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Feasibility of Characterizing Reproductive Events in Large Nondomestic Species by Transrectal Ultrasonic Imaging

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The feasibility of using transrectal ultrasonography for imaging the in situ morphology of the reproductive tract of females of several large nondomestic and endangered species was studied. Two black (*Diceros bicornis*) and 1 white (*Diceros simus*) rhinoceros, 2 Asian (*Elaphus maximus*) and 2 African (*Loxodonta africana*) elephants, 4 banteng (*Bos javanicus*), 1 gaur (*Bos taurus*), 1 giraffe (*Giraffa camelopardalis*), and 1 bactrian camel (*Camelus bactrianus*) were examined. Real-time ultrasonic images were obtained for the following structures: 1) rhinoceros—corpus luteum, ovarian follicles, uterus, cervix, and early conceptus, 2) elephants—posterior uterus and cervix, 3) banteng and gaur—corpus luteum, ovarian follicles, uterus, cervix, and conceptus, 4) giraffe—posterior uterus, placentomes, and late conceptus, 5) camel—posterior uterus, fetal fluids, and fetal membranes. Individual ovarian follicles were identified and monitored over a 34 day observational period in 1 nontranquilized white rhinoceros. Difficulties and limitations in viewing the ovaries in the elephants were attributed to operator inexperience and to the size, positioning, and demeanor of the animals. Pregnancy was detected in 1 black rhinoceros (27 days), 1 banteng cow (48 days), the giraffe (13 months), and in the bactrian camel (approximately 3½ months). Impending embryonic loss was suspected in the banteng cow because a heartbeat was not detected in the embryo proper; the cow was subsequently diagnosed nonpregnant by transrectal palpation 20 days later. It is concluded that the ability afforded by transrectal ultrasonography to detect and measure ovarian structures and changes in morphology of the tubular genitalia and conceptus provides a research methodology for the elucidation of certain aspects of reproductive biology, and a clinical modality for reproductive management and assisted fertilization programs of large nondomestic species.

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INTRODUCTION

Prior to the introduction of real-time transrectal ultrasonography, form and function of the reproductive organs of large domestic animals (e.g., cattle, horses, llamas) were evaluated by transrectal palpation, at surgery, at necropsy, or indirectly through measurement of hormones. The dynamic relationships between form and function of the reproductive system were largely inaccessible. Real-time ultrasonography has provided a noninvasive and nonterminal method for directly imaging the in situ morphology of the reproductive organs and characterizing reproductive events (e.g., ovulation, transition of tubular genitalia from diestrus to estrus, pregnancy, embryonic loss). The technique allows frequent or continuous (e.g., for 30 minutes or more) evaluation of individual animals enabling the study of sequential relationships among structures and events. Clinical and research aspects of large-animal ultrasonography in a number of domestic species have been reviewed in the last several years [equine: Ginther, 1986; Fontjne and Hennis, 1989; bovine: Pierson and Ginther, 1988; Kastelic et al., 1988; caprine and bovine: Buckrell, 1988; llamas: Adams et al., 1989; research applications: Griffin and Ginther, 1991a].

Despite the tremendous potential of ultrasonography, extremely limited use of this technology has been employed in the study of reproduction in wild or endangered species [du Boulay and Wilson, 1988]. Knowledge of the reproductive biology of wild animals is often limited or nonexistent, and basic comparative research in this area is urgently needed. Moreover, information gleaned from such studies is critical for development of successful breeding programs and may provide the basis in captive species for development of assisted fertilization programs (e.g., artificial insemination, in vitro fertilization, embryo transfer).

The purpose of the present study was to determine the feasibility of transrectal ultrasonography for imaging the reproductive organs of several large, captive, nondomestic species.

METHODS

Animals

Two black rhinoceroses (ages, 15 and 18 years) weighing approximately 1,200 kg each were examined once at the St. Louis Zoological Park in St. Louis, Missouri. Both rhinoceroses were immobilized with an intramuscular injection of etorphine (3 mg) and xylazine (25 mg). One white rhonoceros (age, 19 years) weighing 1,800 kg was examined daily for 10 days and every other day thereafter for 24 days at the Henry Vilas Park Zoo in Madison, Wisconsin. The rhinoceros was restrained without tranquilization in a large metal chute (Fig. 1) built prior to the present study. Over a period of 24 months, the female was required to walk through the chute daily to get outside, and by providing feed, she was conditioned on a weekly or monthly basis to stand quietly in the chute. With this prior conditioning she responded well to daily confinement in the chute for transrectal examination.

