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Ultrasonography of the Urogenital Tract in Elephants (*Loxodonta africana* and *Elephas maximus*): An Important Tool for Assessing Male Reproductive Function

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The success rate of captive elephant breeding programs worldwide is poor. Along with undiagnosed reproductive disorders in females and fatal diseases such as the newly discovered herpesvirus infection, male infertility now is considered a major contributing factor in the failure to maintain self-sustaining captive populations. To address questions related to male reproductive dysfunction, approximately 309 ultrasonographic assessments combined with semen collection were performed in captive (n = 10) and wild (n = 4) African (*Loxodonta africana*) and captive (n = 61) Asian (*Elephas maximus*) elephants. Bulls ranged from 4 to 50 years of age and were examined at 9 institutions in North America, 13 in Europe, 2 in Africa, and 7 in Asia. About half of the reproductive assessments were performed in protected contact situations with elephants handled in a restraint device, and half involved assessments of trained Asian bulls managed in free contact. Four wild African and two Asian elephant bulls were evaluated after receiving general anesthesia. Transrectal ultrasound was used to characterize the morphology and functionality of the entire urogenital tract, including the testes and accessory sex organs. Bulls were categorized on the basis of breeding status (breeders vs. non-breeders) and social history (i.e., type of interaction with conspecifics and keepers). Most of the bulls were non-breeders (designated Types I–

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V). Type I (n = 3 African, 6 Asian) and Type V (n = 1 Asian) were immature and castrate, respectively. On the basis of keeper evaluations, Type II bulls (n = 2, 4) were subordinate to older cows and keepers, whereas Type III bulls (n = 4, 28) were dominated by other bulls. Type IV (n = 1, 8) were older bulls of unknown history that exhibited numerous testicular pathologies resulting in poor semen quality. Non-breeding bulls included those that were exposed to females, but failed to breed, as well as those that had no opportunities to breed. Type VI individuals (n = 4, 14) were proven breeders. The percentage of observable reproductive tract pathology in adult males was remarkably low (14%), even in older bulls. However, apparent infertility of non-organic cause (i.e., not due to specific anatomical abnormalities) in these otherwise healthy bulls was high (32%). Semen quality varied markedly in ejaculates collected from the same bull, as well as from different bulls. In conclusion, although many of these bulls could serve as semen donors for natural mating or artificial insemination, the inconsistent production of good-quality ejaculates raises questions as to the reliability of these individuals to participate in breeding programs. The apparent inhibitory effect of suppressive social interactions on reproductive potential also needs to be investigated. Ultrasound examinations combined with semen collection should be conducted periodically to estimate the reproductive value of each bull and determine whether altered management strategies are needed to enhance captive breeding. Zoo Biol 19:333–345, 2000.

Key words: male elephant; reproductive anatomy; ultrasonography; breeding evaluation; accessory sex glands; sperm; testis

INTRODUCTION

Infertility in bull elephants is having a substantial negative impact on the reproductive success of the captive population, perhaps more so than the problems observed in cows [Hildebrandt et al., 2000b] because of the skewed sex ratio. In North America, African elephant (*Loxodonta africana*) bulls comprise only 10.2% and Asian elephant (*Elephas maximus*) bulls only 13.4% of the Species Survival Plan (SSP) population. Moreover, of these small percentages, only a few individuals are siring offspring. Our understanding of the basic biology of male elephants is limited; thus, it is crucial to begin developing a database of reproductive information that can be used to identify and mitigate breeding problems. Ultrasonographic evaluation of bull elephants has become a valuable tool for assessing sexual maturation [Hildebrandt et al., 1998b, 1999; Fritsch et al., 2000], detecting reproductive pathologies [Hildebrandt et al., unpublished data], describing the ejaculatory process [Hildebrandt et al., 1997a,b], and studying the reproductive function of musth [Blottner et al., unpublished data; Pucher et al., unpublished data].

In captive elephants, there appear to be fewer reproductive-tract pathologies occurring in males than in females, suggesting that captivity does not have as significant an impact on the health of male sexual organs as it does on the female reproductive tract [Hildebrant and Göritz, 1998a; Hildebrandt et al., 2000b]. Although males exhibit fewer anatomical pathologies, they nevertheless exhibit a higher rate of reproductive failure caused by a lack of libido or sub-optimal sperm quality. There are unpublished speculations that some causes of reproductive dysfunction could be related to social suppression by dominant bulls, cows, or even animal handlers. Also, the reproductive consequences of exposing bulls to musth-controlling drugs or disease-fighting agents are not known. Regular ultrasonographic examinations combined with semen collection are key to identifying causes of suspected non-organic causes of infertility. Once causes are recognized, it may be possible to take corrective actions to restore fertility. This article describes the basic reproductive tract anatomy and characteristic morphology of bulls that appear to be reproductively normal (i.e., potentially fertile) compared to those with suspected reproductive problems (i.e., suspected or proven infertile). This information can be used as a guideline for elephant specialists to identify which bulls should be considered for breeding programs.

