ULTRASONOGRAPHY OF THE REPRODUCTIVE ANATOMY IN THE SUMATRAN RHINOCEROS (DICERORHINUS SUMATRENSIS)

N. E. Schaffer, D.V.M., Z. Zainal-Zahari, D.V.M., M. S. M. Suri, M. R. Jainudeen, D.V.M., Ph.D., and R. S. Jeyendran, D.V.M., Ph.D.

Abstract: Information on the reproductive status of the Sumatran rhinoceros (Dicerorhinus sumatrensis) can improve the captive management of this critically endangered species. Ultrasonography is an effective tool for gathering such information. This report details the successful application of ultrasonography to describe the reproductive anatomy of one male and four female Sumatran rhinoceroses at the Sungai Dusun Reserve in Selangor, Malaysia. These regularly handled and manageable animals were adequately controlled for reproductive ultrasonography by a cage facility at the reserve. In the male, rectal probe ultrasonography revealed the accessory sex glands. Imaging of the scrotum revealed fluid dilations of the testicular adnexa of both testicles. In the females, rectal probe ultrasonography revealed 1) a cervix containing a complex of luminal folds, 2) the uterus, and 3) the ovaries. The presence of several follicles on the ovaries of some females suggested normal reproductive activity. Several large cysts and tumors were evident in the uteri of three animals. Further analysis would be feasible and is necessary to confirm reproductive events and assess reproductive abnormalities.

Key words: Sumatran rhinoceros, Dicerorhinus sumatrensis, ultrasonography, reproduction.

INTRODUCTION

The Sumatran rhinoceros (Dicerorhinus sumatrensis) is a critically endangered species that needs intensive management to survive.18 With the world population reduced to approximately 500 individuals and with approximately 30 in captivity, 10 it is vital to establish the reproductive potential of the species and the fertility status of individuals to expedite and implement management programs. Reproductive potential can be assessed with ultrasonography in wild animals that are trained to tolerate restraint. With ultrasonography, the accessory sex glands and testicles and the uterus and ovaries have been examined in domestic animals and other rhinoceros species. Ultra-

sonography of the female rhinoceros reproductive tract has revealed evidence of estrous cycles, pregnancy, and abnormalities^{2,26,27} and has aided in confirming reproductive hormone levels by demonstrating normal ovarian development and pregnancy in a black rhinoceros.4 Sumatran rhinoceros females trained to stand in chutes have tolerated initial ultrasonographic examination (mentioned in a videotape, "Reproductive Anatomy and Ultrasound Images in the Rhinoceros," available from the first author).35 Although the feasibility of restraining and using ultrasonography with these animals is apparent, no previous reports are available. These examinations are needed to identify normal anatomy and reproductive events in this species.

In this report, we provide details of the use of this technique and observations made while using it on five Sumatran rhinoceroses: a young male that was immature when caught in the wild in 1988 and four females that were mature when caught in the wild between 1984 and 1988. All of the females had been sexually receptive (stood for mounting by a male) but had not become

From the Milwaukee County Zoo, Milwaukee, Wisconsin 53226, USA (Schaffer); the Northwestern University Medical School, Chicago, Illinois 60611, USA (Schaffer, Jeyendran); the Malacca Zoo, Malacca, Malaysia (Zainal-Zahari); the Department of Wildlife and National Parks, Kuala Lumpur, Malaysia (Suri); and the Department of Veterinary Clinical Studies, University Pertanian Malaysia, Kuala Lumpur, Malaysia (Jainudeen).

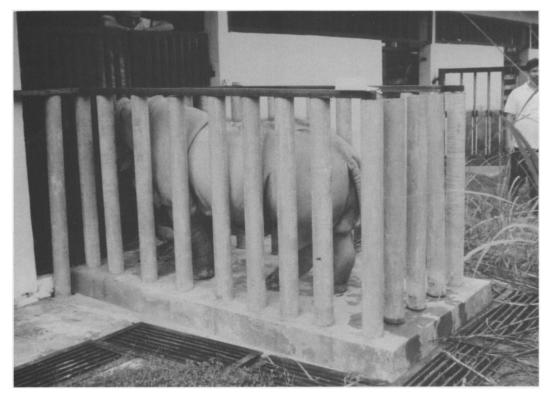


Figure 1. Chute for Sumatran rhinoceroses at Sungai Dusun Reserve, Selangor, Malaysia.

pregnant. Their estrous status at the time of examination was unknown. A 25-day estrous cycle had been observed in one female, as determined by receptivity to a male. The male had a history of phimosis, which was treated and resolved in 1989.³⁴ It had mounted the females but failed to complete intromission. Ultrasonographic examinations were performed in December 1991 and October 1992 at the Rhino Breeding Center at the Sungai Dusun Reserve in Selangor, Malaysia.