Two Asian elephants (ages, 19 and 22 years) weighing 2,700 kg and 3,100 kg, respectively, were examined at the St. Louis Zoological Park, and 2 African ele-

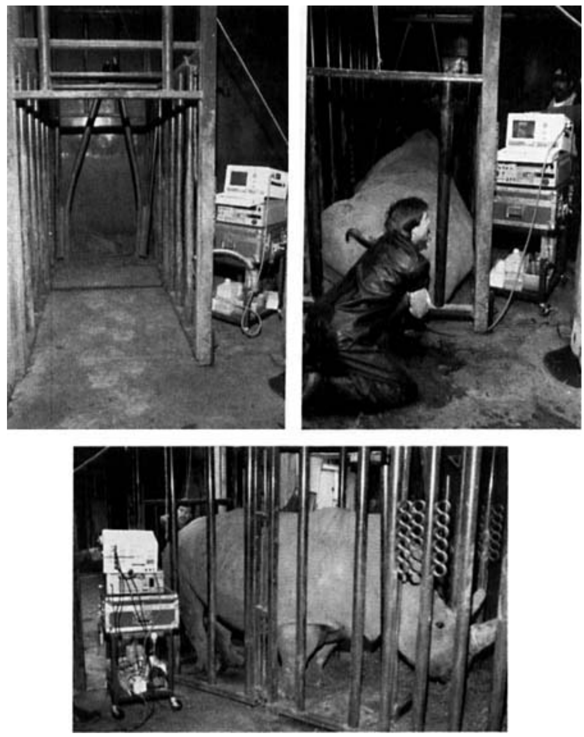

Fig. 1. Restraining chute and examination of a nontranquilized white rhinoceros. The female's position alternated between standing and sternal recumbency during transrectal ultrasound examinations.

phants (ages, 27 and 36 years) weighing approximately 3,300 kg and 3,600 kg, respectively, were examined at the Henry Doorly Zoo in Omaha, Nebraska. The elephants were restrained with leg chains and were trained by the zookeeper to remain stationary; they did not require tranquilization.

Four banteng cows (ages, 2 to 17 years) weighing between 300 and 400 kg were examined at the St. Louis Zoological Park. The banteng were restrained in a conventional cattle chute and did not require tranquilization. One gaur cow (age, 4 years) weighing 500 kg was examined after being immobilized with an intramuscular injection of etorphine (11 mg), xylazine (150 mg), and ketamine (300 mg) and placed in lateral recumbency at the St. Louis Zoological Park.

One female giraffe (age, 14 years) was examined at the Henry Doorly Zoo. She was restrained without tranquilization in a semicircular pen between 2 large swinging gates.

One female bactrian camel (age, 12 years) weighing 780 kg was examined after being immobilized with etorphine (4 mg) and xylazine (120 mg) and placed in sternal recumbency at the St. Louis Zoological Park.

Examinations

Ultrasound examinations were made with the use of a real-time B-mode scanner equipped with either a 5.0 MHz linear-array or a 3.5 MHz convex-array transducer

(Aloka 500, Corometrics Medical Systems Inc., Wallingford, CT) or a 7.5 MHz linear-array transducer (Tokyo Keiki 1000, Products Group International, Denver, CO). A plastic sleeve was placed over the examiner's arm and after manual removal of the feces the hand-held transducer was inserted into the rectum and directed over the reproductive tract as described in the mare [Ginther, 1986] and cow [Pierson and Ginther, 1988]. Structures (corpus luteum, follicles, uterus, cervix, conceptus) were identified on the basis of morphology, location, and similarity to ultrasonic images of such structures in large domestic species. The heights of the ultrasonic images of the cervix, uterine body, uterine horns, and ovarian structures (follicles and corpora lutea) were measured by using the integrated electronic calipers. In the white rhinoceros, individual follicles were identified and monitored as described previously in cattle [Knopf et al., 1989]. Ultrasound images were recorded by a 3/4 inch video cassette recorder (Sony U-Matic SP VO 9600, Sony Corp., Itasca, IL) connected to the ultrasound scanner. Still photographic images were made with a 35 mm camera from a high-resolution monitor during review of the videotapes. Rectal probe extensions (lengths, 70 and 140 cm) made of hollow plastic tubing (inside diameter 3.4 cm; outside diameter 4.2 cm) were prepared in the event that the cranial portion of the reproductive tract could not be reached in any of the species by using the examiner's arm alone. For an extension in the nontranquilized white rhinoceros, a semirigid length of nasogastric tubing (length, 30 cm; inside diameter, 1.3 cm; outside diameter, 2 cm) was cut along its length and placed around the transducer cord and taped in place just behind the transducer head.