METHODS

Animals

During the last 6 years, approximately 309 ultrasonographic examinations were performed in 10 captive and 4 wild African and 61 captive Asian male elephants by the Institute for Zoo Biology and Wildlife Research (Berlin). The age ranged from prepubertal (~4 years) to ~50 years of age. The bulls examined belonged to institutions in North America (n = 9), Europe (n = 13), Africa (n = 2), and Asia (n = 7). The examination of four wild African and two captive Asian elephants was performed with the animals in lateral recumbency under general anesthesia. The rest of the assessments were performed in nearly equal numbers in either protected contact situations with the elephant in a restraint device, or in free contact (trained Asian bulls).

An important prerequisite for good-quality imaging was intensive cleaning of the rectum (~2.5 m of the caudal intestine) before assessment. Feces were removed manually and the rectum was irrigated with lukewarm water from a hose with a smooth tip (diameter 1–2 cm) at a flow rate of 5–15 L/min. The hose and rectal gloves of the examiner were lubricated with commercial ultrasound gel. Generally, bulls that accepted direct contact with examiners were more tolerant of rectal palpation than females were. However, the tail of an adult bull elephant is stronger than that of a female and can break the arm of an examiner. Accordingly, a second person should hold the tail during examination if the bull will tolerate it. Immature bulls reacted strongly to their first rectal palpation and tended to swing their feet from side to side, which can be dangerous. In this study, most bulls were habituated to rectal palpation by keeper staff at least 2 weeks before the first ultrasonographic evaluation. As a safety precaution, there always was one elephant handler at the head and two handlers at the tail during each ultrasound evaluation. Positive reinforcement was provided by feeding favored foods during the procedure.

Ultrasonography

During the course of the study, two ultrasound systems were used. One involved a portable B-mode ultrasound unit (Oculus CS 9100, Hitachi, Physia GmbH, Neu-Isenburg, Germany) for routine evaluations and a stationary color flow Doppler system (HDI 1000, ATL Inc., Seattle, WA) for maturation studies associated with puberty assessment. Most examinations were performed with the B-mode ultrasound scanning system equipped with a 3.5-MHz convex, 5.0-MHz microconvex, and a 7.5-MHz linear transducer. The color flow system was equipped with 4-2 MHz convex array and an 11-9 MHz linear array transducer. In addition, systems included special features, such as probe extenders, cable extensions, monitor helmet or video glasses as described previously [Hildebrandt and Göritz, 1994, 1995; Hildebrandt et al., 1997a, 1998b, 2000a]. The transducers were waterproofed with silicone seal, and the ultrasound machines were protected with plastic during the examinations.

The evaluations started with a transcutaneous ultrasound scan of the bulbourethral glands and the root of the penis, performed using a low-frequency transducer. The outer skin was washed before the application of ultrasound gel to improve the penetration of the ultrasound waves through the thick skin. The rest of the internal urogenital tract was evaluated transrectally using a variety of probes. All ultrasound examinations were recorded using a high-quality VCR, such as S-VHS or digital, for review. Each ultrasound examination took no more than 15 minutes to complete.

Semen Collection

After the ultrasound evaluation, semen was collected using one of three collection techniques (electroejaculation, post-mating collection, manual stimulation), depending on the status of the bull. Four bulls had to be anesthetized (three wild African, one captive Asian) and were electroejaculated using a newly developed transrectal probe [Hildebrandt et al., 2000a]. Two "hands off" males (one African, one Asian) were assessed by collecting ejaculate that dripped out of the female genital tract to the ground after mating. All other bulls (n = 59) were manually stimulated using the method described by Schmitt and Hildebrandt [1998]. Immature bulls generally were stimulated transrectally, but the total volume of ejaculate was low (Table 1).

Urine contamination can be a problem if the collection is not properly conducted and will result in samples containing dead sperm. To avoid urine contamination, the following steps should be carried out during manual-manipulation collections. Initial rectal stimulation should be performed carefully until the bull urinates spontaneously. This occurred in 95% of the collections before the actual semen collection. The penis should then be washed with clear, tepid water and dried with a sterile towel. Rectal stimulation should be performed always in response to the reaction of the bull. Overstimulation (too much fist pressure and fast movements) can easily cause urine contamination. The person collecting the semen has to communicate with the person performing the stimulation and remove the collection vessel quickly if there are indications of forthcoming urination (e.g., rectal contraction or reduction in cooperation of the bull). In general, collection bags should be changed at least five times during semen collection to avoid urine contamination. Urine contamination is easy to detect based on the difference in odor between ejaculate and urine. If a sample became urine contaminated, it was not included in the data analysis, and another collection was performed several hours or days later.

