Eur J Wildl Res (2006) 52: 23–27
DOI 10.1007/s10344-005-0012-4

REVIEW

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Ultrasonography: an important tool in captive breeding management in elephants and rhinoceroses

Received: 15 August 2005 / Accepted: 25 August 2005 / Published online: 18 November 2005

Abstract Nearly two decades ago, modern wildlife medicine started to gradually use the advantages of the non-invasive ultrasonography, which was already well established in human and classical veterinary medicine. For more than one decade now, the application of imaging ultrasound for reproductive assessments and as a supportive tool during assisted reproduction procedures such as artificial insemination (AI) in elephants and rhinoceroses has dramatically improved the breeding success in captive breeding programmes. The opportunity for identifying potential breeding candidates on the basis of their reproductive health status is widely used for natural mating or for AI, today. The longitudinal sonographic monitoring of pathological processes on the internal female genital tract allowed the identification of pathogenetic causes for the rapid infertility development in older nulliparous females. The factors causing temporary infertility in captive male elephants and rhinoceroses were also identified by the use of ultrasound. Today, ultrasonography is the golden standard for reproductive assessments in megavertebrates such as elephants and rhinoceroses in captive management settings and also in the wild.

Keywords Proboscidea · Rhinoceroses · Reproduction · Ultrasound · Breeding evaluation

Introduction

Over the last few decades, the elephants and rhinoceroses have become important icons in the saga of wildlife conservation. Recent surveys estimate the wild Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephant populations to be, at most, 50,250 and 637,600 individuals, respectively. The population estimates for the five rhinoceros species with 3,610 black (*Diceros bicornis*), 11,330 white (*Ceratotherium simum*), 2,400 Indian (*Rhinoceros unicornis*), 300 Sumatran (*Dicerorhinus Sumatrensis*) and 60 Javan (*Rhinoceros sondaicus*) are of even greater concern.

The development and broad application of reproductive assessment and artificial insemination (AI) techniques have become critical to the successful breeding management of elephants and rhinoceroses and are the top priorities of the captive conservation programmes. Significant advances in endocrine analyses, ultrasonographic evaluations and behavioural profiling have resulted in a number of remarkable discoveries about reproductive biology in these megavertebrates that have led to major breakthroughs in assisted reproduction. The development of reproductive assessment techniques such as ultrasonography now allows zoo managers to identify candidates for breeding by natural means or by AI. These recent advances have sharpened the focus of elephant and rhinoceros propagations and are already significant in their impact on management and conservation.

Ultrasonography is mainly a reflection imaging technique in contrast to radiography as a transmission imaging technique or magnetic resonance imaging (MRI) as an emission imaging technique. Diagnostic ultrasound (2.0 to 50.0 MHz) produces a cross-sectional anatomical picture (sonogram) of how sound waves reflect, refract and are absorbed by different tissues. The more advanced 3D ultrasonography based on computerized summation of single sonograms is generated by special volume transducers or specific software programmes. Ultrasonography is generally non-invasive, except for the applications listed in Fig. 1. Ultrasonic energy waves themselves can cause minimal bioeffects like mild tissue warming especially if colour flow Doppler mode is used for extended periods of time (>30 min). However, there has been no report of patient injury or ultrasound-induced discomfort when examinations were performed under the international ultrasound safety guidelines. The necessity for animal handling and, potentially, sedation may add invasive components to the application of ultrasonography in non-domestic species. The first attempt to utilize non-imaging ultrasound for a practical application was used in an unsuccessful search for

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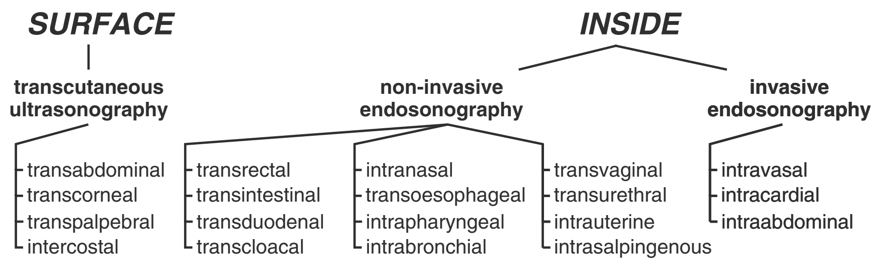

Fig. 1 Overview of the ultrasound applications in mammals. Some approaches are not practicable for megavertebrates like elephants and rhinoceroses

the sunken "Titanic" in the North Atlantic in 1912. Ultrasound was routinely applied in the 1940s in World War II, as a non-imaging Doppler technique for military purposes (Sound Navigation and Ranging, SONAR). The first medical application of ultrasonographic techniques was developed for soft tissue characterization in humans in the 1950s (Donald et al. 1958). Subsequently, there has been a dramatic development of imaging ultrasound, which has been used in a wide range of applications in many fields of human and veterinary medicine (DuBolay and Wilson 1988). This image modality allows non-surgical, transcutaneous and transrectal approaches for exploring of topography and monitoring of biological and diagnosing of pathological processes in humans and animals.