RESULTS

Rhinoceroses

The cervix, uterine body, and uterine horns of both immobilized black rhinoceroses were imaged by using a 5 MHz linear-array transducer without an extension. The right ovary of each animal was also imaged but the left ovary was not visualized. One female was in left lateral recumbency and the other was in sternal recumbency during examination. Abdominal viscera, too heavy to displace, covered the left ovary in the rhinoceros which was in left lateral recumbency. The reproductive tract was more easily examined in the sternally recumbent rhinoceros due to a more normal orientation and suspension within the abdominal cavity. However, the animal's hind legs were tucked cranially under the abdomen, causing the perineal region to face ventrally and nearly contact the floor. The examiner was required to assume a supine position and reach dorsally and cranially in the rectum. The examiner's position precluded access to the left ovary. The use of an extension was of no benefit in the immobilized rhinoceroses. Actual scanning time was about 12 minutes for each black rhinoceros. The cervix and uterus of the nontranquilized white rhinoceros were accessible by using the operator's arm alone, but the ovaries could not be reached without the aid of an extension. The demeanor of the white rhinoceros was variable but she permitted complete examinations, including both ovaries, during all but 2 of the 22 examinations. Her position alternated between standing and sternal recumbency within and among examinations; both positions were satisfactory for using the transrectal approach (Fig. 1).

The morphologic appearance of the reproductive tract was similar among the 3 rhinoceros females (Fig. 2). The cervix, viewed in sagittal section, had bands of

