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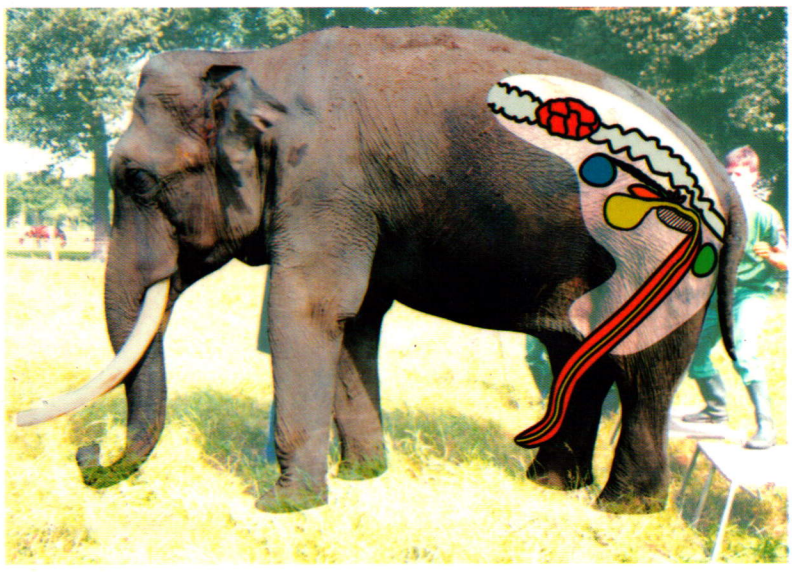

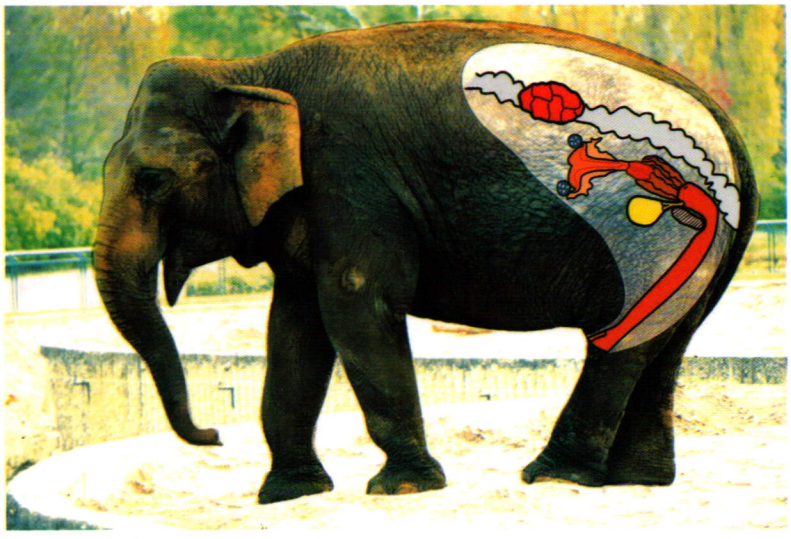

Assessment and Management of Reproductive System in Asian Elephants

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Proceedings of the Workshop, Lectures and Survey in Reproduction Biology of Asian Elephants
May 3-16, 2000
Thailand

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ISBN 974-7772-79-5

Published by **The National Identity Office, Secretariat of the Prime Minister**

First published 2000 : 1,000 copies

Supported by **The National Identity Office, Secretariat of the Prime Minister** and **The Committee on Coordination of Elephant Conservation in Thailand,** Chairman : **Dr. Suvit Yodmani**

Printed by **Srimuang Printing Co.,Ltd.** 5/37-41 Rongmuang Soi 5, Patumwan, Bangkok 10330

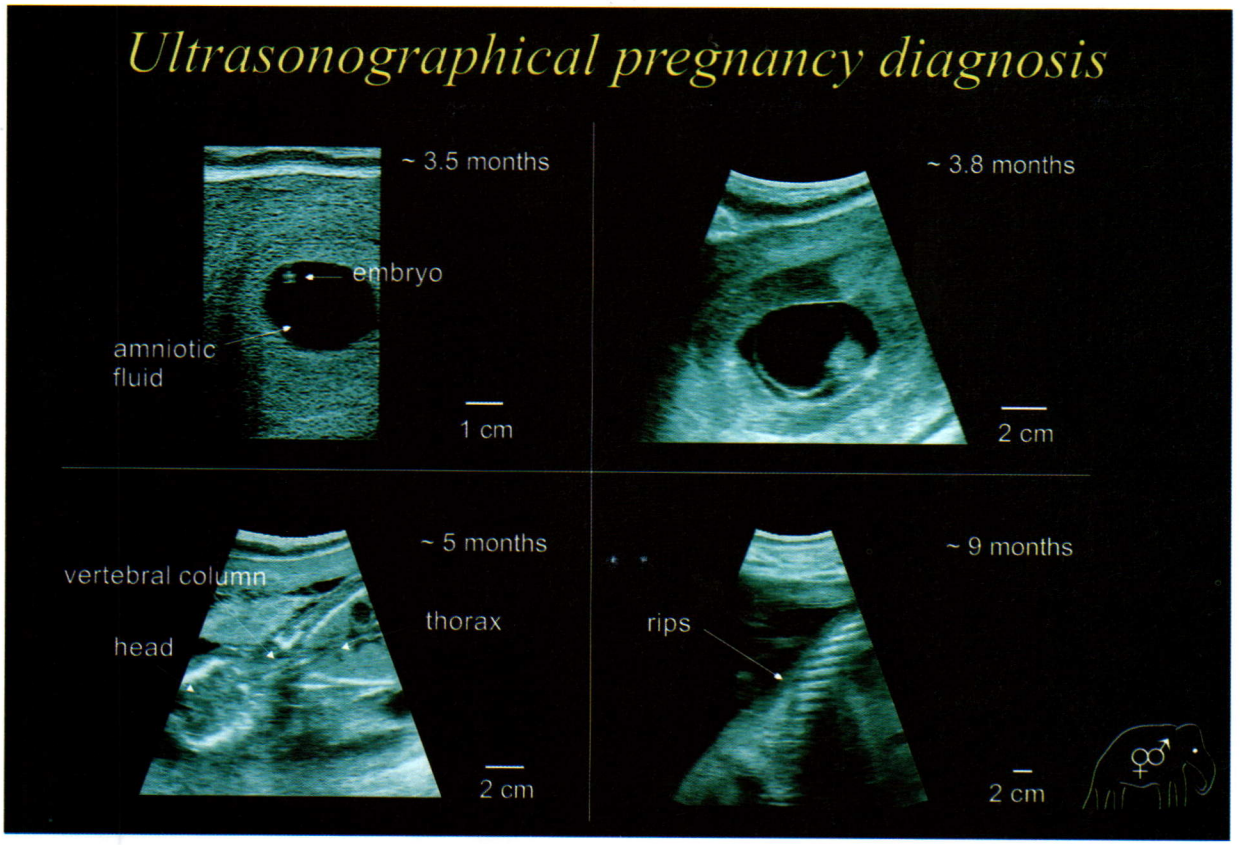


Fig. 9 Sonograms of different stages of pregnancy.

Fig. 10 Application of silicon rubber implants (n=5) behind the ear at the ear base which releases small but effective amounts of 17β-estradiol (ca. 300µg per animal per day) over a period of 1.5 to 2 years.

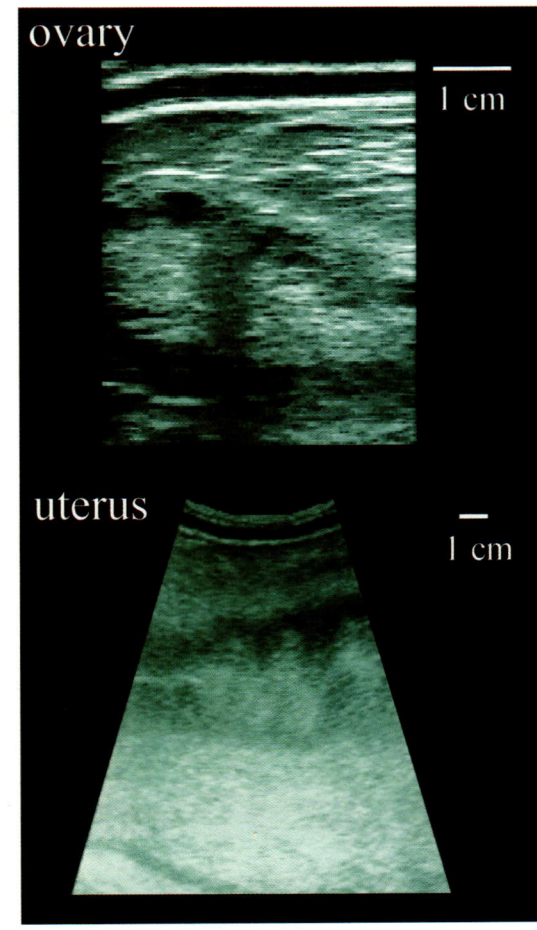
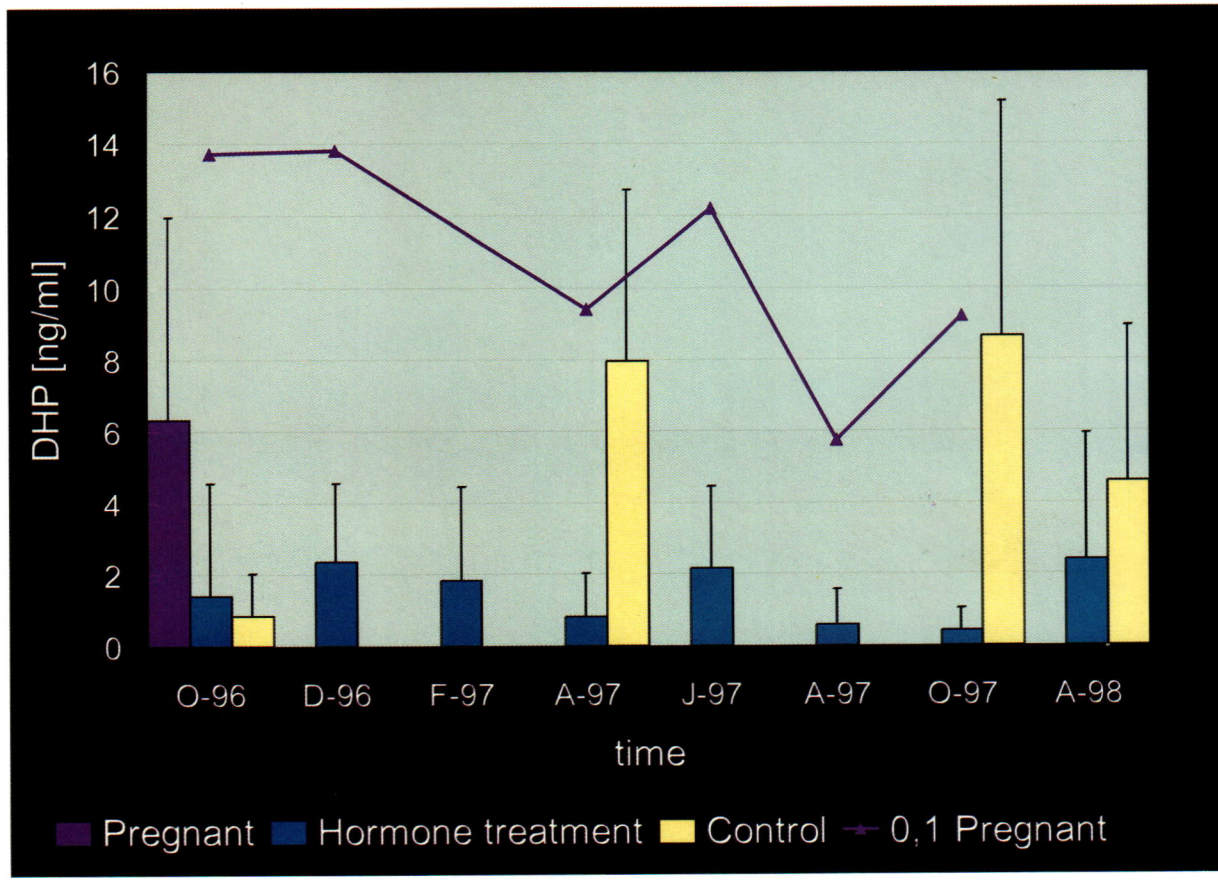


Fig. 11 Sonogram of an inactive ovary and uterus of a hormonal treated animal (one year after implantation) documenting the contraception effect desired ("down-regulation").

Fig. 12 Concentrations of fecal gestagens (DHP) measured by EIA in pregnant, hormonal treated, and control animals.



TRAINING ELEPHANTS FOR REPRODUCTIVE ACCESSMENT PROCEDURES

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Training Elephants for Reproductive Accessment Procedures

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Introduction

People consistently rank elephants as number one on their list of the most fascinating and popular animals. The African elephant is endangered, the Asian elephant is on the brink of extinction and the breeding results of elephants in captivity are still far from being sufficient for maintaining the population.

The keeping of this species by man, whether in zoos, safariparks, trekking or working camps, is very demanding and very elaborate if done seriously and responsibly. It has long been insufficient to simply attain, or maintain, a trained animal situation in which handling or working with them is safe for the zookeeper or mahout. In addition to maintaining the health of the animals via routine inspections, further aspects that are necessary for medical supervision, treatment and research must be considered. Especially new cases of diseases like Herpes and Tuberculosis result in the need of higher medical performance.

Body and Health care for elephants:

The high level of effort and responsibility of our elephant keepers at the Vienna Zoo has resulted in well trained elephants and made projects like artificial insemination possible. In March 1999 the Vienna Zoo, in cooperation with specialists from other institutions, started a series of workshops called the **"European workshop of training elephants for medical care, treatment and research".** The first three workshops with a limited number of participants was attended by nearly 100 keepers, curators and veterinarians from Europe, the United States and Asia. The topics included: foot care, skin care, mouth examination, body measurements, blood samples collection, x-rays, ultrasound procedures and preparation for artificial insemination. Other workshops are planned and will be organised.

Opening Statement by His Excellency Privy Councillor Mr. Ampol Senanarong at the Conference on Reproduction Biology of Asian Elephants On Thai Elephant Day 13 March 2000

Chairman of the Committee on Coordination of Elephant Conservation in Thailand
Distinguished guests;

It is a pleasure and a great honor for me to join in the opening ceremony of the Reproduction Biology of Asian Elephants Conference on Thai Elephant Day. I am also very pleased to see all of you who have come to participate in this event here today from both the government and the private sector.

As we know from the report, the aim of the Conference is to encourage the exchange of ideas and to improve the basic knowledge of the reproduction biology of Asian elephants. The Conference also hopes to build a cooperative network on elephant reproduction both at the national and the international level which is believed will be of great importance in the conservation of this endangered species.

I am sure that the Conference will be of great benefit for all of us gathering here today as it will improve our ability in increasing elephant population of the nation.

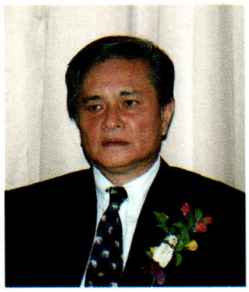
I would like to thank all of you for coming to join in the event. Special acknowledgments are also due to all of those who supported the organization of this year's academic conference and to the Committee on Coordination of Elephant Conservation in Thailand who initiated the Thai Elephant Day for the benefit of the elephants in our country.

In conclusion, I would like to pray under the beneficent power of the Triple Gem and all the holy spirits of the universe that all of you will enjoy prosperous lives. I would also like to wish you all a very fruitful and enjoyable conference and that it will bring substantial benefits for the people in our country. I look forward to seeing the proceeding of this conference shortly and would now like to declare the conference on Reproduction Biology of Asian Elephants open.

Report by Dr.Suvit Yodmani, Chairman, Committee on Coordination of Elephant Conservation in Thailand for the Opening Ceremony of the Conference on Reproduction Biology of Asian Elephants On Thai Elephant Day 13 March 2000

Your Excellency Privy Councillor Mr. Ampol Senanarong,

On behalf of the Committee on Coordination of Elephant Conservation in Thailand. I would like to thank you for your kindness in allowing me to preside over the opening ceremony of the Reproduction Biology of Asian Elephants Conference today.



The Conference aims to encourage the exchange of ideas and to improve the basic knowledge of the reproduction biology of Asian elephants courtesy of guest speakers from Germany and Austria. It is also hopes to create enthusiasm and build a cooperative network on elephant reproduction both at the national and the international level. This is believed will be of great importance as the number of wild and domestic elephants has decreased dramatically in recent years and without some intervention they might become extinct in the not to distant future. The Conference will focus on the role of veterinarians, scientists and researchers in the country and hopes to enable them to develop a good understanding about reproduction biology of Asian elephants and for them to adapt this knowledge for the benefit of the country in the future.

On 29 May 1998, the Office of the Prime Minister announced that Thai Elephant Day would be held annually on 13 March. The Conference is a major activity of this year's Thai Elephant Day and is supported by the Faculty of Veterinary Science, Mahidol University, Thai Airways International, Thai Veterinary Medical Association, Asian Elephant Foundation of Thailand, Elephant Alliance of Thailand and guest speakers from Germany and Austria.

At this point, I would like to invite Your Excellency Privy Councillor Mr. Ampol Senanarong to give the inaugural address.

Activities

- Assesment of Reproductive Tract Health Status and Survey of Endotheliotropic Elephant Herpes Virus (EEHV) Distribution in Captive Asian Elephants in Thailand.
- Special Lecture Programme and Demonstration on Reproduction Biology of Asian Elephants.
- Workshop on Diagnostic Ultrasonography of Reproductive Tract in Asian Elephants.

Organized by

- Committee on Coordination of Elephant Conservation in Thailand, The National Identity Office, Secretariat of the Prime Minister.
- Faculty of Veterinary Science, Mahidol University.

Supported by

- Institute for Zoo Biology and Wildlife Research (IZW), Berlin.
- Asian Elephant Foundation of Thailand (AEFT).
- Thai Airways International.
- Thai Veterinary Medical Association.
- Elephant Alliance of Thailand.

Programme coordinator : Asst.Prof.Dr. Parntep Ratanakorn

Assesment of Reproductive Tract Health Status and Survey of Endotheliotropic Elephant Herpes Virus (EEHV) Distribution in Captive Asian Elephants in Thailand

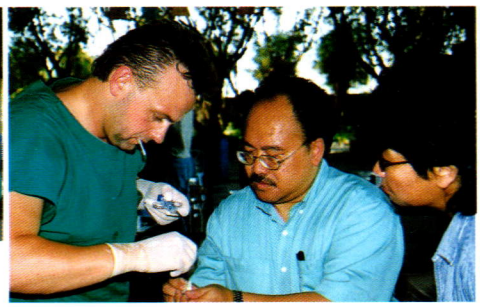
Working Programme

3	March 2000	Audhya, Audhya Elephant Camp
4-5	March 2000	Lumpang, Elephant Conservation Center
7	March 2000	ChiangMai, Maesa Elephant Camp
9-12	March 2000	Chonburi, Nongnuch Park, Sriracha Zoo and Pattaya Elephant Camps
14	March 2000	Nakornpathom, Samphran Elephant Ground
15-16	March 2000	Kanchanaburi, Safari Park and Taweechai Elephant Camp

Special Lecture programme and Demonstration on "Reproduction Biology of Asian Elephants" 13-14 March 2000 at Salaya Pavilion, International College, Mahidol University and Samphran Elephant Ground

13 March 2000

08.30 - 09.00	Registration at Salaya Pavilion
09.00 - 09.15	Opening ceremony
09.15 - 09.30	Introductory lecture, Assistant Prof. Dr. Parntep Ratanakorn
09.30 - 10.30	Introduction to Biology of Reproduction in Asian Elephant, Dr. T. Hildebrandt
10.30 - 10.45	Coffee Break
10.45 - 11.15	Training of elephants for "Assisted Reproductive Technologies" (ART) at the Vienna Zoo, Dr. H. Schwammer
11.15 - 12.00	Bull performance test and sperm collection, Drs. Hildebrandt, Göritz, Rietschel
12.00 - 13.00	Lunch
13.00 - 14.00	Aspects of elephant health care in Europe, Dr. W. Rietschel
14.00 - 14.20	Reproduction health assessment and estrus monitoring, Drs. Hildebrandt, Göritz
14.20 - 14.40	Artificial Insemination (AI) technique, Drs. Göritz, Hildebrandt
14.40 - 15.00	Pregnancy diagnosis, Drs. Hildebrandt, Göritz
15.00 - 15.15	Coffee Break
15.15 - 16.00	Birth management and birth disorders, Drs. Göritz, Hilderandt
16.00 - 16.30	Herpes virus infection in Asian Elephant, Drs. Hildebrandt, Göritz


14 March 2000

08.00 - 08.30	Registration at Salaya Pavilion
08.30	Depart for Samphran Elephant Ground
09.00 - 12.00	Demonstration
	- Restraint for examination, Drs. Rietschel, Schwammer
	- Examination of male reproductive tract, Drs. Hildebrandt, Göritz
	- Ultrasonography examination of female reproductive tract, Drs. Hildebrandt, Göritz
	- Lymph node biopsy, Drs. Hildebrandt, Göritz and Rietschel
12.00 - 13.00	Lunch
13.00 - 14.00	Continuation of clinical demonstration
14.00 - 16.00	Discussion
16.00 - 16.30	Closing remark

Workshop on Diagnostic Ultrasonography of Reproductive Tract in Asian Elephants

Workshop programme

15 March 2000

08.00	Depart for Safari Park at Kanchanaburi
09.30	Arrive
09.30 - 10.30	Lecture : Diagnostic Ulrasonography in Asian Elephant; Principle, Orientation and Instrumentation
10.30 - 10.45	Break
10.45 - 12.00	Practice in Bulls Elephant
12.00 - 13.00	Lunch
13.00 - 16.30	Practice in Cows Elephant

16 March 2000

08.00	Depart for Taveechai Elephant Camp at Kanchanaburi
09.30	Arrive
09.30 - 12.00	Practice
12.00 - 13.00	Lunch
13.00 - 16.00	Practice
16.00 - 16.30	Discussion, Conclusion and Closing remark

CONTENT

Female Elephants

Artificial Insemination

Annex

REPRODUCTION PROBLEMS OF ASIAN ELEPHANTS *(Elephas maximus)* IN THAILAND

P. Ratanakorn[1,2]

[1]Asian Elephant Foundation of Thailand (AEFT)

[2]Faculty of Veterinary Science, Mahidol University at Salaya, Nakornpathom 73170, Thailand.

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Reproduction Problems of Asian Elephants *(Elephas maximus)* in Thailand

Parntep Ratanakorn

Elephants are one of creatures those Thai people keep as domestic animal for thousand of years. We utilize elephant in many ways such as transportation, logging, tourism business and even-though in the military task. Number of captive Asian elephant in the pass was rather high. In 1948, there were 13,470 domestic elephants all over Thailand (DLD, Yearly Statistic Reports). But now a day, in 1998, only 2,118 domestic elephants left (DLD, Yearly Statistic Reports). It means that 85 percent of domestic or captive elephants are decreasing with a rapid rate, in the average of 227 elephants lost per year (1948-1998). This fact frightened individuals who are concerned about elephant conservation as well as Thai society very much. Many causes of declining in number of captive elephants are declaired for example car accident, infectious diseases, land mines, aging and etc. One of the major threat to those elephants is "Reproduction problems" which most people overlooked.

Reproduction problems of captive Asian elephants in Thailand are caused by 3 major problems as follows :-

Difficulty in fertility, infertility and less chance for copulation. These problems are dued to inappropriate breeding management for example;

Pathology of reproductive tract such as "Leiomyoma" which has never been diagnosed. This can be lead to infertility.

No assessment of reproductive tract soundness in both male and female elephants is used to reveal the readiness of breeders. For example sperm quality has never been checked in all breeding bulls before going to breed.

In certain area of Thailand, some small elephant villages, almost 40 elephants, are female. Not a single bull in that place. This situation can reduce chances of elephant to breed too frequently.

Domestic Asian Elephant Population in Thailand Registered to Department of Livestock Development (DLD) (1948-1998)

Year	Number	Year	Number
1948	13,470	1974	8,736
1949	14,128	1975	6,915
1950	13,397	1976	5,152
1951	13,397	1977	6,208
1952	12,695	1978	6,311
1953	13,520	1979	5,843
1954	13,226	1980	4,874
1955	12,471	1981	3,705
1956	12,467	1982	3,419
1957	12,562	1983	2,988
1958	12,438	1984	3,413
1959	12,300	1985	3,381
1960	12,595	1986	3,216
1961	12,613	1987	3,390
1962	12,099	1988	3,147
1963	11,789	1989	3,243
1964	11,428	1990	3,145
1965	11,192	1991	2,938
1966	11,277	1992	2,954
1967	11,276	1993	2,665
1968	11,149	1994	2,502
1969	11,022	1995	2,692
1970	NA	1996	3,514
1971	9,665	1997	2,180
1972	8,438	1998	2,118
1973	9,492		

From Yearly Statistic Reports (DLD)

Domestic Asian Elephant Population in Thailand
Registered to Asian Elephant Foundation of Thailand (AEFT)

age (Y)	sex				dead**	no I.D.	total
	male* $\overline{w}$ tusk	male $\overline{wo}$ tusk	male	female			
0-5	7	22	4	38	3		
6-10	0	15	11	63	1		
11-15	3	12	2	47	1		
16-20	0	18	6	77	1		
21-25	2	7	5	47	0		
26-30	2	10	4	93	1		
31-35	2	11	4	96	1		
36-40	1	13	4	92	1		
41-45	2	15	1	59	1		
46-50	1	8	3	62	1		
51-55	3	4	0	20	1		
over 55	1	5	1	48	4		
no I.D.	0	3	2	9	2	2	
total	24	143	47	751	0	2	967***
dead	1	3	3	11			18

* *no record ($\overline{w}$ tusk or $\overline{wo}$ tusk)*

** *only those reported to AEFT*

*** *from AEFT database, 31 Jul. 2000*

Mortality and Morbidity of Domestic Asian Elephant in Thailand (1996-1999)*

		Year			
		1996	1997	1998	1999
dead	**cause**				
	accident	2	1	3	2
	aging	2	3	-	-
	infection	3	1	2	2
	unknown	-	-	3	1
	killed	-	4	4	3
	total	7	9	12	8
born	**sex**				
	male	-	1	2	3
	female	2	1	4	8
	total	2	2	6	11

* *registered to AEFT by transponder identification only, from AEFT database, 31 Jul. 2000*

According to a rapid mortality rate, we are awaring of extinction of captive elephant in Thailand. Morbidity rate of this creature is very low, as well as calf survival rate. Most calves have grown to reach puberty, not more than the first 3 years of life. To increase number of elephant in order to replace and balance of the lost is a must. Application of modern technology in reproduction of livestock and other wildlife such as ultrasonography assessment of reproductive tract for breeders, embryo transfer, artificial insemination, in vitro fertilization, sperm banking and etc., are performed in many European countries and America with promising results.

We do hope that collaboration and transfering of appropriate technology in elephant reproduction can be very helpful to conserve our elephants. Workshop, survey and research to solve reproduction problems are starting with the help from German veterinarians and scientists for the benefit of elephants in Thailand.

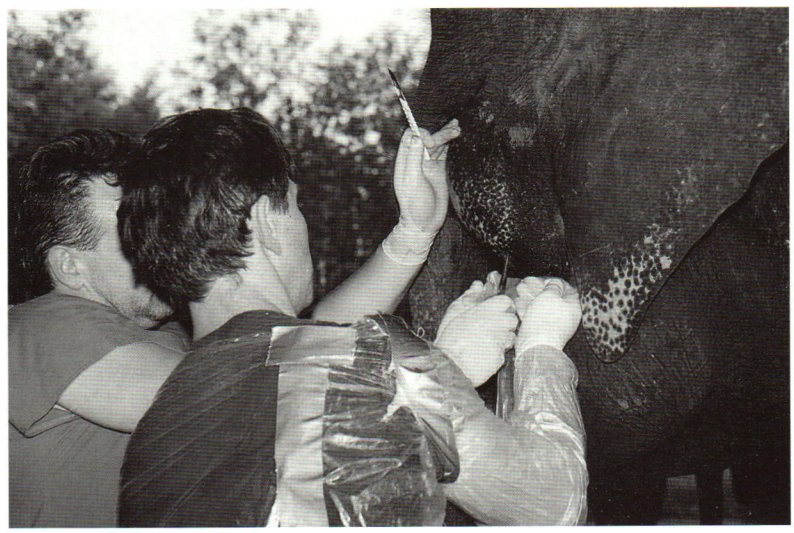
NEW ASPECTS OF BREEDING MANAGEMENT IN CAPTIVE ASIAN AND AFRICAN ELEPHANTS

Thomas B. Hildebrandt[1]

[1]**Institute for Zoo Biology and Wildlife Research (IZW), Berlin**

New Aspects of Breeding Management in Captive Asian and African elephants

Thomas B. Hildebrandt



The difficulty to establish self-sustaining captive populations in both species is mainly due to low reproduction rates. Beside the lack of sufficient breeding partners, there is also high level of reproductive disorders in the captive elephant population. The development of scientific-guided reproduction programs in elephants will greatly enhance the potential for creating self-sustaining populations in captivity. Especially for megavertebrates like elephants, it was critical that methods for evaluation of reproductive capacity be developed, including development and health status of female and male urogenital tracts as well as sperm parameters. Based on results of reproductive assessment in over 280 females and 75 bulls in different management systems and countries there were different types of infertility in captive elephants identified. Female infertility occurs temporary or permanent in captivity and is always linked with changes on the internal genital organs. These reproductive alterations are clearly related to aging and nulliparous status of the females. Older cows with a breeding history of several pregnancies did not show any of the typical lesions found in old nulliparous females. However, the internal axis of the hypothalamus, the piturity gland and the gonads was not affected and there was no difference in sexual cycle activity in the fertile and infertile cows. In contrast to the situation in females, the male infertility was mainly characterized by a functional reproductive disorder. The infertility risk in bulls is normally not age-related and becomes especially high if more than one bull is kept in the same facility. The plasma testosterone level was not an useful parameter to evaluate male breeding capacity. In general, the negative impact of male infertility for breeding programs is more than 10 times higher as reproductive disorders in females due to the sex ratio in captivity. Techniques for identifying male and female infertility and a classification system will be presented and possible causes for the infertility syndromes and options for treatment discussed.

TECHNIQUES OF ASSISTED REPRODUCTION - NEW POSSIBILITIES TO SUPPORT AND TO CONTROL THE REPRODUCTION IN ASIAN AND AFRICAN ELEPHANTS

Frank Göritz[1] et. al

[1]IZW, Berlin.

Techniques of Assisted Reproduction - New Possibilities to Support and to Control the Reproduction in Asian and African elephants

Frank Göritz, Robert Hermes and Thomas B. Hildebrandt

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Introduction

Dear Colleagues,

Cooperative research on wild and zoo animals, by developing and applying modern methods and techniques for wildlife management is one of the main targets of our institute (IZW).

Elephants, particularly their reproduction, have been high on the IZW priority list right from beginning and we soon found out, how few basic data were available beyond respectable empiric records provided by zoo vets and keepers, or wildlife managers. But elephants had to be attended to, with the Asian population on constant decline, its captive population with an appallingly low recruitment rate, and the African population highly controversial.

In consequence, development and application of new technologies adapted to elephants are required either to increase the reproductive success in captivity (e.g. reproductive assessment using transrectal ultrasonography; artificial insemination, AI) or to control reproduction (e.g. hormonal contraception of genetically over represented individuals in captivity; or population management of wild or free ranging African elephants).

I. Artificial insemination in captive elephants

Background:

The total number of free-ranging Asian elephants *(Elephas maximus)* and African elephants *(Loxodonta africana)* is estimated to be from 40,000 to 49,000 (WWF, 1995). As the number of free-ranging individuals of this endangered species continues to decline, successful genetic management of captive elephants becomes more and more imperative. The current world population of captive elephants (11,000; WWF, 1995) results primarily from export out of Africa and Asia. The difficulty in establishing self-sustaining captive populations in both species is mainly due to low reproduction rates. Besides the scarcity of breeding bulls, there has also been a high occurrence of undetected reproductive disorders in the captive elephant cow population **[Hildebrandt et al., this issue].** Successful captive elephant management is a priority among zoo and wildlife organizations worldwide. Captive populations have been maintained by collecting from the wild, longterm breeding loans, and to a lesser extent, by management of males onsite. Conservation, safety concerns, and the belief that removing females from their familiar social groupings for breeding

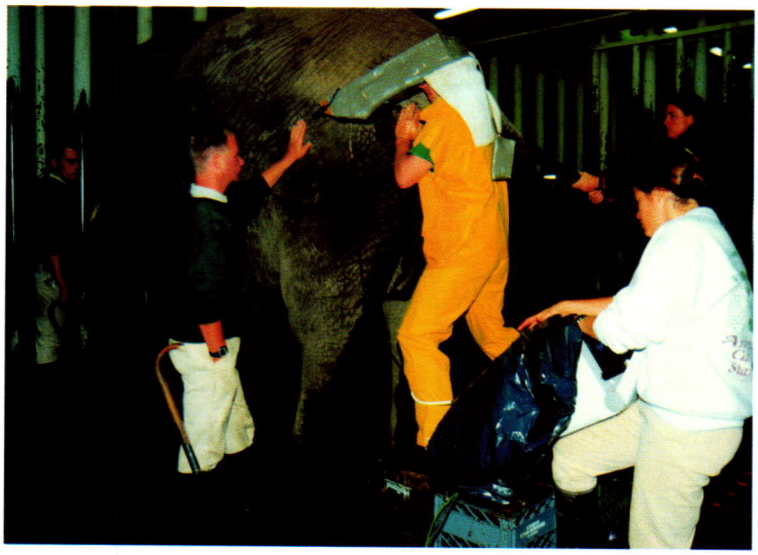


Fig. 1 Transrectal ultrasound examination in a unrestrained female African elephant at the Indianapolis zoo.

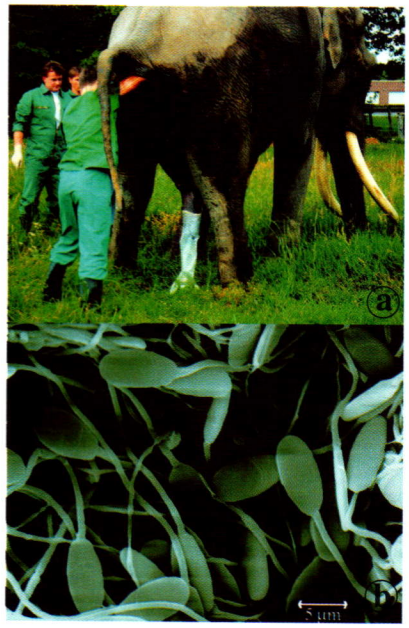


Fig. 2 ***a)*** *Manual stimulation of the ejaculatory process in a trained bull elephant;* ***b)*** *Scanning electron microscope image of the spermatozoa from a processed ejaculate.*

loans can cause distress and transmission of diseases **[e. g. fatal elephant herpes; Hildebrandt et al., this issue]**, have all contributed to the need for development of Artificial insemination (AI).

Successful application of AI would enhance captive elephant management, including the collection of genetic material from the wild **[Göritz et al., this issue]** for integration into captive populations once semen cryopreservation techniques have been perfected.

Ultrasonography:

A new technique involving the application of ultrasonography for reproductive assessment and AI has been recently implemented at different zoos in Indianapolis, USA; Washington DC, USA and Vienna, Austria. The technology was developed at the Institute for Zoo Biology and Wildlife Research in Berlin. Transrectal ultrasonography using a real-time B-mode ultrasound scanning system **(FIG. 1)** is performed in the standing or laying position without the use of sedatives or restraint devices. Feces are removed manually with the use of lubrication. The rectum is irrigated with lukewarm water. For visualizing the caudal component of the urogenital tract (vestibule, urethra, vagina, urinary bladder, cervix, caudal corpus uteri) a 3,5 MHz transducer is introduced into the rectum with ultrasound gel for coupling. To visualize the cranial component of the genital tract (cranial corpus uteri, uterine horns, ovaries, surrounding tissues) a 5.0 - 7.5 MHz transducer is attached to a specially adapted extension.