RESULTS AND DISCUSSION

Figure 1A shows a schematic diagram of the urogenital tract of a male African elephant. There were no differences in the morphology or size of the urogenital system between male African and Asian elephants, except in shape and dimension of the prostate gland [Hildebrandt et al., 1998b]. The descriptions of the structures are given as average sizes derived from adult individuals examined in this study.

Table 1 summarizes the accessory sex gland status and ejaculate characteristics of bulls classified on the basis of breeding history and social conditions (designated Types I–VI). Types I–V were not in breeding situations, and included bulls that were immature (Type I) and castrated (Type V). Mature, otherwise normal non-breeding bulls were categorized by their interactions with conspecifics and handlers using subjective behavioral profiles. Type II bulls appeared to be subordinate to older cows

TABLE 1. Accessory sex gland and ejaculate characteristics of African and Asian elephant bulls evaluated using transrectal ultrasonography and semen collection techniques[a]

Breeding activity	Bull social status and history (n)	Testis characteristics: Diameter (cm)	Testis characteristics: Integrity	Status of ampullae[b]	Status of seminal vesicles[b]	Ejaculate characteristics: Volume (ml)	Ejaculate characteristics: Sperm motility	Remarks
Non-breeders								
I	Immature bulls (3 African, 6 Asian)	>10	Ecogenic parenchyma	0–1	0	0–5	—	No semen produced; potential semen donors or breeders
II	Bulls dominated by older females, keepers or single bulls (2 African, 4 Asian)	10–16	Normal	1–3	1–2	≤40	30–90%	Not consistent semen quality; potential semen donors for AI
III	Bulls dominated by other bulls (4 African, 28 Asian[d])	10–16	Normal	1–3	1–2	≤40	0–90%[C]	Less consistent semen quality than Type II; potential semen donors for AI
IV	Older bulls with unknown history (1 African, 8 Asian)	8–12	Testicular pathology	0–1	2–3	>40	0% (mostly aspermatic)	No viable semen; spermatogenic cells irreversibly damaged
V	Surgically castrated (1 Asian)	—	—	1	0	≤5	Aspermatic	Useful only for display
Breeders								
VI	Bulls in breeding centers (4 African, 14 Asian)	10–18	Normal	3[e]	3[e]	>40	75–90%	All produced viable semen; need to prevent overrepresentation

AI, artificial insemination.

[a]Bulls were classified (Types I–VI) on the basis of breeding status (breeder vs. non-breeder) and social/history status as determined by keeper behavioral profiles.

[b]Accessory sex gland status: 0, no detectable lumen; 1, only 25% fluid content of the estimated total volume; 2, 25–75% fluid content of the estimated total volume; 3, 75–100% of the estimated total volume.

[c]Inconsistent motility rates up to 90%.

[d]Includes two bulls with chronic prostatovesiculitis.

[e]Less fluid volume if examined after mating.