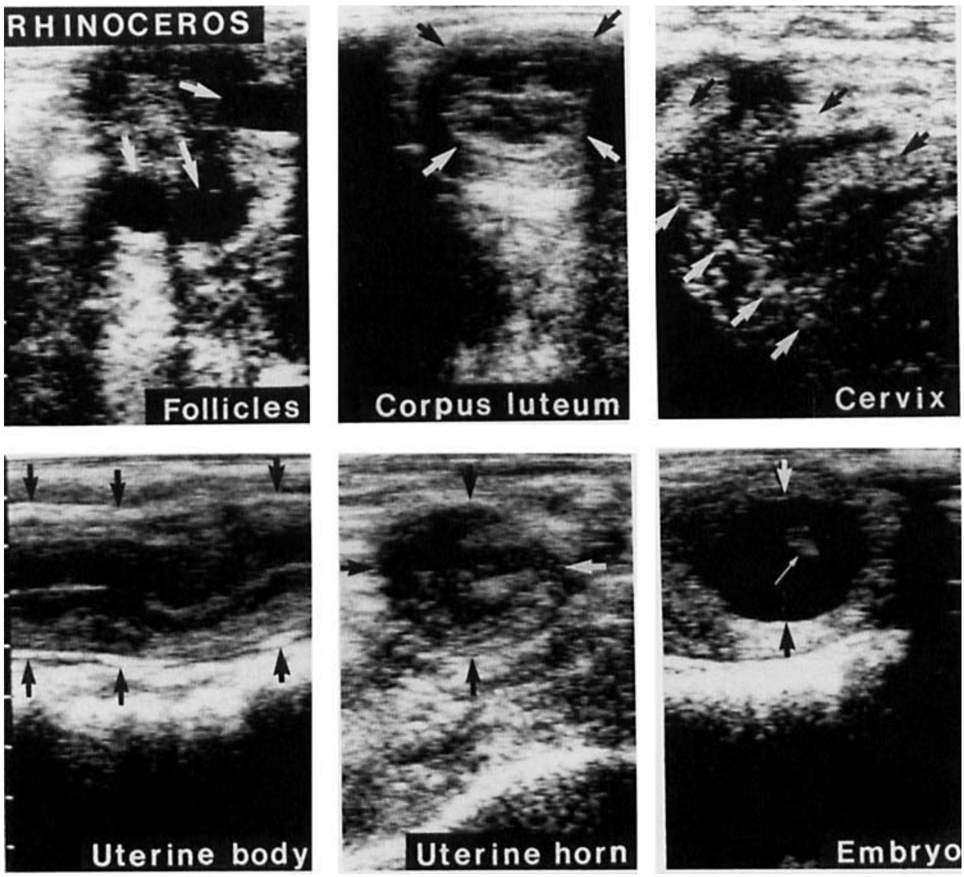

Fig. 2. Ultrasonic images of the internal genitalia of the female rhinoceros obtained by using a 5 MHz linear-array transducer. Arrows point to, or delineate, indicated structures. The cervix viewed in sagittal section (white arrows indicate lower border) had roughly vertical echogenic bands (black arrows). The uterine body was viewed in sagittal section and the uterine horns in transverse section. The approximately 27 day embryonic vesicle (arrows) containing the embryo (small arrow) was spherical and located at the base of the uterine horn near the corpus-cornual junction. The cranial direction is to the right. The graduation marks on the left margin indicate 1 cm increments.

intense echogenicity roughly oriented in a dorso-ventral plane giving the impression of annular rings typical of the bovine and llama cervix. The diameter of the cervix was between 4 and 6 cm. The uterine body was viewed in sagittal section and the horns in transverse section. The vertical diameters of the uterine body and the uterine horn near the corpus-cornual junction were between 3.5 and 4.0 cm. A roughly spherical (24 × 30 mm) accumulation of fluid was detected at the base of the left uterine horn near the corpus-cornual junction in the younger black rhinoceros. Further examination revealed the presence of an embryo proper with a heartbeat (Fig. 2). The embryo proper was 13 mm in length. The endometrial echotexture was a relatively homogeneous light gray, and the right ovary contained a follicle 28 mm in diameter and a single corpus luteum 30 mm in diameter (Fig. 2). In the older black rhinoceros, no fluid was detected in the lumen of the uterus. Endometrial echotexture was

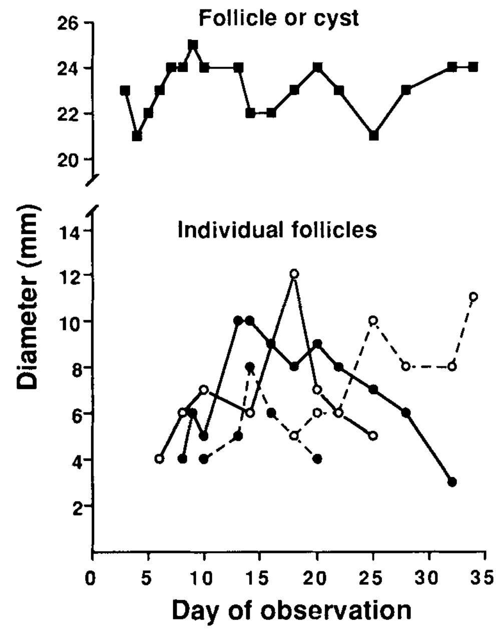

Fig. 3. Diameter profiles of the 2 largest follicles in each ovary and a large follicle or cyst in, or immediately adjacent to, the left ovary in a white rhinoceros over a period of 34 days (solid line = left ovary; dashed = right ovary).

moderately heterogeneous; some light gray areas were interspersed with dark or nearly black areas giving the impression of mild to moderate accumulation of interstitial fluid. The 3 largest follicles detected in the right ovary were 17, 15, and 12 mm in diameter; several smaller follicles between 3 and 7 mm in diameter were also present (Fig. 2). A corpus luteum was not detected in the right ovary. In the white rhinoceros, no intrauterine fluid was detected and endometrial echotexture remained homogeneous light gray throughout the 34 day observational period. Changes in the diameter of the largest and second-largest follicle in each ovary are shown in Figure 3; follicles ranged in diameter from 4 to 12 mm. A large follicle or cyst was detected in, or immediately adjacent to, the left ovary at each examination; diameter of the structure ranged from 21 to 25 mm (mean, 23.1 mm) over the observational period (Fig. 3). The number of follices >4 mm in diameter detected in both ovaries ranged from 4 to 11 (mean, 6.9). A corpus luteum was not detected in either ovary.