Semen collection and semen processing:

Semen was collected by rectal palpation **(FIG. 2a, b)** of the accessory glands with manual penile stimulation. Ejaculate volume, concentration and pH, and sperm motility and viability were assessed at collection and prior to the insemination. Samples were fractionated, and each assessed separately prior to combining. Semen was diluted 1x1 with TL Hepes solution. A microbiological evaluation of the semen was performed to monitor

the potential risk of pathogenicity. Extended semen was transported by air at 4°C in a refrigerated vessel. Preliminary trials revealed semen maintained good motility for 6 days and 8 hours after collection for African and Asian elephants, respectively.

AI-procedure:

This project involved simultaneous imaging by ultrasonography and endoscopy to verify semen placement. These components have never been accomplished together in an elephant AI. The insemination technique is noninvasive and has resulted in sperm deposition directly into the cervix. Ultrasound guided AI has been attempted, to date, in three African and one Asian elephants. Reproductive hormone levels have been monitored from blood samples from ear veins regularly. The cows were determined to be excellent candidates for several reasons:

1. They are of prime breeding age.
2. They are extremely calm, tractable and well-trained.
3. They are in very good general and reproductive health.
4. They have been palpated both vaginally and rectally on a routine basis.

Ultrasonographic examinations revealed no indications of reproductive pathology in the urogenital tract or ovaries. Several small cysts were visualized, one in particular serving as a landmark near the opening of the cervix. There was no evidence of injury or inflammation due to these ultrasound procedures. Both endocrine data and ultrasonographic images were used to determine the timing of AI trials. The AI series was attempted as close to the predicted time of ovulation as possible, ideally inseminating at least

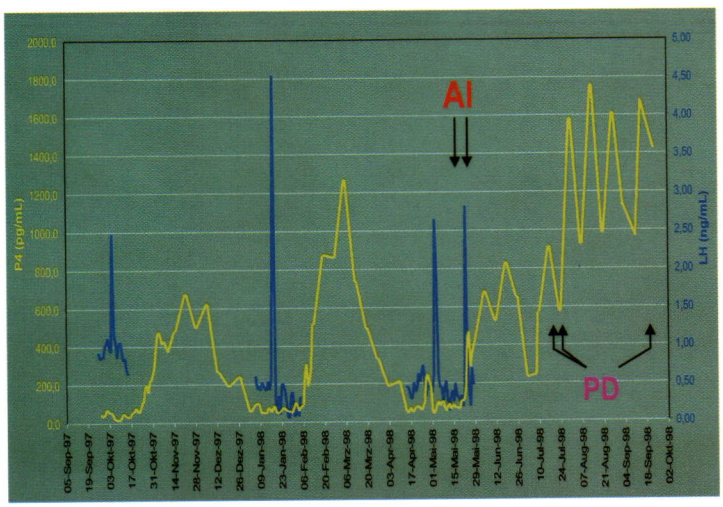


Fig. 3 Schematic diagram shows the P4 and LH hormone profiles of the African cow "Kubwa". Arrows indicate AI timing and numbers of trials and ultrasonographic pregnancy diagnosis (PD) at 9 weeks gestation.

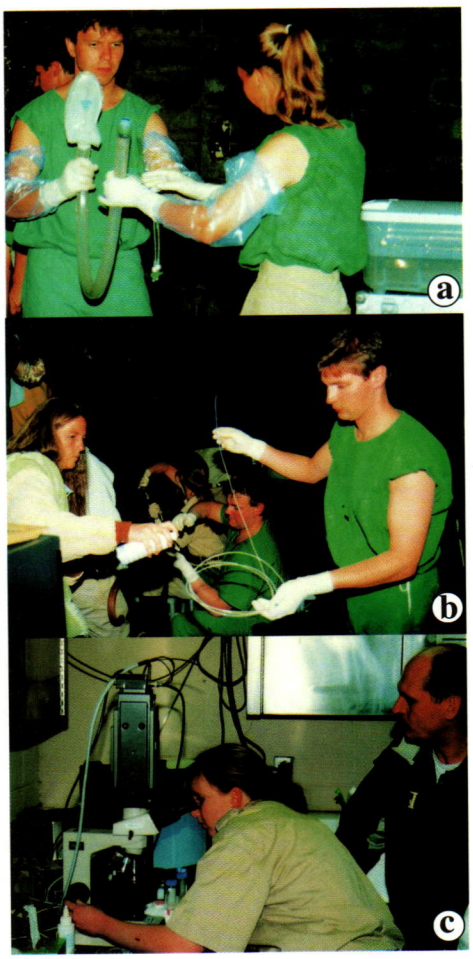
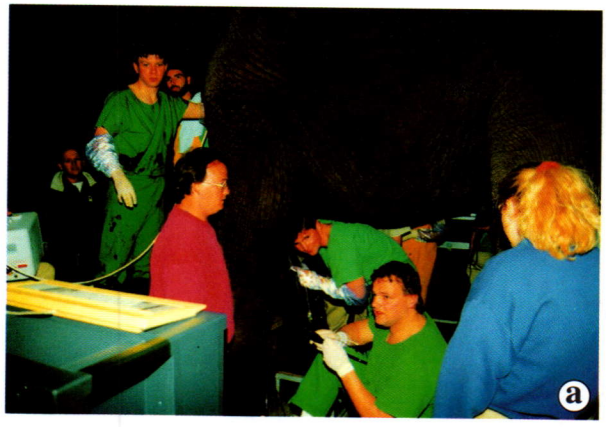
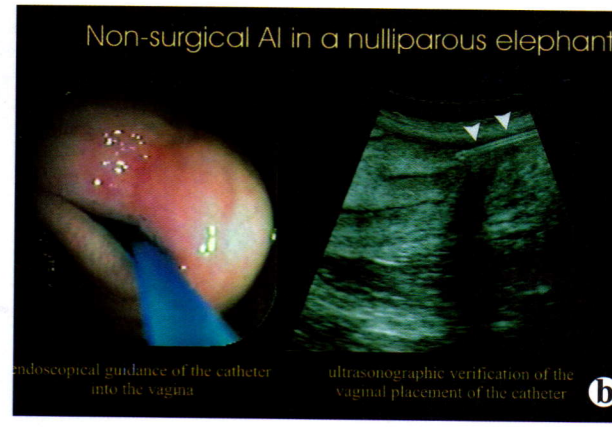


Fig. 4 a) Balloon catheter is lubricated prior to install into the urogenital tract; b) AI catheter (length 3m, diameter 2.2 mm) is placed into the working channel of endoscope; c) microscopical semen assessment prior to place into the cervix via catheter.





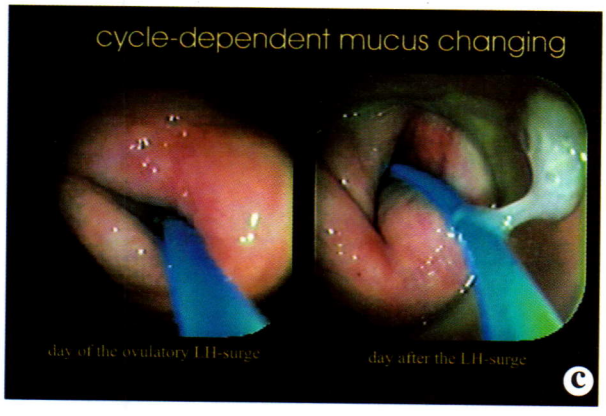


Fig. 5 *a) Endoscopical and ultrasonographic guidance of the AI catheter; b) Endoscopical image (left) of the 2.2mm sized catheter passing through the cervical portio, corresponding sonogram (right) shows the inserted AI catheter in the cervix appearing as a white line; c) Two endoscopical images of the vaginal os showing the cycle-dependent mucus changing.*

one try before and after the actual day of ovulation. Females were monitored daily for circulating levels of progesterone (P4) and luteinizing hormone (LH). Two LH peaks, separated by 20 days, were detected, with the second peak being the ovulatory LH surge. Detection of the first peak provided a three week window to prepare for the inseminations **(FIG. 3)**. Daily transrectal ultrasonography identified morphological changes in the vagina and endometrium and characterized developing ovarian structures during the follicular phase. For the first time, ultrasonography visualized follicle growth and maturation and the development of Graafian follicles by the detection of cumulus oophorus. The ruptured ovulatory follicle and corpus hemorrhagicum could also be visualized.

No sedation or restraint was used during the procedures. A sterile balloon catheter **(FIG. 4a)** was inserted in the vestibule to slightly distend the reproductive tract for optimal visualization and placement of endoscope and insemination catheter **(FIG. 4b)**. Concurrently, the semen was warmed to 37 °C prior to insemination **(FIG. 4c)**. Samples used for AI trials ranged in motility from 20-95%. Introduction of semen was monitored endoscopically and ultrasonographically to verify placement **(FIG. 5a - c)**. The individuals were inseminated 2-3 times (once a day), due to the availability of semen. The entire procedure required only 1.5 hours. Olfactory cues were presented to the AI candidates before and after AI. They showed strong reactions, rumbling and pelvic thrusting.

Pregnancies (n = 4) were confirmed ca. 9 weeks after AI by transrectal ultrasonography **(FIG. 6)**.

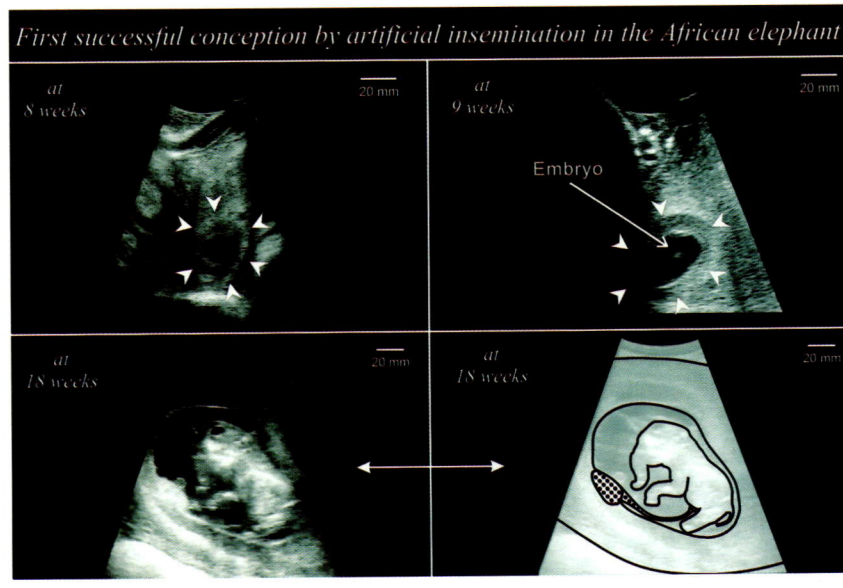


Fig. 6 Ultrasonographic pregnancy diagnosis in the 22-year old female African elephant "Kubwa" 8, 9 and 18 weeks after the AI; AI baby "Amali" born 6th March 2000.

Conclusions:

Artificial insemination (AI) and semen preservation techniques improve our ability to breed elephants in captivity. The development of assisted reproduction techniques might be future key to elephant conservation by amplifying genetic diversity and breeding success in captive and wild populations, e.g. in reintroduction programs for endangered subspecies of elephants.

II. Hormonal contraception in wild African elephants

Most effective game-management policies and intense protection led to a steady increase of the elephant population at Kruger National Park (KNP), South Africa. To prevent an elephant overpopulation that would destroy habitats and threaten the survival of many other species, the National Parks Board (NPB) was urged for many years (1968-1995) to cull entire elephant herds (average 517 elephants per year). Responding to international discussions NPB started in 1996 to evaluate alternative management measures to ensure the habitats balanced ecosystem and biodiversity. Translocation of elephant groups **(FIG. 7)** to other protected reserves with less density offered only a short term alternative to culling due to limited availability of new habitats. Another alternative of controlling population growth was the implementation of contraceptive methods. The aim of our two year study was to investigate the use of hormonal contraception for prolongation of the four year inter-calving interval estimated in elephants at the KNP.

In October 1996, a total of 66 female elephants were examined by ultrasonography **(FIG. 8)**. This technique has been modified successfully to determine the reproductive status in elephants under field conditions within 15-20 minutes. Fourteen animals diagnosed as pregnant **(FIG. 9)** were excluded from the study. The other animals (n=52) were divided into (i) an immunocontraception group (USA/RSA project, n=21), (ii) a hormonal contraception group (n=10), and (iii) a control group (n=21). Elephants in the hormonal group were treated with subcutaneous

Table 1 Sonographically diagnosed pregnancy rates

	No. of animals examined			controls (n=21)		treatment (n=10)	
	total	gravid	non-gravid	gravid	non-gravid	gravid	non-gravid
October 96	66	16*	50		21	(1)°	9
April 97	20	7	13	6	4	(1)°	9
October 97	46	25	21	15	2	(1)°	9
August 98	14	6	8	1	1	1	7**

* 14 pregnant females were excluded from the study in October 1996.

** Graafian folicle detected.

° accuracy of ultrasonographical pregnancy diagnosis approx. 95%; in one female, the pregnancy was missed in October 1996 and the female was included in the hormone group and released in October 1997.

silicone rubber implants (**FIG. 10**), 5 implants per animal, (Compudose™) which slowly releases minute amounts of 17β-oestradiol (300µg/animal/d). Blood samples had been taken in two-monthly intervals to determine their plasma levels of oestradiol (E_2) and dihydroprogesterone (DHP).

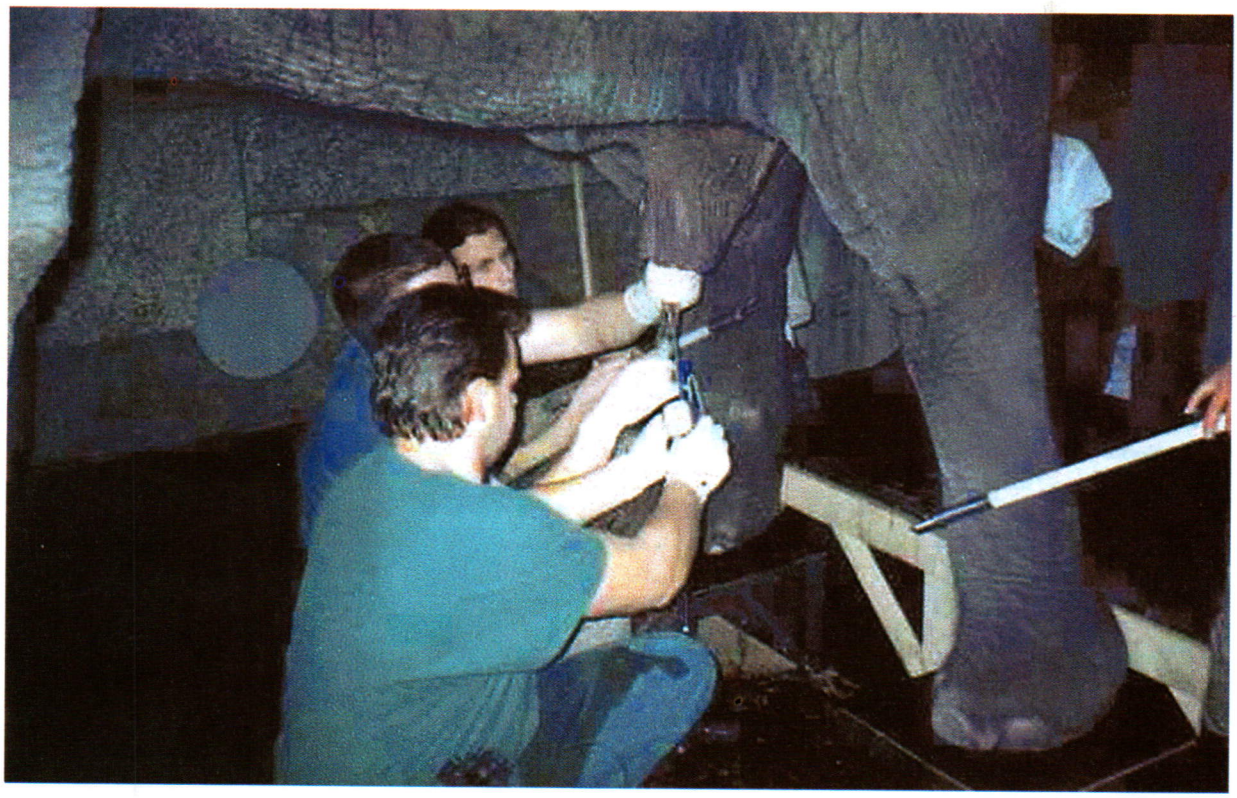
After one year none of the animals treated was diagnosed sonographically to be pregnant (100% contraception success rate) when compared to a 94% pregnancy rate in individuals of the control group investigated (n=18). After two years, contraception success rate was still at 87.5% compared to a 100% pregnancy rate in the control group. One conception after 16 months (approx. 8 months gestation) and one documented ovulation 24 months after the treatment prove the reversibility of the hormonal contraceptive method applied (**TABLE 1**). Serum analysis within the

Fig. 7 Immobilization and translocation of an African elephant at the Kruger National Park, South Africa.

Fig. 8 Ultrasound examination in the field for pregnancy detection in a free ranging, immobilized African elephant at the Kruger National Park, Skukuza, RSA.

first 12 months revealed constant E_2-levels in treated animals that were in average lower than E_2-levels in control animals in follicular phase or in pregnancy (treated: 0.4-5.3 pg E_2/ml, controls non-pregnant: 0.1-10.5 pg E_2/ml, pregnant controls: 2.1-3.6 pg E_2/ml). E_2-implants caused a down regulation of the ovarian function documented in three ultrasound examinations throughout the study **(FIG. 11)** and in serum analysis for DHP (treated: 0.0-2.8 ng DHP/ml controls non-pregnant: 0.1-26.4 ng DHP/ml, pregnant controls: 7.2-71.6 ng DHP/ml) **(FIG. 12)**. No pathological alterations possibly caused by the treatment were detected during regular ultrasound examinations of the urogenital organs (four exams per animal 1996-1998). All animals treated (n=10) were healthy and lactating with calves as before. After two years the animals examined (n=8) still nursed their own or adopted calves (n=1) aged over two years at the termination of the project.

Further studies to improve the effectiveness and practicability of hormonal contraception can provide a safe and reversible solution to population control for overrepresented individuals in captive breeding programs or in small reserve populations in Africa, where culling is totally unacceptable.



AI: *Artificial insemination in an african elephant.*
Photos: *Harald Schwammer*

Basic requirements:

With exclusive regard to the situation in zoos it can be said that two elephant handling strategies are dominant. There has been a great deal of professional discussion within the elephant management community worldwide regarding elephant handling systems and programs. This discussion is based on questions of ideology and ethics. At present there is an increasing awareness of constructive discussion, collaboration and a growing understanding that free contact, protected contact and confined contact are all necessary to successfully manage elephants in captivity.

In our workshop here in Bangkok we will restrict ourselves to a discussion of the free contact system. Achievement of the necessary level of elephant training requires perfectly trained elephant handlers. In "the West" practical training is provided by the zoos themselves, including voluntary work in other zoos, as well as in a several week long practical training course available in Arcansas (USA).

It is just these specific training methods, however, it becomes the target of criticism by animal welfare organizations all over the world. It is for this reason that at the Vienna Zoo we are taking care to ensure that the training program is based on the most modern results, meaning that positive and negative reinforcement is used. A major component involves rewarding the animal, i.e. positive learning. At any rate, it is based on the building up of trust between the animal and the human, where the latter must, of course, retain control over the elephant.

Body care:

Daily showering is the basis of every routine, whereby the condition of the skin, sole of the foot, toes, eyes, ears and the entire rest of the body is checked. Showering primarily serves

Ultrasound: *Elephant laying down.*
Photos: *Harald Schwammer*

skin care, but is additionally important as a daily training routine.

Foot care is of particular importance as many fatal outcome are still the result of dangerous infections or diseases that stem from poor foot care.

It is for this reason that the animals are trained to wait patiently with their feet on small pedestals so that the sole of the foot and the toenails can be cared for and treated with special tools, such as hoof knives and rasps.

African elephants are somewhat easier to care for, with regard to foot care, than Asian elephants, which require constant care. It is also helpful if the elephants are trained to lie on both sides, facilitating the foot care task.

It is readily apparent that this work requires well grounded knowledge of the foot anatomy of elephants inorder to be successful. This means that a course in elephant care has to start with anatomy.

Veterinary supervision:

For a specific monitoring of the animals it is recommended that the blood be sampled weekly. In this way the state of health of the animal can be monitored, as well as the oestrus-cycle in females for successful breeding management. It is important that these blood samples be taken at different spots and sides of the body, e.g. the ear or leg veins. Utmost cleanliness is essential.

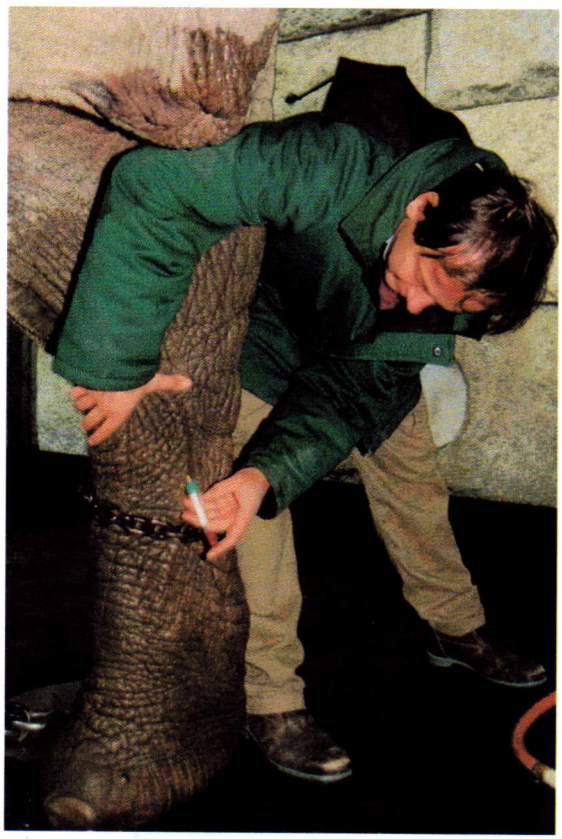
For monitoring the health condition and to test T.B. disease, the "trunk wash" technique has been developed. The elephant has to be trained to allow filling fluid into the trunk, keep it there for some moments and then blow the fluid into specific container. This sample is sent to laboratory for testing of T.B.

All these things are normally easy to carry out on animals under control, and, after a brief acquaintance period, in a short time. Ultrasound

inspections and eventually artificial insemination place further and more complicated demands on the training status of the animal and require patient training sessions.

Advanced requirements:

The repeated use of ultrasound and the eventual resulting artificial insemination initially involves restraining the animals, but later they can stand completely free and quietly on two double pedestals. The procedure can take between 30 min. and one and a half hours. The animal is first to be accustomed to standing quietly on the pedestals, then to the presence of strange persons both beside, as well as underneath, it. Various pieces of equipment, and the noises they make, is to become accustomed to as well. Finally, the animal stands completely relaxed on these pedestals, eating tidbits e.g. carrots, and the veterinarians can carry out the examinations without problem. During these examinations particular care is to be placed on the safety of the persons involved, therefore it is recommended that this training be done step-by-step and with patience. It should also be considered that modern equipment of enormous value is being used that is easily damaged or ruined by an untrained animal. The highpoint of the training, but also of the technical organization and coordination, is artificial insemination. As the reliable storage of elephant sperm has still not been achieved, only the use of fresh sperm stored less than 10 hrs. is possible. Obtaining sperm is



Blood sample: *Taken from the lateral saphenous vein.*
Photos: *Harald Schwammer*

Foot care: *Daily control and trimming sole and nails.*
Photos: *Harald Schwammer*

the other problem that still is in need of solving. The more approachable the bull is, however, the easier it is. Secure restrainment of the bull with four chains is essential.

As mentioned, in our case insemination can be performed between 30 min. and 1.5 hrs. Four elephant keepers secure the free standing elephant while two veterinarians inseminate from below. A further veterinarian controls the procedure via rectal sonography. Monitors, recorders and other expensive equipments document the procedure. This short description should clearly show how much effort in terms of manhours, material and organization are involved in this sort of project.

General Safety Aspects for Veterinarians:

1) Before beginning, the plan must be discussed with all the involved persons and it is to be clarified what the elephant is capable of doing, and what can be expected of it. An exact plan of the procedure must be unmistakably agreed to by the mahout or keeper.

2) At every inspection or treatment of elephants even the best trained must be secured by chains (min. two, better four).

3) During the whole procedure the elephant has to be kept under steady control by one specific elephant handler. All other persons have to be instructed about their job, clearly.

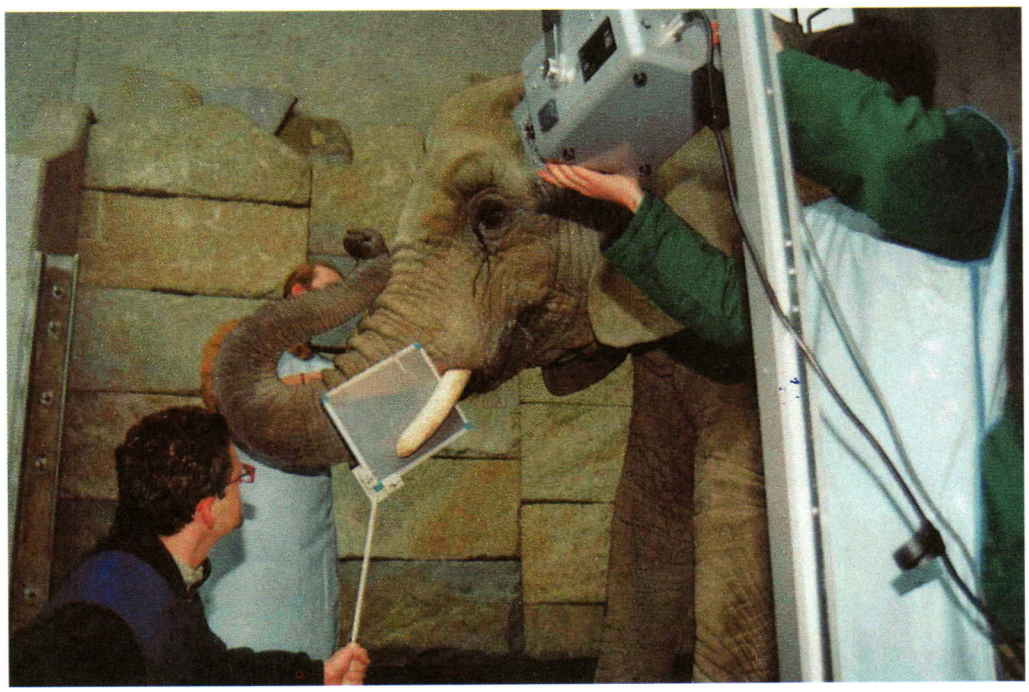
4) The surrounding area has to be free of persons not involved. Other free ranging elephants have to be removed or restrained, too.

5) All equipments have to be kept under best condition and cleaned properly after every use.

6) Only calm behaviour and following exact the plan enables safe procedures and successful projects.

The theoretical discussion of this topic only can inform about these problems and it is necessary to organize additional workshops for showing and teaching people the different technics of training elephants due to the described requirements.

Tusk x-ray ***Photos:*** *Harald Schwammer*



SEDATION OF THAI WORKING ELEPHANTS WITH XYLAZINE AND ATIPAMIZOLE AS A REVERSAL

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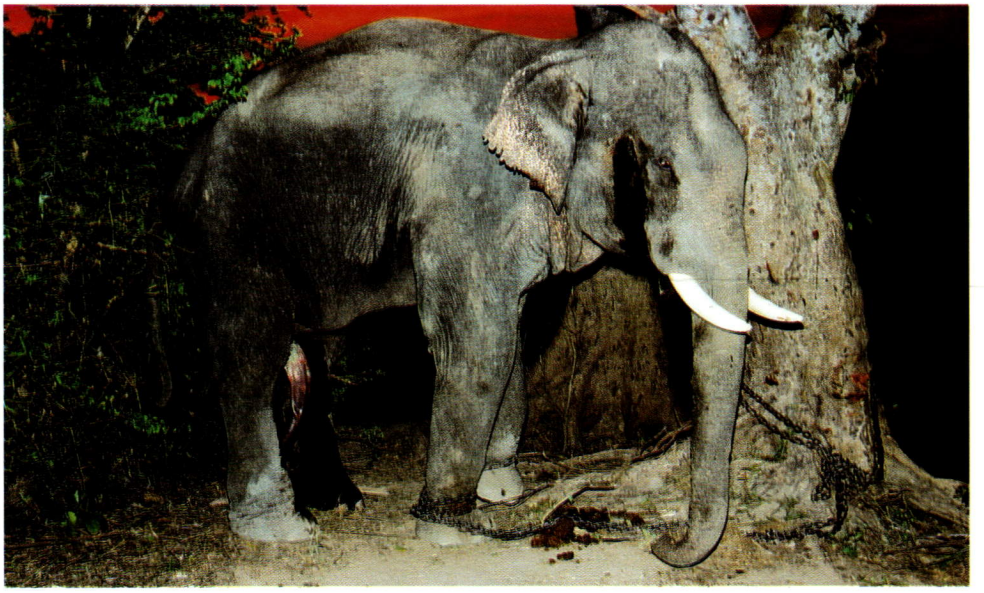
Sedation of Thai Working Elephants with Xylazine and Atipamezole as a Reversal

Wolfram Rietschel, Thomas Hildebrandt, Frank Göritz and Parntep Ratanakorn

Introduction:

In March 2000, a joint Thai-German project was started with the aim to analyse a possible infection of Asian elephants in Thailand with a novel Endotheliotropic Herpes virus (ETHV). A team of veterinarians from the Institute for Zoo Biology and Wildlife Research Berlin, the Wilhelma Zoological and Botanical Garden Stuttgart (Germany) and the Mahidol University Nakhonpathom (Thailand), performed health checks, treatments, ultrasound examinations of the genital organs of male and female elephants, collected blood samples, lymph node biopsy samples, parasites and semen in all together 83 Asian elephants in various places in Thailand: Ayudhya Elephant Camp, March 2-3, Elephant Conservation Centre Hang Chat, March 4-5, Maesa Elephant Ground, March 6-7, Nong Nooch Tropical Garden, March 9-10, Sriracha Tiger Zoo, March 11, Samphran Elephant Ground, March 14 and 17, Safari Park Kanchanaburi, March 15, Taweechai Elephant Camp, March 16 and Khao Kheow Open Zoo, March 21. Semen collection by manual rectal stimulation, collection of blood samples and ectoparasites, most of the ultrasound examinations, the foot care and some minor treatments could be performed without anaesthesia. 21 elephants had to be sedated by intravenous injection of Rompun® (10% xylazine) to allow the necessary diagnostic procedures as lymph node biopsy, ultrasound examination of the internal genital tract and painful treatments like flushing of abscesses. One very excited female and her 3 year old calf had to be tranquillized by the use of a blowpipe. Additional one elephant bull in musth was sedated the same way to allow the mahout to fix the loose chain. For the first time, Antisedan® (atipamezole) was used as a reversal in Thai elephants.

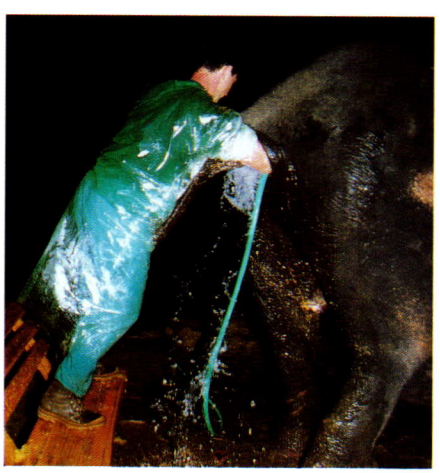


Trunk immobilization, prolapsed penis in an elephant bull in musth after sedation with 1000 mg xylazine by blowpipe.

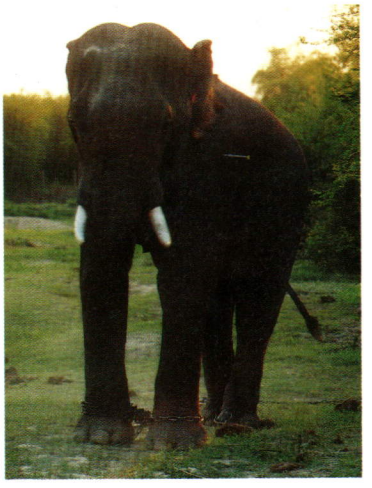
Material and methods:

Table 1 *Elephants, drugs (xyl. = xylazine, a. = atipamezole) and dosages. All injections i.v. or by blowpipe (*).*

Date	Location	Sex	Age (y)	Name	xyl. (mg)	a. (mg)	Remarks
3.3.	Ayudhya	0.1	35	Maruay	200	10	
3.3.	Ayudhya	0.1	30	Lumruy	200	10	Tympanic colic after sedation
3.3.	Ayudhya	0.1	40	Dokmak	300	15	Abscess treatment
4.3.	Hang Chat	1.0	40	Somboon	300	20	Penis retracted 4h after a.
4.3.	Hang Chat	1.0	50	Plaikaew	250	25	Penis retracted. 2h after a.
4.3.	Hang Chat	1.0	28	Tadang	200	25	Penis retracted. 1h after a.
5.3.	Hang Chat	0.1	50	Pungbee	200	20	
5.3.	Hang Chat	0.1	47	Pongiang	200+100	25	
5.3.	Hang Chat	0.1	21	Lannah	200	20	
5.3.	Hang Chat	0.1	27	Tantawan	200		
5.3.	Hang Chat	0.1	43	Saeb	200+50	20	Abscess right ear
5.3.	Hang Chat	0.1	37	Pamae	250	20	
7.3.	Maesa	1.0	27	Sombum	250	20	Penis retracted 3 min after a.
7.3.	Maesa	0.1	13	Maesani	150+75	15	
7.3.	Maesa	0.1	39	Buakum	200	15	
7.3.	Maesa	1.0	25	Boonpaea	200	15	Bact. infect. of the gen. tract
14.3.	Samphran	0.1	30	Benz	200	5	
14.3.	Samphran	1.0	19	Ole	100	10	Penis retracted 10min after a.
15.3.	Safari park	0.1	25	Jim	600*	30	Abscess treatment
15.3.	Safari park	1.0	3	Tongtoe	100*	10	Son of Jim
15.3.	Safari park	1.0	15	Sompong	1000*	50	Musth, penis retr. 5min after a.
16.3.	Taweechai	0.1	19	Wasana	100	10	6 weeks pregnant
16.3.	Taweechai	1.0	21	Kamo	150	15	Penis retracted 5 min after a.
16.3.	Taweechai	0.1	20	Pungperm	100	10	Early pregnancy



Emergency treatment with fecal evacuation and enema of and elephant cow with tympany after xylazine sedation and overfeeding with corn.



Elephant in musth attacking one of the authors. A Telinject® dart is visible at the left shoulder.

Trunk immobilization in a female elephant after intravenous injection of 200 mg xylazine.

Results and discussion:

Xylazine seems to be a suitable drug to calm down Asian elephants for diagnostic procedures and some veterinary treatments. Intravenous injection of 100-300 mg in adult elephants (approximately 4-10 mg/100 kg bodyweight) will cause immediate sedation visible by trunk immobilization, prolapse of penis or external female genitalia (praeputium feminale). The animals remain standing and are unable to use trunk, legs and feet in a coordinated manner. According to Fowler (1) who recommends 8-15 mg/100 kg bodyweight intramuscularly, the valuable effects of xylazine in elephants are: low therapeutic dose, smooth induction, smooth recovery, excellent analgesia and excellent sedation. Fowler warns that there is a greater risk of the elephant's falling down because of the drug rapidly affects the brain when xylazine is administered intravenously. According to our experience with 21 healthy elephants, xylazine injected slowly intravenously is safe and causes immediately a satisfying sedative, analgesic, and muscle relaxing effect. It is strongly recommended to keep a syringe with a reversal in hand. Yohimbine 1% or atipamezole are effective reversals and can be used in overdosed animals or accidentally injected mahouts (1mg of xylazine/kg body weight can be lethal in man).

