donors.

Cell Lines

Skin sampling followed by in vitro culture of fibroblasts to establish and cryopreserve cell lines is another approach to preserving rhinoceros genetic diversity. The reprogramming of fibroblasts into omnipotent stem cells is one goal of cell line cryopreservation.[42]

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