Elephants

Three of the 4 elephants remained in the standing position during examination and an elevated platform or a stepping-stool (approximately 40 cm high) was required for the operator to adequately reach the rectum. By using a 5 MHz linear-array transducer with a 140 cm extension, the cervix and caudal portion of the uterine body were visualized in 1 of the 2 standing African elephants, but the reproductive tract of the other was not satisfactorily imaged with or without a rectal probe extension. The demeanor of the standing Asian elephant precluded adequate examination. The smaller Asian elephant maintained a kneeling position (olecranons and stifles resting

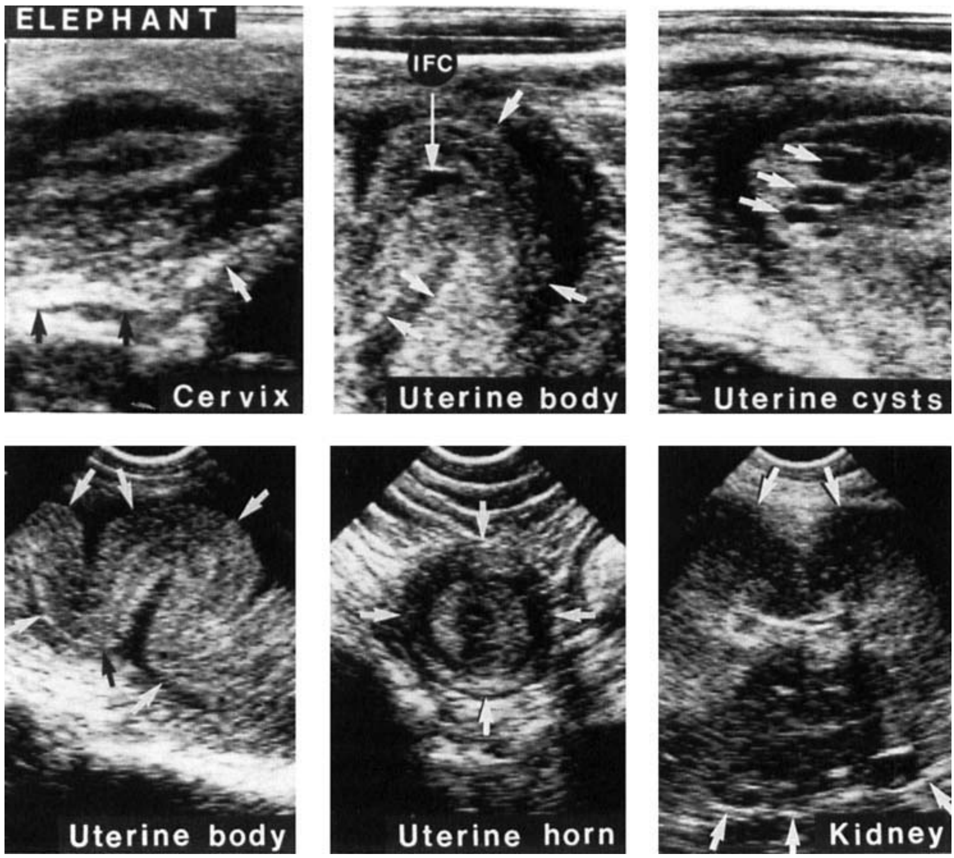

Fig. 4. Ultrasonic images of the cervix, uterus, and kidney in the elephant obtained by using a 5 MHz linear-array (**upper panel**) and a 3.5 MHz convex-array (**lower panel**) transducer. Arrows point to, or delineate, indicated structures. Cervix and uterine body were viewed in sagittal section. Note the intrauterine fluid collection (IFC) in the upper middle image. The cranial direction is to the right.

on the floor) during examination. The rectum was at a comfortable height (approximately 140 cm) for the standing operator and neither an elevated platform nor a rectal probe extension was required to image the cervix, uterine body, and uterine horns.

Examination of the kneeling elephant was initially done with a 5 MHz linear-array transducer (Fig. 4) and subsequently with a 3.5 MHz convex-array transducer (Fig. 4). The cervix and uterine body were approximately 5 cm in diameter; the junction between the cervix and uterine body was not well delineated. The uterine body, viewed in sagittal section, appeared to be thrown into serpentine folds (Fig. 4). A small (5 × 20 mm) intraluminal fluid collection was observed in the uterine body (Fig. 4) and several small fluid-filled structures resembling uterine cysts were seen in the caudal uterine body (Fig. 4). Cross-sectional images of the uterine horns were seen by moving the transducer cranially and laterally from the uterine body (Fig. 4). The horns were followed to the tip and beyond in an attempt to visualize the ovaries. An organ, believed to be the left ovary, was visualized, briefly, adjacent to the caudal aspect of the left kidney but a clear image was not obtained. The apparent ovary