In 1991, Adams et al. described the first use of ultrasonography in rhinoceroses and elephants. Special accessories and protocols were developed over the next few years to increase the efficacy of ultrasound in these megavertebrates (Hildebrandt et al. 2000a). Since 1993, the Institute for Zoo and Wildlife (IZW) research team has performed more than 3,000 individual ultrasound examinations in approximately 400 elephants and more than 150 reproductive assessments in more than 70 rhinoceroses under different management settings and in the wild.

In megavertebrates, the application is mainly limited to transcutaneous and transrectal ultrasound. However, the use of ultrasonography offers new opportunities in these species to evaluate parts of internal organ systems and joints which are inaccessible by other means (e.g. conventional/digital radiography, magnetic resonance, endo/arthroscopy) due to the enormous size and the location and dimension of the organs. Additionally, in contrast to other imaging procedures, clinical ultrasonography has several advantages: (1) it is safe due to minimal bioeffects and therefore repeatable; (2) it provides real-time information; (3) it generates high-resolution characterization of soft tissue and bone surface as well as morphometrics of organs, implants or other foreign bodies; (4) it produces sectional images and 3D reconstructions of tissues and organ structures; (5) it permits examining motion and direction (heartbeat, vascular flow, fetal movement); (6) it operates economically efficient; (7) it facilitates documenting and preserving data on storable media; and (8) it is portable and compatible with zoo and field conditions.

In general, only approximately 15% of all ultrasound applications in elephants are performed transcutaneously compared to 85% transrectal applications. In rhinoceroses, the transcutaneous approach is limited to only 5% of the applications such as monitoring of mammary gland development in pregnant rhinoceroses. The cause for this difference in utilization of these two application forms in these pachyderms is due to the anatomy of the integument and the restricted penetration depth of commercially available systems (max. average depth=220 mm). Transcutaneous approach is predominantly used for obstetrics (late-term pregnancy), orthopedics, cardiology, dentistry, ophthalmology and for the visualization of cranial abdominal organs and mammary gland development and function. Transrectal examinations already stated above require a higher level of patient preparation and specific customized ultrasound accessories to overcome the anatomical obstacles of the adult elephant. Nevertheless, transrectal ultrasound is an ideal tool for imaging the urogenital system, rectal wall, intestinal loops, peritoneal–abdominal cavity, and early to mid-term pregnancies (Fig. 2). Furthermore, the transrectal approach has the added advantage of improved coupling with the rectal wall, which allows for the use of a variety of transducers with different frequencies ranging from 2.0 to 10.0 MHz compared to transcutaneous ultrasound that can only be performed with a low-frequency probe (e.g. 4–2 MHz) (Hildebrandt et al. 1997). Therefore, more diagnostic information is available from the detailed imaging generated by transrectal ultrasound. For this specific application, the commercial ultrasound system has been modified to incorporate special features, including probe and cable extensions and a monitor helmet with video glasses.

Reproductive disorders are the main obstacle for successful captive management plans (Hermes et al. 2004;

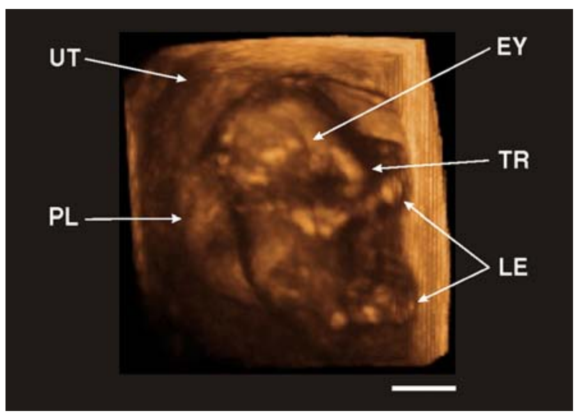

Fig. 2 Transrectal 3D-sonogram (7–5 MHz) of a 168-day-old elephant fetus. The 3D-mode allows the visualization of the fetal eye (*EY*), trunk (*TR*), front and hind legs (*LE*), as well as part of the placenta (*PL*) and the uterus (*UT*). *Bar*=20 mm