MATERIALS AND METHODS

A thorough description of this facility and the care of these animals at the Reserve has been reported.²¹ Animals were restrained for ultrasonography in a rectangular chute consisting of 24 galvanized-iron pipes (10–15 cm in diameter and 120 cm in height) that were imbedded in a cement slab base (120

× 130 × 252 cm) (Fig. 1). The spacing averaged 20 cm on the sides and 13 cm between end pipes. The three end pipes were removed from holes in the cement and reset after each animal was coaxed with fruit into the chute. The animals had been previously introduced to the chute and trained to tolerate medical examination.³³ Both the male and females were restrained in the chute and continually fed fruits and vegetables during reproductive examination that occurred either in the morning or late afternoon.

A real-time ultrasound unit (Aloka SSD-210, DXII, Aloka Co., Tokyo, Japan) with a linear array transrectal transducer (5.0 MHz) was used for ultrasonographic examinations. The transducer was hand held for external scrotal imaging and held by a plastic-sleeved, lubricated arm to image the reproductive anatomy through the wall of the rectum. One male rhinoceros was examined once in 1992, and dates for exam-

Animal	Date of evaluation			Follicles*			
		Ovarian size (mm)		Left		Right	
		Left	Right	n	Diameter (mm)	n	Diameter (mm)
F1	Dec 91	35 × 38		3	5, 13, 28		
	Oct 92	12×20		3	8, 13, 20		
	Oct 92	20×30	25×50	3	10, 20, 27	0	
F2	Oct 92	$30 \times 40 \times 60$	$10 \times 15 \times 55$	2	12, 31	1	15
F3	Oct 92	$25 \times 30 \times 50$	$30 \times 45 \times 65$	2	6, 10	5	7–29
F4	Dec 91	$22 \times 30 \times 70$	$20 \times 40 \times 65$	3	8, 10, 18	3	5, 12, 14
	Oct 92	$35 \times 45 \times 55$	$35 \times 43 \times 58$	12	6–15	1	10
	Oct 92	40×45	40×50	9	6–20	2	7, 10

Table 1. Results of the ultrasonograms of the ovary in four Sumatran rhinoceroses.

ination of the four female rhinoceroses are given in Table 1.

RESULTS

The accessory glands of the male were identified through the ventral floor of the rectum within the pelvic canal. The paired, dense bulbourethral glands were irregularly shaped, with large blood vessels coursing through the body, and lay immediately cranial to the anus under the ventrolateral wall of the rectum (Fig. 2). The prostate on lateral-sagittal cross-sectional imaging was an angular structure surrounding the neck of the bladder (Fig. 3). The paired, long, flattened vesicular glands protruded craniolaterally from between the prostate and bladder. The cross-sectional images of the glands were hypoechogenic with hyperechogenic specks (Fig. 4). The testes were in a pendulous scrotum between the hind legs caudal to the penile sheath and could be examined ultrasonographically through the scrotal skin. The long axis of the testes was horizontal. The midtesticle cross-sectional vertical images of both testicles were circular (50 mm maximum diameter), and the echotexture was homogeneous with small (2-4 mm) hyperechogenic central areas (mediastium testis). The epididymis could be palpated and was hypoechoic in comparison to the testicle. A fluctuate bulge (approx. 4×8 cm) was externally visible dorsolateral to each testicle. These bulges appeared to be nonechoic fluid accumulations of 5–8 mm between the tunica vaginalis and tunica albuginea that surrounded the head and body of each epididymis (Fig. 5).

The reproductive tract was palpated and imaged with ultrasonography in all four females. The cervix was located immediately over the brim of the pelvis usually dorsocranial to the bladder. Its ultrasonographic images were 6–8 cm in length and 4–5 cm in depth and width. The images consisted of alternating hyperechogenic and hypoechogenic vertical lines (Fig. 6).