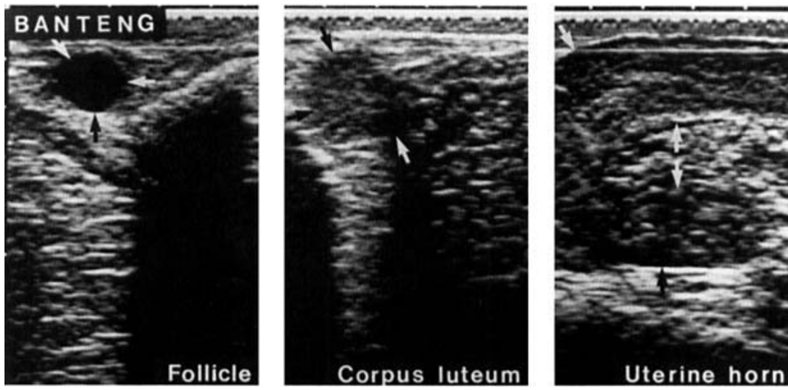

Fig. 5. Ultrasonic images of the internal genitalia of the banteng obtained by using a 7.5 MHz linear-array transducer. Arrows delineate indicated structures. Note the curled shape of the uterine horn (sagittal section). The cranial direction is to the left.

appeared to be acoustically shadowed perhaps by a loop of bowel, uterine horn, or ovarian bursa. The right ovary was not visualized. The ultrasound examination took 14 minutes and was well tolerated by the elephant. The higher-frequency transducer (5 MHz linear-array) provided more detail, but size and overall configuration of the uterus were more easily appreciated with the lower-frequency transducer (3.5 Mhz convex-array). In addition, the convex-array transducer afforded more flexibility in directing the ultrasound beam; the beam could be directed cranially and provided images of structures beyond the arms-length reach of the operator (e.g., ovary and kidney).

Banteng and Gaur

The entire reproductive tract of all 4 banteng cows and the gaur cow was readily imaged by using a 5 MHz linear-array transducer without an extension. Critical measurements of the cervix, uterus, and ovarian structures were not made in all cows but overall size and shape were similar to those reported for holstein heifers [Pierson and Ginther, 1987]. Cervical diameters ranged from 2.5 to 3.5 cm, and uterine body and horn diameters (near corpus-cornual junction) ranged from 2.0 to 3.0 cm. The uterine horns were curled in a ram's-horn-like arrangement (Fig. 5). Follicles from 3 mm to 13 mm in diameter were imaged (Fig. 5). A corpus luteum was detected in 3 of the 4 banteng and in the gaur; luteal diameters ranged from 16 to 23 mm (Fig. 5). One banteng cow which was artificially inseminated 48 days prior to examination had intrauterine fluid extending through the right and part of the left uterine horns. The largest luminal fluid diameter (22 mm) was at the caudal one-third of the right uterine horn corresponding to the location of an apparent embryo proper (length, 9 mm) (Fig. 6). An embryonic heartbeat was not detected and embryonic loss was anticipated. A 23 mm corpus luteum was detected in the right ovary. The cow was subsequently confirmed nonpregnant by transrectal palpation 20 days after ultrasound examination (68 days post-insemination). The banteng cow in which a corpus luteum was not detected had a 13 mm follicle in the right ovary, pronounced uterine tone, and a dark endometrial echotexture.

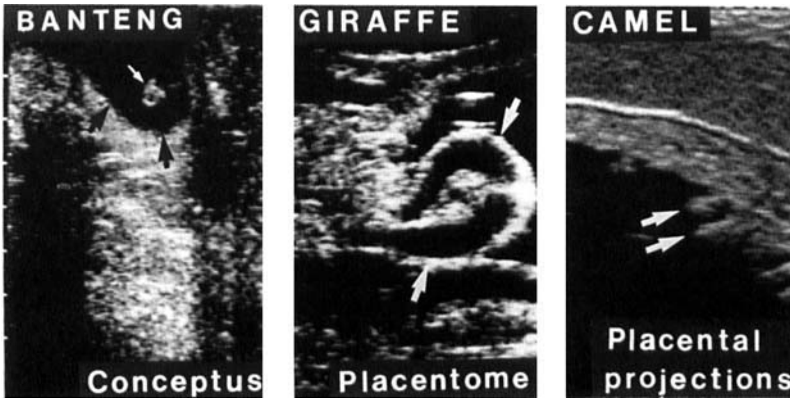

Fig. 6. Ultrasonic images of pregnancy in the banteng (48 days), giraffe (13 months), and bactrian camel (3½ months). Black (nonechogenic) areas represent fetal fluids within the uterus, and arrows point to indicated structures. The conceptus in the banteng did not have a heartbeat and was subsequently lost.