If no intravenous injection is possible and the drug has to be given intramuscularly, an increased dose of xylazine and a minimum of 25 minutes of undisturbed induction is necessary. In excited animals and bulls in musth, the required doses can be much higher. At the Safari park, Kanchanaburi an exited female and one bull in musth had to be darted and needed 600mg and 1000mg to calm down. Overdosing of xylazine seems not to be a serious problem, provided that the animal has no circulatory disease and if a suitable antidote is in hand. One hundred mg. of xylazine can be reversed by 5-10mg of atipamezole. Another effective reversal is Yohimbine in 1% solution (100-150mg/1000kg bodyweight). Atipamezole hydrochloride (Antisedan®) is produced as a reversal of medethomidine

(Domitor®) for cats and dogs. Additional it can be used as a reversal for xylazine in elephants (3) and many species of wildlife (2). In elephants, atipamezole should be injected intramuscularly or very slow intravenously.

It is very important not to allow the animal to eat large quantities of food before and after the sedation. In Ayudhya, one female elephant was overfed with fresh corn after the sedation and showed serious symptoms of colic and tympany. Emergency treatment included several fecal evacuation and intensive enemas, additional injections of atipamezole and moving of the animal for several hours.

In elephant bulls it seems to be very important that prolapsed penis is completely retracted after the immobilization. If not, there is the danger of injuries and oedema if the animal walks back to the forest. The mahout has to be informed about this side effect and advised to observe the condition of the organ. In Hang Chat, a bull sedated with 300 mg xylazine and reversed with 20 mg atipamezole needed four hours to retract his penis. Another bull sedated with 250mg xylazine and reversed with 25mg atipamezole retracted his penis after one hour. This period of time can be reduced to few minutes when in addition cold water is poured on the organ.

Literature:

1. Fowler, M. E.: Zoo and Wild Animal Medicine. 2nd Edition. W.B. Saunders Company. Philadelphia-London-Hong Kong. 1986
2. Göltenboth, R. u. Klös, H.G.: Krankheiten der Zoo-und Wildtiere. Blackwell Wiss. Verlag Berlin. 1995
3. Rietschel, W.: Veterinary care of Asian Elephants in Europe. In "Minimum requirement for health status and management of Asian elephants in South East Asia." Proceedings of the First Regional Workshop on Asian Elephant Health Care. Chiang Mai. The National Identity Office Secretariat of the Prime Minister.1999

Drugs and equipments mentioned in the text:

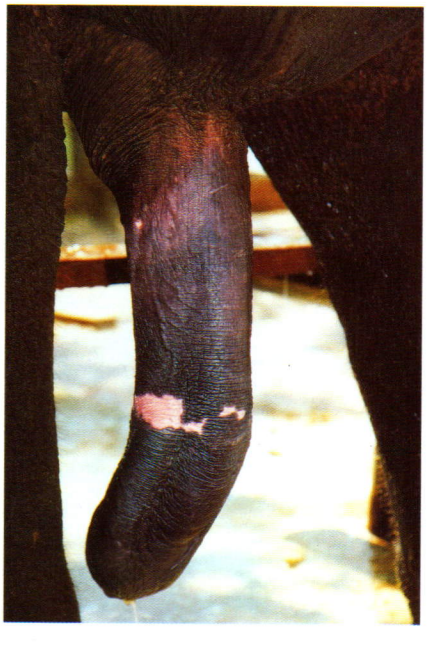
Antisedan®, atipamezole hydrochride, Orion Corporation, Finland

Domitor®, medethomidine, Orion corporation, Finland

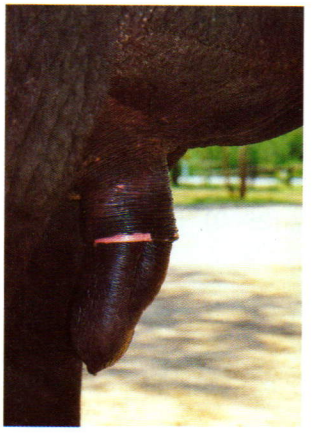
Rompun®, xylazine, Bayer, Germany

Yohimbine 1% solution

Telinject®, Germany



Protrusion of the penis of a 21 years old elephant bull after 150 mg. xylazine injection i.v. at Taweechai Elephant Camp.



Retraction of the penis 3 minutes after 15 mg atipamezole injected i.v.

ULTRASONOGRAPHY AS AN IMPORTANT TOOL FOR THE DEVELOPMENT AND APPLICATION OF REPRODUCTIVE TECHNOLOGIES IN NON-DOMESTIC SPECIES*

Thomas B. Hildebrandt [1] et. al

*Theriogenology (2000), 53(1), 73-84

[1]IZW, Berlin.

Ultrasonography as an Important Tool for the Development and Application of Reproductive Technologies in Non-Domestic Species

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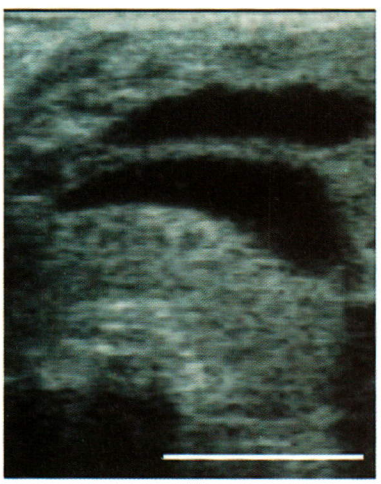
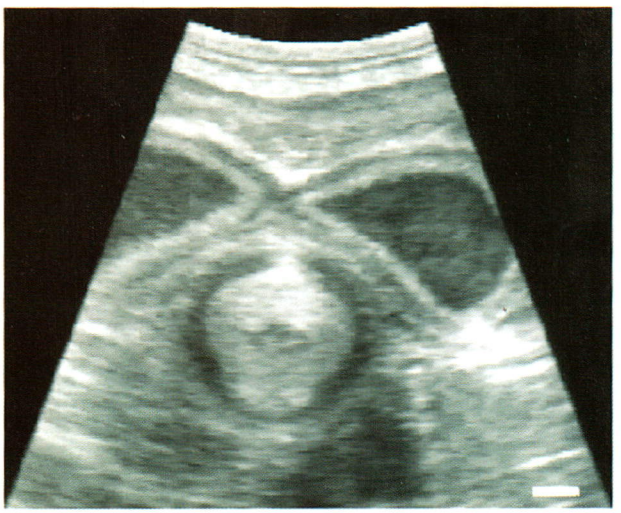
Abstract

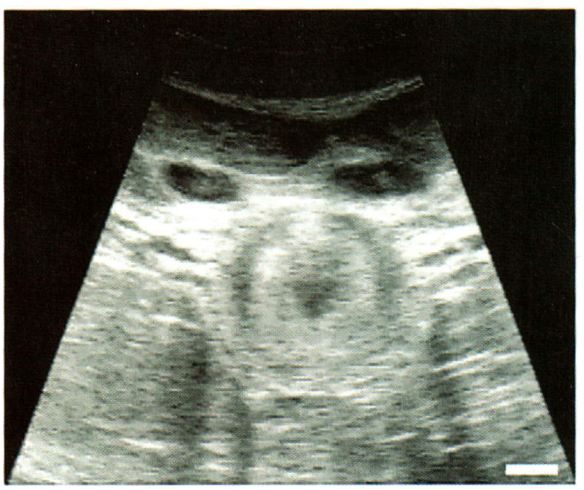
Ultrasound imaging in reproductive science offers new opportunities regarding optimization of induction of the sexual cycle and ovulation, superovulation regimes, contraception programs, semen collection and testicular sperm extraction techniques, ovum pick up and ovarian transplantation procedures, as well as the application of artificial insemination, embryo collection and transfer. In non-domestic species, most of which lack basic data, ultrasonography is an ideal tool to study reproductive biology in both captive and wild populations. The use of this imaging modality led us to develop new or modify established reproductive technologies. Ultrasonography has been an integral part of over 200 assisted reproduction procedures in 17 mammalian species performed by our research team between 1992 and 1999. These procedures included the initial characterization of sexual cycles, hormonal cycle induction, semen collection by electroejaculation or manual stimulation, nonsurgical artificial insemination (AI), nonsurgical embryo transfer and temporary hormonal contraception. For these investigations, a variety of newly developed equipment was applied and species-specific hormonal treatments designed. We used several commercial and customized ultrasound systems with a variety of technical features. Some relevant improvements of these applications will be described and the role of ultrasonography elucidated to.

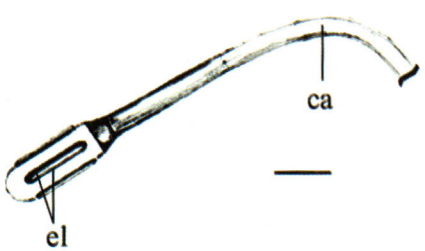
Acknowledgments

We thank our post-doc students A. Broich, M. Fassbender, G. Fritsch and M. Quest for their contributions to the research presented in this manuscript. Unpublished studies were in collaboration with Dr. J.Brown, M. Gilles, Professor P.S. Glatzel, Dr. R. Goltenboth, Dr. D. Grobler, Professor R.R. Hofmann, Dr. A. Lange, Dr. N. Loskutoff, Dr. J. Maltzan, Professor H.H.D. Meyer, Dr. R. Montali, D. Olson, Dr. S. Quandt, Dr. D. Schmitt, Professor F. Schwarzenberger, Dr. Ch. Walzer and Professor H. Wiesner. In addition, the authors would like to acknowledge H. Gerhards, Dr. S. Seager and A. Schnorrenberg as representatives of contributing companies. We are thankful to Dr. M. East for reviewing the manuscript.









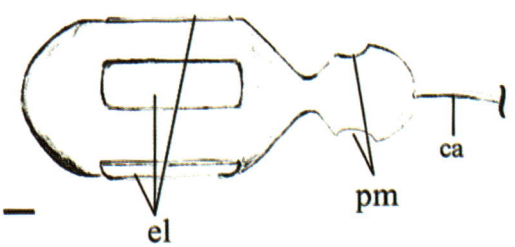


Figure 1: *Sonomorphological studies of testes (upper panel, bar = 1 cm) and accessory sex glands (middle panel, bar = 1 cm) resulted in the design of an electroejaculation probe for European brown hare (lower panel, bar = 2 cm).*

Figure 2: *Sonograms show ampullae (dark areas) above the urethra (round echogenic structure) in cross-section in a wild African elephant before (upper panel) and after electroejection (middle panel, bar=1 cm.) Emptied ampullae after stimulation proved the success of semen collection techniqye. Schematic drawing of the handheld electroejection probe developed for elephants (lower panel, bar=2 cm).*

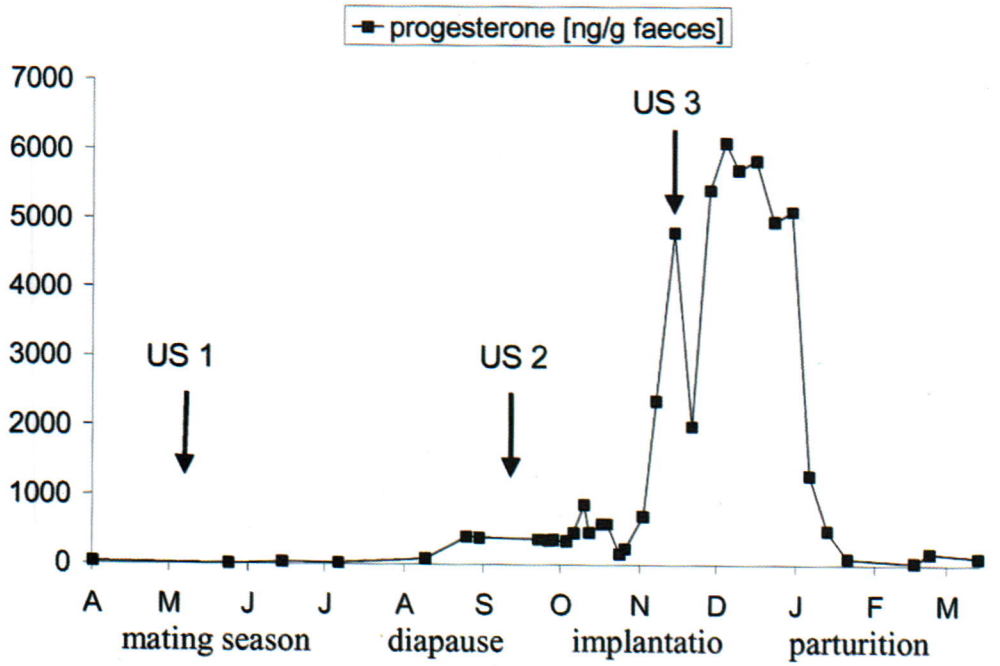


Figure 3: *Concentrations of progesterone in brown bear measured by enzymeimmunoassay were correlated with the results of ultrasound examinations (US 1 - US 3) describing structural changes of the ovaries and uterus during annual sexual cycle. See Figure 4 .*

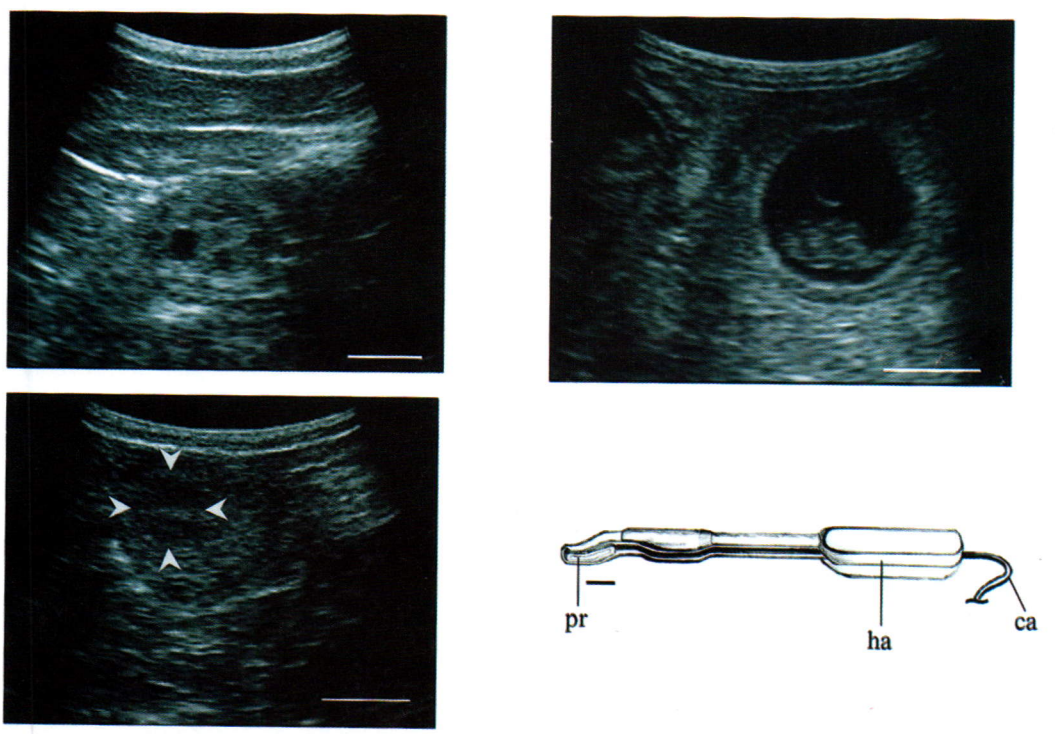


Figure 4: *Sonograms of an ovary in a brown bear showing one dominant as well as several smaller follicles (round dark areas) during mating season (US 1, upper panel left, bar = 1 cm) and Corpus luteum (arrowheads) during diapause (US 2, lower panel left, bar = 1 cm). After implantation (late Nov./early Dec.) pregnancy was detected by ultrasonography (US 3, upper panel right, bar = 1 cm). Visualization of ovaries and non-pregnant uterus was possible by transrectal ultrasonography only. For this application a specific probe extension (Type II) was developed (lower panel right, bar = 2 cm).*

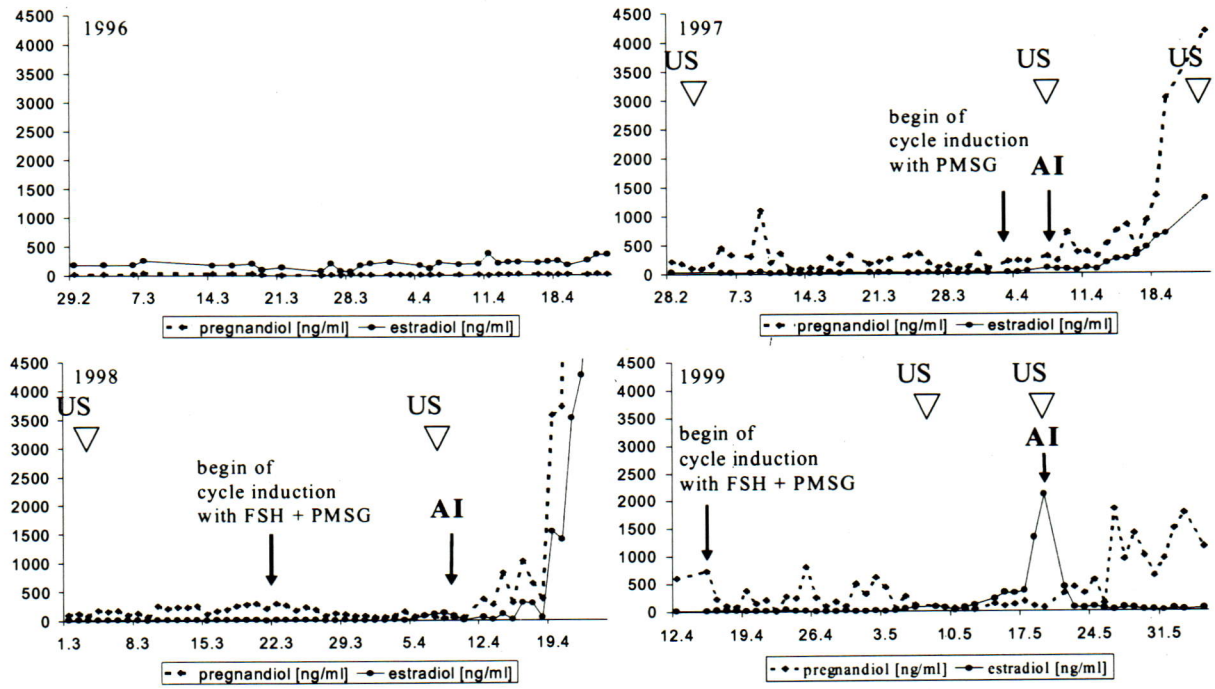


Figure 5: *Different hormone (1997-1999) regimes for estrous cycle induction in a non-cycling giant panda (1996) were accompanied by regular ultrasound examinations. Hormone regimes were optimized based on ultrasonographic (US) and endocrinological findings.*

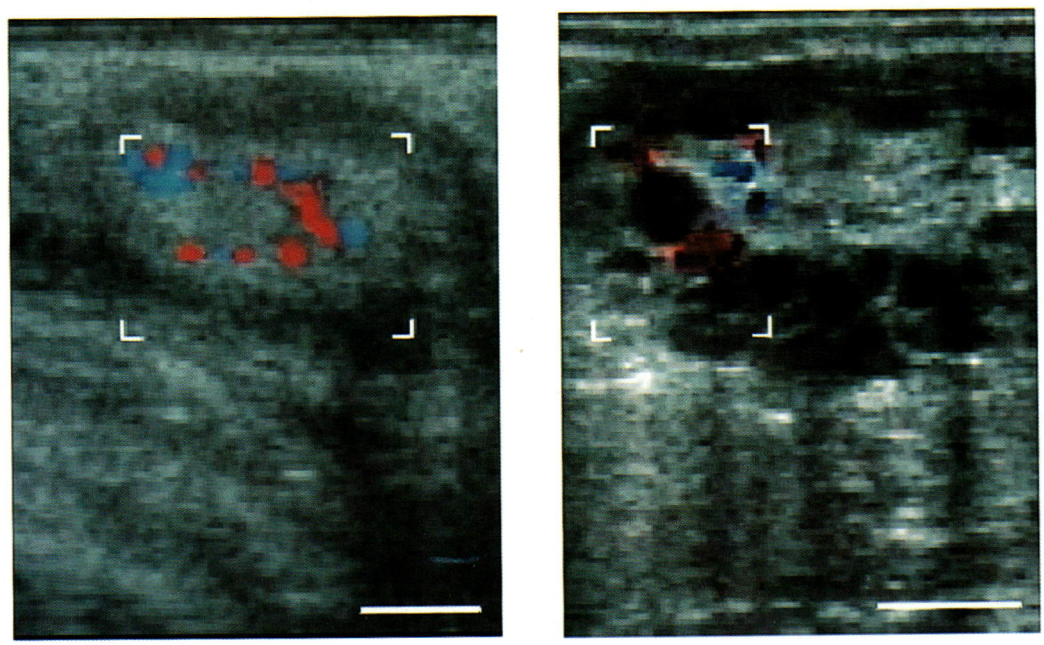


Figure 6: *Sonograms (Doppler color flow, performed 1999) of an ovary of a giant panda before (left) and after (right) hormonal cycle induction. In the inactive ovary no follicles and no cortical blood flow were detectable. A dominant and several antral follicles as well as increased blood supply of the ovarian cortex indicating the success of the advanced hormone treatment (Figure 5, 1999) and accuracy of AI timing, bars = 1 cm.*

Introduction

The reproductive anatomy and physiology in the 20 orders of mammalia with over 3500 species are so divergent that the direct use of methods developed in model species for assisting or studying reproduction are not always applicable[29, 30]. Post-mortem studies on selected species are helpful for identifying unique anatomical features, however, physiological mechanisms of regulation cannot be easily detected by sporadic dissections. Knowledge of reproductive anatomy and physiology are likely to be of particular importance for successful assisted breeding programs in endangered species.

The introduction of ultrasonography in reproductive medicine in the late 1950's[4] revolutionized this entire field. It offered new opportunities for the exploration of morphology and biological processes as well for ultrasound-guided interventions for infertility treatment or biotechnology. Despite the significant advances made in ultrasonographic applications in human and veterinary medicine, the use of this technology has been limited in zoo and wildlife medicine. Ultrasonography was first applied to zoo animals as a diagnostic tool in 1978[33]. Subsequently, several ultrasonographic descriptions of various species were published, but the use of sonographic techniques for the development of technologies for the management of reproduction in non-domestic species has been sporadic[1,5]. The application of ultrasonography to non-domestic animals confronts us with a number of specific problems like size, positioning and demeanor of the subject, which are usually not encountered in human or mainstream veterinary medicine[22]. However, the use of newly developed components including modification of commercial ultrasound system configurations, as well as new methods of examination, provide an advanced tool for assisted reproduction in endangered species. The purpose of this paper is to document new applications of ultrasonographic techniques in zoo and wildlife medicine.

We investigated 17 divergent species (hare to elephant) and developed modifications of the employed ultrasound system and/or new innovative hardware components. These components included miniaturization or re-design of transducers, several probe extenders for internal applications, monitor helmet for the examiner, cable extensions and the conversion of stationary to portable systems. The improved ultrasonic imaging of internal reproductive tract was fundamental for designing essential tools for assisted reproduction, like AI catheters[9,19], embryo flushing and transfer catheters[16,34] and species-specific electroejaculation probes[6]. Continuous ultrasonographic monitoring was applied during medical and hormonal treatments (cycle induction, superovulation or contraception) of non-domestic species. The different reproductive applications involving ultrasonography are separately described in the following chapters.

Characterization of the Female Reproduction Status and Sexual Cycle Patterns

Limited data are available on sexual maturation and reproductive cycle patterns in most non-domestic species in natural populations. Additionally, zoo animals often show pathological alterations of the genital organs (22) and irregular patterns of reproductive activity (3,28,38). The development of reliable methods for visualizing the reproductive tract and monitoring the reproductive cycle of exotic species is needed to optimize breeding management in captivity. Application of new techniques of assisted reproduction, including contraception, require diagnostic tools for attendant reproductive assessment.

Ultrasound examinations were undertaken for distinguishing adult from juvenile animals, both male and female, in species in which sexual maturation is not related to phenotype as is the case in the naked mole rat[24], spotted hyena[17], two-toed sloth[8], Asian and African elephants[22,24]. Follow-up examinations allowed the detailed characterization of the length of the female sexual cycle including seasonal changes, and events like the formation of follicles and/or corpora lutea. The main limitation of repeatable ultrasound examinations in non-domestic animals is the risk of restraint (physical or chemical) or the time-consuming conditioning training necessary for animals to accept transrectal manipulation. Accompanying endocrine analyses, particularly non-invasive urine and fecal assays, can provide valuable complementary support for ultrasonographic findings. We developed ultrasound equipment and standardized procedures for monitoring annual sexual cycle patterns using transrectal ultrasonography and hormone analyses in European brown bears (Figure 1)[11], roe deer[18] and anoa[7] under frequent chemical restraint.

Ultrasonography on captive Asian and African elephants[15,20] serves as an example, how post-mortem anatomical studies, species-specific modification of technical equipment and examination procedures were fundamental for the development of a standardized reproductive assessment protocol performed in over 300 individual elephants with nearly 2000 single exams. The anatomy of elephants requires the use of probe extenders varied from 45 to 60 cm to reach the ovaries at distance of 1 - 1.5 m from the rectum[37]. The US-examiner needs a monitor helmet equipped with a digital or TV screen (Figure 2) for real-time visualization of the genital tract together with other technical innovations, like sunlight protection cover, transducer cable extension and reconfiguration of heavy color flow Doppler machines by weight re-movement and repositioning of the monitor. The data of sonomorphological studies on reproductive status and cycle of elephants were essential for assisted reproduction procedures described below.

A very important field for ultrasonographical monitoring of reproductive status is the application during contraception programs. The improvement of animal husbandry and wildlife conservation has enhanced the breeding success of several species. Reversible contraception is essential in the establishment of genetically variable captive populations within the constraints of limited captive habitat. In our contraception research on captive brown bears and free-ranging elephants we used ultrasonography for determining the reproductive status and monitoring sexual cycles during contraceptive interventions[10,11,13]. Sonomorphological changes during contraception (down regulation of ovarian activity, prevention of implantation or possible pathological alterations) were correlated with pharmacokinetics and sexual hormone analysis. Accompanying ultrasonography to endocrine monitoring was utilized to evaluate efficiency and reversibility of the contraceptive method applied[13,14].

Ultrasonography in Male Reproductive Assessment and Semen Collection

As in females, basic data on morphology, physiology and pathological alterations in males of non-domestic species are also quite limited. Ultrasonographic description of topographical anatomy of the male reproductive tract was fundamental to the development of techniques for semen collection by electroejaculation or manual stimulation in a wide range of species, e.g. Asian and African elephants, black and white rhinoce-

roses and tapir. Applied ultrasonography and semen collection has been used for characterization of puberty[26] and stages of the ejaculatory process[20,21,36], for reproductive health assessment[23], for identification of seasonal changes in testes and spermatogenesis[12] and for artificial breeding programs[27,36].

Performance of ultrasound prior to semen collection provided the operator with a topographic overview of the size and developmental status of the testicles and the accessory sexual glands (Figure 3 and 4). Identification of position of the culliculus seminalis and of the accessory sexual glands is necessary prior to a either manual stimulation or electroejaculation, as they anatomical area represent the point of maximum sensitivity for stimulation. Additionally, estimation of the contents of the sex glands allowed predictions of expected ejaculate volume and avoided over stimulation in subfertile or infertile individuals. Furthermore, the accidental stimulation of the urinary bladder with subsequent urine contamination of the ejaculate can be prevented by correct positioning of the ejaculatory probe. Finally, the success of semen collections was assessed by post collection ultrasound. Imaging and measurement of the dimension of accessory glands after a stimulation confirmed whether the entire ejaculate was collected or further stimulation were required. Based on newly generated information on the physiology of the ejaculatory process advanced types of probes have been designed for several species, including elephants, rhinoceroses, tapirs and hares.

The application of ultrasound and semen collection as reproductive tools in North American captive male elephant populations revealed an alarming number of infertile and subfertile adult bulls. In response, regular ultrasonographic evaluation combined with semen assessment of potential and proven breeders has been adopted by the American Association of Zoos and Aquariums (AZA) and incorporated into management strategies of the Elephant Species Survival Plan (SSP). The authors highly recommend the systematic expansion of intensive reproductive assessment in other endangered species breeding programs.

Induction of Estrus and Superovulation

Captive populations of endangered species are often not self-sustaining because of their relatively low reproductive success. Beside the lack of sufficient breeding partners and male infertility, there is also a lack of female cyclicity in many species[3,28,31,38]. Multiple factors have been discovered to cause ovarian inactivity in females of prime breeding age, however some factors are still unknown. Ultrasonographic assessment could provide the unique feasibility of distinguishing between inactive healthy and inactive pathological altered individuals, as it was shown for elephants[20] and giant pandas (Figure 5). For managing these sub or infertil animals ultrasonography was used to control the impact of cycle induction by changing social and/or environmental factors or by hormonal treatment (Figure 5). The individuals were classified as treatable or nontreatable, with nontreatable animals being excluded from reproduction management plans[32].

In contrast to the treatment of infertility the use of superovulation is currently limited to basic research[2], improvement of reproductive rate and disease control in endangered species. Information on ovarian response to estrous induction and superovulation allowed the adjustment of hormonal regimes or management actions. In general, standard hormone regimes used in domestic species or humans were applied transferred to exotic species. In this cases ultrasono-

graphy provided the fastest information on the success or failure of estrous induction, as well as the formation of pathological structures.

Utrasound-Guided Insemination and Embryo Transfer

There are several reports of successful AI and embryo transfer in a variety of wild animals[25,27,35]. However, in most non-domestic species non-surgical insemination and embryo transfer programs have not been successfully established. Due to the lack of efficient catheter systems, anatomical obstacles and problems of timing the manipulations, these two reproductive technologies are still under-utilized. The type of insemination technique applied is determined by specific female reproductive anatomy in conjunction with the accessible volume and quality of semen. Based on the deposition of the ejaculate during natural breeding, the applied technology should guarantee the placement of the semen in the equivalent compartment of the genital tract. Due to the loss of semen quality during collection, preservation and transport, the optimal placement is the posterior portion of the uterine body. Correct positioning of the catheter in the uterine body is obligatory for successful embryo flushing and transfer.

Ultrasound has provided crucial information on female anatomy, timing of ovulation, guidance of the catheter deep into the genital tract and the correct deposition of semen or embryos (Figure 6). Accompanying images of the catheterization process have helped the handle difficult urogenital structures like the urethral os, vaginal hymen and folds, cervical os and cushions[9,27]. For the collection of in vivo-embryos, larger animals typically require long catheters which are difficult to insert into the uterus.

Ultrasonography has been essential to the development and application of insemination for unique species such as giant panda, swamp and roe deer, white rhinoceros (Figure 6), Malayan tapir, cheetah and elephant[6,8,25]. Utilization of commercial equipment for AI and embryo transfer was restricted to closely related species like yak or tapir[34,35]. Customization of embryo transfer sets proved to be more complicated due to the high technical requirements of these systems in comparison to AI equipment. Future development of non-surgical AI and embryo transfer techniques in non-domestic species relies on basic anatomical and physiological knowledge, much of which can be provided by ultrasonography.

Conclusion

Ultrasonography is a basic approach for non-invasive exploration of reproductive anatomy and physiology of non-domestic animals. This imaging modality represents a practicable, reliable, and accurate tool for monitoring and managing the sexual cycle, semen collection, AI and embryo transfer and contraception. A number of recent technical advances in ultrasonography were applied to breeding programs of endangered species with resultant improvements in the success rate of assisted reproduction techniques. Ultrasonographic monitoring and guidance allowed non-surgical procedures to replace surgical approaches in a wide range of applications. This has not only enhanced the acceptance of applied biotechnology in the scientific community but is more preferable from an animal welfare point of view.

References

1. **Adams GP, Plottka ED, Asa CS, Ginther OJ.** Feasibility characterizing reproductive events in large non-domestic species by transrectal ultrasonic imaging. J Zoo Biol 1991;10:247-259.
2. **Broich A, Hildebrandt TB, Lange A, Gilles M, Hermes R, Jewgenow K, Lengwinat T, Baugatz C Göritz F.** Experimental investigation of embryonic diapause in European roe deer (Capreolus capreolus). J Reprod Fertil 1998;22:33 abstr..
3. **Brown JL, Hildebrandt TB, Theison W, Neiffer DL.** Endocrine and ultrasound evaluation of non-cycling African elephant: Identification of a follicular ovarian cyst. Zoo Biol (in press).
4. **Donald I Macvicar J, Brown TG.** Investigation of abdominal masses by pulsed ultrasound. Lancet 1958;1:1189-1191.
5. **Du Boulay GH, Wilson OL.** Diagnosis of pregnancy and disease by ultrasound in exotic species. Symp Zool Soc Lond 1988;60: 135-150.
6. **Göltenboth R, Seager SWJ, Göritz F, Blottner S, Ochs A, Hildebrandt TB.** Fertility evaluation in males of different endangered species at the Berlin Zoo. Proc 2. Scient Meet Europ Assoc Zoo Wildl Vet Chester, UK, 1998;373-386.
7. **Göritz F, Hildebrandt TB, Nötzold G, Eulenberger K, Gottschalk J, Frank S, Correia H.** Untersuchungen zum reproduktiven Status und Zyklusgeschehen beim Anoa (Bubalus depressicornis) mittels transrektaler Ultrasonographie. Verh ber Erkrg Zootiere 1994;36:107-119.
8. **Göritz F, Hildebrandt TB, Thielebein J.** Transrektale Ultraschalluntersuchung beim Faultier. Bildgebung Imaging 1994; 61(Suppl2):98abstr.
9. **Göritz F, Hildebrandt TB, Lengwinat T, Meyer, HHD.** Ultrasound guided artificial insemination in roe deer (Capreolus capreolus). Reprod Domest Anim 1996;30:460 abstr.
10. **Göritz F, Hildebrandt TB, Jewgenow K, Hermes, R, Strauss G, Rohleder M, Meyer HHD.** Reproductive assessment in captive brown bear. Proc Ann Meet Am Assoc Zoo Vet 1997;204-207.
11. **Göritz F, Hildebrandt TB, Jewgenow K, Wagner N, Hermes R, Strauss G, Meyer HHD.** Transrectal ultrasonographic examination of the female urogenital tract in nonpregnant and pregnant captive bears (Ursidae). J Reprod Fertil 1997;51:303-312.
12. **Göritz F, Hildebrandt TB, Lange A, Gilles M, Lengwinat T, Hermes R, Broich A, Blottner S.** Experimental investigations on seasonality of spermatogenesis in roe deer (Capreolus capreolus). Proc 50th ICAR Gametes: Development and Function 1998;540 abstr.
13. **Göritz F, Hildebrandt TB, Hermes R, Quandt S, Grobler D, Jewgenow K, Rohleder M, Meyer HHD, Hofmann RR.** Results of a hormonal contraception programme in free-ranging African elephants. Verh ber Erkrg Zootiere 1999;39:39-40.
14. **Göritz F, Quest M, Hildebrandt TB, Maltzan J, Wiesner H, Meyer HHD, Rohleder M, Kolter L, Jewgenow K.** Antigestagene - eine neue Möglichkeit der Reproduktionskontrolle von Bären (Ursidae) in Menschenhand. Verh ber Erkrg Zootiere 1999;39:295-296.