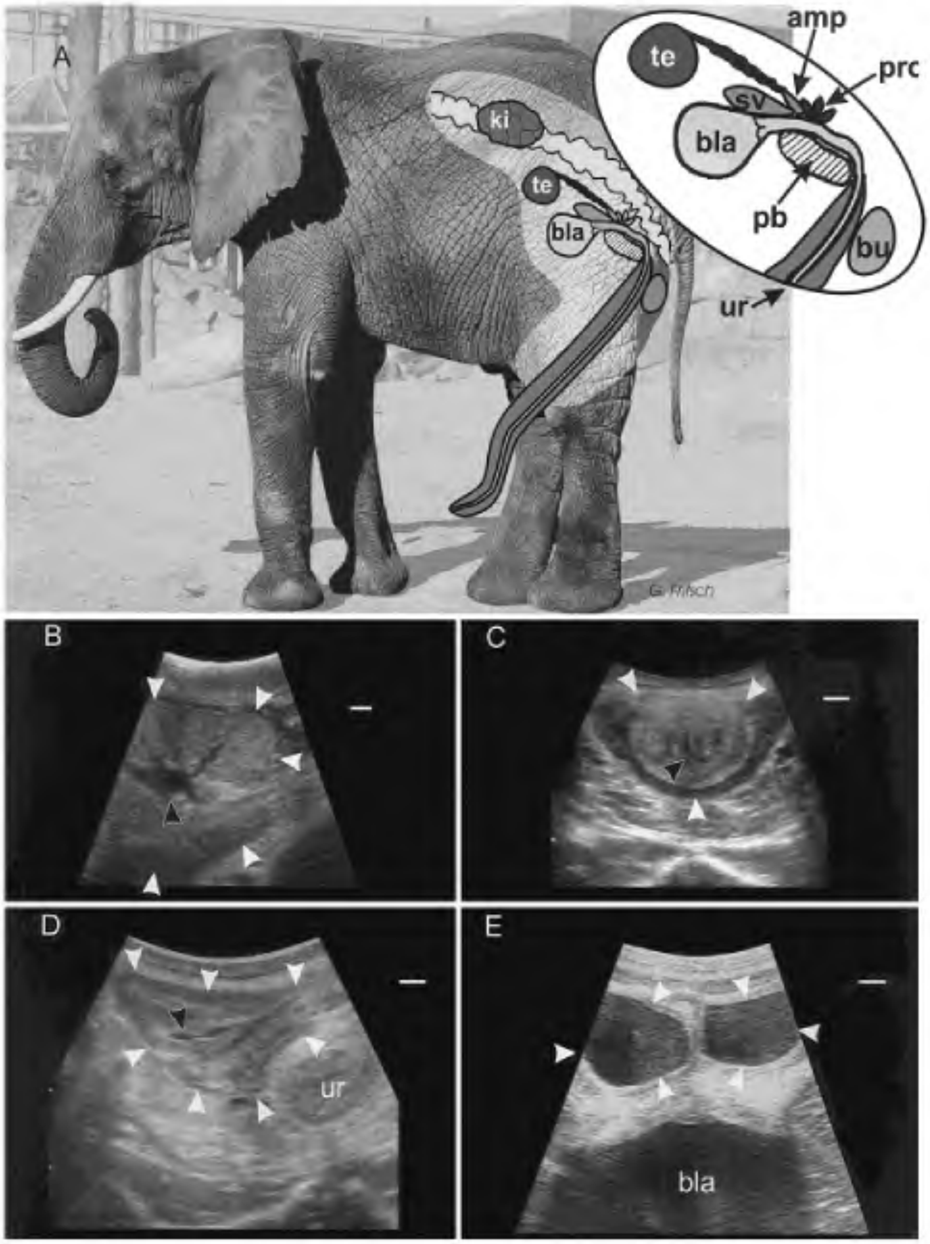

Fig. 1. **A:** Schematic diagram of the urogenital tract in a male African elephant. The magnification shows the main parts of the reproductive tract. ki, kidney; te, testis; sv, seminal vesicle; amp, ampullae; pro, prostate; bu, bulbourethral gland; bla, urinary bladder; ur, urethra; pb, pelvic bone. **B:** Sonogram (transcutaneous, 3.5 MHz) of the bulbourethral gland. White arrowheads mark the border of the gland. The black arrowhead indicates the internal cavity filled with anechoic secretion. **C:** Cross-sectional sonogram (transrectal, 3.5 MHz) of the urethra (pelvic part), with the external borders marked by white arrowheads. The urethra is located below the rectum and above the echoic pelvic bone. The black arrowhead indicates the colliculus seminalis. **D:** Cross-sectional sonogram (transrectal, 3.5 MHz) of the prostate of an African elephant, with the external borders marked by white arrowheads. The prostate of African elephants is lobulated and located above the urethra (ur) as in Asian bulls. However, this gland contains a sonographically detectable cavity in each lobe only in the African species (indicated by black arrowheads). **E:** Cross-sectional sonogram (transrectal, 3.5 MHz) of both ampullae (white arrowheads) filled with sperm. These accessory sex glands are situated primarily above the caudal part of the urinary bladder (bla).