The bicornuate uterus had a short uterine body, and the cornu appeared to be initially joined. Midcornual cross-sectional ultrasonographic images of the uterus were $20 \pm 5 \ (\bar{x} \pm \text{SD}) \ \text{mm}$ in diameter. Also, folding of the endometrium and layers of the myometrium was apparent. In the caudal cornu and body of the uterus, one female (F1) had several 2–5-mm cysts and two masses, one centrally hypoechogenic mass (15 mm) in the myometrium and one hyperechogenic mass (20 mm) in the endometrium (Fig. 7). A second female (F2) had 11 5–15-mm cysts dispersed throughout the endometrium of the uterine cornua, along with at least four

^a Follicles ≥5 mm in diameter.

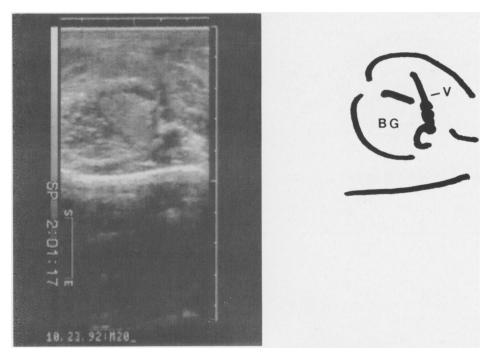


Figure 2. Bulbourethral gland of a Sumatran rhinoceros. Left. Lateral sagittal ultrasonographic image. Right. Line drawing: BG = bulbourethral gland; V = blood vessel.

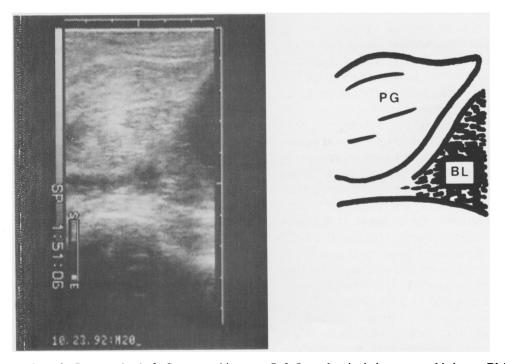


Figure 3. Prostate gland of a Sumatran rhinoceros. Left. Lateral sagittal ultrasonographic image. Right. Line drawing: PG = prostate gland; BL = bladder.

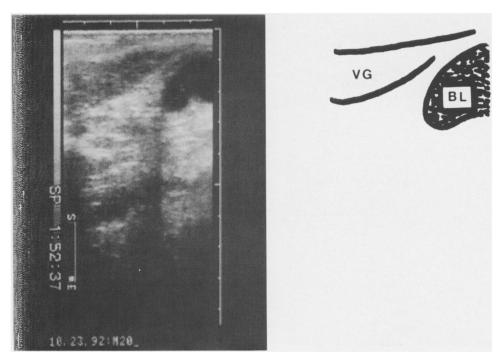


Figure 4. Vesicular gland of a Sumatran rhinoceros. Left. Lateral sagittal ultrasonographic image. Right. Line drawing: VG = vesicular gland; BL = bladder.

circumscribed masses (10-48 mm). The largest mass (48 mm) had an irregular non-echogenic center. A third animal (F3) had at least three cysts in the endometrium of the caudal uterine cornua that were 3, 5, and 6 mm in diameter.

The uterine cornua could be grasped through the rectum and followed craniolaterally to the ovaries. The ovoid ovaries were located approximately 50-55 cm from the vulva. The ovaries were 50-70 mm long, 15-50 mm wide, and 10-40 mm deep and contained circular nonechogenic structures of various sizes (Table 1). Although size and location of most of these structures suggested that they were follicles, this hypothesis was not confirmed. The first examination of rhinoceros F1 revealed two large follicles (28 and 13 mm) in the middle and a small follicle (5 mm) at one pole of the left ovary. The right ovary was not identified. A year later the left ovary had a few small follicles (<5 mm), two follicles at the posterior pole (8 and 13 mm), and one 20mm follicle at the anterior pole. The parenchyma of the right ovary was irregular, consisting of two echogenic areas, 15 mm and 20 mm, and no follicles. F2 had extensive evidence of pathology in her uterus and had a large irregular follicle (31 mm) on the left ovary adjacent to a 12-mm follicle. She exhibited only a few intermediate and small follicles on either ovary. The right ovary was determined by palpation to be smaller, with some superficial firm nodules and an approximately 15-mm follicle at the posterior pole. F3 had a large irregular follicle (29 mm) on the posterior pole of the right ovary and several intermediate and small follicles. The left ovary had two intermediate (6 and 10 mm) and several small (<5 mm) follicles. At each examination during both years, the fourth female (F4) consistently had numerous follicles of various sizes, including several < 5-mm follicles (Fig. 8). F4 also had a large (23 \times 20 mm) hy-