15. **Hermes R, Olson D, Göritz F, Schmitt DL, Hagan D, Peterson J, Brown J, Fritsch G, Hildebrandt TB.** Ultrasonographical characterisation of the sexual cycle in female African elephants (Loxodonta africana). Verh ber Erkrg Zootiere 1999;39:35-38.
16. **Hildebrandt TB, Pitra C, Reinsch A.** Ultraschallgestützte Embryonengewinnung und Trächtigkeitsuntersuchungen bei Macaca fascicularis. Tierärztl Praxis 1993;(Suppl.1): 66-67.
17. **Hildebrandt TB, Göritz F, Göltenboth R, Pitra C.** Sonomorphologische Geschlechtsdiagnose bei der Tüpfelhyäne (*Crocuta crocuta Erxleben*). Fertilität (1996);12:46-50.
18. **Hildebrandt TB, Göritz F, Hermes R, Lengwinat T, Baugatz C, Pinkowski M, Meyer HHD.** Diagnosis of ovarian activity and pregnancy in roe deer (Capreolus capreolus) by transrectal sonography. Reprod Domest Anim 1996;30:341abstr.
19. **Hildebrandt TB, Schnorrenberg A.** Besteck zur künstlichen Besamung von Elefanten. Dtsch. Patentamt, Offenlegungsschrift DE1906925A1, 1996; 1-8.
20. **Hildebrandt TB, Göritz F, Pratt NC, Schmitt DL, Lehnhardt J, Hermes R, Quandt S, Raath J, West G, Montali RJ.** Assessment of health and reproductive status in African and Asian elephants by transrectal ultrasonography. Proc Ann Meet Am Assoc Zoo Vet 1997;207-213.
21. **Hildebrandt TB, Göritz F, Schmitt D, Pratt NC, Howard JG, Jewgenow K, Blottner S, Montali RJ.** Ultrasonographic and spermatological investigation of the ejaculatory process in male Asian elephants. Proc 2nd Ann Elephant Res Symp, Pittsburgh, PA 1997; 18-19.
22. **Hildebrandt TB, Göritz F.** Use of ultrasonography in zoo animals. In: Fowler ME, Miller RE. (eds) Zoo and Wild Animal Medicine: Current Therapy 4. Philadelphia: WB Saunders Company, 1998;41-54.
23. **Hildebrandt TB, Göritz F, Pratt NC, Schmitt DL, Quandt S, Raath J, Hofmann RR.** Reproductive assessment of male elephants (Loxodonta africana and Elephas maximus) by ultrasonography. J Zoo Wildl Med 1998;29: 114-128.
24. **Hildebrandt TB, Göritz F, Stetter MD, Hermes R, Hofmann RR.** Applications of sonography in vertebrates. Zoology 1998;101: 64-73.
25. **Hildebrandt TB, Göritz F, Schnorrenberg A, Hermes R, Schmitt DL, Hagan D, Peterson J, Brown J, Loskutoff N, Pratt NC, Lehnhardt JL, Miller G, Montali RJ, Olson D.** Successful artificial insemination of African nulliparous elephants at the Indianapolis Zoo. Verh ber Erkrg Zootiere 1999;39:41-46.
26. **Hildebrandt TB, Fritsch G, Hermes R, Jewgenow K, Rohleder M, Rudolph M, Maltzan J, Wiesner H, Pratt NC, Neiffer D, Göritz F.** Charakterisierung der Pubertät männlicher Elefanten mit Hilfe ultrasonographischer, morphologischer, endokrinologischer und spermatologischer Untersuchungsmethoden. Verh ber Erkrg. Zootiere 1999;39:27-34.
27. **Hildebrandt TB, Göritz F, Schnorrenberg A, Hermes R, Schmitt DL, Hagan D, Peterson J, Brown, JL, Loskutoff NM, Pratt NC, Lehnhardt JL, Miller G, Montali RJ, Olson D.** Successful artificial insemination of African nulliparous elephants at the Indianapolis. Zoo Verh ber Erkrg Zootiere 1999;39:41-46.
28. **Howard JG.** Assisted reproductive techniques

in non-domestic carnivores. In: Fowler ME, Miller RE. (eds) Zoo and Wild Animal Medicine: Current Therapy 4. Philadelphia: WB Saunders Company, 1998;449-457.

29. **Lasley BL, Shideler S.** Methods for assessing reproduction in non-domestic species. Fowler ME. (ed) Zoo and Wild Animal Medicine: Current Therapy 3. Philadelphia: WB Saunders Company, 1993;597-604.
30. **Loskutoff NM.** Biology, technology and strategy of genetic resource banking in conservation programs for wildlife. Proc 50th ICAR Gametes: Development and Function 1998;275-286.
31. **Meyer HHD, Rohleder M, Streich WJ, Göltenboth R, Ochs A.** Sexualsteroidprofile und Ovaraktivitäten des Pandaweibchen Yan Yan im Berliner Zoo. Berl Münch Tierärztl Wochenschr 1997;143-147.
32. **Montali RJ, Hildebrandt TB, Göritz F, Hermes R, Ippen R, Ramsay E.** Ultrasonography and pathology of genital tract leiomyomas in captive Asian elephants: Implications for reproductive soundness. Verh ber Erkrg Zootiere 1997;38:253-258.
33. **O'Grady JP, Yeager CH, Thomas W.** Practical applications of real time ultrasound scanning to problems of zoo veterinary medicine. J Zoo Anim Med 1978;9:52-56.
34. **Pitra C. Hildebrandt TB, Reinsch A, Strauss G, Pohle C, Kanitz W.** Non-surgical embryo recovery in the Yak (Bos mutus grunniens) using induction of ovulation. Proc 12. ICAR, The Hague 1992;2:805-807.
35. **Pope CE, Loskutoff NM.** Embryo transfer and semen technology from cattle applied to non-domestic artiodactylids. In: Fowler ME, Miller RE. (eds) Zoo and Wild Animal Medicine: Current Therapy 4. Philadelphia: WB Saunders Company, 1998;597-604.
36. **Schmitt DL, Hildebrandt TB.** Manual collection and characterization of semen from Asian elephants (Elephas maximus). Anim Reprod Sci 1998;53:309-314.
37. **Schnorrenberg A.** Ultraschallkopftraeger. Dtsch. Patentamt, Offenlegungsschrift DE4422220A1, 1995;1-6.
38. **Schwarzenberger F, Walzer C, Tomasova K, Zima J, Göritz F, Hermes R, Hildebrandt TB.** Can the problems associated with low reproductive rate in captive white rhinoceroses (Cerathoterium simum) be solved within the next 5 years? Verh ber Erkrg Zootiere 1999;39: 283-292.

ASSESSMENT OF HEALTH AND REPRODUCTIVE STATUS IN AFRICAN AND ASIAN ELEPHANTS BY TRANSRECTAL ULTRASONOGRAPHY*

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* **Proc Am Assoc Zoo Vet (1997), 207-212**

[1] **IZW, Berlin.**

Assessment of Health and Reproductive Status in African and Asian Elephants by Transrectal Ultrasonography

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Abstract

Transrectal ultrasonography was performed on 10 male and 85 female African elephants *(Loxodonta africana)* and on 5 male and 39 female Asian elephants *(Elephas maximus)* in order to develop standards for assessment of reproductive health and status.

Captive and wild African males and females as well as captive Asian males and females were examined. The entire internal urogenital tract was visualized ultrasonographically by using a 3.5 MHz, a 7.5 MHz and a 10.0 MHz transducer in combination with two probe extensions specially adapted for elephant anatomy. The findings were verified by post-mortem *ex situ* ultrasound examinations in each species. Each part of the internal urogenital tract was sonographically detectable except for main parts of the late pregnant uterus (> 13 month p.c.) in females and the bulbo-urethral glands and the cranial portion of the ureters (in both sex) and ductus deferentes in males. A variety of pathological alterations were found but mostly in the captive population of African and Asian elephants. There was a very

Acknowledgements

The authors are grateful for assistance from the staffs at the following institutions: Circus Frankello; Circus Althaus; Circus Sarrasani; Circus Scholl; Dickerson Park Zoo; Fort Worth Zoo; Indianapolis Zoo; Knoxville Zoo; Kruger National Park Board; Pittsburgh Zoo; private zoo (Virginia); Ringling Bros. and Barnum & Bailey; St. Louis Zoo; Tierpark Carl Hagenbeck, Hamburg; Woodland Park Zoo; Zoo Wroclaw; Zoological Garden, Berlin; Zoological Garden, Münster; Zoological Garden, Erfurt; Zoo Zürich.

Financial support was provided in part by the Institute for Zoo Biology and Wildlife Research, Berlin and the Smithsonian Institution Short Term Visitor Program. The work on the wild African elephants was supported by a grant of the Society for Technical Collaboration, Germany.

high incidence of uterine leiomyomas in the female genital tract of captive Asian elephants (35.9%). In contrast, wild and captive African elephants never develop leiomyomas but frequently have endometrial cysts (11.3% wild, 14.3% captive) and ovarian cysts (1.4% wild, 21.4% captive). This study presents results which indicate that transrectal ultrasonography may be used as an effective, non-surgical tool for reproductive and health assessment of elephants which has implications for management, population control and assisted reproduction.

Introduction

Reproduction in African elephants (*Loxodonta africana*, La) and Asian elephants (*Elephas maximus*, Em) is often less successful than in natural populations, inhibiting the establishment of self-sustaining captive populations.[3,11] Infertility due to reproductive disorders and mismanagement can have a devastating effect on captive breeding programs.[5,8] There is a death of knowledge regarding the reproductive anatomy and physiology of these both species.[2,3,6,12,14] Sonographic imaging techniques have had a beneficial impact on reproductive and veterinary studies in a wide range of domestic[13] and wild species;[4,8] however, the feasibility of this technique as a routine diagnostic procedure in elephants has not been overly successful.[1] Some of the difficulties in viewing intra-abdominal testes, ovaries and monitoring fetal development were attributed to difficulties in positioning the instruments, and to the size and demeanor of these large pachyderms. Major anatomical obstacles during routine andrological, gynecological and obstetrical ultrasonography include locating components of the reproductive tract which in elephants lie deeply within the abdominal cavity, and the marked inclination of the axis of the proboscid pelvic inlet.[15] Recently, the instrumentation for transrectal sonographic examinations in elephants has been greatly improved to overcome these obstacles.[7,9]

Methods

Transrectal ultrasonography were performed on 10 male and 85 female La and on 5 male (including 1 castrated individual) and 39 female Em to develop standards for assessment of reproductive health and status. This study included 5 wild male and 71 female La from the Kruger National, Skukuza, South Africa as well as captive La and Em kept in 19 different facilities with a variety of management systems. The age of the individuals ranged from 7 to 62 years. The majority of the 139 elephants examined were in the reproductive age. The number of ultrasono-graphic examination per individual ranged from 1 to 62. However, the most elephants had only one examination.

Ultrasonography was performed either in the nontranquilized captive La and Em in standing position (often chain- or chute-restraint) or in the immobilized wild African elephants (M99®, etorphine 10.0 to 15.0 mg, i.m., Reckett & Coleman, Hull, U.K.; reversed by M50-M50®, diprenorphine 30.0 mg, i.v., Reckett & Coleman, Hull, U.K.) which were in lateral recumbency. Both positions were satisfactory for using the transrectal approach. After manual removal of the feces and extensive irrigation in combination with the application of ultrasound gel (Aquasonic 100®, Parker Laboratories Inc., Orange, NJ 07050, USA), the transducer was inserted into the rectum and directed carefully over the reproductive tract. The internal urogenital tract was visualized ultrasonographically by using a real-time, B-mode ultrasound scanning system (CS 9100 Oculus, Picker International GmbH, Espelkamp, D-32339, Germany) equipped with either a 3.5 MHz,

Table 1 Utrasonographic imaging of the urogenital organs in comparison to the applied transducers

	organ	adapter	3.5 MHz			7.5 MHz			10.0 MHz*		
			overview	details	surface	overview	details	surface	overview	details	surface
male	testes	yes	+++	+++	++	-	++	+++	-	-	-
	epidymides	yes	+	-	-	++	++	++	-	-	-
	ductus deferentes (cranial portion)	yes	-	-	-	-	-	-	-	-	-
	ductus deferentes (caudal portion)	no	++	+	+	+++	++	++	++	+++	+++
	ampullae	no	+++	++	+	+++	++	++	++	+++	+++
	seminal vesicles	no	+++	++	++	++	+++	++	+	++	+++
	prostate	no	+++	++	+	+++	+++	++	++	++	+++
	bulbo-urethral glands	no	-	-	-	-	-	-	-	-	-
Female	ovaries	yes	++	+	-	+++	+++	+++	-	-	-
	uterine horns	yes	+++	++	+	+++	+++	+++	-	-	-
	uterine body	no	+++	+++	++	++	+	++	-	+	+++
	cervix	no	+++	+++	++	++	++	++	+	++	+++
	vagina	no	+++	+++	++	++	+++	++	+	++	+++
	vestibule (cranial portion)	no	+++	+++	++	++	+++	++	-	++	+++
General	kidneys	yes	+++	+++	++	+	++	+++	-	-	-
	urinary bladder	no	+++	++	+	+	+++	++	-	++	+++
	ureters (cranial portion)	yes	-	-	-	-	-	-	-	-	-
	ureters (caudal portion)	no	+++	++	+	++	+++	++	-	++	+++
	urethra	no	+++	+++	++	++	++	+++	++	+++	

* = this type of transducer did not fit in an adapter

+++ = excellent

++ = good

\+ = insufficient

\- = impossible

7.5 MHz, or a 10.0 MHz transducer in combination with two different probe extensions adapted for elephant anatomy (Ultraschallkopftraeger II and III, A. Schnorrenberg Chirurgiemechanik, Woltersdorf, D-15569, Germany). These adapters were an essential part of our equipment which allowed good contact between the transducer and the rectal mucosa and can be extended to the cranial segment of the reproductive tract to view the kidneys, testes or ovaries. In addition to the main ultrasound monitor, we adapted a small monitor to a helmet that was worn by the examiner for orientation of the ultrasonogram during the procedure. Measurements were taken from the reproductive structures using the electronic caliper provided on the ultrasound unit. Actual scanning time was 15 to 25 min. for each individual. The findings were verified by post-mortem *ex situ* ultrasound examinations on the isolated urogenital organs in each species (63 La, 6 Em).

Results

Each part of the internal urogenital was sonographically detectable except for main parts of the late pregnant uterus (> 13 month p.c.) in females and the bulbo-urethral glands and the

Table 2 Pathological findings detected by transrectal ultrasound in wild and captive elephants

	pathological findings	**wild La**		**captive La**		**captive Em**	
		#	%*	#	%	#	%
general	ascites	-	-	1f(14)	7	2f(39)	5.1
	rectal papillomatosis	-	-	1f(14)	7	1f(39)	2.6
	cystitis (urinary bladder)	-	-	-	-	1f(39)	2.6
	calcification of the urethra	-	-	1m(5)	20	1f(39)	2.6
male [m]	immature genital tract*	-	-	1(5)	20	-	-
(total 15)	atrophic genital tract	-	-	-	-	1(5)**	(20)
	malformation of the prostate	-	-	-	-	1(5)	20
female [f]	immature genital tract*	-	-	3(14)	21.4	1(39)	2.6
(total 124)	atrophic genital tract	-	-	-	-	1(39)	2.6
	prolapsed uterus	-	-	-	-	2(39)	5.1
	fetal death (delayed pregnancy)	-	-	-	-	1(39)	2.6
	leiomyomas in the uterus	-	-	-	-	14(39)	35.9
	cystic structures in:						
	- urogenital tract (vestibule)	-	-	-	-	2(39)	5.1
	- vagina	-	-	-	-	3(39)	7.7
	- cervix	-	-	-	-	4(39)	10.3
	- uterus	8(71)	11.3	2(14)	14.3	3(39)	7.7
	- ovary	1(71)	1.4	3(14)	21.4	-	-

* = in comparison to the normal age-related development

** = caused by a surgical castration

( ) = brackets contain total number of individuals investigated

cranial portion of the ureters (in both sex) and ductus deferentes in males (Tab.1). The use of all three ultrasound transducers in combination with the two adapters was necessary to get optimal information about the urogenital organs. The quality of the ultrasonographic images of each probe were evaluated on the basis of the criteria *overview*, *detail* and *surface* of the organ examined (Tab. 1). This knowledge concerning the appearance of healthy genital structures was necessary for clear recognition of pathological alterations. Ultrasonography was used to detect the following pathological alterations listed in Table 2.

Discussion

Assessment of the reproductive capacity of an individual is based on the health of the internal genital tract. It is therefore important to detect pathological alterations of the inner genital tract and to determine the importance or influence they may have on reproductive performance before forming breeding groups.[8]

Generally, exotic animals show signs of diseases or disorders very late in their progression. Sonographic examination of the urogenital organs, may provide useful criteria to appraise the fitness or breeding potential of an individual. Detection of subclinical changes in these organs indicates that a clinically apparent metabolic disorder may occur under the physiological burden of breeding activity and pregnancy which could have possible lethal consequences for mother and/or fetus.

There was a very high incidence of uterine leiomyomas in the female genital tract of captive Asian elephants (35.9%). In contrast, wild and captive African elephants never develop leiomyomas but frequently have endometrial cysts (11.3% wild, 14.3% captive) and ovarian cysts (1.4% wild, 21.4% captive). These findings correlated with a post mortem study between 1975 and 1995 of approximately 30,000 exotic mammal cases at the Institute for Zoo Biology and Wildlife Research Berlin, and the Smithsonian Institution, Washington DC, leiomyomas were found in the uterus, cervix, and vagina in 14 species of exotic mammals. These animals originated from the Smithsonian's National Zoological Park (NZP) and from multiple zoos in Europe. This study indicated a very high necropsy prevalence of genital tract leiomyomas in Asian elephants as compared to other species.[10]

Findings regarding the general health of the animal and those specific to the genital tract should be considered inorder to make appropriate decisions regarding which animals to breed.[4,8] This study presents results which indicate that transrectal ultrasonography may be used as an effective, non-surgical tool for reproductive and health assessment of elephants which has implications for management, population control and assisted reproduction.

Literature cited

1. **Adams, G.P., E.D. Plotka, C.S. Asa, and O.J. Ginther. 1991.** Feasibility of characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging. Zoo Biol. 10: 247-260.
2. **Balke, J.M.E., I.K. Barker, M.K. Hackenberger, R. McManamon, and W.J. Boever 1988a.** Reproductive anatomy of three nulliparous female Asian elephants: The development of artificial breeding techniques. Zoo Biol. 7: 99-113.
3. **Balke, J.M.E., W.J. Boever, M.R. Ellersiek, U.S. Seal, and D.A. Smith 1988b.** Anatomy of the reproductive tract of the female African elephant (Loxodonta africana) with reference to development of techniques for artificial

breeding. J.Reprod. Fertil. 84: 485-492.

4. **Du Boulay, G.H., and O.L. Wilson 1988.** Diagnosis of pregnancy and disease by ultrasound in exotic species. Symp. Zool. Soc. (Lond.) 60: 135-150.
5. **Göritz F. 1996.** Sonographie bei Zoo- und Wildtieren. DVM Diss, Freie Univ Berlin, Germany.
6. **Hess, D.L., A.M. Schmidt, and M.J. Schmidt 1983.** Reproductive cycle of the Asian elephant (Elephas maximus) in captivity. Biol. Reprod. 28. 767-783.
7. **Hildebrandt, T.B., and F. Göritz 1995a.** Sonographischer Nachweis von Leiomyomen im Genitaltrakt weiblicher Elefanten. Verh ber Erkrg Zootiere 37: 287-294.
8. **Hildebrandt, T.B., and F. Göritz 1995b.** Ultrasonography as a Tool in Zoo Animal Management. In: Zoological Library - Research and captive Propagation. Ed. Ganzloser, U., J.K. Hodges, and W. Kaumanns. Filander Verlag GmbH, Fürth, Pp. 49-58.
9. **Hildebrandt, T.B., F. Göritz, S. Quandt, N.C. Pratt, J. Lehnhardt, R.J. Montali, and C. Pitra 1996.** Ultrasonography as a tool to evaluate the reproductive tract in female Asian and African elephants. J. Ultras. Med. 15: S59.
10. **Hildebrandt, T.B., R. Ippen, H.E. Kaiser, and R.J. Montali 1995.** Leiomyomas in the genital tract of captive exotic mammals. Anticancer Research 15: 1754.
11. **Kurt F. 1995.** Die Haltung des Asiatischen Elefanten in Menschenobhut - ein Vergleich zwischen verschiedenen Haltungssystemen in Südasien und Europa. Zeitschr. Köln. Zoo 38: 91-113.
12. **Mikota, S.K., E.L. Sargent, and G.S. Ranglack 1994.** The Reproductive System. In: Medical Management of the Elephant. Ed. Mikota, S.K., Sargent, E.L., and G.S. Ranglack. Indira Publishing House, Michigan, Pp. 159-186.
13. **Nyland, T.G., and J.S. Mattoon 1995.** Veterinary diagnostic ultrasound. Philadelphia, W.B. Saunders Company.
14. **Schmidt, M.J. 1982.** Studies on Asian Elephants reproduction at the Washington Park Zoo. Zoo Biol. 1: 141-147.
15. **Watson, M. 1881.** On the anatomy of the female organs of the Probocidea. Trans. Zool. Soc. (Lond.) 11: 111-130.

REPRODUCTIVE ASSESSMENT BY ULTRASONOGRAPHY IN ELEPHANTS I SONOMORPHOLOGY OF THE MALE UROGENITAL TRACT*

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* Journal of Zoo and Wildlife Medicine (1998), 29(2), 114-128

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Reproductive Assessment by Ultrasonography in Elephants I Sonomorphology of the Male Urogenital Tract

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Summary

Transrectal ultrasonography was performed on seven African and three Asian male elephants in order to develop standards for assessment of reproductive health and status. Their ages ranged from a neonate to reproductively-active adults. Captive and wild African males and captive Asian males were examined. The entire internal urogenital tract was visualized ultrasonographically by using a 3.5 MHz and a 7.5 MHz transducer in combination with a probe extension specially adapted for elephant anatomy. The findings were verified by post-mortem *ex situ* ultrasound examinations in each species. Each part of the internal urogenital tract was sonographically detectable except for the bulbourethral glands, which were only visualizable in the neonate, and the cranial part of the ureters. Each structure visualized was measured and described. The size and morphology of the urogenital structures, especially the accessory glands, were reliable indicators of breeding status. There was a notable difference between African and Asian males in the size and morphology of the prostate gland and a slight difference in the shape of the ampullary glands. All other structures showed no significant species differences. The detection of the location and description of the testes provides critical information for modifying present castration procedures. Furthermore, ultra-sound examination of the male accessory glands aids in the identification of semen donors for assisted reproduction programs in captive elephants.

Acknowledgement

The authors are grateful for assistance from the staffs at the following institutions: Kruger National Park Board; Dickerson Park Zoo; Tierpark Carl Hagenbeck, Hamburg; Knoxville Zoo; Pittsburgh Zoo. We also thank Prof. Keith J. Hodges for the initial idea applicating ultrasound in elephants. Special thanks to Dr. Richard J. Montali of the National Zoological Park and Dr. Edward C. Ramsay of the University of Tennessee for their expertise and A. Reinsch, R. Hermes, Institute for Zoo Biology and Wildlife Research, Berlin for their photographical work. Financial support was provided in part by the Institute for Zoo Biology and Wildlife Research, Berlin and the Smithsonian Institution Short Term Visitor Program.

Introduction

The two extant members of the order Proboscidae, the Asian (*Elephas maximus*) and subpopulations of the African (*Loxodonta africana*) elephant, are classified as endangered (CITES, 1995) due to poaching and habitat loss. Although zoos and private reserves have become increasingly proactive in their elephant management programmes, establishing conditions necessary for successful captive reproduction has proven to be difficult (Hess *et al.*, 1983; Balke *et al.*, 1988a,b; Niemüller *et al.*, 1993; Hodges, 1995; Hodges *et al.*, 1995). The number of breeding bulls in captivity is limited, as they require specially-modified enclosures and present an inherent safety risk. Additionally, the ability to assess reproductive health and status of bulls has been restricted to a few studies on hormone and semen analyses (Jainudeen *et al.*, 1971; Jones, 1973; Jones *et al.*, 1974; Ruedi and Kuepfer, 1981; Ruedi *et al.*, 1983; Howard *et al.*, 1984; Cooper *et al.*, 1990; Niemuller and Liptrap, 1991; Mar *et al.*, 1992).

The development of assisted reproduction programmes in elephants will greatly enhance the potential for creating self-sustaining populations in captivity. An integral component of any such programme would be the practical and effective reproductive assessment of all individuals presently in captivity, including the detection of disease, reproductive cyclicity and maturity. For bull elephants, it is critical that methods for evaluation of reproductive capacity be developed, including development and status of accessory glands and integrity of the testes. Recent methods, based on semen collection via electroejaculation, do not provide a complete description of reproductive capacity. Additionally, this method of semen collection includes an inherent risk due to the anaesthesia required, and is not so readily accepted by most elephant managers. Semen collection by manual manipulation is now limited to only a few well-trained bulls (Jainudeen *et al.*, 1971; Schmidt, 1982; Price *et al.*, 1986). While this method provides for more accurate semen analysis than elctroejaculation, it still does not elucidate the status of the accessory glands. Transrectal ultrasonographic imaging provides the non-invasive means for locating and measuring reproductive structures which are the basis for reproductive assessment in elephants.

Sonographic imaging techniques have had a beneficial impact on reproductive and veterinary studies in a wide range of domestic and wild species. However, the feasibility of this technique as a routine diagnostic procedure for the visualization of gonads in elephants has not been overly successful (Adams *et al.*, 1991). Some of the difficulties in viewing the total urogenital tract and monitoring ontogenetic development have been attributed to difficulties in positioning the instruments and to the size and demeanour of these large pachyderms. Major anatomical obstacles during routine reproductive ultrasonography include the marked inclination of the axis of the proboscid pelvic inlet (Watson, 1881) and the depth at which the urogenital structures are situated. Recently, the instrumentation for transrectal sonographic examinations in elephants has been greatly improved to overcome these obstacles (Hildebrandt and Göritz, 1995a, b; Hildebrandt *et al.*, 1996). This paper presents results from the use of this new non-invasive imaging technique for visualizing the male urogenital tract in both African and Asian elephants.

Materials and Methods

Animals

A total of 10 male elephants were examined in this study: 7 African (*Loxodonta africana;* La)

Table 1 *List of male elephants examined by transrectal ultrasonography. LA* = Loxodonta africana*; EM* = Elephas maximus*; KNP = Kruger National Park, Republic of South Africa; KZ = Knoxville Zoo, Knoxville, Tennessee, USA; PZ = Pittsburgh Zoo, Pittsburgh, Pennsylvania, USA; TCH = Tierpark Carl Hagenbeck, Hamburg, Germany; DPZ = Dickerson Park Zoo, Springfield, Missouri, USA.*

ID	Location	Age	Weight (kg)	Wild / Captive	Status	in situ exam	ex situ exam	proven breeder
La1	KNP	adult	4500	wild	culled	yes	yes	suspected
La2	KNP	adult	4500	wild	culled	yes	yes	suspected
La3	KNP	adult	4000	wild	culled	no	yes	suspected
La4	KNP	adult	3500	wild	culled	no	yes	no
La5	KNP	adult	5000	wild	immobilised	yes	no	yes*
La6	KZ	17 yr	4500	captive	sedated	yes	no	no
La7	PZ	12 yr	2100	captive	restrained	yes	no	no
Em1	DPZ	32 yr	5200	captive	restrained	yes	no	yes
Em2	DPZ	17 yr	4800	captive	restrained	yes	no	no
Em3	TCH	stillborn	160	captive	stillborn	yes	yes	no

** This wild bull was radiocollared and monitored by park staff. It had been observed mounting female elephants in the past.*

and 3 Asian (*Elephas maximus;* Em) elephants. The individuals and examinations performed are listed in Table 1.

Verification of the Sonomorphology of the Urogenital Tract in Male African and Asian Elephants

The reproductive anatomy of four culled wild adult male African elephants (La1-La4), weighing 3500-4500 kg, was examined in Kruger National Park. Ultrasonographic examinations of the reproductive tract were performed in lateral recumbency according to the protocol described below. The reproductive tracts of two of the four culled animals (La1 and La2) were examined *in situ* immediately after being shot and the genital organs from all four males were also examined *ex situ.* The genital organs along with the rectum and kidney of each of the four males were isolated from the carcass at an abattoir approximately four hours after culling. The reproductive organs were prepared according to the methods described by Schulte (1937) and Short et al. (1967) .

Additionally, one fully-developed, neonate Asian elephant (Em3), weighing 160 kg, from the Tierpark Carl Hagenbeck was examined by transrectal ultrasonography as described below and the results were also verified by necropsy and *ex situ* sonographic examinations.

The caudal part of the urogenital tract (urinary bladder, urethra, penis, bulbo-urethral glands, prostate glands, ampullary glands, part of the ductus deferentes, and seminal vesicles) along with the attached rectum was dissected from La1-La4 and Em3. Structures of interest in the dissection were measured. The rectum was then opened longitudinally and all remaining faeces were removed. For examining the cranial part of the urogenital tract, including kidneys and testes and attached ductus deferentes, a small square

piece of the rectal wall was cut out and laid over this area. Ultrasonographic examinations of the isolated urogenital organs were made through the rectal wall by the protocol described below.

Ultrasonographic Examinations of the Urogenital Tract in Male African Elephants

The urogenital tracts of three African males (La5-La7) were examined by transrectal ultrasonography. One wild adult bull, La5, (weighing approximately 5000 kg) in Kruger National Park was immobilized with Large Animal Immobilon® (etorphine 11.25 mg + acepromazine 50 mg, i.m., C-vet Ltd.) and examined in lateral recumbency. Revivon® (diprenorphine 30.0 mg, i.v., C-vet Ltd.) was used for drug reversal. One 17 year-old male, La6, (weighing approximately 4500 kg) at the Knoxville Zoo was examined under standing sedation (xylazine 800 mg, i.m., Rompun®, Bayer; +carfentanil 0.5 mg, i.m., Wildnil®, Wildlife Laboratories) and reversed with Antagonil® (yohimbine 400 mg, i.m., Wildlife Laboratories), and Naltrexone® (naltrexone 25 mg, i.m., Du Pont). The third live African male was an 11 year-old (weighing 2100 kg), La7, at the Pittsburgh Zoo which was examined standing, under chain-restraint, without sedation. All three males were healthy and in good condition. Faeces were removed manually with the use of ultrasound gel (Aquasonic 100®; Orange, New Jersey, USA) for lubrication and the rectum was then irrigated with water. They were all examined by the ultrasonographic method described below.

Ultrasonographic Examinations of the Urogenital Tract in Male Asian Elephants

Two adult male Asian elephants at the Dickerson Park Zoo were examined with transrectal ultrasound. Each non-sedated, chute-restrained animal was examined in the standing position. One male (Em1) was an approximately 32 year-old, wild-born proven breeder (weighing 5200 kg). The other male (Em2) was a 17 year-old inexperienced bull (weighing 4800 kg) from whom good-quality semen has been collected by electroejaculation under sedation (Schmitt, unpublished data). Faeces were removed manually with the use of ultrasound gel and the rectum was then irrigated with water. Transrectal ultrasonography was performed by the protocol described below.

Transrectal Ultrasonography Protocol

A real-time, B-mode ultrasound scanning system (CS 9100, Picker International GmbH; Espelkamp, Germany) equipped with a customized, water-sealed 3.5 MHz convex transducer or a 7.5 MHz linear intraoperative transducer was then introduced into the rectum with ultrasound gel for coupling. The low frequency probe was initially oriented to provide cross-sectional images of the urogenital tract, and subsequently rotated to provide longitudinal images of the structures of interest. For all *ex situ* examinations, both probes were manually-guided over the structures of interest in the isolated organs. The *in situ* ultrasound examination of the stillborn Asian elephant was performed only with the intraoperative high frequency probe, extended with a 450 mm-long adapter (A. Schnorrenberg Chirurgiemechanik; Schönwalde, Germany) to visualize the entire urogenital tract (Fig. 1 a). The diameter of the rectum in this individual was too small to allow insertion of the 3.5 MHz probe, since the examiner's hand had to be inserted also to guide this probe. Only the 3.5 MHz probe was used in the two adult Asian bulls. In the African elephants, the lower frequency probe was used to gain an overview of the urogenital tract, to measure the size of the

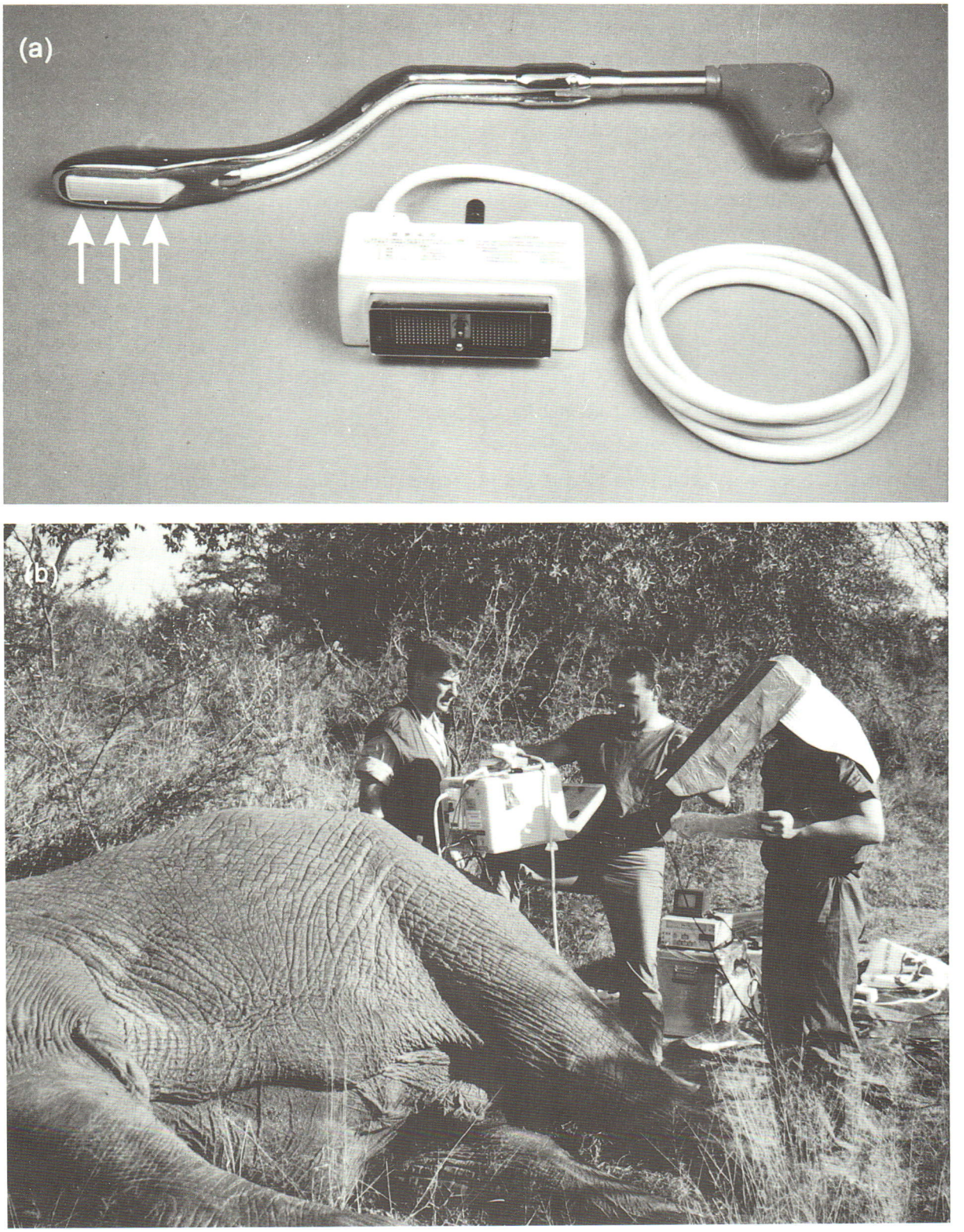

Fig. 1 *(a) 450 mm-long adapter (weighing 1.6 kg) is customized to elephant anatomy. The position of inserted linear transducer is indicated by arrows. (b) Ultrasound examination of immobilized La5 under field conditions in Kruger National Park, RSA. One investigator (right) uses monitor and microphone equipped helmet. Another investigator (middle) operates portable ultrasound unit.*

accessory glands, and to visualize the testes. The higher frequency probe was used to visualize the accessory glands with greater resolution, and, in the three wild African elephants, this probe was extended with the 450 mm-long adapter to reach the testes and kidneys.

One examiner scanned the reproductive tract with the probes while viewing the ultrasound images with a monitor and microphone equipped helmet (Fig. 1 b). The helmet design prevented glare on the screen and provided this examiner with greater mobility in guiding the transrectal probe to the internal structures. A second examiner monitored the same images on the ultrasound machine and operated the computer sonograph to optimise the images and measure the structures of interest with an internal calliper system. The entire examinations required 15-25 minutes. All ultrasonographic imaging and accompanying descriptive narrative were audiovisually recorded for further examination after the procedure.