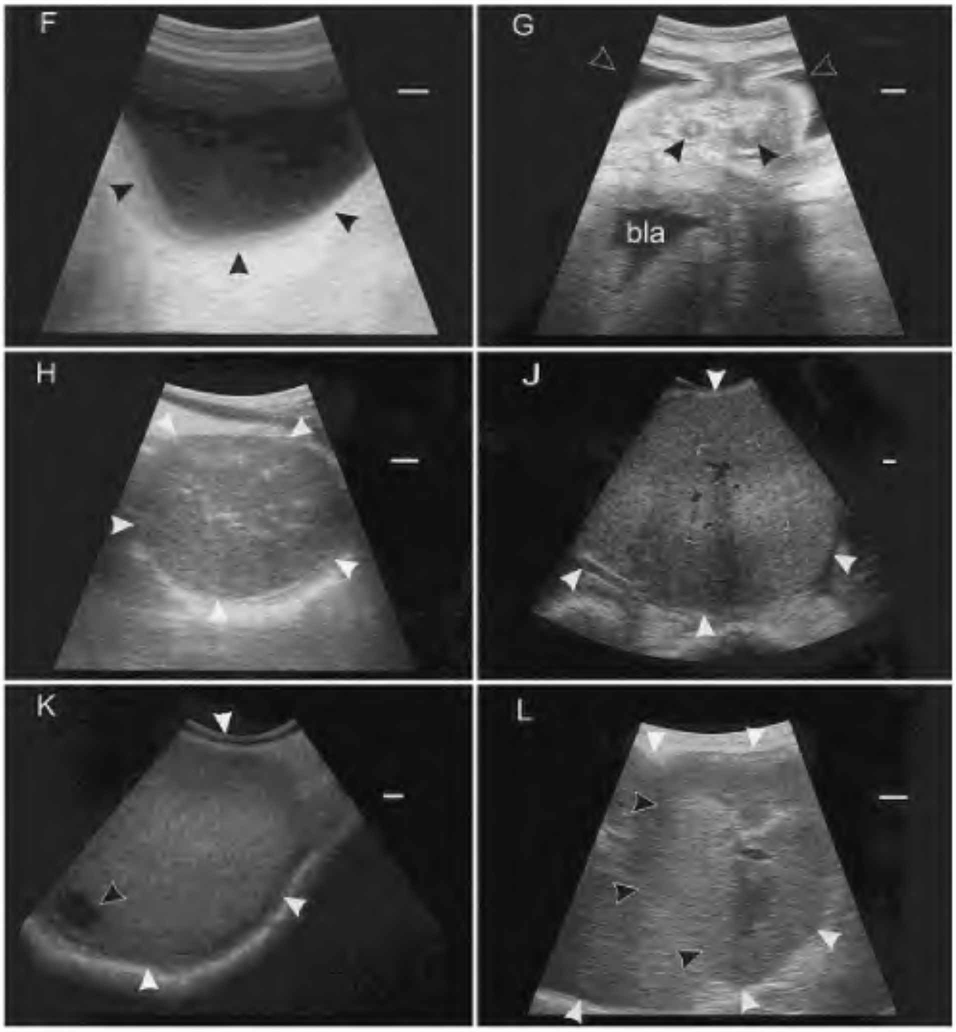

Fig. 1 (continued). **F:** Cross-sectional image (transrectal, 3.5 MHz) of the fluid-filled seminal vesicles of a breeding bull. The outer border of the glands is indicated by black arrowheads. **G:** Cross-sectional sonogram (transrectal, 3.5 MHz) of both ductus deferens (black arrowheads), which are positioned between the seminal vesicles (marked by white-bordered black arrowheads). Bla, urinary bladder. **H:** Sonogram (transrectal, 3.5 MHz) of the testis of a juvenile male (white arrowheads). The immature testis is small and the parenchyma appears inhomogeneous and echogenic. **J:** Sonogram (transrectal, 3.5 MHz) of the testis of an adult, sexually active bull (white arrowheads). The parenchyma of a mature testis appears homogeneous with a low echogenicity. It is characterized by a distinct blood supply (red and blue spots in the center) imaged by color flow Doppler sonography. **K:** Sonogram (transrectal, 3.5 MHz) of the testis of a non-breeding bull (white arrowheads) with a cyst in the parenchyma (black arrowhead). **L:** Sonogram (transrectal, 3.5 MHz) of part of the lobulated kidney with a dominant echoic capsule marked by white arrowheads. Black arrowheads indicate the internal border of one kidney lobe. White bar at right (B–L) = 1 cm.

and keepers. Type II bulls also included those housed alone. Type III bulls were those that appeared to be dominated by other bulls. Type IV were older bulls of unknown history that exhibited various testicular pathologies resulting in poor semen quality. Non-breeding bulls included those that were exposed to females, but failed to breed, as well as those that had no opportunities to breed. Type VI individuals were those in breeding situations that had sired offspring.

Bull ages within the various groups varied. Ages in the Type I group ranged from 4 to 12 years. The older individuals in this group were exclusively circus elephants that appeared to have an extended puberty because of their hard daily performance routine. In contrast, some Asian bulls in other groups produced ejaculates at 6 years of age, although the volume and composition of these early manually collected ejaculates suggested that the bulls probably would not be useful for natural breeding. There was one example of an Asian bull at the Houston Zoo that mated successfully with his sister (3.5 years old) at the age of 6. He produced a second pregnancy at the age of 10 in Berlin before he died of a herpesvirus infection in August 1998. His testicular diameter was 13 cm on postmortem examination. At the African Lion Safari Park in Canada, a 10-year-old bull (Calvin) was responsible for several pregnancies and was thought to contribute to the infertility of a 30-year-old conspecific (Rex). The mean life span for breeding bulls in Europe generally is only 35 years (they often die during anesthesia, of foot problems, or by accident).