Hildebrandt et al. 2005). So far, none of the captive elephant and rhinoceros propagation programmes reached the level of being self-sustaining (Hermes at el. 2004; Olson and Wiese 2000; Wiese 2000; Wiese and Willis 2004). The consequence is that imports from the range countries are necessary for the zoo populations. In general, reproductive pathologies in female elephants and rhinoceroses mainly involve changes in the internal genital organs, primarily uterine tumours (leiomyomas), endometrial cysts and ovarian cysts (Fig. 3). There is a higher incidence of genital tract leiomyomas in Asian compared to African species, whereas ovarian cysts occur frequently in African but rarely in Asian species (Hildebrandt and Göritz 1995, 1999; Hildebrandt et al. 2000b, 2003a; Montali et al. 1997; Hermes et al. 2001). Cystic endometrial degeneration has been found in all species (Agnew et al. 2004; Hildebrandt et al. 1997). These pathologies in an advanced stage affect reproduction dramatically. Ovarian cysts have been associated with acyclicity in elephants and rhinoceroses (Brown et al. 1999; Hermes et al. 2004; Hermes et al. 2005b). It is now known that the incidence of these reproductive disorders increases with age and is greater in nulliparous than in similarly aged parous individuals (Hermes et al. 2004; Hildebrandt et al. 2000b). An additional reproductive disorder complex in elephants is the high percentage of more than 50% dystocia cases in older primiparous cows (>20 years old) resulting in stillborn calves and in about 5% of the cases in the death of the cow due to retained conceptus and intoxication (Hildebrandt et al. 2003b; Lange at el. 1999; Schaftenaar 1996). The use of ultrasonography during a dystocia treatment can optimize the medical intervention and therefore helps save the life of the cow (Schaftenaar et al. 2001). These findings suggest that breeding elephants and rhinoceroses at a young age (before onset of pathogenesis) should be a high priority, and that females should be assessed periodically by ultrasound and longitudinal hormonal analyses (usually progesterone) to assess reproductive fitness.

Male elephant infertility appears to be related to poor semen quality and low libido rather than any specific anatomical abnormality. To date, evaluating circulating testosterone has been a non-informative index for diagnosing gonadal dysfunction. Rather, ultrasonography remains the most reliable method of assessing breeding potential in bull elephants. Transrectal ultrasound assessments combined with manual semen collection (Hildebrandt et al. 2000c; Schmitt and Hildebrandt 1998) were performed in more than 100 bull elephants. In that process, the morphology and functionality of the entire urogenital tract have been characterized, including testes and accessory sex organs as well as the sperm parameters (Hildebrandt et al. 1998, 2000c, 2003a). Most bulls were non-breeders even those exposed to females. Percentage of reproductive tract pathologies detected by ultrasound was low (14%), however, even in older animals. In contrast, semen quality varied markedly, and sperm motility often was less than 50%. Interestingly, more than half of the 'infertile' bulls appeared to be in social situations where they were subordinate to older cows or other bulls. Thus, although many of these bulls could serve as semen donors, they inconsistently produce good quality ejaculates that could have resulted from physiological disorder(s), behavioural problems or perhaps suboptimal semen collection methods. Regardless, periodic ultrasound examinations combined with semen collection have been recommended as routine management tools to monitor the reproductive value of bull elephants.

Despite profound differences in social systems of elephants and rhinoceroses, unsuitable social environments have similar impact on reproductive performance of male rhinoceroses showing long-term detrimental effects on fertility and resulting in 50% of the male captive population being subfertile or infertile (Seror et al. 2002; Hermes et al. 2005a). Ultrasonography of the accessory sex glands combined with semen collections by using electro-ejaculation in white rhinoceroses documented that semen quality is associated with group size and social rank (Hermes et al. 2005a). Reproductive ageing processes and tumour devel-

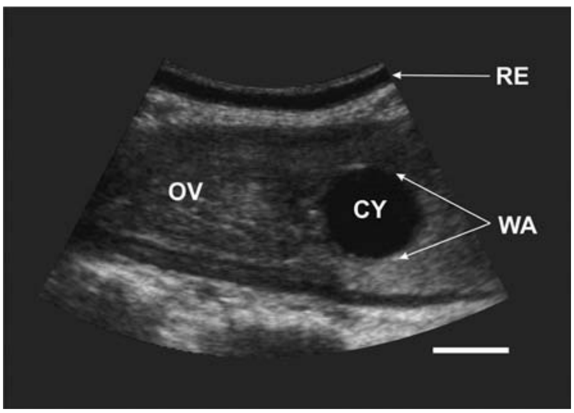

Fig. 3 Transrectal sonogram (4–2 MHz) of an ovary (*OV*) containing an ovarian cyst (*CY*). The cyst can be clearly distinguished from a follicular structure by the presence of a prominent wall (*WA*). Ovarian cysts are often combined with acyclicity in adult elephants. The rectal wall (*RE*) appears as a moderately echoic strip at the top of the sonogram. *Bar*=20 mm