Results

Sonomorphology of the Urogenital Tract in Male African Elephants

The ultrasound system described allowed successful endosonographic examinations of the entire internal urogenital tracts of male African elephants, except the penis and bulbo-urethral glands. Verification of the structures identified in the sonographic images generated *in situ* was accomplished *ex situ* by performing ultrasonographic examinations of isolated urogenital tracts (Fig. 2 a-f). There were no significant differences found between the ultrasonographic images generated *in situ* with those generated *ex situ* four hours later (Figs. 3 a and b). Reverberation artefacts were occasionally created in the *ex situ* examination images by air interposition between the rectal wall and the urogenital tract. However, these artefacts appeared only on the outside edges of the ultrasonographic images and all structures could be viewed clearly by repositioning the transducer.

Urethra

In the cross-sectional sonogram (Fig. 3 c), the urethra was recognised as a round structure with a diameter of 35-55 mm, surrounded by a distinct layer (2-8 mm) of hypoechoic tissue (muscle). Most of the urethra appeared hyperechoic, except for an irregular, V-shaped hypoechoic centre (mucosal folds) which was about 5 mm in diameter. In La1 and in La5, the lumen of the urethra contained an unechoic area of about 8 mm in diameter, probably caused by the presence of fluid (urine). The organization of the urethra appeared identical in longitudinal sonograms. La7 showed two, 1x3 mm hyperechoic structures attached to the urethal mucosa, characterized by ultrasound shadows (Fig. 3 d). The seminal colliculus was detectable only in cross-section as a hypoechoic area on the dorsal wall of the urethra. It measured 27 mm wide and 15 mm deep.

Urinary Bladder

In all La males, the urinary bladder was pear-shaped (2 a): 150-250 mm long, 100-150 mm wide and 100-150 mm deep (Fig. 3 e). Urine appeared mostly unechoic and sometimes slightly cloudy. The hyperechoic bladder wall was about 5-10 mm thick; the differentiation of the three smooth muscle layers was evident only in the captive males. The ultrasonographic visualization of the urinary bladder of the wild males was hindered by the larger size of their ampullary and seminal vesicles, which were positioned between the rectal wall and the bladder. In the two younger males, La6 and La7, these glands were not very well-developed yet, so the dorsal bladder wall was much more visible, allowing detection of the ureters.

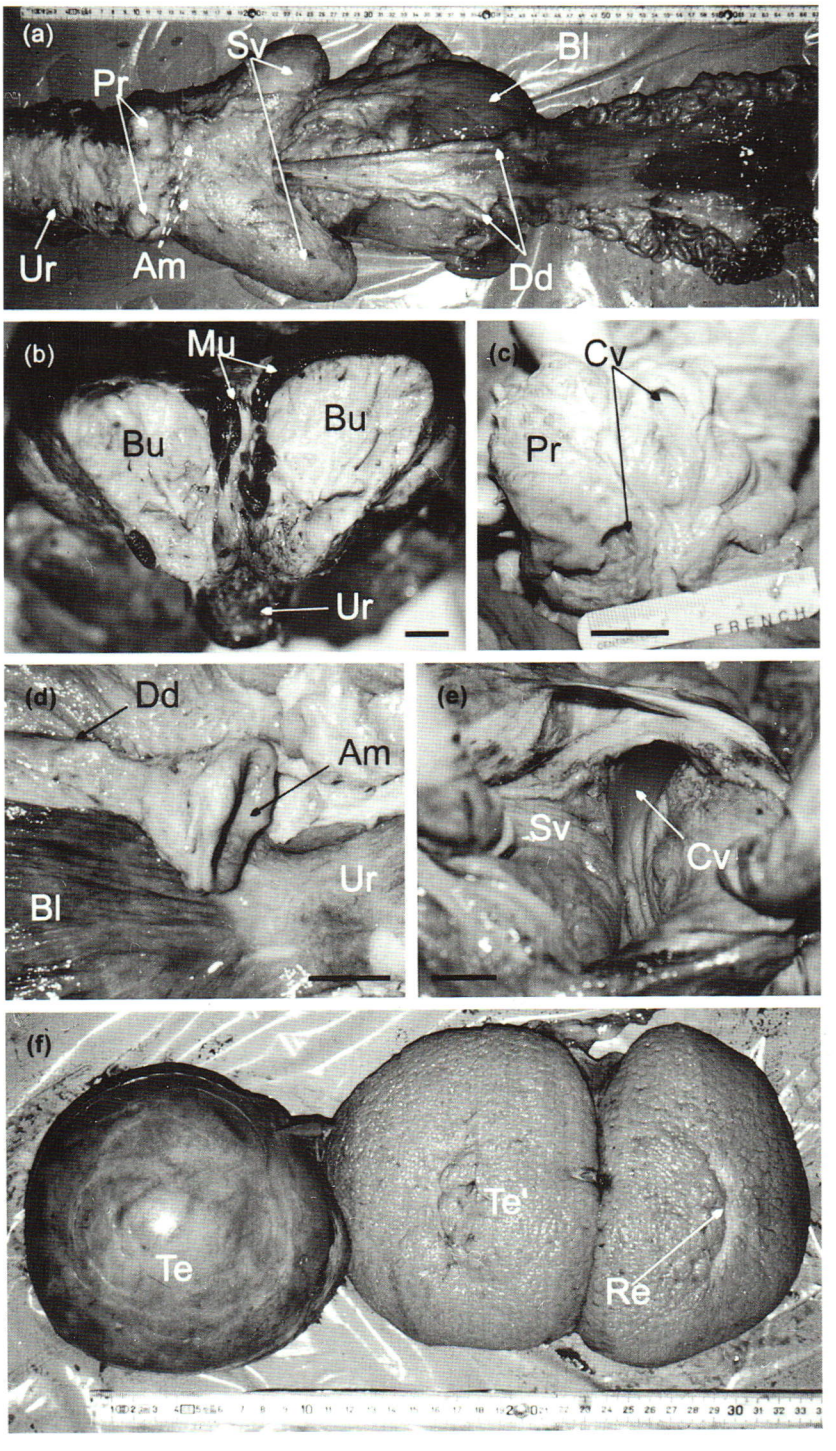

Fig. 2 *(a-f) Isolated parts of the urogenital tract of an African elephant (La4). (a) Pelvic part of the urogenital tract: urethra (Ur), prostate glands (Pr), ampullary glands (Am), seminal vesicles (Sv), urinary bladder (Bl), ductus deferentes (Dd). (b) Cross section of the bulbo-urethal glands (Bu) surrounded by muscle layer (Mu) laying dorsal the urethra (Ur). (c) Cross section of prostate gland (Pr). Note the irregular central cavity (Cv) observed only in African elephants. (d) Isolated ampullary gland (Am) and caudal part of the ductus deferens (Dd). Urinary bladder (Bl) and urethra (Ur) are dissected. (e) Dissected seminal vesicle (Sv) with fluid-filled cavity (Cv). (f) Intact left testis (Te) and cross section of right testis (Te'). Note elongated rete testis (Re). Magnifications are indicated in (a) and (f) by rulers (cm) and in (b), (c), (d), (e) by scale bars (20 mm).*

The ureters were not visible in the wild adult African males.

Kidneys

Both kidneys were located *in situ* by moving the 7.5 MHz probe laterally in the rectum, 850-1300 mm from the anus. In all adult African males examined *in situ*, parts of the kidneys were visualized using the 450 mm probe extension. In La7, the kidneys were visible with both the 3.5 MHz probe and the 7.5 MHz probe without an extension, because of their short distance (850 mm) from the anus. The most remarkable characteristic for identifying the kidney was the smoothly curved, hyperechoic capsule (2 mm thick) (Fig. 3 f). The kidney capsule captured much of the energy of the ultrasound waves, hindering the visualization of the internal structure. Ultrasonography revealed the moderately echoic, homogenous cortex (20-40 mm thick) and part of the heterogeneous medulla with hyper and hypoechoic regions. The ultrasonographic image of the kidney was interrupted by shadows created by the high echogenicity of the septa which divide the renal lobes. Visualization of the kidney did not improve by using the 3.5 MHz probe on the isolated organ. The length of the isolated kidneys from La1-La4 ranged from 200-300 mm.

Bulbo-urethral Glands

The paired bulbo-urethral glands (2 b) were not visible by *in situ* transrectal ultrasonography. The bulbo-urethral glands were positioned near the ischial arch between the pelvic bones and the skin. Consequently, there was very limited space in which to manoeuvre the ultrasound probe in this region to search for these distal glands. An attempt to visualize the oval glands by transcutaneous ultrasound was also unsuccessful because of the thickness and high echogenicity of the skin. The bulbo-urethral glands were visualized only by *ex situ* examinations. They were approximately 50-100 mm in diameter, surrounded by a hypoechoic muscular capsule, and embedded in connective tissue continuous with that of the urethra. The parenchyma had moderate echogenicity. Many secretory channels emptied into an irregular medullary cavity. The lumen of these channels and the cavity contained thick, clear secretions but appeared surprisingly unechoic.

Prostate Gland

The prostate gland in adult African males (2 a,c) appeared as a two-lobed structure, each lobe measuring 50-80 mm in diameter. The entire lobe was visible only with the 3.5 MHz probe (Fig. 3 g), but the 7.5 MHz probe was required to detect its fine structure. The lobes were situated dorsolaterally on both sides of the urethra, approximately 80 mm from the urinary bladder. The prostate consisted of a thin, hyperechoic capsule of connective tissue surrounding a 1-2 mm hypoechoic, muscle layer which was attached directly to the stroma. The moderately echoic, highly-folded stroma surrounded a large, irregularly-shaped, fluid-filled unechoic cavity. The prostate of La7 appeared much less developed than that of the adults. Each lobe of the prostate in this male measured 25 mm in diameter and was characterised by a round, unechoic, fluid-filled cavity surrounded by unfolded stroma. In all males, the anterior pole of the prostate covered the posterior part of the ampullary and seminal vesicles.

Ampullary Glands

In the African elephants examined, these glands (2 d) appeared as paired structures on the posterior ends of the ductus deferentes (Fig. 3 h). The topography of the glands was visible only with the 3.5 MHz probe, but the fine differentiation required the use of the 7.5 MHz probe. The

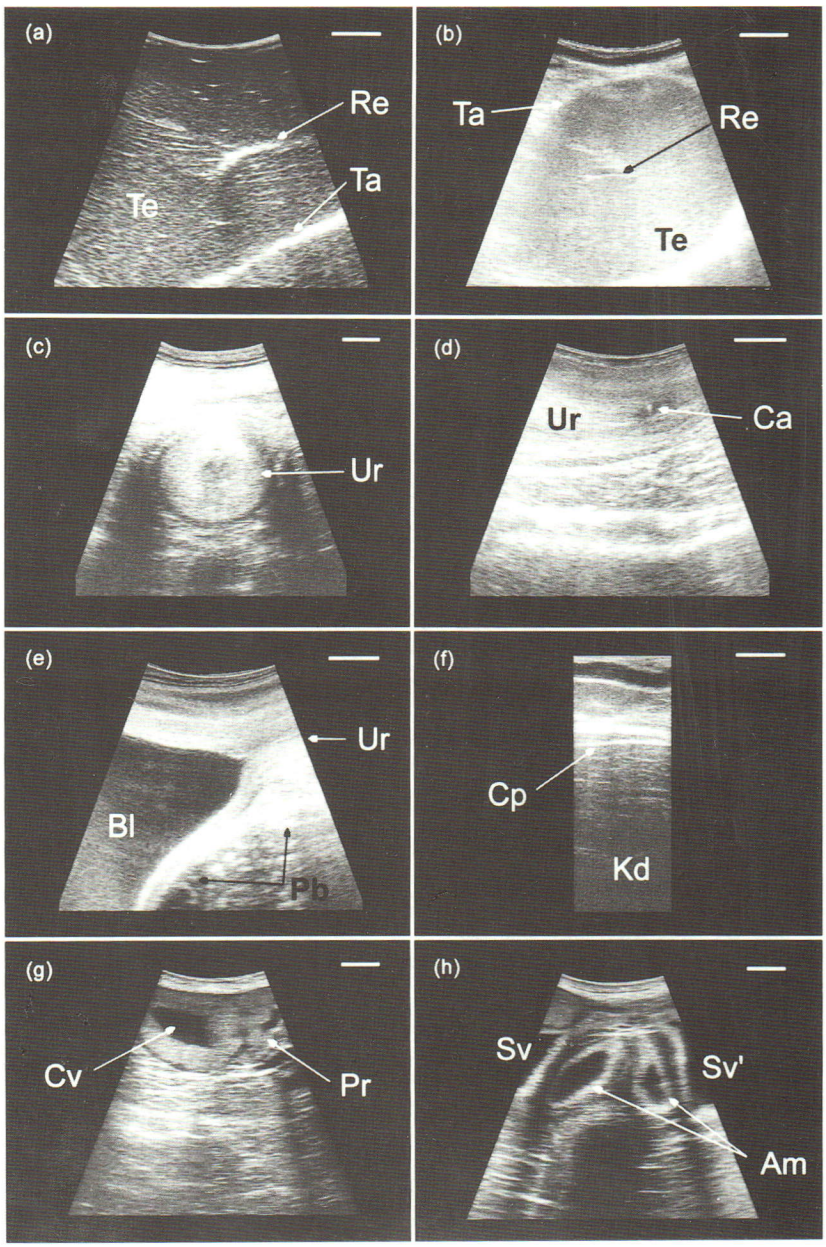

Fig. 3 *(a) and (b) Ex situ and in situ sonograms (3.5 MHz) of the testes (Te) in La1 and La7. Note elongated hyperechoic rete testis (Re). Fanlike septa are only detectable in ex situ ultrasound examinations as moderately echoic structures within the hypoechoic parenchyma (a). The tunica albuginea (Ta) appears generally as a hyperechoic border of the testis. In subadults a major hypoechoic blood vessel is embedded in Re (b) in contrast to adults (a). (c) Sagittal sonogram (3.5 MHz) of the urethra (Ur) with V-shaped hypoechoic centre (mucosal folds) in La5. (d) Longitudinal sonogram (3.5 MHz) of the urethra (Ur) in La7. Note arrow-marked calcification (Ca) surrounded by hypoechoic inflammatory area. (e) Longitudinal ultrasonographic image (3.5 MHz) of the caudal part of the urinary bladder (Bl) and beginning of urethra (Ur) directly above the pelvic bone (Pb). (f) Sonogram of a part of the kidney (Kd) made using a 7.5 MHz transducer inserted in an adapter. Note the hyperechoic renal capsule (Cp) capturing much of the energy of the ultrasound beams. (g) Sonogram (3.5 MHz) of left prostate gland (Pr) of La6 with irregular-shaped, fluid filled cavity (Cv), surrounded by highly folded, moderately echoic stroma followed by a thin hypoechoic muscle layer. (h) Sagittal sonogram (3.5 MHz) of both ampullary glands (Am) bordered by the left and right seminal vesicle (Sv and Sv') in La1. The ampullary glands are located above the neck of the bladder (black area at the bottom).*

ampullary glands were positioned directly under the midline of the rectum, between the seminal vesicles and the urethra or urinary bladder. They were trumpet-shaped ran dorsally from the posterior end of the bladder to the beginning of the urethra. In the wild adults, they were 50-70 mm long with a maximum diameter of 30-40 mm and contained a well-visualized fluid-filled cavity. In La6 and La7, they were 40 and 20 mm long with a maximum diameter of 2 and 1.4 cm, respectively, and contained only very small cavities (Fig. 4 a). The well-developed walls of the ampullary glands in the wild adults were composed of a 3 mm hypoechoic muscle layer and a 3-5 mm thick hyperechoic stroma. No hyperechoic connective tissue was found along the dorsolateral borders of these glands, making them very distinct in the ultrasonographic images.

Ductus Deferentes

The ductus deferentes (2 d) were positioned ventromedial to the seminal vesicles and dorsal to the urinary bladder and were surrounded by connective tissue. Sonographic cross-section was the best technique to visualize these tubular structures because they were tightly coiled, which made longitudinal imaging difficult. The posterior end of each ductus deferens appeared to be 5-8 mm in diameter in the adult males (Fig.4b). Two regions were distinguishable: the hyperechoic, irregular centre, which included the epithelium; and the hypoechoic, 2 mm thick muscle layer. The middle portion of the ductus deferens was outside of the field visible by transrectal ultrasound. The anterior part of the ductus deferens, near the epididymis, could only be visualized with the use of the high-frequency probe in the adults. This part was 5-8 mm in diameter and was generally hypoechoic without clear distinction between epithelium and muscle layers. In La7, the ductus deferentes were similar in organizational structure to those of the adults, but the diameter was only 3.5 mm. The whole of both structures was visible with the 7.5 MHz probe without an extension because of the shorter distance from the anus in this individual.

Seminal Vesicles

These paired, cigar-shaped glands (2a,e) were the largest accessory glands detected. The seminal vesicles of La1- La5 measured 150-250 mm long with a maximum diameter of 100-150 mm. In La6, these glands were only 110 mm long with a maximum diameter of 50 mm. In contrast, the seminal vesicles in La7 were not cigar-shaped, but more flattened, and measured 45 mm long, 20 mm wide and 10 mm deep. A portion of both seminal vesicles is shown in Fig.4c; imaging of the whole gland in one scan was not possible because of its large size in adults. The glands were distinguishable from the surrounding highly echogenic connective tissue. The wall was composed of a hypoechoic muscular coat (2-3 mm thick) with a hyperechoic mucous membrane (3-5 mm thick) which had an irregular internal surface around a fluid-filled cavity. The organizational structure of this glandular wall, especially its thick, irregular epithelium, allowed its distinction from the urinary bladder. The image of the glands in La1-La5 indicated a large amount (approximately 300 ml/gland) of cloudy fluid in the lumen. This fluid appeared cloudier than the urine in the bladder. In contrast, in La6 and La7, there was only a small amount (approximately 60 and 15 ml/gland, respectively) of less cloudy fluid in the lumen.

Epididymides

Ultrasonographic imaging of the epididymides *in situ* was only possible with the 450 mm extension with the 7.5 MHz linear probe. The elephant epididymis is very small compared

to the testis. Additionally, the epididymis is not well-demarcated from the rest of the genital duct in the elephant, making it difficult to locate (Schulte, 1937; Perry, 1953; Short *et al.*, 1967; Jones *et al.*, 1974). In La7, the epididymis was not detectable sonographically. In La1-La6, the epididymis was found to be approximately 100 mm long and 10-20 mm wide, surrounded by a large amount of hyperechoic connective tissue (Fig.4d). The epididymis was characteristically hypoechoic, due to the moderately coiled nature of the structure. The lumen of the tube was evident in each section visualized by a small centre of lower echogenicity. The attachment to the testis was characterized by a white, 1 mm thick line (tunica albuginea) which interfered with visualization of the deeper part of the testicular tissue. Sonographically, there was no apparent differentiation between the caput, corpus and cauda epididymides.

Testes

In La1-La6, testicular tissue was visualized only with the 7.5 MHz frequency linear probe with the 450 mm adapter, because the intraabdominal testes (2 f) were located caudoventral to the kidneys, 950-1300 mm from the anus. The ventral side of the testis was the most clearly visualized part of this organ during *in situ* ultrasonography. Ultrasonographic beams could not penetrate deeply enough to visualize the rete testis (corpus fibrosum) in the centre of the gonad in adults (La1-La5). The hypoechoic testicular parenchyma appeared homogenous, divided by fanlike projections of moderately echoic septa, and was bordered by the highly echogenic, slightly convex tunica albuginea (Fig. 4 e). During the *ex situ* ultrasound examinations of La1-La4, it was possible to visualize the entire testicular structure with the 3.5 MHz scanner (Fig. 3 a). The isolated testes were 15-23 cm in diameter. The rete testis (corpus fibrosum) was visualized as an irregular, hyperechoic region, approximately 30 mm long and 10 mm wide with moderately echoic septa branching toward the periphery. In La7, it was possible to visualize each entire testis (approxi-mately 100 mm in diameter) with the 3.5 MHz probe because of the shorter distance from the anus to the testes (750 mm). The internal structure of the subadult testis showed remarkable differences to that in the adult. In the subadult, a central major blood vessel (2 mm in diameter), embedded in the rete testis, was detectable with the 3.5 MHz probe only (Fig. 3 b). It was not detectable by *ex situ* ultrasound or in the gross dissection of the testes in the adults, La1-La4.

Sonomorphology of the Urogenital Tract in Male Asian Elephants

Only the 3.5 MHz probe was used to examine Em1 and Em2, due to safety concerns for the non-sedated, chute-restrained animals. It was necessary to minimize the time required for these examinations. The bulbo-urethral glands were visualized in Em3, the neonate, as were the seminal vesicles. The prostate and ampullary glands were not distinguishable from each other in the neonate.

Urethra

The urethra in Em1 and Em2 measured approximately 50-55 mm in cross-section. The internal structures showed no significant differences from those visualized in the African males. In Em3, the urethra was 4 mm thick and the lumen appeared uniformly rounded in cross-section, rather than V-shaped as in the adults.

Urinary Bladder

Only the dorsal part of the urinary bladder was visible in Em1 because of the large accessory

glands in this region. In Em2, the bladder appeared relatively flat, probably because of urination during the restraint procedure. The hyperechoic bladder wall in both males was 15-20 mm thick. The three muscle layers of the wall of the urinary bladder in the adult Asian males were only visible between the cranial part of the ampullary glands and the caudal part of the seminal vesicles, because the distance between the rectum and the bladder was shortest at this point. Both ureters were visible within the wall of the urinary bladder in Em2. The urine in the bladder appeared cloudier than the fluid in the seminal vesicles in both Asian males. This pattern contrasted with that found in the African males. The urinary bladder appeared empty in Em3. It was filled with water retrogradely through the urethra to better distinguish the bladder from the accessory glands. The position and texture of the filled bladder appeared similar to those of the adult Asian elephants, except that the wall was only 5-7 mm thick.

Kidneys

The kidneys were not visualized in Em1 and Em2 because the 7.5 MHz probe was not used. In Em3, the kidneys were partly visualized with the 7.5 MHz probe *in situ*. The smoothly-curved capsule was hyperechoic and the intrarenal connective tissue septa demarcated the lobes of the kidney, unvisualizable in the adult elephants. The moderately-echoic cortex was distinguishable from the hypoechoic medulla. Parts of the hyperechoic pelvis renalis were detectable in the deeper regions of the kidney. In the post-mortem preparation of Em3, the isolated right and left kidneys measured 150 and 135 mm long, respectively.

Bulbo-urethral Glands

The bulbo-urethral glands were only visible in Em3, because they were positioned caudo-ventral to the pelvis and were not reachable in the older elephants with the ultrasound frequencies used. In Em3, they were each 29 x 19 mm in size and were located dorsolateral to the urethra. The parenchyma was hypoechoic with a moderate echoic border and several internal hyperechoic septa (Fig. 4 f). No lumen was distinguishable in this individual in contrast to the findings in the post-mortem preparations of the adult African elephants.

Prostate Gland

The lobes of the prostate gland in the Asian elephants appeared egg-shaped. Each lobe in Em1 measured approximately 30 x 18 mm (4 g) and each lobe in Em2 measured 62 x 28 mm. The lobes were situated dorsolaterally on both sides of the urethra, approximately 60 mm from the urinary bladder. The prostate was covered only by a thin, hypoechoic muscle layer, no layer of connective tissue was detected sonographically. In both males, the anterior pole of the prostate covered the laterally-lying posterior part of the ampullary glands and the medially-lying posterior part of the seminal vesicles. The stroma in the Asian males appeared more echoic than in the African males. There was also no visible cavity in the prostate of either Asian male. The small centre of the prostate appeared moderately echoic and had an irregular border, indicating it was filled with connective tissue. In Em3, the neonate, the prostate and ampullary glands were not distinguishable from each other. They were visualized, both *in situ* and *ex situ*, together as a hypoechoic package, 13 x 16 mm in size, dorsolateral to the urethra. In the post-mortem preparation, the glands were identified, but the connective tissue between the two glands was still undeveloped.

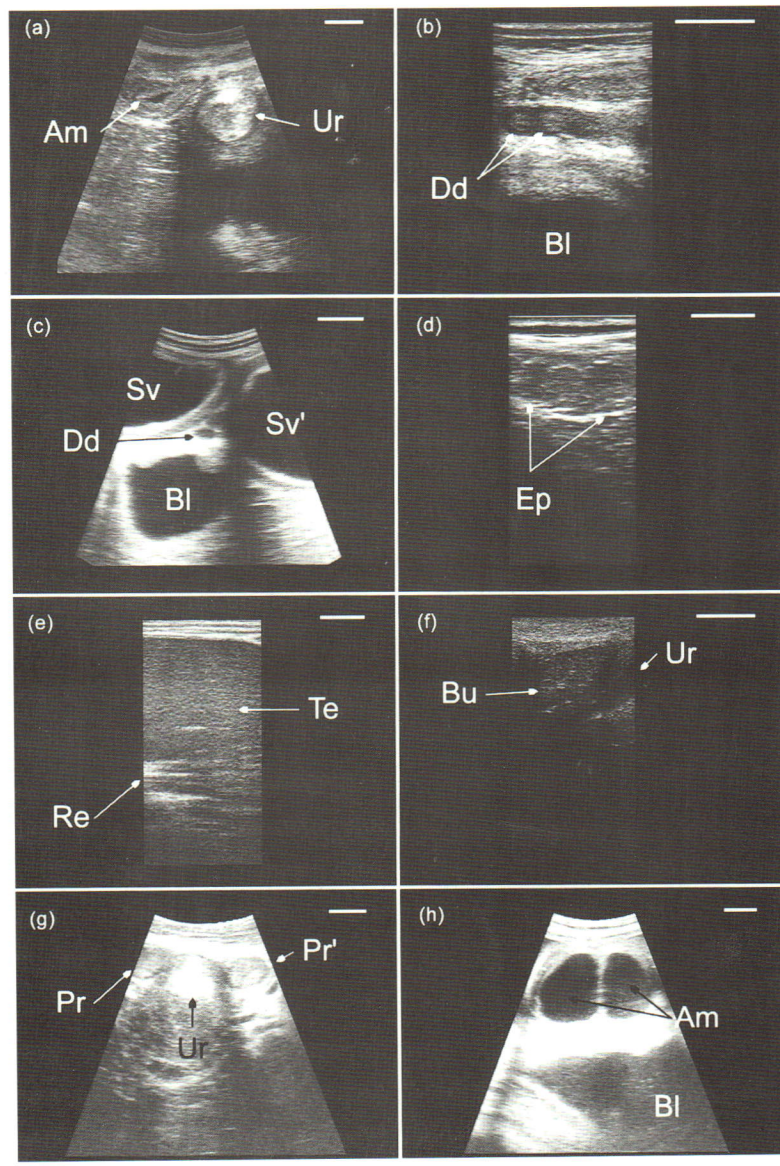

Fig. 4 *(a) Sagittal ultrasonographic image (3.5 MHz) of the left ampullary gland (Am) and urethra (Ur) in La6. Note the undeveloped status of this gland characterized by its small size and the absence of a big cavity. (b) Sagittal sonogram (7.5 MHz) of the paired dutus deferens (Dd) of La2 near the seminal colliculus. In this region the two ductus deferentes are not separated from each other. (c) Ultrasonographic image (3.5 MHz) of parts of both seminal vesicles (Sv and Sv') of La5 located dorsolateral to the neck of the bladder (Bl) and enclosing the ductus deferentes (Dd). (d) Longitudinal sonogram of the corpus epididymidis (Ep) of La1 made using a 7.5 MHz transducer inserted in an adapter. Note the hyperechoic wavelike epididymal border created by its coiled nature. The attachment to the testis is characterized by a white line (arrow heads) below the epididymis. (e) Ultrasonographic image (7.5 MHz) showing a part of the left testis (Te) of La6. The central hyperechoic rete testis (Re) is clearly distinguishable from the homogenous parenchyma characterized by lower echogenicity. (f) Longitudinal sonogram (7.5 MHz) of the bulbo-urethral gland (Bu) of the neonate (Em3) shows the hypoechoic parenchyma with several hyperechoic internal septa. The gland is attached to the urethra (Ur). (g) Sagittal ultrasonographic image (3.5 MHz) of the paired prostate gland (Pr and Pr') of Em1 enclosing the urethra (Ur). Note the small dimension and absence of a cavity in this moderately echoic organ in contrast to the prostate gland in African elephants. (h) Sagittal ultrasonographic image (3.5 MHz) of both ampullary glands (Am) of Em2. Note the large internal unechoic cavities of these glands laying above the urinary bladder (Bl).*

Ampullary Glands

The ampullary glands appeared more truncated in the adult Asian males than in the adult African males (Figs. 3 h and 4 h). In cross section, the ampullary glands of Em1, an active breeder, appeared as folded, spheres measuring 35 mm in diameter. Those of Em2 appeared very large (57 x 70 mm at maximum) and unfolded. In both males, the ampullary glands were filled with a slightly cloudy fluid. The muscle wall of the ampullary gland was 2 mm thick and the stroma was 3-7 mm thick. The anterior portion of the ampullary glands connected to the ductus deferentes.

Ductus Deferentes

In Em1 and Em2, the ductus deferentes ranged from 6-8 mm in diameter and were characterized by a hypoechoic periphery and a 2-3 mm thick hyperechoic central epithelium. The whole structure was embedded in hyperechoic connective tissue which was attached ventrally to the urinary bladder and dorsolaterally to the respective seminal vesicles. The ductus deferentes showed the same internal structure as described for the African males, above (see Fig. 4 b). The ductus deferentes were difficult to find in Em3, because the 7.5 MHz probe could only be moved longitudinally in the small rectum. In the *ex situ* examination, they were not well-defined due to their underdevelopment, but appeared to be approximately 1 mm in diameter.

Seminal vesicles

These cigar-shaped glands were the largest accessory glands detected in the Asian males as well. The maximum diameter of the seminal vesicles was approximately 90 mm, but the pressure of the probe on the rectal wall may have slightly compressed the glands. There were about 200-250 ml/gland of unechoic fluid in the lumen. The glands were slightly smaller than in the African males, but the total length of the structure was not measurable. The internal structure was characterized by a 5-6 mm thick hypoechoic muscle layer and a 3 mm thick hyperechoic mucous membrane. In Em3, the seminal vesicles were detectable because they were isolated from the other glands, dorsolateral to the urinary bladder. They were 65-70 mm in length and 14-21 mm in diameter and appeared homogenous without a clear hyperechoic border. There was no secretory fluid inside the flattened lumen. The *ex situ* examination revealed no further details of the seminal vesicles in Em3.

Epididymides

Visualization of the epididymides was not attempted in the adult Asian males, because the 7.5 MHz probe with the adapter was not used for safety concerns. In Em3, the epididymides were not detectable in the *in situ* and *ex situ* ultrasound examinations. An epididymal structure was located in the post-mortem preparation of Em3, but it was deeply embedded in connective tissue and could not be isolated or well-distinguished macroscopically from this tissue.

Testes

Visualization of the testes was also not attempted in the adult Asian males, because of the same concerns in using the 7.5 MHz probe with the adapter. In the neonate, Em3, the rounded, intraabdominal testes were detected caudoventral to the kidney. The right testis measured 17x28 mm and the left 15x25 mm. Each was characterized by a homogenous, moderately-echoic parenchyma. The tunica albuginea was well-demarcated from the parenchyma except in the area of the mesorchium, where the tunica albuginea is not attached to the peritoneum. The central blood vessel found in the testis of the subadult African

male was not clearly visualized in the Asian neonate Em3. It was visible as a very thin (<1 mm) blood vessel in the post-mortem preparation.

Discussion

These results indicate that transrectal ultrasonography is an effective method for developing standards for the assessment of reproductive status in male elephants. This technique has already been proven useful for evaluating female elephants (Adams *et al.*, 1991; Hildebrandt and Göritz, 1995a; 1995b; Hildebrandt *et al.*, 1996). Based on post-mortem studies in male elephants of both species performed by the authors and by others (Stukeley, 1723; Hofmann, 1925; Schulte, 1937; Perry, 1953; Ottow, 1955; Johnson and Buss, 1967a; 1967b; Short *et al.*, 1967; Jones and Brosnan, 1981; see Micota *et al.*, 1994), each part of the internal urogenital tract was sonographically identifiable. The size and morphology of the urogenital structures, especially the accessory glands, were correlative with the breeding history of each individual (Table 1).

There is a very broad range for the timing of the onset of sexual maturation and breeding activity in captive elephant bulls and, at present, no reliable method for predicting these events. The youngest age at which a captive African bull has been known to sire a calf is approximately 10 years; for a captive Asian bull it is approximately 9 years (Schweiger, 1993a, b, Haufellner, 1996). Additionally, in some zoos, apparently healthy, mature bulls unexplainable show no breeding activity when housed with cows over several years (Haufellner *et al.*, 1993). Developing methods for the determination of reproductive health and status in male elephants has a high priority among elephant managers. Recent evaluation methods for behaviour and physiology have proven insufficient for captive management needs. The phenomenon of musth in wild and captive bull elephants of both species and its relationship to breeding activity remain subjects of varying hypotheses and speculation (Eisenberg *et al.*, 1971; Poole and Moss, 1981; Poole *et al.*, 1984; Cooper *et al.*, 1990; Kurt 1995). Studies using electroejaculation for semen collection have produced inconclusive results regarding reproductive capacity due to the high proportion of morphological abnormalities in the samples and the lack of consistent repeatability (Jones, 1973; Ruedi and Kuepfer, 1981; Ruedi *et al.*, 1983; Mar *et al.*, 1992; but see Howard *et al.*, 1984).

Evaluation of the size and morphology of the urogenital tract, especially the accessory glands, by non-invasive transrectal ultrasonography can detect congenital abnormalities, pathological alterations and individual differences which may affect successful reproduction in several exotic species (Hildebrandt and Göritz, 1995b; 1995c). For example, in this study, ultrasonography of La7 revealed calcifications indicative of a previous infection in the urethra (Fig. 3 d). Especially notable among the African males was that La5, a proven breeder, had a body size within the same range as that of La6, which was kept with two females and has never shown any mating behaviour. The prostate glands of these two individuals showed no significant differences. However, the ampullary glands, which appear to be the most important glands indicating sexual maturity (Hildebrandt *et al.*, unpublished data), differed in size between the two males. The breeding male, La5, had much larger, well-developed ampullary glands, each containing a fluid-filled cavity; La6 had small, underdeveloped ampullary glands, each with only a very small cavity (Figs. 3 h and 4 a). In contrast to this finding in the two African bulls, a comparison of

the two Asian bulls, Em1 and Em2, showed that the accessory glands of the younger, inexperienced male appeared even larger than those of the older, proven breeder. This breeder, Em1, has sired five calves, is a regular semen donor for artificial insemination programs and frequently exhibits copulatory behaviour with several different females. The younger bull, Em2, appears physiologically sexually mature, but breeding is hindered by behavioural inexperience.

In this study, the prostate gland in the African elephant was found to be much larger (approximately three times as large in diameter) than that found in the Asian elephants. The La prostate also contained a large, irregularly-bordered cavity, which was absent in the Em prostate (Figs. 3 g and 4 g). These differences have not been described in the literature. The prostate may serve different functions in the two species. In addition, the shape of the ampullary gland also differed between the two species, being more trumpet-shaped in the African males. However, the differences were not dramatic; this finding may only indicate individual, and not functional variation.

Both ultrasonographically and according to other post-mortem examinations (Hildebrandt *et al.*, unpublished data), the internal structure of the African subadult testis appeared significantly different from that of the adult. In the subadult, a central major blood vessel was embedded in the rete testis. A similar structure was present in the neonate Asian testis. This phenomenon has not previously been reported for either species in the literature (Hofmann, 1925; Schulte, 1937; Perry, 1953; Johnson and Buss, 1967 a; 1967 b; Short *et al.*, 1967).