Penis

The root of the penis (Fig. 1A) was heavily vascularized and had a diameter of approximately 20 cm. It was only partly visible by ultrasound because of its position and diameter. Most of the central urethra could be imaged transcutaneously. The penis complex and associated Cowper's muscle formed a bulge under the tail in a few individuals. Generally, this bulge became more prominent during musth; there was no correlation between the size of the bulge and fertility.

Bulbourethral Glands

These fist-sized, paired glands were situated about 2 cm under the skin surface, immediately caudal to the root of the penis (Fig. 1A,B). These glands appeared as primarily solid tissue, with a central irregularly shaped cavity. Bulbourethral secretions, visualized within the gland cavity before ejaculation, are clear and sticky, averaging 5–20 mL per pair of glands. The function of these secretions may be to cleanse and balance the pH of the urethral environment as well as to support emission of the penis into the female's vestibule [Hildebrandt et al., 2000b].

Urethra

Sonographically, the pelvic urethra was similar to that of female elephants, except for the presence of the colliculus seminalis, which was located about 1 cm caudal to the opening of the urinary bladder (Fig. 1C,D). The colliculus seminalis was an integral part of the dorsal urethral wall, approximately 1.5 cm long and 8 mm in diameter. This structure contained the joint ducts of the ampullae and paired seminal vesicles and several individual ducts (at least three from each side) from the prostate lobes. These ducts had a maximum diameter of 1 mm and could be imaged inside the colliculus by use of high-frequency transducers, but could not be distinguished from each other. Primary pathology was not detected in this area of the urogenital tract, except for infections of the prostate glands and seminal vesicles (see prostate glands section). Urethral alterations such as calcification of the mucosa were relatively uncommon [Hildebrandt, 1998]. However, if present, these lesions should be monitored closely to diagnose possible metabolic diseases. In one adult castrated Asian elephant, a 4-cm × 2-cm urinary calculus led to a urethral obstruction close to the bulbourethral glands [M. Hackenberger, personal communication]. Urethrotomy performed for surgical removal of the stone resulted in a permanent urethral fistula similar to that in females after episiotomy for dystocia treatment [Lange et al., 1999].

The ureters and urinary bladder (Fig. 1E,G) were identical in appearance to those described by Hildebrandt et al. [2000b] for the female elephant. None of the bulls examined in this study showed ultrasonographic evidence of urinary bladder infection.

Prostate Glands

The elephant prostate gland is a paired structure, with each half consisting of three lobes joined by a bridge of tissue. It is located above the pelvic urethra, caudal to the ampullae (Fig. 1D). The prostate is the only accessory sex gland that differs in shape and size between the two elephant species. The prostate glands in African elephants were larger, up to 5 cm in total diameter on each side, in contrast to the 2-cm prostate in Asian elephants. African elephant prostate glands also contained irregularly shaped internal cavities in each of the three lobes on both sides, which were lacking in Asian elephants. Further studies may reveal the cause of these differences, and whether they are related to the apparent species differences observed in the viability and longevity of sperm in vitro [Loskutoff, 1998]. There were no obvious characteristics of the adult prostate detectable by ultrasound that could be used as indicators of fertility. However, there was a dramatic developmental difference in size and complexity of the glands between immature and adult males.

The prostate as well as the other accessory sex glands can be involved in urogenital infections such as *Escherichia coli*. Two captive Asian elephant bulls exhibited pathological changes caused by a chronic prostatovesiculitis (Table 1). The infected prostate was characterized by an enlarged and less echoic parenchyma. The concurrently infected seminal vesicles were thick walled (>10 mm) and contained a cloudy secretion. The definitive diagnosis of urogenital infections was only possible by microbiological analysis of the ejaculate. In both cases, ejaculates were chocolate colored in contrast to the normal off-white to amber appearance.

Ampullae

In elephants, the cone-shaped ampullae contain spermatozoa directly from the testes; there are no well-developed epididymides. In this study, ampullae had a maximum diameter of 5 cm and were located on the ends of the ductus deferens above the urinary bladder at the cranial part of the urethra (Fig. 1E). The circumference of the ampullae, when measured in cross-section, indicates their fullness, and the echogenicity of the fluid indicates the sperm concentration. The characteristics of the ampullae can be used as predictors of the amount of sperm obtainable through manual collection, thus making these glands one of the most important structures to assess during ultrasonographic evaluations of testicular activity. Immature bulls (Type I; six Asian, three African) were easily identified by the undeveloped status of the ampullae (Table 1). Semen collections were mainly unsuccessful or produced small amounts of ejaculate with immature sperm cells. More than half of the mature bulls (Types II and III; n = 38) had well-expanded ampullae that appeared highly echogenic, but they ejaculated mostly immotile sperm after stimulation. A few bulls (Type IV) and one former proven breeder (included in the Type IV category) exhibited flat ampullae on ultrasound examination and produced nearly aspermic ejaculates. There was clear evidence of testicular pathology in the Type IV group (Table 1, see testes section). One adult male that had been surgically castrated as a juvenile also had fluid-filled ampullae (Type V), but other accessory glands were barely detectable. The manually collected ejaculate (~3 mL) from this animal was aspermic and had an amberlike color.