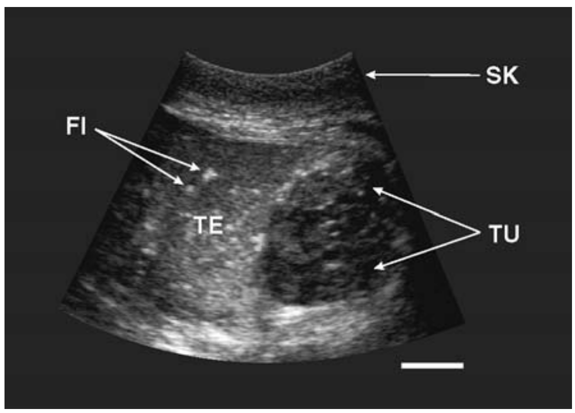

Fig. 4 Transcutaneous sonogram (4–2 MHz) generated through the inguinal skin (*SK*) of the cigar-shaped testis (*TE*) in an approx. 40-year-old white rhinoceros bull affected by a medium-sized seminoma and fibrosis (*FI*) in the testicular parenchyma. The malignant tumour (*TU*) appeared hypoechoic and had a spherical structure. *Bar*=20 mm

opment in the genital tract play only a minor role in male rhinoceroses. In the Indian and the white rhinoceros, progressive changes in testicular morphology attributable to ageing occur in males older than 15 years of age.

Different from the females, this ageing process does not overtly affect functional reproductive parameters as older males prove to be the best semen donors despite considerable testicular fibrosis (Endo et al. 1996; Hermes et al. 2005a). In contrast to frequent neoplastic processes in the female genital tract, testicular tumours in a male rhinoceros are rare findings (Fig. 4). The establishment of a social hierarchy and low-grade fighting may involve sustained testicular trauma. The subsequent exposure of spermatozoa and seminal fluid to the surrounding testicular tissue induce seminoma development (Portas et al. 2005). Despite seminomas fast-growing character, the fertilizing potential of affected males may remain high until in the advanced stage of tumour growth.

Conclusions

This short review has endeavoured to provide credible applications of the value of ultrasonography as a practical diagnostic tool in elephants and rhinoceroses. The technology still is vastly underutilized, and its many advantages (non-invasiveness, reproducible real-time images, cross-sectional images of tissues/organs and ability to measure morphometry) certainly argue for more widespread use. In the field of embryology alone, real-time ultrasonography expands our ability to study embryonic development, uterine and ovarian function in ways that are impossible using conventional techniques.

Applying ultrasound to the systematic examination will undoubtedly help find new solutions to current physiological problems. But it will also promote the technology and provoke the design of new and more specialized, species-specific instruments. Continued miniaturization of complete high-end ultrasound systems and development of probes with compact, contoured shapes will improve the imaging possibilities and increase the amount of diagnostic information extractable from ultrasonography. Many new systems offer colour flow imaging that allows direct evaluation of blood vessel architecture in reproductive organs. This is a promising area of research, providing insight into the marked changes related to seasonality, maturation, sexual activity and pregnancy or tumour development. 3D ultrasonography also will further expand our ability to characterize reproductive soundness while facilitating a better understanding of complex physiological processes and creating near life-like images.

A high priority is to incorporate this technology into more comprehensive, integrated investigations involving multiple scientific disciplines. Successful collaborations already exist, especially collaborations amongst ultrasonography specialists, endocrinologists and behaviourists. Such scholarly partnerships will create new databases, helping to develop mitigating treatments for overcoming reproductive failure. It is the hope of the authors that more scientists and zoo veterinarians will accept the challenge of conducting integrated studies that cross-discipline for the pleasure of pure scientific discovery as well as for developing practical solutions to managing elephant and rhinoceros species. Ultimately, our goal is to understand the reproduction biology and physiology of these megavertebrates in the hopes of improving management to establish or maintain self-sustaining populations, both in situ and ex situ.

Acknowledgements The authors dedicate this article to Professor Christian Pitra for his upcoming 65th birthday in 2006. The new field of reproduction management including the development of assisted reproduction technologies for non-domestic species was originally inspired and started by Christian Pitra at the Institute for Zoo and Wildlife Research before his heart discovered evolutionary genetics. T. B. H. would like to thank personally my former teacher and mentor for his ideas and support which had an important impact on my own professional career.

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