The detection of the location and description of the testes provides critical information for modifying present castration procedures (Fowler, 1973; Fowler and Hart, 1973; Gehring and Schröder, 1982; Foerner *et al.*, 1994). Transrectal ultrasonography can facilitate efficient castration by determining the precise size and location of the intraabdominal testes prior to surgical incision. The entire testicular structure of an 11 year-old African elephant (La7) was ultrasonographically visualizable without the use of a probe extension in this study. The general practice of performing two laparoscopic incisions in large elephants (older than five years), the second several months after the first (Fowler, 1973; Fowler and Hart, 1973; Gehring and Schröder, 1982; Foerner *et al.*, 1994), may be discarded by using transrectal and intraabdominal ultrasound during the surgery to detect the testis on the side contralateral to the incision. Ultrasound-guided surgery reduces the risks associated with large incisions and is standard practice in some human medical procedures (Savader et al., 1990; Fleischer *et al.*, 1995; Kastan *et al.*, 1995).

Semen collection in elephants by electroejaculation requires anaesthesia and by manual manipulation requires a long-term investment in training. The methods present hazards both to the animal and to the handlers. It is therefore critical to perform a reproductive assessment on each bull considered for semen collection before subjecting it to such procedures. The results of this study indicate that transrectal ultrasonography provides the means for efficient, non-invasive reproductive assessment for selection of breeding candidates.

Although zoos and private reserves have recently become more proactive with elephant management programs, establishing conditions for successful reproduction has proven difficult. Breeding bulls are limited in number and methods for evaluating the reproductive capacity of bulls have been inconsistent in their effectiveness.

Sonographic imaging techniques have had a beneficial impact on reproductive and veterinary studies in a wide range of domestic and wild species; however, the feasibility of this technique as a routine diagnostic procedure in elephants has not been overly successful in the past. Some of the difficulties in viewing reproductive structures were attributed to difficulties in positioning the instruments so that the ultrasound beams would penetrate deep within the abdominal cavity, and to the size and demeanour of these large pachyderms. The instrumentation described in this study can overcome the anatomical barriers to successful visualization of the urogenital tract, and can reduce the amount of time needed for an ultrasonographic examination. This study presents results which indicate that transrectal ultrasonography may be used as an effective, non-invasive tool for reproductive assessment of male elephants which has implications for management, population control and assisted reproduction.

References

Adams GP, Plotka ED, Asa CS, and Ginther OJ (1991) Feasibility of characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging *Zoo Biology* **10** 247-260

Balke JME, Barker IK, Hackenberger MK, McManamon R, and Boever WJ (1988a) Reproductive anatomy of three nulliparous female Asian elephants: The development of artificial breeding techniques *Zoo Biology* **7** 99-113

Balke JME, Boever WJ, Ellersiek MR, Seal US, and Smith DA (1988b) Anatomy of the reproductive tract of the female African elephant (Loxodonta africana) with reference to development of techniques for artificial breeding *Journal of Reproduction and Fertility* **84** 485-492

CITES (1995) Convention on International Trade in Endangered Species Appendix **I** p.6

Cooper KA, Harder JD, Clawson DH, Fredrick DL, Lodge GA, Peachy HC, Spellmire TJ and Winstel DP (1990) Serum testosterone and musth in captive male African and Asian elephants *Zoo Biology* **9** 297-306

Eisenberg JF, McKay GM, and Jainudeen MR (1971) Reproductive behavior of the Asiatic elephant (*Elephas maximus maximus L.*) *Behaviour* **38** 193-225

FleischerAC, Burnett LS, Jones HW, and Cullinan JA (1995) Transrectal and transperineal sonography during guided intrauterine procedures *Journal of Ultrasound in Medicine* **14** 135-138

Foerner JJ, Houck RI, Copeland JF, Schmidt MJ, Byron HT and Olsen JH (1994) Surgical castration of the elephant (*Elephas maximus and Loxodonta africana*) *Journal of Zoo and Wildlife Medicine* **25** 355-359

Fowler ME (1973) Castration of an elephant *Journal of Zoo Animal Medicine* **4** 25-27

Fowler ME and Hart R (1973) Castration of an Asian elephant, using etorphine anesthesia *Journal of the American Veterinary Medicine Association* **163** 539-543

Gehring H, and Schröder HD (1982) Teilkastration eines Elefantenbullen (*Elephas maximus*) *Zoologischer Garten* **52** 365-368

Haufellner A, Schilfarth J, and Schweiger G (1993) Elefanten in Zoo und Zirkus *Bongo* **22** 35-46

Haufellner A (1996) Personal communication

Hess DL, Schmidt AM, and Schmidt MJ (1983) Reproductive cycle of the Asian elephant (Elephas maximus) in captivity *Biology of Reproduction* **28** 767-783

Hildebrandt T, and Göritz F (1995a) Sonographischer Nachweis von Leiomyomen im Genitaltrakt weiblicher Elefanten *Verhandlungsbericht Erkrankungen der Zootiere* **37** 287-294

Hildebrandt TB, and Göritz F (1995b) Ultrasonography as a tool in propagation of zoo animals *Proceedings of the American Association of Zoo Veterinarians* 356-364

Hildebrandt T, and Göritz F (1995c) Ultrasonography as a Tool in Zoo Animal Management. In *Zoological Library - Research and captive Propagation* 49-58 Ed. U Ganzloser, JK Hodges, W Kaumanns. Filander Verlag GmbH, Fürth

Hildebrandt TB, Göritz F, Pratt NC, Quandt S, Lehnhardt J, and Montali RJ, and Pitra C (1996) Ultrasonography as a Tool to Evaluate the Reproductive Tract in Female Asian Elephants ultrasound in elephants *Journal of Ultrasound in Medicine* **15** Suppl **3** S59

Hodges JK (1995) Reproductive Biology and Captive Propagation: An overview In Zoological Library - Research and captive Propagation 29-35 Ed. U Ganzloser, JK Hodges, W Kaumanns. Filander Verlag, Fürth

Hodges JK, van Aarde RJ, Heistermann M, and Hoppen HO (1995) Progestin content and biosynthetic potential of the corpus luteum of the African elephant (*Loxodonta africana*) *Journal of Reproduction and Fertility* **102** 163-168

Hofmann L (1925) Zur Anatomie des münnlichen Elefanten-, Tapir- und Hippopotamus-Genitale *Zoologisches Jahrbuch, Abteilung Anatomie und Ontogenese* **45** 161-179

Howard JG, Bush M, deVos V, and Wildt DE (1984) Electroejaculation, semencharacteristics and serum testosterone concentrations of free-ranging African elephants (*Loxodonta africana*) *Journal of Reproduction and Fertility* **72** 187-195

Jainudeen MR, Eisenberg JF, and Jayasinghe JB (1971) Semen of the Ceylon elephant, *Elephas maximus Journal of Reproduction and Fertility* **24** 213-217

Jones RC (1973) Collection, motility and storage of spermatozoa from the African elephant (*Loxodonta africana*) *Nature* **243** 38-39

Jones RC, Rowlands IW, and Skinner JD (1974) Spermatozoa in the genital ducts of the African elephant, *Loxodonta africana Journal of Reproduction and Fertility* **41** 189-192

Jones RC and Brosnan MF (1981) Studies of the deferent ducts from the testis of the African elephant, *Loxodonta africana. I.Structural differentiation Journal of Anatomy* **132** 371-386

Johnson OW and Buss IO (1967a) The testis of the African elephant (*Loxodonta africana*) I. histological features *Journal of Reproduction and Fertility* **13** 11-21

Johnson OW and Buss IO (1967b) The testis of the African elephant (*Loxodonta africana*) II. development, puberty, and weight *Journal of Reproduction and Fertility* **13** 23-30

Kastan DJ, Nelsen KM, Shetty PC, Burke MW, and Sharma RP (1995) Combined transrectal sonographic and fluoroscopic guidance for deep pelvic abscess drainage *Journal of Ultrasound in Medicine* **15** 235-239

Kurt F (1995) Die Haltung des Asiatischen Elefanten in Menschenobhut - ein Vergleich zwischen verschiedenen Haltungssystemen in Südasien und Europa *Zeitschrift des Kölner Zoo* **38**, 91-113

Mikota SK, Sargent EL, and Ranglack GS (1994) The Reproductive System In Medical Management of the Elephant 159-186 Ed. SK Mikota, EL Sargent, GS Ranglack. Indira Publishing House, Michigan, USA

Mar UK, Thein M, Khaing AT, Tun W, and Nyunt T (1992) Electroejaculation and semen characteristics in myanmar timber elephants

In: Forestry Science Research Paper, Union of Myanmar, Minstry of Forestry 1-10

Niemüller CA and Liptrap RM (1991) Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (*Elaphas maximus*) *Journal of Reproduction and Fertility* **91** 139-146

Niemüller CA, Shaw HJ, and Hodges JK (1993) Non-invasive monitoring of ovarian function in Asian elephants (*Elephas maximus*) by measurement of urinary 5b-pregnantriol *Journal of Reproduction and Fertility* **99** 617-625

Ottow B (1955) Proboscidae In Biologische Anatomie der Genitalorgane und der Fortpflanzung der Säugetiere pp 127-130. Verlag Fischer, Jena

Perry JS (1953) The reproduction of the African elephant *Philosophical Transactions of the Royal Society of London Series B. Biological Sciences* **643** 93-149

Poole JH and Moss CJ (1981) Musth in the African elephant, Loxodonta africana *Nature* **292** 830-831

Poole JH, Kasman LH, Ramsay EC and Lasley BL (1984) Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*) *Journal of Reproduction and Fertility* **70** 255-260

Price P, Bradford J and Schmitt D (1986) Collection and semen analysis in Asian elephants. *Proceedings of the American Association of Zoological Parks and Aquariums* 310-313.

Ruedi D and Kuepfer U (1981) Semen collection in the African Elephant (Loxodonta africana): A step towards Artificial Insemination *Proceedings of the American Association of Zoo Veterinarians* 142-143

Ruedi D, Kuepfer U, Girard J, and Gutzwiller A (1983) Untersuchungen zur Fortpflanzungsphysiologie beim Afrikanischen Elefanten (*Loxodonta africana*): Samengewinnung bei Wildbulle, Anatomiestudien an Wildkühen, weitere Schritte hinsichtlich künstlicher Besamung *Verhandlungsbericht Erkrankungen der Zootiere* **25** 341-350

Savader BL, Hamper UM, Sheth S, Ballard RL, and Sanders RC (1990) Pelvic masses: Aspiration biopsy with transrectal US guidance *Radiology* **176** 351-353

Schmidt MJ (1982) Studies on Asian Elephants • reproduction at the Washington Park Zoo *Zoo Biology* **1** 141-147

Schulte TL (1937) The genito-urinary system of the *Elephas indicus American Journal of Anatomy* **61** 131-156

Schweiger G (1993a) Geburtenubersicht Asiatischer Elefanten in Europa In Elefanten in Zoo und Zirkus, Dokumentation Teil 1: Europa 6-21 Eds. A Haufellner, F Kurt, J Schilfarth and G Schweiger. Karl Wenschow, München

Schweiger G (1993b) Geburtenubersicht Afrikanischer Elefanten in Europa In Elefanten in Zoo und Zirkus, Dokumentation Teil 1: Europa 80-83 Eds. A Haufellner, F Kurt, J Schilfarth and G Schweiger. Karl Wenschow, München

Short RV, Mann T and Hay MF (1967) Male reproductive organs of the African elephant, *Loxodonta africana Journal of Reproduction and Fertility* **13** 517-536.

Stukeley WMO (1723) An essay toward the anatomy of the elephant In Of the Spleen, its Description and History pp 89-108 Ed. WMO Stukeley. The author, London.

Watson M (1881) On the anatomy of the female organs of the Probocidea *Transactions of the Zoological Society (London)* **11** 111-130

MANUAL COLLECTION AND CHARACTERIZATION OF SEMEN FROM ASIAN ELEPHANTS *(Elephas maximus)**

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Manual collection and characterization of semen From Asian elephants *(Elephas maximus)*

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Abstract

The implications of collecting semen from elephants for use in artificial insemination programs are profound in the context of propagating captive elephants. Using a manual manipulation technique, semen was collected and characterized from five adult Asian elephants *(Elephas maximus)* and ejaculate fluid was obtained from one castrated elephant bull. The penis was stimulated to protrusion and erection by rectal massage of the pelvic portion of the urethra. During an ejaculatory response, massage was also directed onto the area of the ampulla of the ductus deferens. Sperm rich ejaculates were usually collected as a result of each ejaculatory contraction. Ejaculates were evaluated for spermatozoal concentration and pH (when possible) and sperm rich fractions combined for determination of total volume. Mean total volume of each collection was 27.5±4.4 ml. Mean concentration of the first and second ejaculatory responses from a collection was 2.05±0.17x109 and 1.34±0.19x109 sperm/ml, respectively. Measurement of seminal pH revealed no significant differences between the fractions. Mean pH of the first and second ejaculatory responses were 7.05±0.07 and 7.04x0.13. This method of collecting elephant sperm can be utilized for semen evaluation of bulls of unknown reproductive status in conjunction with other evaluation techniques (i.e. ultrasonographic, endocrinologic). It also has the potential for providing valuable genetic material for genome resource banks and for use with assisted reproductive techniques like artificial insemination.

Introduction

Due to the precarious status of the Asian elephant in the wild, it is vital that further knowledge and techniques for captive elephant reproduction be developed if the species is to continue to exist. The implications of successfully collecting and preserving elephant semen are profound, especially when considered in the context of propagating Asian elephants in captivity. The ability to transport semen to fertile elephant cows, circumventing the severe logistical problems of transporting elephants for breeding purposes is essential. Assessment of semen quality to determine the breeding potential of males is routinely performed in domestic animals (Hafez, 1993). The anatomy of male reproductive organs has been described for Asian *(Elephas maximus)* and African *(Loxodonta africana)* elephants (Mikota et al., 1994), but only a few observations have pertained to semen characteristics. The earliest study on semen characteristics (Landowski and Gill, 1964) was performed with semen obtained from the urogenital tract immediately after copulation. A later study (Jainudeen et al., 1971) described seminal characteristics of a male Asian elephant teased with a female in oestrus, with passive collection of semen for evaluation. Several

investigators have used electroejaculation (Jones, 1973; Ruedi and Kuepfer, 1981; Schmidt, 1982; Ruedi et.al., 1983; Howard et al., 1984; Mar et al., 1992; Schmidt, 1993) to collect semen from elephants. However, the risks of frequent anaesthesia and possible injury during induction and recovery may preclude its frequent use in most captive situations. Using manual rectal stimulation, sperm rich samples can be obtained without dilution of the fluids from the accessory sex glands, and without extensive training of the animal to accept this method of semen collection. Semen collected by rectal massage was first described by Heath et al. (1983) with the use of a modified equine artificial vagina. Price et al. (1986) subsequently described semen collection by manual massage with a condom made from a palpation sleeve to collect the sample. The ability to collect semen from a high percentage of bulls, without prior semen collection training, is needed to evaluate their potential for breeding recommendations. The application of a collection procedure that can be applied to bulls in a variety of environments is essential.

Materials and methods

The method used to collect semen by rectal stimulation was previously reported by Price et al. (1986). In brief, the protrusion and erection of the penis was accomplished by rectal massage of the pelvic portion of the urethra, near the seminal colliculus. Following protrusion, the penis was cleaned and dried to reduce environmental contamination of the semen sample. A collection sleeve was then placed over the end of the penis and massage of the pelvic urethra begun. As an ejaculatory response was detected, massage included the region of the ampulla of the ductus deferens and expulsion of the sperm rich fraction was obtained.

Immediately following each ejaculatory response, the collection sleeve was removed, identified and placed into a warmed 35°c holding container for immediate transport to the laboratory. Semen volume was recorded and, when possible, the concentration and pH were determined. Seven bulls were evaluated in this study. Two were maintained at the Dickerson Park Zoo (Springfield, MO, USA); a 32-year-old male (Em1) that has collected over 250 times in a 10-year period, and a 19-year-old male (Em2) thet has been maintained at the facility for 17 years. The third bull is a 9-year-old proven sire (Em3) maintained at African Lion Safari (Cambridge, Ontario, Canada). Semen from these three males were collected by the senior author (Schmitt). The other four males were collected using the same procedure by the other author (Hildebrandt). One 38-year-old European circus elephant (Em4) without prior training for semen collection was collected four different times in a one-year period. Two males in Myanmar, captured a few months before the study and estimated to by ~20 years old (Em5 and Em6), were each collected once. Additionally, a male castrated at 5 years of age, and 11 years old at the time of collection (Em7), was maintained at the Ringling Brothers Elephant Conservation Center (Polk City, FL, USA) and used to assess ejaculatory responses.

Results

A total of 250 collections over a 10-year period from Em1 were consolidated and semen characteristics presented in Table 1. Good quality and adequate quantities of ejaculate were collected in 80% of the attempts. Semen characteristics were not influenced by season or the presence or absence of 'musth' (determined by drainage from the temporal glands). Mean total ejaculate volume was 27.5±4.4 ml. Good quality semen was

Table 1 *Mean ejaculate characteristics of Em1 (250 ejaculates over 10 year period).*

Fraction	Volume (ml)	Concentration ($\times 10^9$ / ml)	pH
1	10.5 ml	2.05 ± 0.17	7.05 ± 0.07
2	17.0 ml	1.34 ± 0.19	7.04 ± 0.13
Total	27.5 ml	1.61 ± 4.4	

indicated by its white colour, and usually the first fraction collected was the most concentrated. The third fraction was often distinctly yellow and contained lower concentrations of sperm cells and numerous urine salts upon microscopic examination. These fractions were discarded and were not analyzed in this study. Early in the collection of Em1, during a period of 12 successive months, three collections per week were attempted. Table 2 summarizes the volumes of semen collected during a selected 30-day period from Em1 during the 12 months of frequently scheduled collections. In addition to the scheduled semen collections, artificial insemination attempts were ongoing so that occasionally successive days of semen collection occurred as demonstrated by Table 2. While demonstrating the volumes that were possible with frequent collection, most of the collections of Em1 during the 10 year study period were not performed as frequently as this 30-day period. During the 10 years of semen collection of Em1, a change from 'free contact' to 'restricted contact' was accomplished for Em1 and Em2. Semen collection attempts in 'restricted contact' were performed in an elephant restraint device. All attempts to collect Em2 were performed after the change to restricted contact. Attempts

Table 2 *Semen volumes from a selected 30 day period from Em1.*

Day	Volume (ml)
1	4
2	5
5	28
6	160
7	200
8	190
9	200
10	55
11	3
17	0
19	0
20	2
24	8
26	6
27	25

to collect Em2 six times with manual rectal massage were unsuccessful, except for one additional trial when 2 ml was collected under sedation. Em3, the nine year old proven breeder, was collected on two successive days with low volumes (3 ml and 3.5 ml, respectively) and concentrations ($890x10^6$/ml and $900x10^6$/ml, respectively). Em4 was collected four times in a one-year period from June 1996 through June 1997 and the volumes and concentrations are listed in Table 3. Em5 and Em6 both responded with orgasmic contractions of the penis. Em 5 produced no fluid and Em 6 produced 3 ml of yellowish-white fluid containing numerous sperm, but concentration was not determined. On evaluation of a sample of

the ejaculate from Em6, no motility was observed. Em7, the castrated male, ejaculated 6 ml of clear fluid which contained no sperm cells.

Discussion

The collection of semen was demonstrated in several males in varying captive environments. The success of this procedure in five elephants from different backgrounds is an important tool to be added to the repertoire of assisted reproduction in elephants. The use of manual rectal massage has several advantages over two other methods currently employed, electroejaculation and artificial vagina. The use of electroejaculation often results in semen samples with gelatinous accessory gland contamination, and usually requires anaesthesia. Employing the use of an artificial vagina requires extensive training and habituation to the device, time which is often not available for a quick evaluation of a potential breeding male. The use of ultrasound to evaluate the status of the ampulla glands before attempting to collect semen from bulls of unknown status was helpful in evaluating the ejaculatory response. Ultrasound of the ampulla glands before and after ejaculation can be used to verify emptying of these glands was completed (Hildebrandt et al., 1997). The emptying of the ampulla was monitored ultrasonographically in four of the collections of Em1, one collection attempt in Em2, both collections of Em3, the four collections in Em4, and the attempt in the castrate (Em7). Additionally, the technique of rectal stimulation allows fractionation of the ejaculate into sperm rich fractions. The use of other semen collection techniques do not usually provide this option.

In sum, this study demonstrates that semen can be safety and easily collected from unanaesthetized bull elephant managed under protected or free-contact systems. This technique has tremendous application for improving the genetic management of captive elephants, and is a necessary prerequisite for the efficient utilization of assisted reproductive techniques like artificial insemination.

Table 3 *Ejaculate characteristics of Em4 over one year period.*

Collection date	Fraction	Volume (ml)	Concentration ($\times 10^6$ / ml)
June 1996	1	4	87.8
	2	13.5	67.5
	3	300	21.3—Urine contaminated
September 1996	1	11	300
	2	30	120
	3	150	*
November 1996 (musth)	1	15	all sperm dead
	2	50	all sperm dead
	3		
June 1997	1	30	868
	2	36	235
	3	100	Urine contaminated
	4	80	Urine contaminated

References

Hafez, E.S.E., 1993 Artificial insemination, In : Hafez, E.S.E.(Ed.), Reproduction in Farm Animals. 6th edn., Lea and Febiger, Philadelphia, pp. 424-439.

Heath, E., Jeyendran, R.S., Graham, E.F., 1983. Ultrastructure of spermatozoa of the Asiatic Elephant *(Elephas maximus)*. Zbl. Vet. C. Anat. Histol. Embryol. 12, 245-252.

Hildebrandt, T., Göritz, F., Pratt, N.C., Schmitt, D.L., Quandt, S., Raath, J., Hofmann, R.R., 1997. Reproductive assessment by ultrasonography in elephants *(Loxodonta africana and Elephas maximus)*: I. Sonomorphology of the male urogenital tract. J. Zoo Wildlife Med., In press.

Howard, J.G., Bush, M., de Vos, V., Widt, D.E., 1984. Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants *(Loxodonta africana)*. J. Reprod. Fertil. 72, 187-195.

Jainudeen, M.R., Eisenberg, J.F., Jayasinghe, J.b., 1971. Semen of the Ceylon elephant, Elephas maximus. J. Reprod. Fertil. 24, 213-217.

Jones, R.c., 1973. Collection, motility and storage of spermatozoa from the African elephant *(Loxodonta africana)*. Nature 243, 38-39.

Landowski, V.J., Gill, J., 1964. Einige Beobachtungen uber das Sperma des Indischen Elefanten *(Elephas maximus L.)* Zoologische Garten Lpz. 29, 205-212.

Mar, U.K., Thein, M., Khaing, A.T., Tun, W., Nyunt, T., 1992. Electroejaculation and Semen Characteristics in Myanmar Timber Elephants. Forestry Science Research Paper, Union of Myanmar, Ministry of Forestry, pp. 1-10.

Mikota, S.K., Sargent, E.L., Ranglack, G.S., 1994. The reproductive system. In: Mikota, S.k., Sargent, E.L., Ranglack, G.S. (Eds.), Medical Management of the Elephant. Indira Publishing House, MI, pp. 159-186.

Price, P., Bradford, J., Schmitt, D., 1986. Collection and semen analysis in Asian elephants, Proc. Am. Assoc. Zool. Parks Aquar. pp. 310-313.

Ruedi, D.,Kuepfer, U., 1981. Semen collection in the African Elephant *(Loxodonta africana)*: a step towards artificial insemination, Proc. Am. Assoc. Zoo Vet. pp. 142-143.

Ruedi, D., Kuepfer., U., Girard, J., Gutzwiller, A., 1983. Untersuchungen zur Fortpflanzungsphysiologie beim Afrikanischen Elefanten *(Loxodonta africana)*: Samengewinnung bei Wildbulle, Anatomiestudien an Wildkuhen, weitere Schritte hinsichtlich kunstlicher Besamung. Verh. Ber. Erkrankg. Zootiere 25, 341-350.

Schmidt, M.J., 1982. Studies on Asian elephant reproduction at the Washington Park Zoo Biol. 1, 141-147.

Schmidt, M.J., 1993. Breeding Elephants in Captivity. In: Fowler, M.E. (Ed.), Zoo and Wild Animal Medicine, Current Therapy 3. W.B. Saunders, Philadelphia, pp. 445-448.

SEMEN COLLECTION IN WILD AFRICAN ELEPHANTS BY ELECTROSTIMULATION (ES)

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Semen Collection in Wild African Elephants by Electrostimulations (ES)

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The significance of collecting semen from fertile bulls has increased with the recent success of artifical insemination in Asian and African elephants. Using a manual manipulation technique is most recommended for captive bulls (free contact, protected contact). However, the proportion of males in the North American SSP population is 13.4% for Asian and 10.2% for African bulls (AZA Elephant Masterplan 1999). Only a few individuals of this total male population are currently included in breeding programs and there are strong evidences for a high rate of infertility. Therefore, application of a collecting procedure which can be applied to wild bulls is essential in the context of long-term conservation of the elephant. The method of choice is collecting semen by means of Electro-stimulation (ES) under general anesthesia. However, only few publications refer to the anatomical and physiological preconditions required. Former trials of ES were described often unsuccessful. Based on anatomical and sonographical studies, a new handheld 11-cm-long rectal probe plated with three raised longitudinal placed electrodes was designed in co-work with Prof. S. Seager (National Rehabilitation Hospital in Washington D.C.). In 1998 three mature bull elephants were immobilized in the Kruger National Park by Dr. Douw Grobler (National Park Board) for translocation to another park. After transrectal examination assessing their reproductive capability. ES was used to collect semen. The probe was inserted in the rectum placed with the electrodes direct on the internal accessory glands above the *cumulus seminalis*. Electrical stimulation's (max. 15 V, 800 mA) were performed using the apparatus type Seager, model 14. The number of stimulation's differed individually (10 to 25). Semen samples were obtained in all instances within a time of 10 to 20 min. This method in connection with further development of reliable cryopreservation techniques of elephant sperm can provide valuable genetic material for use with artificial insemination and for genome resource banks.

ULTRASONOGRAPHY OF THE UROGENITAL TRACT IN ELEPHANTS (*Loxodonta africana* and *Elephas maximus*) : AN IMPORTANT TOOL FOR ASSESSING FEMALE REPRODUCTIVE FUNCTION*

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*Zoo Biology (2000), 19, 321-332.

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

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Abstract

Presently, the worldwide captive elephant population is not self-sustaining. The major reason for low reproductive rates is the heretofore undiagnosed reproductive disorders of nulliparous females of prime breeding age. Recent advances in ultrasound technology have facilitated the detection of these disorders in non-sedated animals. Approximately 2000 ultrasonographic examinations were performed in more than 280 captive and wild African and captive Asian female elephants. The entire urogenital tract was scanned, measured and documented to provide a reference for ultrasound specialists involved in elephant breeding programs. The primary pathological lesions which influence reproductive rates in these females were uterine tumors and endometrial cysts, and ovarian cysts which resulted in acyclicity. The detection of these disorders and their stage of development can be used by elephant managers to make decisions about which animals to include in breeding programs. Ultrasonography can be used as an effective tool for assessing the reproductive fitness of female breeding candidates in both African and Asian elephants.

Introduction

The current world population of captive elephants results primarily from imports out of Africa and Asia. The difficulty in establishing self-sustaining captive populations of both species is mainly due to low reproduction rates. Besides

Acknowledgments

This study was funded, in part, by the German Society of Technical Collaboration. The authors wish to thank all the institutions and elephants that participated in this study. We also thank Dr. Sybille Quandt and Dr. Douw Grobler for their support and assistance of the work conducted in the Kruger National Park.

the scarcity of breeding bulls, there has also been a high occurrence of undetected reproductive disorders in the captive elephant population. Some of these disorders may be attributed to continuous estrous cyclicity in nulliparous females of prime breeding age (18-30 yrs). After approximately 10-15 years of cyclicity with roughly 40 non-fertile ovulations, pathological alterations like uterine tumors (leiomyoma) and endometrial cysts (hyperplasia) often develop. These lesions usually remain undetected because of a lack of symptoms (e.g., estrous cyclicity appears normal) or outward signs, except that no pregnancy results from breeding.

The breeding guidelines of the American Zoo and Aquarium Association Elephant Species Survival Plan (AZA Elephant SSP, 1997) and the European Association of Zoos and Aquariums Elephant Studbook (EAZA Elephant Studbook, 1999) now recommend an annual ultrasonographic reproductive assessment of potential breeding candidates to distinguish between reproductively healthy and pathologically-altered individuals of prime breeding age. This information would allow elephant managers to make more accurate decisions about population management, including breeding arrangements such as animal movements and artificial insemination, as well as offer the opportunity for clinical treatment in some cases.

Presently, there are too few elephant specialists trained in ultrasonography to assess all of the elephants in the captive population. The three ultrasound articles in this special issue of Zoo Biology provide a basic introduction to elephant reproductive ultrasonography for animal care professionals interested in assessing the status of their animals. This paper presents the summary of ultrasonographic assessments of over 280 female African and Asian elephants. Normal and pathological anatomical structures are described and illustrated with corresponding sonograms. The findings are discussed in terms of their relevance to breeding success and management programs.

Methods

Animals

Over the last six years, approximately 2000 ultrasonographic examinations have been performed in more than 280 captive and wild African and captive Asian female elephants by the Institute for Zoo Biology and Wildlife Research (Berlin, Germany). Captive animals were housed at 17 facilities in North America and 13 facilities in Europe. Free-ranging African elephants were evaluated in the Kruger National Park (South Africa). African elephants also were evaluated at Elephant Back Safari game park (Botswana). Asian elephant cows were evaluated in 7 park facilities in Thailand. Most assessments were performed in free contact situations, with the elephant standing or recumbent without the use of tranquilizers, anesthetics or restrictive devices. There were no remarkable differences in image quality or accessibility to the internal urogenital tract when the examinations were performed in restraint chutes (protected contact) or under anesthesia (no contact, field studies).

An important prerequisite for good quality imaging was intensive cleaning of the rectum in the range of the ultrasonographic exploration (approx. 2.5 m) prior to the investigation. Feces were removed manually and the rectum irrigated with lukewarm water from a hose with a smooth tip (diameter, 1-2 cm) at a flow rate of 5-15 liter per min. The hose and rectal gloves of the examiner were lubricated with commercial ultrasound gel for overcoming the anal tonus painlessly. If the individual appeared nervous or had a lot of anal and rectal contraction, additional

lubricant was applied and the water flow rate was reduced to avoid damage or pain. In general, most elephants became calm and lifted their tails within a few minutes in response to rectal palpation and the enema. As a precaution in unanesthetized animals, there was always one elephant keeper at the animal's head and one keeper at the tail during each ultrasound investigation. Positive reinforcement was provided by feeding favored foods during the procedure. Discomfort was assumed if the animal stopped accepting treats at any time during the examination. By following these general guidelines, daily rectal examinations could be performed over several weeks without any behavioral problems or rectal lesions [Hermes et al., this issue]. In some cases, the ultrasound examinations became part of the daily routine like bathing, foot care, and blood sampling and was welltolerated.

Ultrasonography

Over the course of the study, several ultrasound systems were tested, including stationary color flow Doppler systems and a three-dimensional ultrasound machine. However, the portable ultrasound systems equipped with a variety of ultrasound transducers proved to be the most practical for use in the field and in typical elephant barns. Most commonly, a real time, B-mode ultrasound scanning system (Oculus CS 9100, Hitachi, Physia GmbH, Neu-Isenburg, Germany) equipped with a 3.5 MHz convex, 5.0 MHz microconvex, and a 7.5 MHz linear transducer was used. The systems were modified with special features, such as probe extenders, cable extensions, monitor helmet or video glasses as described in earlier publications [Hildebrandt and Göritz, 1994; 1995; Hildebrandt et al., 1997; 1998; 2000]. The transducers were silicone-sealed to waterproof them for use in the transrectal ultrasound applications. Similarly, the ultrasound machines were protected with plastic during examinations.

Caudal sections of the urogenital tract (vestibule, urethra, ureters, urinary bladder, vagina, cervix, corpus uteri and caudal aspects cornua uteri) were accessed longitudinally and cross-sectionally using a 3.5 MHz convex ultrasound probe. The cranial aspects of the uterine horns, oviducts, ovaries and kidneys were visualized longitudinally using a 3.5 MHz convex or 7.5 MHz linear ultrasound probe fitted to a 45 cm Z-shaped steel adapter (Schnorrenberg Chirugiemechanik GmbH, Schönewalde,Germany). This adapter was specifically designed for the elephant to overcome anatomical obstacles associated with the pelvic inclination, the distance from the anus to the ovary and the varying position of the adnexes caused by extended ligamenta of the uterine horns and ovaries [Hildebrandt and Göritz, 1994; 1995]. In immature females, the cranial part of the urogenital tract was visualized using a hand-held 3.5 or 5.0 MHz convex scanner. All ultrasound examinations were recorded on high-quality S-VHS or digital tape for subsequent viewing and evaluation. Each ultrasound examination took less than 30 minutes to complete.

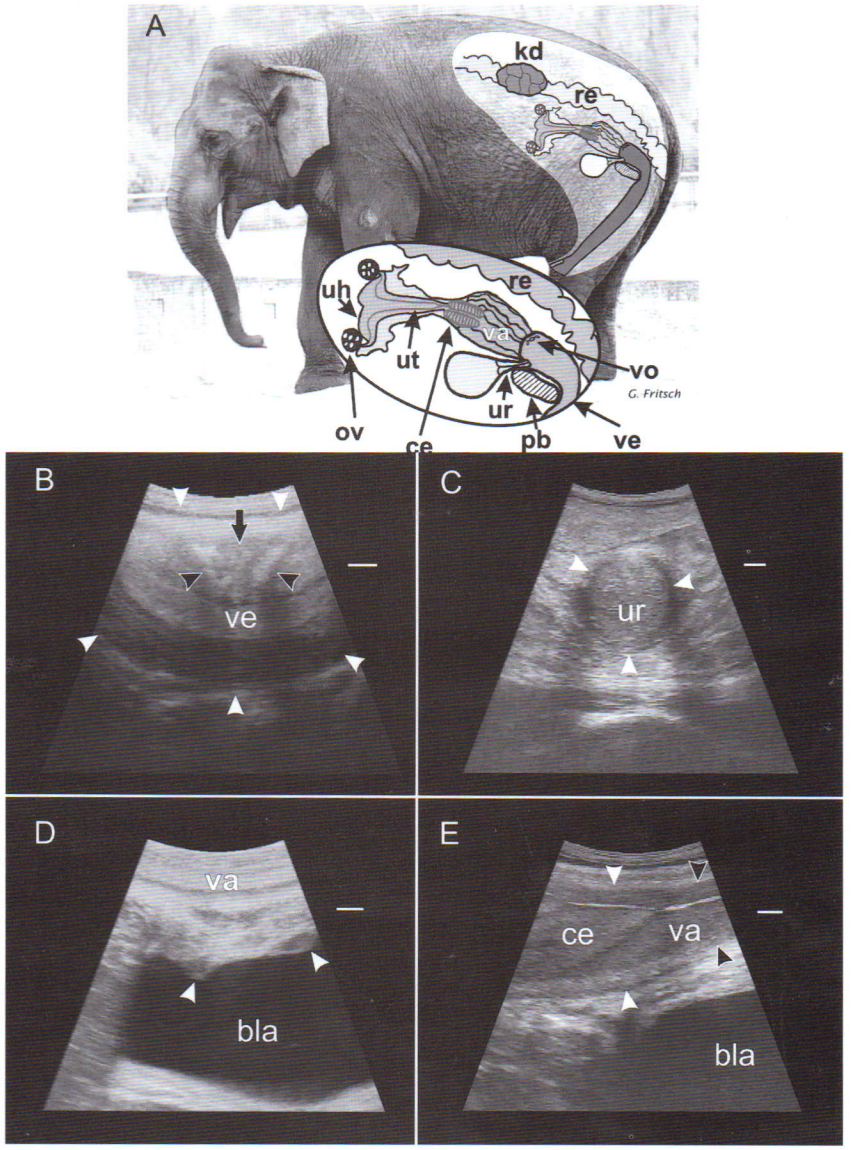

Figure legends

All sonograms (Figs. B-V) contain a white bar on the right side of the image representing 1 cm.

Fig. A. Schematic diagram of the urogenital tract in a female Asian elephant. kd = kidney; re = rectum; ov = ovary; uh = uterine horn; ut = uterine body; ce = cervix; va = vagina; ur = urethra; vo = vaginal os; ve = vestibule; pb = pelvic bone.