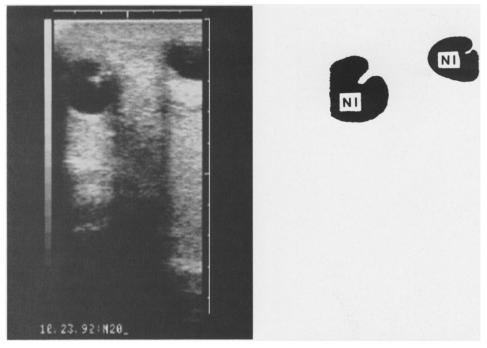


Figure 5. Epididymides of a Sumatran rhinoceros. Left. Bilateral nonechoic cross-sectional ultrasonographic images in association with the body of each epididymis. Right. Line drawing: NI = nonechoic image.

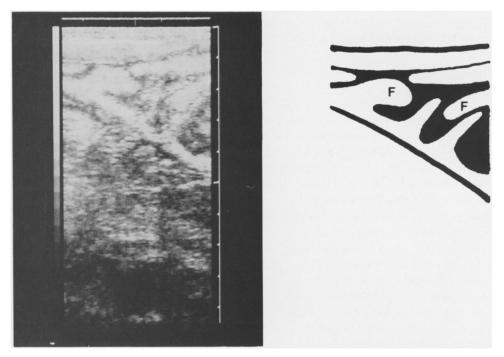


Figure 6. Cervix of a Sumatran rhinoceros. Left. Midsagittal ultrasonographic image of caudal cervix. Right. Line drawing: F = luminal fold in cervix.

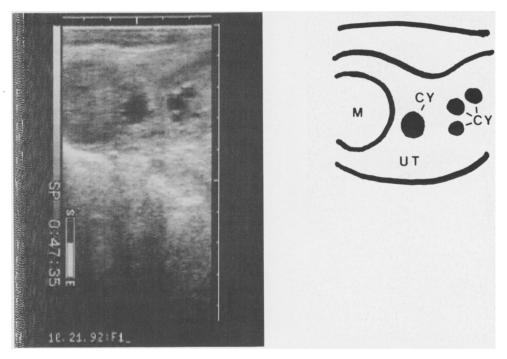


Figure 7. Uterus of a Sumatran rhinoceros, with a mass and several cysts. Left. Longitudinal ultrasonographic image. Right. Line drawing: UT = uterus; M = mass; CY = cysts.

perechogenic mass with a defined border (corpus luteum) that flattened ($15 \times 20 \text{ mm}$) in 5 days (Fig. 9).

DISCUSSION

The chute was effective in restraining these Sumatran rhinoceroses for reproductive examinations, particularly because they had become highly manageable through "psychological restraint," which consisted of light manual contacts (pushing and guiding) and voice commands.33 The chute was slightly short in height, but they did not attempt to climb out. They would also remain in the chute while the heavy pipes were replaced into holes in the cement floor. Some excessive length in the chute was corrected by a second row of removable pipes located 50 cm from one end. The animals became increasingly tolerant of procedures and even reentered the chute immediately following examination.

The accessory glands and testes of the Sumatran rhinoceros could be easily evaluated with a hand-held rectal probe. The vesicular glands were dorsolateral to the bladder and were similar to the irregular hypoechogenic lobules of the bull.³⁰ The prostate, however, was similar to the isthmus-connected bilobed gland of the stallion.19 The appearance of the prostate and the large, irregular shape of the bulbourethral gland were comparable to those of the African species of rhinoceros.26 Because detailed images are possible in the Sumatran rhinoceros, ultrasonography can be used for examination of the accessory glands for evidence of disease.31 Ultrasonography has also been used in domestic animals to evaluate emission of semen into the posterior urethra during ejaculation,²⁹ a response that has been noted in the black rhinoceros during penile massage (pers. obs.). Because the urethra of this Sumatran male rhinoceros was apparent on ultrasonography, it is possible that seminal