There was only one abnormality of the ampullae observed in this study. This adult bull had a pea-shaped, fluid-filled structure 1cm in diameter with a clear border between the two medial ampullae walls. The structure did not empty or change shape after ejaculation as in the other bulls, nor did it affect semen quality. The hypothesized diagnosis of this structure was vestigial embryonic uterine tissue (i.e., müllerian duct, so called *utriculus masculinus*).

Seminal Vesicles

The seminal vesicles (Fig. 1F,G) are normally the largest accessory glands of an actively breeding bull and can contain up to 400 mL fluid in each gland. These glands are characterized by a single cavity with a wall 5–10 mm thick. An outer muscular and an internal mucosal layer can be clearly distinguished by ultrasound. The seminal vesicles produce the bulk of the seminal fluid, which provides nutrients and a vehicle for the ejaculated sperm. These glands showed the most variation of any of the structures examined in this study and were important criteria, in combination with the ampullae, for characterizing breeding potential (Table 1).

In a healthy bull, the seminal vesicles combined empty 25–150 mL of fluid per ejaculation, up to five times per day, during natural breeding. Manual collection or collection by artificial vagina [Schmidt, 1982, 1993] can mimic these results. Electroejaculation under anesthesia can completely empty the seminal vesicles, producing ejaculates approaching 800 mL [B. Wolfe, personal communication], although ejaculate volumes in the range of 100–200 mL are probably more common [Howard et al., 1984, 1986].

Ductus Deferentes

The strongly coiled ductus deferens complex was about 1 m long and 2 cm in diameter at its widest. The total length could not be imaged sonographically because the middle section was embedded in large fat pads that were craniolateral to the pelvic bone, outside the exploration field of the transducer, even with the use of an adapter. The straight, caudal part, embedded between the seminal vesicle and the urinary bladder (Fig. 1G), could be easily assessed by a hand-held probe. The diameter of these structures in adult bulls ranged from 5 to 10 mm. The cranial part, adjacent to the testis, could only be visualized using an adapter. In actively breeding bulls (Type VI), the caudal part contained an anechoic lumen, indicating sperm. In the castrated bull, they were nearly undetectable. However, the ductus deferens were not as effective an indicator of breeding potential as the ampullae and seminal vesicles.

Testes

Elephants have internal testes (Fig. 1H–K) situated close to the kidney. They are nearly round and can reach a maximum diameter of about 15–23 cm [Hildebrandt et al., 1998]. Testicular morphology was usually a good indicator of reproductive status; however, in many bulls it was not possible to visualize the gonads due to interference from ingesta-filled intestinal loops or cecum. Sometimes both testes could be visualized by examining the bull in lateral recumbency. In general, only slight differences in diameter between right and left testes were observed, due mainly to the position of the scan head in relation to the organ. However, there was one Asian bull in which the right testis diameter was considerably larger (16 cm) than the left (10 cm), and this difference was also evident in the filling degree of the right and left ampullae. This bull was otherwise physically mature and a proven breeder; the cause of this disproportion was unknown.

The immature testis (Fig. 1H) ranged from 2 cm (newborn) to ~9 cm. Sperm-rich ejaculates were collected in this study only in individuals with a testis size of more than 10 cm in diameter (Fig. 1J). In addition to the difference in size between inactive and active gonads, there was a remarkable difference in the echogenicity of the parenchyma. The parenchyma of an active testis appeared less echoic due to the higher degree of blood circulation than in an inactive testis. However, concentrations of testosterone measured in peripheral blood often does not reflect the differences in size and echogenicity between small inactive and large active testes, suggesting that this measurement alone may not be useful in determining the reproductive status of a bull elephant [Hildebrandt et al., 1999; Fritsch et al., 2000].

Figure 1J shows a color flow Doppler image of the internal blood circulation of the testicular parenchyma (inside the white frame) that illustrates the high tissue metabolism in a sexually active bull. However, the results of the color flow Doppler examinations were not markedly different from those using the B-mode ultrasound images. This advanced ultrasound technique was used primarily for basic studies of sexual maturation of immature bulls and only sporadically in breeding bulls as reference. This was done because the color flow Doppler machine is too heavy, fragile, and expensive for routine examinations in comparison to a portable B-mode ultrasound machine.