Fig. B. Cross-sectional sonogram (3.5 MHz) of the vestibule (ve) at the position of the vaginal os (indicated by the black arrow). White arrowheads mark the border of the vestibule. Black arrowheads indicate the blind pouches.

Fig. C. Cross-sectional sonogram (3.5 MHz) of the urethra (ur), with the external borders marked by white arrowheads. The urethra is located below the vagina and above the echoic pelvic bone.

Fig. D. Cross-sectional sonogram (3.5 MHz) of the vagina (va) and urinary bladder (bla). Ureters are integrated into the dorsal bladder wall and are indicated by white arrowheads.

Fig. E. Longitudinal image (3.5 MHz) of the caudal part of the cervix (ce) (white arrowheads) and the cranial part of the vagina (va) (black arrowheads) which is filled with a thick mucous appearing as a white line. These structures are situated above the bladder (bla).

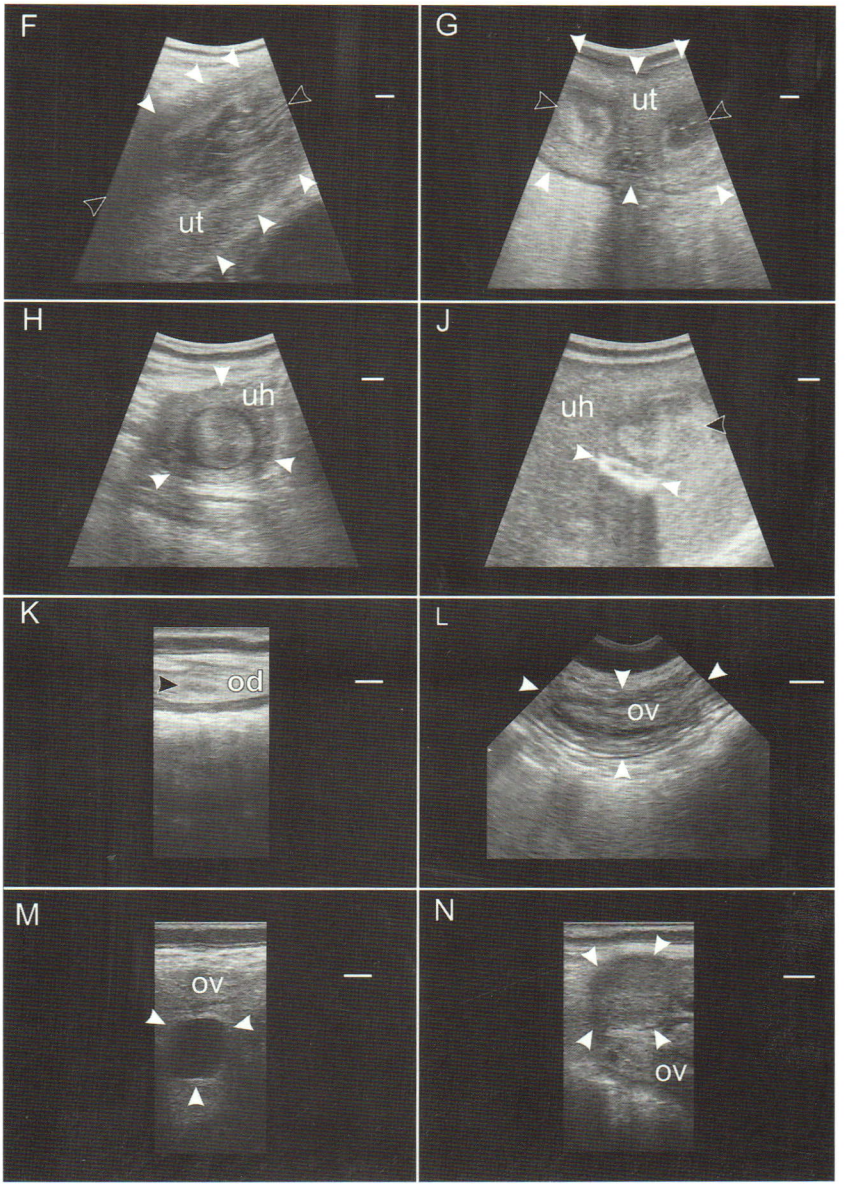

Fig. F. Longitudinal image (3.5 MHz) of the uterine body (ut), with a highly folded mucosa (black arrowheads). The outer border of the uterus is indicated by white arrowheads.

Fig. G. Cross-sectional sonogram (3.5 MHz) of the uterine horns (black arrowheads), which are fused in the uterine body (ut). The outer border of the uterus is indicated by white arrowheads.

Fig. H. Cross-sectional sonogram (3.5 MHz) of the cranial part of the uterine horn (uh) (white arrowheads) with a well-defined endometrium.

Fig. J. Cross-sectional sonogram (3.5 MHz) of the uterine horn (uh) post-partum with a regressing placental scar (white arrowheads). The black arrowhead indicates the endometrium.

Fig. K. Cross-sectional sonogram (7.5 MHz) of the relatively short oviduct (od) (black arrowhead) embedded in connective tissue.

Fig. L. Longitudinal image (5.0 MHz) of an immature ovary (ov) without any functional structures. The ovarian cortex appears less echoic than the parenchyma. The white arrowheads mark the outer border of the ovary.

Fig. M. Sonogram (7.5 MHz) of a Graafian follicle integrated into the ovary (ov), two days before ovulation (white arrowheads).

Fig. N. Sonogram (7.5 MHz) of a corpus luteum of pregnancy (white arrowheads) situated on the edge of the ovary (ov).

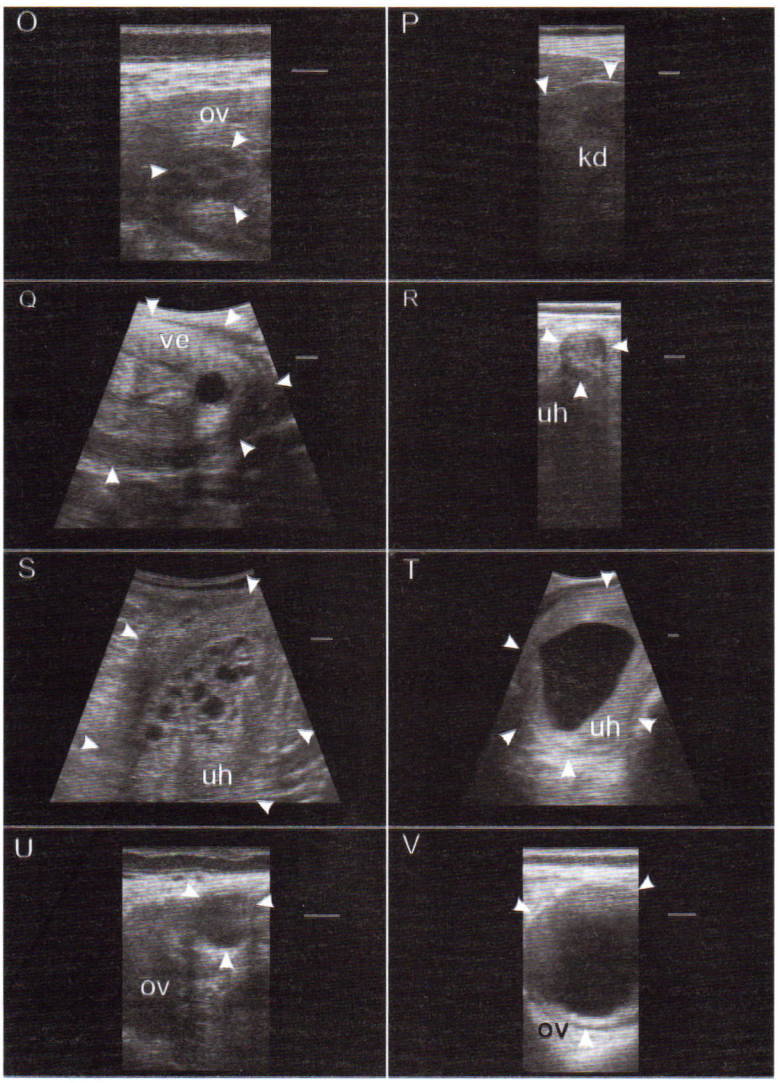

Fig. O. Sonogram (7.5 MHz) of ovary (ov) with accessory corpus luteum (white arrowheads) with a rarely detected central cavity. These structures are embedded deeper into the ovarian tissue than the corpora lutea derived from ovulation.

Fig. P. Sonogram (7.5 MHz) of a part of a kidney (kd) lobe with a dominant echoic capsule (white arrowheads).

Fig. Q. Cross-sectional image (3.5 MHz) of the vestibule (ve) near the vaginal os, containing a single mucosal cyst filled with anechoic fluid. The white arrowheads indicate the outer border of the vestibule.

Fig. R. Sonogram (7.5 MHz) of a uterine horn (uh) containing a small benign uterine muscle tumor (leiomyoma) with a typical moderately echogenicity and sharp round border (white arrowheads). Leiomyomas are generally multiple tumors, and in elephants, are found only in the Asian species, exclusively in the uterine wall.

Fig. S. Sonogram (3.5 MHz) of a uterine horn (uh) (white arrowheads), containing cystic endometrium (endometrial hyperplasia). These cysts develop mostly in older, nulliparous African elephants, but can also be found in Asian elephants. This degree of hyperplasia causes infertility.

Fig. T. Sonogram (3.5 MHz) of a uterine horn (uh) (white arrowheads) with pyometra caused by resorbed embryonic/early fetal tissue. The cloudy appearance of the internal fluid indicates non-infective pus.

Fig. U. Sonogram (7.5 MHz) of an ovary (ov) containing a thick-walled cyst (white arowheads) with the dimension of a medium-sized follicle (~1.5 cm). These ovarian structures can be coincident with acyclicity.

Fig. V. Sonogram (7.5 MHz) of a paraovarian cyst (white arrowheads) close to the ovary (ov). These cysts can reach up to 5 cm in diameter, but have no deleterious effect on the reproductive cycle or fertility.

Results and Discussion

Figure A shows a schematic diagram of the urogenital tract of a female Asian elephant. There was no difference in shape and size of the urogenital system between African and Asian elephants, except in the type of pathological lesions, which will be discussed below. Generally, ultrasound images obtained from African elephants were more distinct than those from Asians because of the differential absorption of ultrasound waves by fat and connective tissue which tends to be greater in Asian elephants [Hildebrandt et al., 1998]. Descriptions of structures are given as average sizes derived from adult individuals.

Vestibule

The vestibule (Figs. A, B, Q) or urogenital canal is extremely long in elephants (1.0-1.4 m). It is a tube-like structure which begins between the hind legs, runs vertically up towards the tail, and then curves horizontally at the cranial end, creating a sac (20-40 cm) situated above the bony pelvis. Urethra and vagina open into the cranial sac. The clitoris is integrated in the muscular wall of the vertical part and reaches a length of 0.6-0.8 m. The glans clitoris measures 7-12 cm and is situated close to the external opening of the vestibule. Figure Q shows a sagittal sonogram of the vestibule of an 18-year-old nulliparous Asian cow with a singular mucosal cyst. These vestibular cysts were found frequently in captive elephants of both species. In contrast to the cystic lesions, vestibular polyps, up to 5 cm in diameter, occurred only in older captive African cows (in about 70% of African elephant females >30 years of age). These alterations apparently have no direct impact on reproductive health; however, they are a clear indicator of advanced age (Figs. R, S). Vestibular scars were found in the pelvic rim region, the narrowest part of the vestibule, located directly below the anus, in 12 captive cows. These alterations in the vestibular mucosa and/or muscle originated from different causes such as urogenital infection, mechanical injuries during mating or birth, episiotomy for artificial insemination or injury from the incorrect use of an elephant hook. The relevance of the lesions in affecting fertility is unknown. However, afflicted females may experience discomfort since they sometimes reacted strongly to rectal and vestibular palpation or avoid natural mating. Two Asian elephant females each had a permanent vestibular fistula caused by an episiotomy for dystocia treatment [Merkt et al., 1985, Lange et al., 1999]. These major lesions appeared to cause less discomfort than the internal vestibular scars.

Bladder

The urinary bladder (Figs. A, D, E) is pear-shaped, and has a relatively small volume capacity (4-6 L) in comparison to total body size. That explains why elephants urinate frequently. The bladder is well-fixed by ligamenta as well as connective tissue and does not prolapse in contrast to the genital tract [Kuntze, 1989, Hildebrandt at el., 1997]. Sonographically, urine appears mostly unechoic, sometimes cloudy. The hyperechoic bladder wall is 3-10 mm thick, depending on the bladder volume. The differentiation of three smooth muscle layers are sometimes distinguishable on ultrasound examination. Urinary bladder infections are relatively rare in elephants and affect mostly old or infirmed cows with weak immune systems. Two females examined had cystitis purulenta where pus was present in the bladder. Ultrasonographic follow-up exams allowed monitoring of the healing process during treatment.

Urethra

The urethra (Figs. A, C) in elephants is a well-defined structure with a strong internal sphincter. The total length ranges from 8-11 cm and the diameter ranges from 3-4 cm. In cross-section, it appears as a round structure with a less echogenic outer muscle wall and a moderately echogenic mucosa (Fig. C). The longitudinal view is also important for the evaluation of the status of the urethra. In about 10% of the captive females investigated, inflammatory processes inside the urethral mucosa were detected, characterized by one or several echodense regions (1-3 mm) surrounded by nearly anechoic mucosa. These alterations have no direct influence on the ability of the cow to conceive, however they can become so extensive and painful that the cow will not allow a bull to mate, similar to a cow with a urinary bladder infection or vestibular lesions.

The caudal part of the ureters are integrated into the wall of the bladder and are easily detected by ultrasound (Fig. D). They are 4-6 mm thick and appear less echogenic than the dorsal wall of the urinary bladder. In females that are nervous during the ultrasound examination, there is frequent transport of urine via the ureters from the kidneys into the bladder. Pathological alterations of the ureters have not been reported in female elephants; however, it is prudent to check them with every transrectal ultrasound investigation performed.

Vagina

The vagina is characterized by many longitudinal folds. It measures approximately 30 x 15 x 10 cm and serves as the place for natural semen deposition. During pregnancy, the vagina takes on the function of a mechanical and infectious protective barrier by filling up with thick vaginal mucus. Nulliparous females have a hymen-like structure which does not rupture during mating. This vaginal os (Figs. A, B) is only 0.4 x 0.2 cm and is flanked by two blind pouches (relics of the Wolffian ducts). After one year post-partum, the opening is approximately 1 x 1 cm and the blind pouches disappear. The vagina illustrated in Figure E (longitudinal sonogram) contains some mucus (end of the luteal phase) visible as a central white line. In general, cross-sectional images of the vagina and cervix are more difficult to interpret and not very useful. The scan head position showing both vagina and cervix in the longitudinal direction is important for verifying the final position of any instrument, such as an artificial insemination catheter. In nulliparous cows older than 30 years of age (both species), there were relatively frequent cysts detected in the vaginal mucosa. In two cows, cysts were so extensive, they filled the vaginal lumen. In general, these cystic alterations have no direct impact on conception rates; however, they are a clear indicator of progressive aging in addition to other pathological alterations of the upper genital tract.

Cervix

The cervix (Figs. A, E) has a prominent portia (approximately 9 x 7 x 5 cm), but a short total length of about 15 cm. The thickness of the cervical mucosa ranges from 8-15 mm. The main cervical pathology found in both species was cystic lesions. These lesions occurred more frequently in older captive African and Asian elephants (about 15% of those examined) than in wild African females (<1%). Occasionally, small cervical polyps with a maximal diameter of 1 cm were detected in African cows. There was one case of scarification in the cervical tissue caused by rupture during parturition. Another rare birth-associated alteration in an Asian cow involving

the cervix and the caudal part of the uterus was a permanent subcutaneous prolapse (large bulb under the tail) caused by partial rupture of the genital ligamenta. These individuals were more sensitive to the rectal palpation procedure. Female elephants trained too young to perform behaviors that result in non-physiological abdominal contraction also tend to develop pelvic prolapses [Kuntze, 1989]. The pelvic diaphragm is not yet strong enough to accommodate the abdominal pressure and the genital tissue becomes compressed into a bulge under the anus that can cause parturition problems later in life.

Uterus

The uterus (Figs. A, F, G, H, J, S, T) is 0.8-1.5 m in length and characterized by a very short corpus uteri (5-10 cm; Figs. A, F). The mucosa of the corpus uteri is convoluted and not as homogenous as the endometrium in the horns (Figs. G. H). Both horns run parallel for 0.5-0.7 m up to the bifurcation (Figs. A, G). Generally, the endometrium is well-defined and can range in diameter from 12-45 mm. The sonographic appearance of the endometrium changes dramatically over the sexual cycle [Hermes et al., this issue].

Pregnancies were detected only in the joint horn complex (Fig. G) or cranially in one horn, but never in the corpus uteri. The finding that the two horns are widely separated may account for the rare phenomenon in elephants that twin calves can be delivered independently with a long time period between births (up to several months), and with both offspring being non-macerated. Figure J was generated approximately six months after birth in a wild African elephant and illustrates a uterine horn in cross-section with an enlarged endometrium and a placental scar in regression. Permanent placental scars are formed by the invasive attachment of the placenta (zonary placentation) during each pregnancy [Laws, 1967] and are detectable by ultrasonography or post-mortem examination. The total number of placental scars can be quantitated by ultrasonography, even under field conditions, and the time period between the last birth and examination can be estimated.

The two species exhibited clearly different uterine pathologies. Asian elephants have the tendency to develop multiple benign uterine tumors in the myometrium (leiomyomas) after a limited non-fertlile reproductive period (10-15 years) [Hildebrandt and Göritz, 1995; Montali et al., 1997]. In contrast, African elephants have not been observed with these neoformations, but rather often develop a cystic endometrium (endometrial hyperplasia) (Fig. S) [Hildebrandt et al., 1997]. A less common finding, sometimes observed in captivity, but rarely in the wild (~2%), is a resorbed embryo or early fetus that resulted in pyometra (a non-infective, pus-filled cavity) at the end of the uterine horn (Fig. T).

Oviducts

The oviducts are approximately 10 cm in length (Fig. K). The mucosa appears less echoic than the surrounding tissue. In general, the oviducts are important landmarks for locating the ovaries in the abdominal cavity. Three of the African cows investigated, including one wild female, had cystic lesions in this region. One 13-year-old captive African cow, which had a paraovarian cyst 17 mm in diameter near the oviduct, was selected for artificial insemination and became pregnant after two attempts [Hildebrandt et al., 1999]. In contrast to the medium-sized ovarian cysts (Fig. U), paraovarian cysts can reach sizes of up to 5 cm in diameter (Fig. V) and have no effect on the reproductive cycle. Paraovarian cysts often occur at the end of stalk-like structures located near the ovaries in the ligamentum. They can confound the results

of an ultrasound examination, being misinterpreted as ovarian structures.

Ovaries

The relatively small ovaries (Figs. A, L, M, N, O, U, V) are about 7 x 5 x 2.5 cm in adults. At 3-4 years of age, the ovaries develop a convoluted, brain-like surface that can be detected by ultrasonography. Figure L shows a juvenile ovary in median section which is divided into the echoic central *medulla ovarii* and the less echogenic peripheral *cortex ovarii*. In general, there are no large follicles or corpora lutea visible until the female enters puberty. Figure M shows part of an active ovary with a well developed Graafian follicle about two days before ovulation. Follicles are characterized sonographically by their round shape, anechoic appearance and typical white line below fluid-filled structures on the far side of the transducer. However, the distinction between ovarian follicles and fluid-filled cysts often is difficult and subsequent ultrasound examinations are necessary to determine the true nature of the structure [Brown et al., 1999].

Figure N shows part of an active ovary of an Asian elephant with a well-developed corpus luteum of pregnancy. Typical corpora lutea derived from ovulation are large (>25 mm) and prominent on the ovarian cortex, in contrast to the smaller, intra-cortical accessory corpora lutea. In general, corpora lutea in elephants are moderately echogenic, with an elongated echogenic center and a homogenous parenchyma regardless of type. The total number of corpora lutea, including accessory corpora lutea, can range from 0-10 on each ovary, with higher numbers observed during mid and late gestation [Hanks and Short, 1972]. However, there generally appears to be only one large corpus luteum of pregnancy produced. Figure O shows part of an active ovary of a pregnant African elephant with a newly formed accessory corpus luteum containing a fluid-filled cavity 5 mm in diameter. These fluid-filled structures were rare, however, occurring in <5% of cows examined, and in each case resulted from the luteinization of follicles, not ovulation. In general, differences in structure shape, location within the ovary, absence of stigmata and occasionally the presence of a fluid-filled cavity, distinguish accessory corpora lutea from corpora lutea produced after ovulation.

Minor pathologies of the ovary were detected in both species, such as follicular cysts in the cortex up to 25 mm in diameter, characterized by a well-defined wall up to 2 mm thick (Fig. U). Small cysts occurred as follicular structures located in the outer cortex as compared to pre-Graafian follicles which are embedded in the parenchyma. Large cysts were within the size range of normal Graafian follicles; however, the **cyst** wall was thicker than a normal follicle. In this study, ovarian cysts were observed in ~5% of captive Asian and ~15% of captive African elephants, but rarely were observed in free-ranging African females (<1%). At present, no data are available for wild Asian elephants. In a few females with ovarian cysts (2 captive Asian, 2 captive African), longitudinal blood sample analyses of progesterone and estrogens indicated they were acyclic. An earlier study by Brown et al. [1999] also showed no cyclic hormonal patterns in a captive African elephant with a persistant ovarian follicular cyst. Unfortunately, blood data were not available for other elephants to ascertain if all ovarian cysts result in acyclicity. Obtaining that information obviously is important, especially for developing appropriate mitigating therapies.

Kidneys

The dorsal side of the lobed kidneys (Figs. A, P) are situated retroperitoneal, 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, sono-grams of a single kidney lobe easily can be misinterpreted as an ovarian structure, like a large corpus luteum. To avoid misinterpretation, kidneys should be scanned with a 3.5 MHz probe.

Conclusions

There now is clear evidence in both African and Asian elephants that older (>30 years of age) cows have an increased incidence of pathological structures that develop throughout the reproductive tract. Often these pathologies are not suspected because females continue to have regular ovarian cycles and exhibit no other clinical signs of reproductive problems. With ultrasound, using probes specifically designed for the elephant anatomy, these morphological abnormalities are easily identified and their impact on fertility can be studied. Ultrasonographic examinations should be conducted in potential breeding candidates exhibiting no history of pregnancy or long birth intervals.

Based on the age distribution of females examined, the types of pathologies identified and the historical calving records of elephants in captivity, there appears to be a window of 10-15 years from the onset of estrous cyclicity until a dramatic decline in reproductive fitness is observed, particularly in nulliparous cows. For example, in this study several Asian females that started cycling by age 4 were unable to conceive by age 14-19 despite exposure to bulls and observed matings. In other cases, estrous cyclicity is affected and perhaps a quarter of captive elephants do not cycle [Brown, this issue]. What proportion of this acyclicity problem can be attributed to reproductive tract pathologies is under investigation.

In addition to providing new information on basic reproductive mechanisms and diseases, ultrasonography is an important tool for identifying healthy, potential breeders suitable for natural or assisted breeding efforts, and is a requirement of the AZA Elephant SSP Master Plan and EAZA Elephant Studbook breeding recommendation process. The authors further recommend that all institutions participating in elephant breeding programs implement an annual ultrasound examination, not only of breeding candidates, but also elephants exhibiting reproductive problems. Ultrasonography is essential to assessing the efficacy of mitigating treatments for reproductive problems [Brown et al., 1999]. It even has been used in attempts to design contraception protocols for wild elephants that have become overpopulated in certain African regions [Göritz et al., 1999]. Lastly, is imperative that continuing education programs be established to train more veterinarians to perform these ultrasonographic assessments.

References

AZA SSP Masterplan for Elephants, 1997.

Brown JL. Reproductive endocrine monitoring of elephants: An essential tool for assisting captive management. Zoo Biol. (this issue).

Brown JL., Hildebrandt TB., Theison W. and Neiffer DL. 1999. Endocrine and ultrasound evaluation of a non-cycling African elephant. Zoo Biol 18:223-232.

EAZA Studbook for Elephants, 1999.

Göritz F., Hildebrandt TB., Hermes R., Quandt S., Grobler D., Jewgenow K., Rohleder M.,

Meyer HHD. and Hofmann RR. 1999. Results of hormonal contraception program in free-ranging African elephants. Verh ber Erkrg Zootiere 39: 39-40.

Hanks J., Short RV. 1972. The formation of the corpus luteum in the African elephant (*Loxodonta africana*). J Reprod Fert 29: 79-89.

Hermes R., Olson D., Göritz F., Brown, JL., Schmitt DL., Hagan D., Peterson JS., Fritsch G., Hildebrandt TB. Ultrasonography of the estrous cycle in female African elephants (*Loxodonta africana*). Zoo Biol (this issue).

Hildebrandt TB., Göritz F. 1994. Einsatz der transrektalen Sonographie zur Beurteilung des Genitaltraktes weiblicher Elefanten. Imaging 61: Suppl.2., p98, V1.2.

Hildebrandt TB., Göritz F. 1995. Transrectal ultrasonography for ovary and pregnancy in Indian elephant. Verh ber Erkrg Zootiere 37: 261-268.

Hildebrandt TB., Göritz F., Pratt NC., Schmitt DL., Lehnhardt J., Hermes R., Quandt S., Raath J., West G. and Montali RJ. 1997. Assessment of health and reproductive status in African and Asian elephants by transrectal ultrasonography. Proc Am Assoc Zoo Vet p207 - 212.

Hildebrandt TB., Göritz F., Pratt NC., Schmitt DL., Quandt S., Raath J., Hofmann RR. 1998. Reproductive assessment of male elephants (*Loxodonta africana* and *Elephas maximus*) by ultrasonography. J Zoo Wildl Med 29: 114-128.

Hildebrandt TB., Göritz F., Hermes R., Schmitt DL., Brown JL., Schwammer H., Loskutoff N., Pratt NC., Lehnhardt JL., Montali RJ., Olson D. 1999. Artificial insemination of African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. Proc Am Assoc Zoo Vet p83-86.

Hildebrandt TB., Hermes R., Jewgenow K., Göritz F. 2000. Ultrasonography as an important tool for the development and application of reproductive technologies in nondomestic species. Theriogenology 53: 73-84.

Hildebrandt TB., Hermes R., Pratt NC., Fritsch G., Blottner S., Schmitt DL., Ratanakorn P., Brown JL., Rietschel W., Göritz F. Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): An important tool for assessing male reproductive function. Zoo Biol (this issue)

Kuntze A. 1989. Arbeitsbedingte Krankheitsbilder: Hernia perinealis, Bursitis praepatellaris and Tyloma olecrani bei Zirkuselefanten (*Elephas maximus*). Verh ber Erkg Zootiere 31: 185-187.

Lange A., Hildebrandt TB., Strauss G., Czupalla O., Göritz F., Schaftenaar W., Schmitt DL. 1999. Feasibilities and limits of obstetrics in elephants. Verh ber Erkg Zootiere 39: 47-57.

Laws RM. 1967. Occurrence of placental scars in the uterus of African elephant (*Loxodonta africana*). J Reprod Fert 14:445-449.

Merkt H., Ahlers D., Bader H., Brandt H-P., Böer M., Dietrich L. 1985. Der Dammschnitt, eine geburtshilfliche Interventionsmöglichkeit bei einer Elefantenkuh. Dtsch tieraerztl Wschr 92: 428-432.

Montali RJ., Hildebrandt T., Göritz F., Hermes R., Ippen R., Ramsay E. 1997. Ultrasonography and pathology of genital tract leiomyomas in captive Asian elephants: Implications for reproductive soundness. Verh ber Erkrg Zootiere 38: 253-258.

ULTRASONOGRAPHY OF THE ESTROUS CYCLE IN FEMALE AFRICAN ELEPHANT *(Loxodonta africana)*

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Ultrasonography of the estrous cycle in female African elephants (*Loxodonta africana*)

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Sonography of the estrous cycle in elephants

The endocrinology of the elephant estrous cycle has been well characterized, but little emphawsis has been placed on evaluating corresponding changes in the reproductive tract. Ultrasound was used to document changes in reproductive tract morphology throughout the estrous cycle in four cycling female African elephants. Over a 7-month period, frequent ultrasound examinations (n = 190) during the luteal and non-luteal phase were compared with serum progesterone and LH concentrations over a 7-month period. Ultrasonographic images documented vaginal and cervical edema and changes in mucus consistency during the non-luteal phase. The cross-sectional diameter of the endometrium showed a dramatic increase during the non-luteal phase and followed cyclic changes. A different pattern of follicular development on the ovary was associated with the two LH surges. Follicle growth associated with the first, anovulatory LH (anLH) surge was characterized by the formation of multiple small follicles, in contrast to the maturation of a single

Acknowledgements

This project was funded by a Conservation Project Grant from IMLS (IC 70253) and the Elephant Conservation Fund of the Indianapolis Zoological Society. The authors are most grateful to the elephant handlers of the Indianapolis Zoo: Lesley Mackie, Jill Sampson, Barre Fields, Barbara King and Craig Cook. We are especially grateful for the excellent technical assistance and overtime commitment to this project by Astrid Bellem, Jeff Proudfoot, DVM, Nick Kapustin, DVM and Julie Hutt, RVT and the staff of the Indiana University Endocrinology Laboratory. Other members of the Indianapolis Zoo veterinary staff are jointly credited for their assistance in the project's successful outcome. We also are thankful for the administrative support of Dr. Jeffrey Bonner, Roy Shea and Andrea Martin. Special thanks are extended to Eric Sampson, Andrea Krause and Andrea Tscherter for graphic assistance. Dr. Katharina Jewgenow is greatly acknowledged for statistical advice.

large follicle at the second, ovulatory LH (ovLH) surge. Ovulation and the subsequent formation of a corpus luteum (CL) were observed only after the ovLH surge. Ultrasound data in combination with endocrine assessments suggest that the African elephant is monovulatory, although multiple non-ovulatory luteal structures developed during the late non-luteal phase of each cycle. Both ovulatory CL and non-ovulatory luteal structures were present only through one cycle and regressed at the end of the luteal phase in conjunction with the drop in serum progesterone. We conclude that periodic reproductive tract ultrasound assessments in association with continued endocrine monitoring of the estrous cycle should be incorporated into the routine reproductive health assessment of elephants. This information is necessary for determining reproductive fitness before making breeding recommendations. It also has proven to be an invaluable tool for use with assisted reproductive techniques, and has enormous potential for evaluating the efficiency of hormonal therapies used to treat reproductive dysfunction.

Introduction

Captive breeding programs for the African elephant (*Loxodonta africana*) in North America and Europe have fared poorly over the last decades. In addition, the captive population is aging rapidly. In just fifteen years, statistics predict that seventy-five percent of the female captive population will be post-reproductive. Increasing our limited knowledge of reproductive biology is imperative to developing a successful self-sustaining captive population. Endocrine, behavioral and chemosensory research has contributed essential data in recent years towards improving our understanding of the reproductive biology of elephants, and assisting the reproductive management of captive populations [Hodges, 1998, Rasmussen, 1998, Rasmussen and Schulte, 1998]. On the basis of progesterone analysis, the estrous cycle of the African and Asian (*Elephas maximus*) elephant is 13 - 16 weeks in duration with an 8 - 10 week luteal and a 4 - 6 week non-luteal phase [Hess et al., 1983, Plotka et al., 1988, Brown et al., 1991, Kapustin et al., 1996]. Earlier measurements of estrogen-related events [vaginal cytology, Watson and D'Souza, 1975; behavior, Eisenberg et al., 1971; urinary estrogen excretion, Ramsay et al, 1981] suggested an estrous cycle length of 3 weeks. These are now believed to reflect periodic waves of follicular growth that culminate in ovulation only once every 13 - 16 weeks [Plotka et al., 1988].

One unique aspect of the elephant estrous cycle is the presence of two surges of luteinizing hormone (LH) during the non-luteal phase [African, Kapustin et al., 1996; Asian, Brown et al., 1999]. The two surges are quantitatively and qualitatively indistinguishable and occur consistently 19-22 days apart. Ovulation and a subsequent rise in progesterone is observed only after the second LH surge and is termed the ovulatory LH (ovLH) surge. The function of the first, anovulatory LH (anLH) surge is unknown. It may be significant, though, that these surges are ~3 weeks apart, apparently supporting the hypothesis that waves of follicular activity occur at this frequency [Plotka et al., 1988]. Unfortunately, analysis of circulating estrogens have not borne out this pattern, as concentrations are low and fluctuate with no clear pattern phase [Hess et al., 1983, Brannian et al., 1988, Brown et al., 1999].

Until now it has not been possible to address these questions because of an inability to directly observe ovarian activity in elephants [Hodges, 1998]. The adaptation of ultrasonography to examine the elephant reproductive tract provides a new perspective for further investigating

the dynamics of reproductive hormonal relationships in both male and female elephants [Hildebrandt and Göritz, 1994, Hildebrandt and Göritz, 1995, Hildebrandt et al., 1998 a]. The objective of this study was to describe the cyclic changes in the genital tract morphology of the African elephant using transrectal ultrasonography in association with secretory patterns of LH and progesterone. Special emphasis was placed on examining the ovarian dynamics associated with the two LH surges during the non-luteal phase of the cycle.

METHODS

Animals

Four cycling (16 - 29 years) female African elephants housed at the Indianapolis Zoo (Indianapolis, IN) were examined by transrectal ultrasonography in conjunction with endocrine monitoring over a period of seven months. Three of the females were nulliparous and the fourth female had given birth to a calf 17 years prior. All females were managed in a free contact system. All females were calm and tractable, thus no sedatives or restraint devices were used for the 10-15 min ultrasound procedure. Ultrasound examinations were performed once per week during the luteal phase, three times per week during the non-luteal phase and daily around the expected times of the anLH and ovLH surges. To compare ultrasonographic findings throughout the estrous cycle with serum progesterone and LH concentrations, blood samples (20 ml) were taken from ear veins. Blood collection was part of the morning routine and performed weekly during the luteal phase and daily during the non-luteal phase.

Ultrasonography

A real time, B-mode ultrasound scanning system (Oculus CS 9100, Hitachi, Physia GmbH, Neu-Isenburg, Germany) was used. Ultrasonographic images of the reproductive organs were achieved by scanning in a ventral direction through the rectal wall using the technique described by Hildebrandt et al. (this issue). Prior to examination, feces were removed manually. A 5-min warm water enema removed remaining fecal material and ensured acoustic coupling and artifact-free imaging. During the first 2-4 weeks, ultrasound examinations were conducted with the females in the standing position. Thereafter, the female elephants were trained to permit the examination in the lateral recumbent position, resulting in better positioning of the uterine horn and ovary in relation to the rectally inserted ultrasound probe. Caudal sections of the genital tract (vestibule, vagina, cervix, corpus uteri and caudal aspects cornua uteri) were accessed longitudinally and cross-sectionally using a 3.5 MHz convex ultrasound-probe. The cranial aspects of the uterine horns and ovaries were visualized longitudinally using a 7.5 MHz linear ultrasound-probe fitted to a 45 cm Z-shaped steel adapter (Fa. A. Schnorrenberg, Chirurgiemechanik, Wolters-dorf, Germany). This adapter was specifically designed for the elephant to overcome anatomical problems associated with the long distance from the ovary to the anus (1-1.3 m), the mobility of the uterine horns and ovary, and the lateral position of the ovary [Hildebrandt and Göritz, 1994]. Despite these adaptations, intestinal loops inhibited occasionally the visualization of the ovary. However, the identification of the ovary was successful in 168 of 190 exams (88 %). All ultrasound examinations were videotaped using a S-VHS recorder (SVO-9500, Sony, Germany). The longitudinal cross-sectional diameter of the

vagina, cervix and endometrium was measured. The intra-luminal contents or structures of each organ were measured in the same manner at the point of maximum diameter. The ultrasonographical measures were categorized into those recorded in the luteal and the non-luteal phases. The start of the non-luteal phase was defined, when serum progesterone concentration dropped below 100 pg/ml. A total of six luteal and seven follicular phases were monitored in the 7-month period. Values are presented as means ± standard error of the mean (SEM). Data were analyzed using a t-test for dependent samples. All statistical procedures were performed with the software program Statistica for Windows (Release 4.5, copyright StatSoft Inc., 1993).