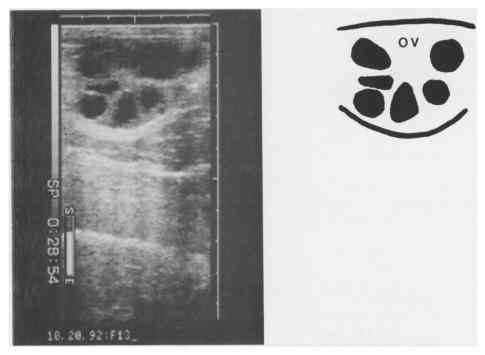


Figure 8. Ovary of a Sumatran rhinoceros, with several follicles. Left. Cross-sectional ultrasonographic image. Right. Line drawing: OV = ovary with follicles.

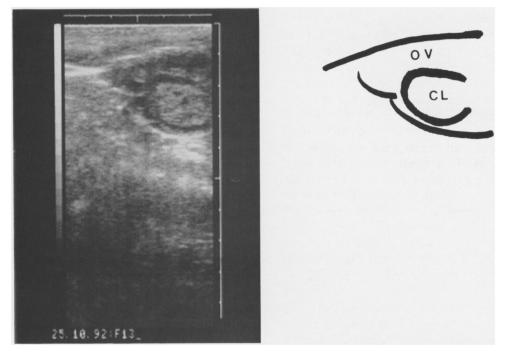


Figure 9. Ovary of a Sumatran rhinoceros, with corpus luteum. Left. Cross-sectional ultrasonographic image. Right. Line drawing: OV = ovary; CL = corpus luteum.

emissions resulting from using different collection methods could be evaluated.

The Sumatran rhinoceros testes are more visible than those of other species of rhinoceros because the scrotum is pendulous. In the African and greater one-horned Asian (*Rhinoceros unicornis*) rhinoceroses, the testes are usually hidden in the large penile sheath, especially when they are drawn more proximally towards the inguinal canal.²⁸

The echotexture and dimensions of the testicles and epididymides in this rhinoceros were similar to normal images of domestic animals.^{6,9} As in other species of rhinoceros, the stallion, and the boar, the long axis of the testicle was horizontal. Therefore, the probe had to be held vertically to image its maximum midsectional diameter. Normal testicular dimensions for Sumatran rhinoceroses are not known; however, the diameter of these testicles (50 mm) was smaller than that of the bull or boar (60–80 mm) but similar to that of the stallion (40–60 mm).

The large nonechogenic images surrounding the head and body of the epididymides that occurred at the location of the bilateral swellings appeared to be dilations of the tunica vaginalis cavities. Because only one male was observed, the condition could be normal. However, although some fluid accumulation can normally occur in the cavity, in the bull, there is usually <2 mm between tunicas.23 Genital and abdominal pathologies can cause fluid accumulation (hydrocele). This dilation may also have been due to a peritoneal hernia. In this male Sumatran rhinoceros, the dilations may be benign secondary occurrences associated with the earlier condition that caused phimosis, or they may be abnormalities possibly associated with its inability to breed. Further investigation of the swelling is needed.

The females of this species could be more safely and easily examined than the larger species of rhinoceroses. Because of their small size, the probe could be hand held as in other wild and domestic animals.^{7,8,12} In

large rhinoceroses and elephants, extensions such as a rubber hose are needed to reach the ovaries for imaging. ^{2,26} Some horses are prone to rectal perforation, and use of extensions can increase the risk. However, rhinoceroses are probably more similar to the cow and have a stronger rectal integrity; in one study, postmortem rhinoceros rectums could not be perforated even under extreme pressure.²

The ultrasonographic images of the anatomy of these female Sumatran rhinoceroses were similar to the images of the three large species of rhinoceroses^{2,26} and distinctly different from those of domestic animals.5,13,24 As in other species of rhinoceroses, the cervix of the Sumatran rhinoceros was found just over the brim of the pelvis and was closely associated with the bladder. Unlike the simple cervix of the horse, rhinoceroses have a convoluted cervix. In the three species of rhinoceroses examined, the interlocking of numerous folds of the luminal surface of the cervix results in closely associated hypoechogenic and hyperechogenic lines on ultrasonography. These lines were also apparent in these Sumatran rhinoceros females. The cornua of the uterus of the Sumatran rhinoceros course cranially as they do in other rhinoceroses, horses, and pigs. Ovaries could be palpated as well as imaged at a distance of about 55 cm cranially from the vulva. Similar to other species of rhinoceroses, inactive postmortem ovaries in one Sumatran rhinoceros were flat ovoids (B. Dresser, pers. comm.). In another female Sumatran rhinoceros, postmortem ovaries were $5 \times 2 \times 1$ cm.¹¹ In this study, the ovaries of these females were irregular in shape and differed greatly in size because of the presence of various large follicles.