Giraffe

The giraffe maintained a standing position and the cervix, placentomes, and fetal limbs were imaged by using a 5 MHz linear-array transducer without an extension. The operator sat on an elevated bar (approximately 150 cm high) that was part of the chute. The giraffe had been bred naturally about 13 months before examination. The distal long bones of the fetal limbs completely reflected the ultrasound beam. Numerous structures, presumably placentomes, were seen and those near the cervix were 2.5 to 3 cm in diameter (Fig. 6). Because of the advanced stage of gestation, no attempt was made to image the ovaries. Scanning time was approximately 10 minutes and was well tolerated by the giraffe. She subsequently gave birth to a live baby 65 days after examination.

Bactrian Camel

A diagnosis of pregnancy was made in the sternally recumbent camel by using a 5 MHz linear-array transducer without an extension. The fetus was apparently too deep to be visualized but fetal membranes in the fluid-filled uterus were detected. The inner surface of the placenta appeared to have short, slender, finger-like projections or folds (Fig. 6). She had been bred approximately 3½ months before examination and subsequently gave birth to a live male baby 256 days after examination.

DISCUSSION

Prior to the present study, we received rectal tissue from 1 black and 1 white rhinoceros and 1 Asian elephant after they had been euthanatized for medical reasons; the excised rectums were used to examine the risk of perforation in using the transrectal approach. The tissues were received on ice within 24 hours of euthanasia and were tested for strength by attempting to force a rectal probe through the wall. Up to 60 lb of pressure was applied in 3 different places on the tissue of each animal and in all instances but 1 the tissue was resistant to the force. In the single exception, an area began to tear after 45 lb of pressure was applied; however, this area was very near a place that appeared to have been cut when the tissue was removed. We concluded that rectal tissues from the rhinoceros and elephant adequately resisted perforation and the transrectal approach posed minimal risk to the animals.

An important objective of the present study was to determine the accessibility of ovaries in the elephant and rhinoceros by the transrectal approach. Detailed monitoring of individual follicles and luteal development has been accomplished with transrectal ultrasonography in horses [Bergfelt et al., 1989; Sirois et al., 1989; Ginther, 1990], cattle [Pierson and Ginther, 1988; Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989], and llamas [Adams et al., 1990, 1991]. One ovary in each of the black rhinoceroses and both ovaries in the white rhinoceros were successfully imaged in the present study. Inability to visualize the second ovary in the immobilized black rhinoceroses was attributed to the animals' awkward positioning and not to the depth of the reproductive tract. In the larger nontranquilized white rhinoceros, the ovaries were not accessible without the use of a rectal-probe extension. The white rhinoceros was nulliparous despite having been housed with a male for 15 years; no breedings have been observed. Reproductive cyclicity was not detected in the white rhinoceros; follicles did not exceed 12 mm in diameter; ovulation and a corpus luteum were not detected; and endometrial echotexture did not change during the 34 day observational period. The large fluid-filled structure in, or immediately adjacent to, the left ovary may have been a static follicle, a follicular cyst, or a paraovarian cyst; apparent changes in diameter likely represent measurement error. Further observation would be required to determine if the female was under seasonal, age, or pathologic influences. Although satisfactory images of the elephants' ovaries were not obtained, an image was obtained that appeared to be of the left ovary; the image was disrupted by shadow artifact. The apparent ovary was adjacent to the caudal aspect of the kidney, which is consistent with necropsy specimens described by Perry [1963]. Identification of the kidney was based on the ultrasonic morphology of kidneys in horses (Fig. 4). Apparent visualization of the kidney indicated that adequate depth had been achieved during examination. The ovarian bursa or ovarial sac [Perry, 1953] is a large, well-developed structure in the elephant which apparently envelops the entire ovary, and it may have been responsible for acoustically shadowing the ovary. Difficulty in detecting the ovaries in the other elephants was attributed to operator inexperience, improper choice of transducer, and the size, position, and restraint of the animal. The best images were obtained in the kneeling elephant by using a 3.5 MHz convex-array transducer without an extension. In retrospect, the 5 MHz linear-array transducer may not have provided adequate tissue penetration. One standing elephant swayed from side to side and pushed her tail against the operator's shoulder, making examination difficult.