In this study, nine adult bulls were identified with testicular pathology of unknown origin and poor semen quality (Type IV). The bulls in this group had small testes (<10 cm) with echogenic parenchyma, except one Asian bull that had testicular cysts (Fig. 1K). Possible causes for the testicular degeneration could be chronic infection, long-term treatments for diseases such as tuberculosis, or inadequate application of anti-musth drugs. The return of these bulls to an active breeding status is questionable due to massive changes in testicular morphology, which in one case was documented by a post-mortem examination.

Kidneys

The dorsal side of the lobed kidneys (Fig. 1A,L) is situated retroperitoneally, close to the rectum and about 1.5–2.0 m cranial to the anus. The kidney can be identified by its smoothly curved hyperechoic capsule, 1–2 mm thick. The parenchyma contains several channels and blood vessels. In general, imaging of the kidneys is limited using a 7.5-MHz probe. Therefore, a sonogram of a single kidney lobe can be easily misinterpreted as the outer part of the testicle. To avoid this mistake, the kidneys also should be scanned using a 3.5-MHz probe for overview.

Semen Collection

Semen collections were mainly unsuccessful or produced small amounts of ejaculate with immature sperm cells. More than half of the mature bulls (Types II and III; n = 38) had well-expanded ampullae that appeared highly echogenic, but they ejaculated mostly immotile sperm after stimulation. A few bulls, even one proven breeder, exhibited very flat ampullae on ultrasound examination, and produced nearly aspermic ejaculates (Type IV bulls). One adult male that had been surgically castrated as a juvenile also had fluid-filled ampullae (Type V), but other accessory glands were barely detectable. The manually collected ejaculate (~3 mL) from these animals was aspermic and had an amberlike color.

Type II–III bulls produced sperm-rich ejaculates, but the motility was highly variable between ejaculates from the same individual and among bulls, ranging from 0 to 90% motile. In these sexually inactive bulls, the developmental status of the accessory glands, especially the seminal vesicles, was disproportionate. Subsequent evaluations of the sperm samples indicated that there were no significant morphological abnormalities, even of the immotile cells. There was a high incidence of precursor cells or immature sperm in the ejaculate of bulls just entering puberty. Immature sperm were cells characterized by incomplete formations such as microcephaly, nuclear vacuoles, and proximal and distal cytoplasmic drops. The adult elephants classified as Type IV were characterized by a mostly aspermic ejaculate due to the testicular pathology described above. Evaluations of sperm quality revealed a difference between sexual inactive bulls dominated by older female elephants or keepers (Type II), in contrast to bulls that were socialized with active breeding bulls (Type III). In this study, 76% of the elephants examined (57 bulls, Types I–V) produced less than 100 mL of ejaculate after manual collection. These bulls were characterized by unsuccessful copulation or lack of libido. The mean range of motility in Type VI bulls ranged from 75 to 90%, with a total volume of 40 to 300 mL. The average sperm motility of the 18 breeding bulls was 85%, and the mean ejaculate volume was 120 mL. The ejaculates of breeding bulls collected by manual stimulation were always composed of three to four fractions [Schmitt and Hildebrandt, 1998]. There was no evidence of immature sperm cells in the ejaculates of proven bulls.

CONCLUSIONS

Poor reproductive performance of captive elephants has been largely attributed to female dysfunction [Brown, 2000; Hildebrandt et al., 2000b]. However, the results of this study suggest that fertility problems with bulls may be of equal or greater importance, especially when considered on the basis of a percentage of the population. Proportionately, there are few elephant bulls in captivity; the finding that the majority of these individuals probably are not capable of producing offspring is of enormous concern. Poor libido aside, otherwise healthy adult bulls are not producing good-quality sperm, so that even artificial insemination is not an option. Ultrasonography is a critical tool for identifying the causes of male reproductive problems and developing mitigating treatments. Ultrasound examinations, combined with semen collection, should be conducted regularly (yearly if possible) to document changes in the reproductive status of captive bull elephants and determine whether altered management strategies are warranted. This approach also is key to identifying bulls in native countries that could serve as semen donors for artificial insemination. From a management perspective, importing semen from wild or semi-wild individuals would allow maximizing of the genetic diversity of captive populations and would add to the flexibility of planned breeding programs. However, for artificial insemination to be practical, especially for imported semen, methods of cryopreserving spermatozoa for long-term storage need to be developed. A program involving routine reproductive assessments of male and female elephants (hormonal and ultrasound), combined with improved management of natural breeding (i.e., breeding centers) as well as use of assisted reproductive technologies such as artificial insemination, may finally result in the desired creation of self-sustaining captive populations.

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