Reproductive cyclicity was suggested by large populations of follicles on the ovaries of some of the females. Rhinoceros F4 demonstrated both a behavioral estrous cycle and a cohort of follicles typical of reproductively active animals. This cohort consisted of a number of follicles that varied in size along with a population of small cysts

(<5 mm). These small cysts are needed for recruiting future developing follicles in reproductively active animals. The other animals had ovaries with a few large, irregular follicles and a minimal number of small cysts. The differences between these animals may be due to individual variation, stage of cycle, suppression of cycle, or occurrence of abnormalities.

Some possible abnormalities associated with the ovary include ovarian cysts and periovarian (bursal) cysts. In ultrasonographic images, these structures are difficult to distinguish from ovarian follicles. All of these structures have been reported in rhinoceroses,27 mares,20 and cows.32 Periovarian cysts can generally be distinguished from ovarian cysts by manual manipulation. Periovarian cysts do not generally affect reproduction unless they interfere with fallopian tube function. Ovarian cysts have pathological significance in domestic animals. Further examination of these structures is needed to establish that the cysts in these animals were developing follicles.

Abnormalities of the uterus of these female Sumatran rhinoceroses could be examined with ultrasonography. Uterine cysts occurred in three of the females (Fig. 7). One possible cause of uterine cysts is cystic endometrial hyperplasia which has also occurred in other species of rhinoceroses. Two black rhinoceros (Diceros bicornis) females, one multiparous and one nulliparous, and two nulliparous white rhinoceros (Ceratotherium simum simum) females that were examined on postmortem had cystic endometrial hyperplasia (G. Foley, pers. comm.). 14 All of these females were > 20 yr old. The multiparous black rhinoceros also had ovarian pathology. Cystic endometrial hyperplasia is more common in the cow and is associated with ovarian pathology. It has also occurred in at least two nulliparous elephants, one African elephant (Loxodonta africana) with ovarian pathology (pers. obs.) and one Asian elephant (*Elephas maximus*) without.25 Luminal uterine cysts are also common in older mares1 but are not associated with ovarian pathology. These cysts do not generally interfere with pregnancy in the mare unless they become numerous.¹⁵ A few of these luminal uterine cysts were observed on ultrasonography in a female rhinoceros that subsequently conceived (pers. obs.).

Two of the females that had uterine cysts also had uterine tumors. Some infectious and noninfectious diseases can cause tumors in the uterus; tumors that have been reported in the rhinoceros include primarily leiomyomas, fibromas, and adenocarcinomas. 16,17,22,28 Adenocarcinomas occur in the uterus of domestic animals, but they are less common and more invasive than the other tumors.³ Leiomyomas and fibromas are age related in cows and have occurred in older rhinoceroses. The general physical condition of F2 suggested that it was an older animal. Age may have been a contributing factor in the development of the tumors and cysts in these animals and possibly in another Sumatran rhinoceros reported with uterine pathology.11 The etiology and prognosis of tumors and cysts in these Sumatran rhinoceroses needs further investigation because this pathology may affect fertility, and three out of the four females of this population were affected.

CONCLUSION

The management and facilities at the Sungai Dusun Reserve and the small size and tractable nature of the Sumatran rhinoceros allowed the successful use of ultrasonography. This technique was easily applied and allowed us to obtain preliminary information on fertility in these animals. Abnormalities were present in both the male and the females, but their effect on fertility remains unknown. Regular examination of these animals would help to clarify these pathologies and provide information on normal anatomy and reproductive events such as estrus and pregnancy. In addition, these techniques can be used for confirmation of the findings of other reproductive procedures, such as hormone analyses. Optimizing the use of ultrasonography in the Sumatran rhinoceros would aid in the efficient management of this species.

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