Determining the accessibility of the tubular genitalia was another important aspect of the present study. The dynamic morphology of the cervix and uterus has been studied by transrectal ultrasonography in the mare [Ginther, 1986; Griffin and Ginther, 1991b], cow [Pierson and Ginther, 1987], and llama [Adams et al., 1989]. Changes in uterine shape, horn diameter, contractile activity, and endometrial echotexture have been characterized in relation to changes in the ovarian cycle (luteal phase vs. follicular phase) and, in effect, provide an instant indicator of the prevailing hormonal status (progesterone or estrogen dominance). The cervix and uterus of all 3 rhinoceros, 2 of 4 elephants, all 4 banteng, and the gaur were accessible and satisfactorily imaged in the present study. The dark heterogeneous endometrial echotexture observed in the nonpregnant black rhinoceros compared to the pregnant rhinoceros, and in the banteng cow in which a corpus luteum was not detected, was attributed to the presence of increased interstitial fluid—similar to what has been described in other species during estrus. The serpentine or accordion-like folding of the uterine body of one Asian elephant was curious and may have been due to compression by the abdominal viscera as a result of the kneeling posture. The importance of intrauterine fluid collections in the elephant is not known but such collections are common in the mare during estrus and indicative of endometritis if present during diestrus [Adams et al., 1987]. Although uterine cysts in the elephant have been briefly described by direct examination at necropsy [Balke et al., 1988], the importance of these structures in elephants is unknown; they are common and age-related in mares [Adams et al., 1987]. If imaging the ovaries of the elephant remains problematic, ultrasonically detected changes in the cervix and uterus alone may provide important information regarding estrous cyclicity in this species.

Diagnosis of pregnancy and study of the conceptus is one of the most obvious applications of ultrasonography and was among the first to be developed in domestic horses [Palmer and Draincourt, 1980] and cattle [Pierson and Ginther, 1984]. The transabdominal approach has been used to diagnose pregnancy in bottle-nose dolphins [Williamson et al., 1990], fallow deer [Mulley et al., 1987], and several other wild ungulates [du Boulay and Wilson, 1988]. The transrectal approach has been described in red deer [Bingham et al., 1990], but application of this technique for imaging the tubular genitalia of other large nondomestic species apparently has not been reported. In the present study using the transrectal approach, pregnancy was detected in a black rhinoceros, a banteng cow, a giraffe, and a bactrian camel. Detection of an embryo in the rhinoceros was not anticipated before the examination; subsequent review of the breeding history revealed that her last breeding had been 27 days before the day of examination. She had been bred 6 times previously at intervals of 18 to 27 days (mean, 22.3 days) and was considered a fertility problem. Although these intervals may represent the length of the estrous cycle of this individual, it is not known whether pregnancy followed by embryonic loss occurred after any of the breedings. The ultrasonic appearance of the rhinoceros embryo was remarkably similar to that of the equine embryo [Ginther, 1986]. The embryonic vesicle was clearly spherical (not elongate) and was at the base of the uterine horn near the corpus-cornual junction contralateral to the side of the corpus luteum. The rhinoceros and the horse both belong to Perrisodactyla and perhaps share similar reproductive processes. The ultrasonic appearance of the embryo detected in the banteng cow was similar to that of the domestic cow [Kastelic et al., 1988]. The embryonic vesicle had elongated sufficiently by 48 days post-insemination to occupy both uterine horns, and an embryo proper without a heartbeat was detected, indicating impending embryonic loss. The banteng cow was part of an assisted fertilization program being developed at the St. Louis Zoological Park and was determined to be nonpregnant by transrectal palpation at 68 days post-insemination. These 2 examples illustrate the potential usefulness of transrectal ultrasonography for breeding management, pregnancy diagnosis, and conceptus monitoring in captive, nondomestic species.

Results of the present study indicate that imaging the internal genitalia of large nondomestic and endangered species is feasible by transrectal ultrasonography. With this technique, images of the reproductive tract of female black and white rhinoceroses, Asian and African elephants, banteng, gaur, giraffe, and bactrian camel were obtained. Limitations of the technique involved operator inexperience and the size, positioning, and demeanor of the subject. The operator had extensive experience with ultrasonography in cattle, horses, and llamas, but had no previous experience in scanning the species studied herein. Moreover, the animals had little or no previous conditioning to transrectal ultrasonic examination. With more experience and animal conditioning, transrectal ultrasonic imaging may provide the window through which we can observe reproductive events and gain badly needed information about the basic reproductive biology of captive nondomestic species.

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