Endocrinology

Blood samples were allowed to clot at 4°c, centrifuged, and serum stored at -20°c until hormone analysis. Progesterone was quantified by a radioimmunoassay (Coat-A-Count, Diagnostic Products Inc., Los Angeles, CA) validated for elephant serum [Kapustin et al., 1996]. When weekly serum progesterone concentrations dropped below 100 pg/ml, daily blood samples were collected to identify the two LH surges. Serum LH was quantified by a double-antibody ^{125}I radioimmunoassay validated for elephants that utilized an anti-bovine LH antiserum (518-B7, provided by Dr. Jan Roser, University of California, Davis, CA) an ovine LH label (LER-1374A, provided by Dr. Leo Reichert, Jr. Albany Medical College, Albany, NY) and NIH-LH-S18 standards [Brown et al., 1999]. Assay sensitivities were 30 pg/ml and 0.3 ng/ml for progesterone and LH, respectively. Intra- and interassay coefficients of variation were < 12% for both assays.

RESULT

Reproductive tract ultrasound and endocrine evaluations

The Vestibule and Vagina

The vestibule and vagina had a homogeneous echogenicity. The total diameter of the vestibule and vagina did not change throughout the cycle. However, there was a higher fluid content of the tissues corresponding to low progesterone concentrations during the non-luteal phase. In addition to this mild edema, the vaginal mucus, imaged as a central layer in the vagina between the mucosal folds, changed in volume and consistency (Fig. 1). At the end of the luteal phase, a considerable volume of anechogenic mucus filled the caudal aspects of the vagina in the three nulliparous females. During the 2-week transition from the luteal to the non-luteal phase, vaginal mucus was observed to concentrate at the vestibular-vaginal orifice. The diameter of the mucus (4.5 + 0.1mm) was significantly greater ($p < 0.001$) during this transition period than during the remaining non-luteal (1.6 + 0.04 mm) or luteal (1.5 + 0.09 mm) phases. During the mid to late non-luteal phase, mucus was characterized as a dense echogenic line within the vagina. However, 1-3 days before ovulation, mucus consistency changed and appeared as a discontinuous white line. In contrast to the nulliparous females, no significant difference in vaginal mucus diameter was observed between the transitional period (1.3 + 0,3 mm) and the non-luteal phase (1.3 + 0.1 mm) in the uniparous animal. During the luteal phase in this animal, folds of the vaginal mucosa were adjacent and vaginal mucus was not detectable by ultrasound (< 0.1 mm).

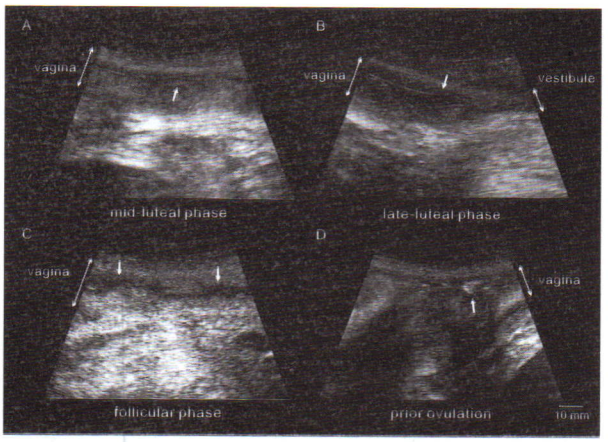

Fig. 1 *Longitudinal sonograms of the vagina in an African elephant at different stages of the estrous cycle. A. Mid-luteal phase, small volume of mucus is present (arrow). B. Late luteal phase, a large volume of anechogenic vaginal mucus (arrows) is situated primarily in the caudal part of the vagina. C. Non-luteal phase, the echogenicity of the vaginal mucosa (arrows) is reduced. D. Before ovulation, the vaginal mucus is echodense and has a broken, shell-like appearance (arrow).*

The Cervix

Sonograms of the triangular shaped portio cervicalis and the cervix revealed a lower echogenicity during the non-luteal phase (Fig. 2) similar to the sonograms of the vagina and the vestibule. The diameter of the central anechogenic cervical canal in the three nulliparous females showed significant cycle-dependent variations in height ($p < 0.001$). During the luteal phase, mean height of the canal was 2.6 + 0.07 mm compared to 4.6 + 0.01 mm during the non-luteal phase. A small cervical cyst was observed in one of the females, the diameter of which varied significantly ($p < 0.035$) between the luteal (7.1 + 0.08 mm) and the non-luteal (8.4 + 0.32 mm) phase of the cycle.

The Uterus

The echogenicity of the uterus changed dramatically between the two phases of the cycle (Fig. 3). Two gross types of uterine ultrasonographic morphology were identified. One type characterized the luteal phase, where the echodense endometrium was barely distinguishable from other components of the uterine wall. The endometrium during this period was "flat" and measured 23.7 + 0.21 mm in cross-sectional diameter. The second type, observed from the onset of the non-luteal phase, was characterized by a low echogenic endometrium. A central white line was indicative of adjacent endometrial folds. The cross-sectional diameter of the endometrium was significantly increased ($p < 0.001$) during the non-luteal phase (35.4+0.21 mm) compared to values during the luteal phase. The cyclic dynamic of the endometrium showed an inverse relationship to serum progesterone concentrations (Fig. 4). The late non-luteal phase was additionally characterized by an increased uterine blood supply and uterine fluid 1-2 weeks before the ovLH surge infrequently observed in all females (Fig. 3). The diameter of detectable blood vessels in the myometrium was significantly increased ($p < 0.003$) during the non-luteal (4.7 + 0.11 mm) compared to the luteal (2.4+0.18 mm) phase. Except for this 1 to 2-week period before ovulation, fluid in the uterus was not observed. One to two days before ovulation, frequently coinciding with a pre-ovulatory rise in serum progesterone, the endometrium became more echogenic compared to that observed during the luteal phase. Shortly after ovulation the echogenicity of the endometrium became echodense and remained so throughout the luteal phase. A sharp decrease in the cross-sectional diameter of the endometrium was observed after ovulation (Fig. 4). In one nulliparous and the primiparous female four and six endometrial cysts (5-10 mm

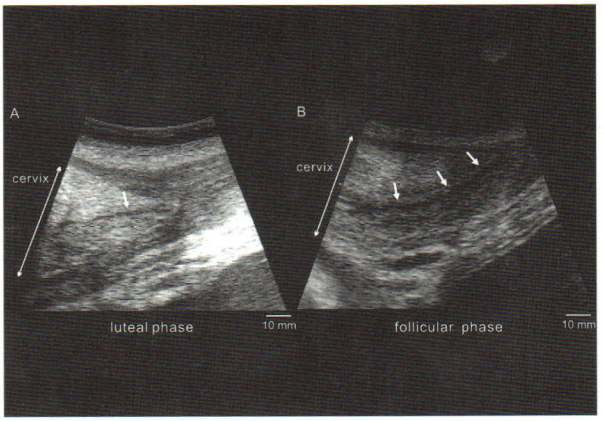

Fig. 2 *Longitudinal sonograms of the cervix in an African elephant. A. Luteal phase, the triangular shaped cervix is echogenic compared to the less echogenic, central cervical canal (arrow). B. Non-luteal phase, the cervix is less echogenic. The cervical canal is well distinguished as an anechogenic band (arrows).*

diameter) were identified. These cysts were anechoic, isolated, round-shaped and located intramural of the uterine body and uterine horns. Changes in the structure or size of these cysts during different phases of the estrous cycle were not observed.

The Ovary

Non-luteal phase

At the onset of the non-luteal phase, when progesterone declined to baseline, the oval-shaped ovary became less echogenic compared with its increased echogenic appearance during the luteal phase. In particular, the ovarian cortex was less echogenic and more clearly distinguished from the denser ovarian medulla. Two distinct waves of follicular development were identified, each of which terminated with an LH surge. However, the follicular growth characteristics differed between the anLH and the ovLH surges. Prior to the first anLH surge, the development of multiple (2-4) follicles was observed. The maximum diameter of the follicles on the day of the anLH surge averaged 13.7 ± 0.73 mm (range, 10 - 19 mm). After the anLH surge, these follicles were replaced by ovarian tissue without the immediate formation of corpora lutea (CL) (Fig. 5 A, B). In contrast to observations at the anLH surge, the development of only one dominant follicle was observed, 5-7 days before the second, ovLH surge (Fig. 5 C). The average diameter of this follicle on the day of the ovLH surge (21.0 ± 0.52 mm) was significantly greater ($p < 0.01$) than those observed on the day of the anLH surge. Ovulation occurred on the day of the ovLH surge or one day after and was characterized by a change in the follicle's round shape to a more oval appearance (Fig. 5 D). In addition, several structures resembling CL were observed 1-5 days before the ovLH surge during each estrous cycle (Fig. 5 D). During the pre-ovulatory period these luteal structures were low echogenic with a central septum. These luteal structures detected before ovulation were significantly smaller in size (14.3 ± 1.2 mm) than the CL derived from the rupture of dominant follicles (22.5 ± 0.81 mm) ($p < 0.005$). Shortly after the ovLH these non-ovulatory luteal structures were clearly distinguishable by their smaller size and slightly increased echogenicity from the larger, low echogenic and septed post-ovulatory CL. The non-ovulatory luteal structures were visualized on both ovaries independent from the ovary the dominant follicle was observed on. The ultrasonographic observation of these luteal structures, possibly the result of luteinization of unovulated follicles, corresponded with a rise in serum progesterone 2-3 days before the ovLH surge.

Luteal phase

Non-ovulatory luteal structures and post-ovulatory CL were imaged on both ovaries repetitively throughout the luteal phase. Non-ovulatory luteal structures and post-ovulatory CL

changed their sonographic appearance within two weeks of the luteal phase. Both were now characterized as round-oval, homogeneous structures with medium or medium-high echogenicity and could not be distinguished sonographically for the rest of the remaining luteal phase. Individual diameters of luteal structures changed throughout the luteal phase and averaged 21.9 + 1.1 mm (range: 10-38 mm). The number of luteal structures observed ranged from three to seven (Fig. 6). However, maximum diameters of 25.8 ± 4.5 mm (range: 23-38 mm) were recorded in week 4 (3.8 weeks ± 0.4) of the luteal phase. Declining serum progesterone concentrations corresponded with a decrease in luteal structure diameter and an increase in echogenicity. Regression of all luteal structures was complete in the early non-luteal phase just before the anLH surge when no luteal structure was detectable by ultrasound within the ovarian parenchyma. No follicular development was observed during the luteal phase of the cycle.

Discussion

This study describes the first longitudinal ultrasound assessment of the elephant reproductive tract in relation to changes in endocrine status throughout the estrous cycle. Of particular interest was the identification of changing ultrasonographic characteristics of the vestibule, vagina, cervix, uterus and ovary between the luteal and non-luteal phases of the cycle. Perhaps even more important was the finding that there are two distinct follicular growth patterns that correspond with the an LH and ovLH surges. The observed maturation and ovulation of only one Graafian follicle per cycle, in addition to the formation of luteal structures resembling CLs suggest an active endocrine mechanism exists in elephants to prevent multiple ovulation while still providing adequate luteal support.

Changes in vestibule, vaginal and cervical echotexture occurred throughout the estrous cycle. Mild cycle edema of these tissues was apparent in the sonograms when progesterone concentrations were at baseline, presumably due to a greater water uptake of the tissue triggered by estrogens. The elephant female has a relatively short, non-convoluted cervix and a long vestibule separated from the vagina in nulliparous females by a hymen-like structure [Balke et al., 1988]. The observed variations in mucus diameter and consistency in the vagina support the earlier proposed cervix-like function of the vagina in elephants [Hildebrandt et al., this issue]. Vaginal mucus was present in considerable amounts during the luteal phase and was reduced during

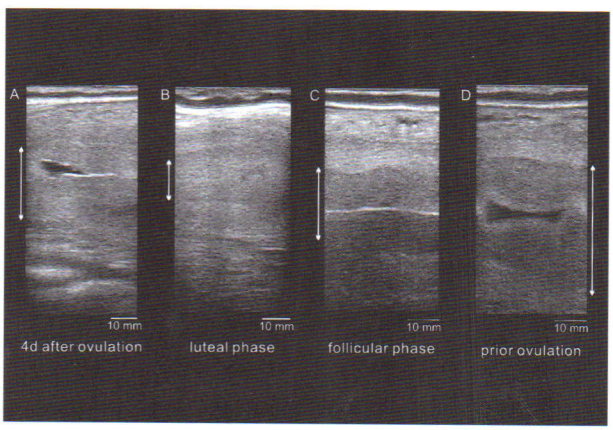

Fig. 3 *Longitudinal sonograms of the uterus in an African elephant. A. Prior to ovulation, the height (↔) of the low echogenic endometrium is at its maximum and fluid is observed in the uterus (arrow). B. Four days after ovulation, the endometrium is more echodense (↔). The endometrium, still large in height (↔), is difficult to distinguish from the myometrium. Fluid (present before ovulation) is still observed in the uterine lumen (arrow). C. Characteristic low echogenic and flat endometrium (↔) during the luteal phase. D. The endometrium during this non-luteal phase is less echogenic and increased in height (↔). Endometrium and myometrium is well distinguished. Blood vessels are observed in the myometrium (arrow). The central white line indicates adjacent endometrial folds.*

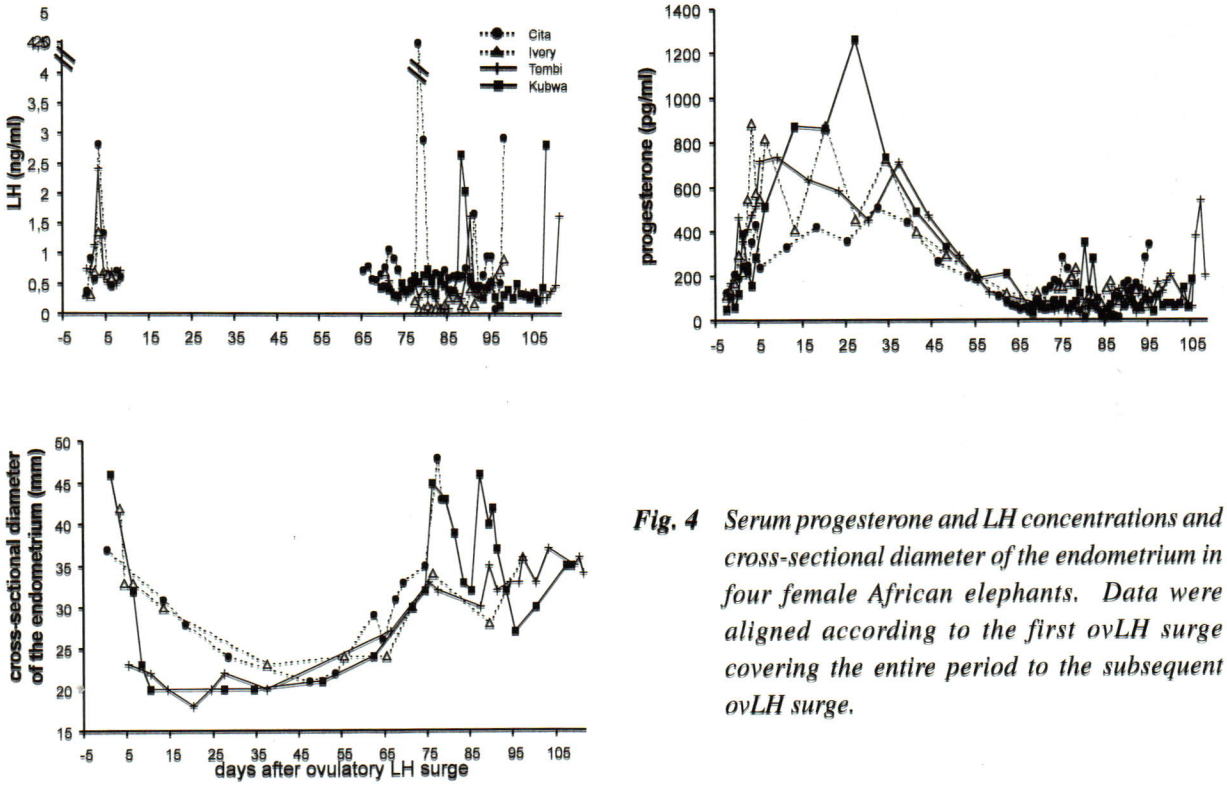

Fig. 4 *Serum progesterone and LH concentrations and cross-sectional diameter of the endometrium in four female African elephants. Data were aligned according to the first ovLH surge covering the entire period to the subsequent ovLH surge.*

the non-luteal phase. Thus, the vagina operates as a natural barrier in elephants protecting the upper genital tract or conceptus from ascending microorganisms. In humans and non-human primates, these variations in vaginal mucus consistency are thought to serve a two-fold function [Tsibris, 1987, Tsibris et al., 1989]. Around the time of ovulation, semen transport and storage is facilitated by loosely structured mucus, whereas at other times of the cycle, the mucus becomes viscous to prevent further entry of spermatozoa or microorganisms. These changes were previously verified in the elephant by endoscopy during artificial insemination trials [Hildebrandt et al., 1999 a,b]. Moreover, changing enzyme activity in human cervical mucus (guaiacol peroxidase) presumably plays an active role in preventing egg fertilization by non-viable spermatozoa that do not reach the oviduct by the time of ovulation [Tsibris et al., 1989].

An increased endometrial wall thickness and myometrial blood supply in conjunction with a decrease in echogenicity of the endometrium were associated with baseline serum progesterone levels. Infrequently, uterine fluid was apparent in all females 1-2 weeks before the ovLH surge. The fertility of estrous cycles in which uterine fluid was observed, was confirmed by two successful conceptions after artificial insemination (Hildebrandt et al., 1999 a,b), suggesting that the uterus is capable of facilitating sperm transport only in the presence of the single dominant follicle that develops at the end of the non-luteal phase. It is therefore concluded that uterine fluid in the pre-ovulatory period is physiological in the African elephant, different from intrauterine fluid collections documented in the mare and rhinoceros in late luteal phase (Ginther, 1992, Radcliffe et al., 1997).

A small number of uterine cysts and one

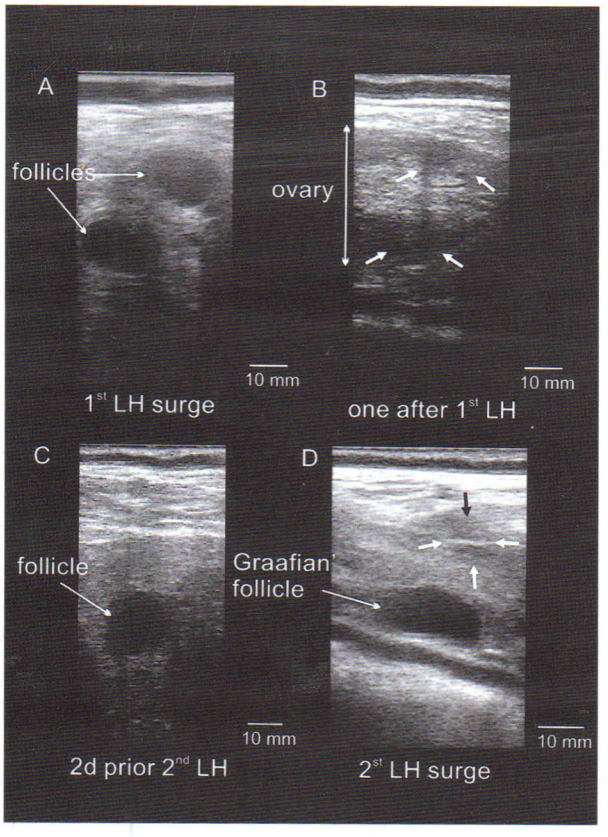

Fig. 5 *Sonograms of the ovary in the African elephant "Tombi" at the first and second LH surge. A. Two follicles are present on the ovary on the day of first, anLH surge. B. One day after the first, anLH surge ovarian tissue (arrows) has replaced previously imaged follicles. C. Dominant follicle prior to the second, ovLH surge. D. Graafian follicle on the day of ovLH surge. Arrows indicate an accessory corpus luteum with a central septum.*

cervical cyst were identified as minor pathological anomalies of the genital tract in three of the four females, compared to those described in captive African elephants [Hildebrandt et al. 1996, 1997, this issue]. The presence of uterine cysts is interpreted as a sign of aging in the elephant, perhaps as a consequence of continuous cyclic ovarian activity without conception. The structural morphology of the uterine cysts was not altered by endocrine status or estrous cycle stage. Therefore, this condition probably represents a slow but progressive pathogenesis, which eventually has a negative effect on pregnancy rates.

Two distinct waves of follicular development were observed during the non-luteal phase when progesterone was low, each terminated by a LH surge. The finding of small but significant estrogen rises (12-15 pg/ml) preceding each LH surge [Kapustin et al., 1996] appears to confirm the ultrasonographic observation of follicular development. However it should be noted that estrogen concentrations in the serum are comparatively low and typically do not fluctuate in a clear pattern indicative of two distinct waves of follicular development. It has been suggested that because circulating estrogens are highly conjugated in the elephant [Czekala et al., 1992, Hodges et al., 1983, Hodges, 1998] that analysis of conjugated estradiol might provide clearer physiological results [Hodges, 1998; Brown, this issue]. But this possibility remains to be determined.

During the non-luteal phase, the first follicular wave involved the formation of multiple follicles, which differed from the second wave where only one large follicle emerged. The inability to detect luteal tissue one day after the anLH surge, in addition to the lack of a subsequent increase in serum progesterone, suggests that these follicles did not ovulate. This first follicular wave was followed by the maturation of a single

large follicle, which did ovulate and form a functional CL after the ovLH surge. At the same time, multiple non-ovulatory luteal structures were observed which may have resulted from the luteinization of follicles from the first wave. The presence and functional significance of multiple CL on the elephant ovary has long been a mystery [Hodges, 1998]. On average 6-8 luteal structures are observed at any one time, some containing ovulation stigmata (30-40%) while others do not. It now is apparent from the ultrasound data that multiple luteal structures do form during the ovarian cycle, with only one CL in response to the ovLH surge. If the non-ovulatory luteal structures are the result of luteinization of follicles from the first wave, it is not clear why these structures are not immediately steroidogenic. It may be significant though, that in the majority of cycles, the luteal phase rise in progesterone precedes the ovLH surge by as many as four days [Kapustin et al., 1996; Carden et al., 1998; Brown et al., 1999]. If adequate CL formation is dependent on the presence of multiple LH surges, then perhaps the first surge acts to initiate a comparatively slow luteinization of the first wave follicles. Alternatively, follicles may luteinize slowly on their own, or respond very rapidly to the steroidogenic actions of the second LH surge. However, the appearance of accessory CL 1-5 days before the ovLH surge, coincident with the pre-ovulatory rise in progesterone, suggests that at least some of this steroidogenic activity may be independent of the ovLH surge.

It was notable that no follicular development was observed during the luteal phase of the cycle. Thus, the speculation of short, 3-week estrous cycles resulting from continuous follicular waves [Plotka et al., 1988] would only apply to the non-luteal phase. In the past, there has been some confusion as to whether this period of low progesterone represented a true follicular phase. On the basis of these new ultrasound data showing waves of follicle development, it does now seem appropriate to refer to this period as a follicular phase. Taken together, the endocrine / ultrasound results suggest that elephants are monovular, corresponding to the low incidence of twinning observed in the wild [Laws, 1969]. However, multiple luteal structures formed during the non-luteal phase of the cycle appear to provide luteal support during both conceptive and non-conceptive cycles. The observation that all luteal structures regressed at the end of the luteal phase eliminates the possibility that multiple CL are the result of

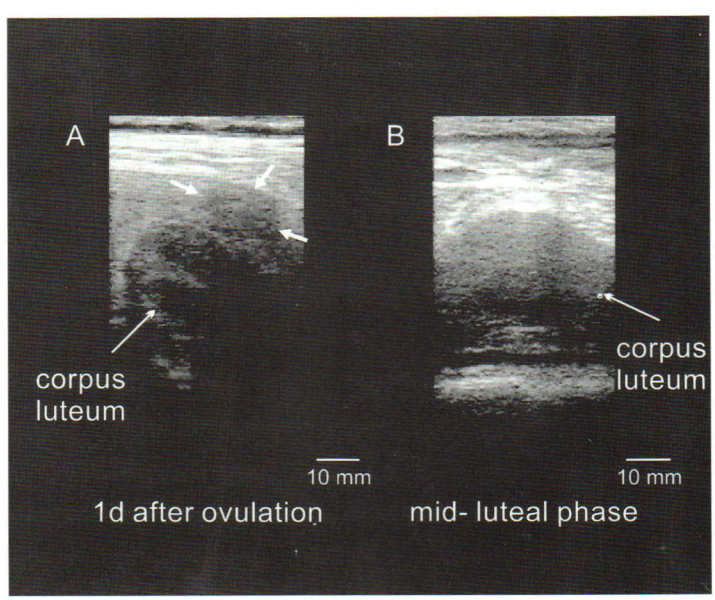

Fig. 6 *Sonograms of corpora lutea in the African elephant "Tombi". A. The corpus luteum one day after ovulation is low echogenic and shows a central septum. The accessory corpora lutea (arrows) are smaller in size and do not have a central septum. B. The corpus luteum during the mid-luteal phase has a homogeneous structure and no septum.*

accumulated structures from cycle to cycle as previously suggested [Hanks and Short, 1972].

The description of sonographic changes associated with the estrous cycle presented in this paper provides further reference data for (1) the reproductive health assessment of individuals, (2) the determination and grading of pathological alterations and their relevance to reproductive soundness [Hildebrandt and Göritz, 1995, Montali et al.,1997, Brown et al., 1999], (3) the identification of potential breeders suitable for natural or assisted breeding efforts, (4) the monitoring of hormone treatment of non-cycling females [Brown et al., 1999], and (5) the evaluation of contraceptive methods in elephants [Göritz et al., 1999].

In addition to providing exciting new information on basic reproductive mechanisms, ultrasonography of the reproductive tract has proven to be an indispensable tool for evaluating the reproductive soundness of female and male elephants [Hildebrandt et al., 1997, 1998]. As a consequence it has become an integral part of the AZA Elephant Species Survival Plan [Keele, 1997] reproductive assessment strategy and will be required for breeding approval. Furthermore, the prediction of ovulation by the use of ultrasonography combined with applied endocrinology, has been essential to four successful artificial inseminations in elephants [Schmitt, 1998, Hildebrandt et al., 1999 a,b].

Conclusions

1. Transrectal ultrasound described morphological changes in the entire genital tract corresponding with the luteal and the non-luteal phases of the estrous cycle in the female African elephant.
2. Two distinct waves of follicular development on the ovary corresponded with the anLH and ovLH surge.
3. Ovulation and subsequent formation of a corpus luteum were observed only after the ovLH surge.
4. Multiple non-ovulatory luteal structures were observed prior to the ovLH surge. Both non-ovulatory luteal structures and ovulatory CL were present only through one cycle and regressed at the end of the luteal phase.
5. Reproductive tract ultrasound and endocrine monitoring of the estrous cycle, incorporated into routine procedures, facilitated the assessment of the reproductive fitness as an imperative basis for elephant breeding recommendations.
6. The physiological, sonographic data of the genital tract is invaluable for the use of assisted reproductive techniques, and has enormous potential for evaluating the efficiency of hormonal therapies used to treat reproductive dysfunction.

References

Balke JME, Barker IK, Hackenberger MK, McManamon R, Boever WJ. 1988. Reproductive anatomy of three nulliparous female Asian elephants: the development of artificial insemination techniques. Zoo Biol 7: 99-113.

Brannian JD, Griffin F, Papkoff H, Terranova PF. 1988. Short and long phases of progesterone secretion during the oestrous cycle of the African elephant (*Loxodonta africana*). J Reprod Fert 84: 357-365.

Brown JL, Citino SC, Bush M, Lehnhardt J, Phillips LG. 1991. Cyclic patterns of luteinizing hormone, follicle-stimulation hormone, inhibin, and progesterone secretion in the Asian elephant (*Elephas maximus*) J Zoo Wildl Med 22: 49-57.

Brown JL, Hildebrandt TB, Theison W, Neiffer DL. 1999. Endocrine and ultrasound evaluation

of a non-cycling African elephant. Zoo Biol 18:223-232.

Carden M, Schmitt D, Tomasi T, Bradfort J, Moll D, Brown J. 1998. Utility of serum progesterone and prolactin analysis for assessing reproductive status in the Asian elephant (*Elephas maximus*). Anim Reprod Sci 53: 133-142.

Czekala NM, Roocroft A, Bates M, Allen J, Lasley BL. 1992. Estrogen metabolism in the Asian elephant (*Elephas maximus*) Zoo Biol 11: 75-80

Eisenberg JF, McKay GM, Jainudeen MR. 1971. Reproductive bevavious of the Asian elephant (*Elephas maximus maximus L.*). Behaviour 34: 193-225.

Göritz F, Hildebrandt TB, Hermes R, Quandt S, Grobler D, Jewgenow K, Rohleder M, Meyer HHD, Hofmann RR. 1999. Results of hormonal contraception program in free-ranging African elephants. Verh ber Erkrg Zootiere 39: 39-40

Hanks J, Short RV. 1972. The formation of the corpus luteum in the African elephant (*Loxodonta africana*). J Reprod Fert 29: 79-89.

Hess DL, Schmidt AM, Schmidt MJ. 1983. Reproductive cycle of the Asian elephant (*Elephas maximus*) in captivity. Biol Reprod 28: 767-773.

Hildebrandt T, Göritz F. 1994. Einsatz der transrektalen Sonographie zur Beurteilung des Genitaltraktes weiblicher Elefanten. Imaging 61: Suppl. 2., p 98, V 1.2.

Hildebrandt TB, Göritz F. 1995. Transrectal ultrasonography for ovary and pregnancy in Indian elephant. Verh ber Erkrg Zootiere 37: 261-268.

Hildebrandt TB, Göritz F, Quandt S, Pratt NC, Lehnhardt J, Montali RJ, Pitra C. 1996. Ultrasonography as a tool to evaluate the reproductive tract in female Asian and African elephants. J Ultras Med 15: 59.

Hildebrandt TB, Göritz F, Pratt NC, Schmitt DL, Lehnhardt J, Hermes R, Quandt S, Raath J, West G, Montali RJ. 1997. Assessment of health and reproductive status in African and Asian elephants by transrectal ultrasonography. Proc Am Assoc Zoo Vet pp 207-212.

Hildebrandt TB, Göritz F, Pratt NC, Schmitt DL, Quandt S, Raath J, Hofmann RR. 1998 a. Reproductive assessment of male elephants (*Loxodonta africana and Elephas maximus*) by ultrasonography. J Zoo Wildl Med 29: 114-128.

Hildebrandt TB, Göritz F, Stetter MD, Hermes R, Hofmann RR. 1998 b. Applications of sonography in vertebrates. Zool 101: 64-73.

Hildebrandt TB, Göritz F, Hermes R, Schmitt DL, Brown JL, Schwammer H, Loskutoff N, Pratt NC, Lehnhardt JL, Montali RJ, Olson D. 1999 a. Artificial insemination of African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. Proc Am Assoc Zoo Vet pp 83-86.

Hildebrandt TB, Göritz F, Schnorrenberg A, Hermes R, Schmitt DL, Hagan D, Peterson JS, Brown JL, Loskutoff N, Pratt NC, Lehnhardt JL, Miller G, Montali RJ, Olson D. 1999 b. Successful Artificial insemination of African nulliparous elephants at the Indianapoils Zoo. Verh.ber Erkrg Zootiere 39: 41-45.

Hodges JK, Henderson C, McNeilly AS. 1983. Circulating oestrogen concentrations during pregnancy in the African elephant (*Loxodonta africana*). J Reprod Fert 67: 121-127.

Hodges JK. 1998. Endocrinology of the ovarian cycle and pregnancy in the Asian (*Elephas maximus*) and African (*Loxodonta africana*)

elephants. Anim Reprod. Sci 53: 3-18.

Kapustin N, Critser JK, Olson D, Malven PV. 1996. Nonluteal estrous cycle of 3 week duration are initiated by anovulatory luteinizing hormone peaks in African elephants. Biol Reprod 55: 1147-1154.

Keele M. 1997. American zoo and aquariums elephant Species Survival Plan masterplan 1997-2002: 21-35.

Laws RM. 1969. Aspects of reproduction in the African elephant, *Loxodonta africana*. J Reprod Fert 6: 193-217.

Leith GS, Kent Allen A. Ultrasonography for reproductive management in the mare. In: Nyland TG, Mattoon JS, editors. Veterinary diagnostic ultrasound. London, UK: W.B. Saunders; 1995. p 305-20

Montali RJ, Hildebrandt T, Goritz F, Hermes R, Ippen R, Ramsay E. 1997. Ultrasonography and pathology of genital tract leiomyomas in captive Asian elephants: Implications for reproductive soundness. Verh ber Erkrg Zootiere 38: 253-258.

Plotka ED, Seal US, Zarembka FR, Simmons LG, Teare A, Phillips LG, Hinshaw KC, Wood DG. 1988. Ovarian function in the elephant: luteinizing hormone and progesterone cycles in the African and Asian elephant. Biol Reprod 38: 309-314.

Ramsay EC, Lasley BL, Stabenfeldt GH. 1981. Monitoring the estrous cycle of the Asian elephant (*Elephas maximus*), using urinary estrogens. Am J Vet Res 42 256-260.

Rasmussen LEL. 1998. Chemical communication: An integral part of functional Asian elephant (*Elephas maximus*) society. Ecoscience 5, 410-426.

Rasmussen LEL, Schulte BA. 1998. Chemical signals in the reproduction of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Anim Reprod Sci 53: 19-34.

Schmitt DL. 1998. Report of a successful artificial insemination in an Asian elephant. Proc 3rd International Elephant Research Symposium. p 7.

Schmitt DL, Hildebrandt TB. 1998. Manual collection and characterization of semen from the Asian elephant (*Elephas maximus*). Anim Reprod Sci 53: 309-314.

Tsibris JCM. 1987. Cervical mucus. In: Gold JJ, Josimovich JB, editors. Gynecologic Endocrinology, New York, NY: Plenum Press. pp 175-183.

Tsibris JCM, Lewis V, Langenberg PW, Chatterton RT, Spellacy WN. 1989. Cervical mucus enzymes as markers of the women's fertile period. Int J Gynecol Obstet 1: 73-82.

Watson PF, D'Souza F. 1975. Detection of oestrus in the African elephant (*Loxodonta africana*). Theriogenology 4: 203-209.

ULTRASONOGRAPHY AND PATHOLOGY OF GENITAL TRACT LEIOMYOMAS IN CAPTIVE ASIAN ELEPHANTS: IMPLICATIONS FOR REPRODUCTIVE SOUNDNESS*

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Ultrasonography and Pathology of Genital Tract Leiomyomas in Captive Asian Elephants: Implications for Reproductive Soundness

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Introduction

Leiomyomas (leiomyomata, fibroleiomyomas, fibroids, myomas) are benign spindle-cell tumors of smooth muscle origin that arise from the involuntary muscle layers usually of hollow viscous organs. In humans, leiomyomas are the most common tumor of the tubular genital tract in 20% of woman over 30 years of age often associated with infertility, menorrhagia and/or dysmenorrhea (ADASHI et al., 1996). In domestic animals, genital tract leiomyomas are most common in the bitch, and are rarely encountered in most other domestic species including goats, ewes, sows, cows and mares.

In a post mortem study between 1975 and 1995 of approximately 30,000 exotic mammal cases at the Institute for Zoo Biology and Wildlife Research (IZW) Berlin, and the Smithsonian Institution, Washington DC, (HILDEBRANDT et al., 1995) leiomyomas were found in the uterus, cervix, and vagina in 14 species of exotic mammals (Table 1). These animals originated from the Smithsonian National Zoological Park (NZP) and from multiple zoos in Europe. This study indicated a very high necropsy prevalence of genital tract leiomyomas in Asian elephants as compared to other species.

Asian elephants are considered endangered by the International Union for the Conservation of Nature and Natural Resources (IUCN) and are the focus of intensive global captive management programs for species survival purposes. Asian elephants are difficult breeders, and establishing conditions for successful reproduction has been difficult. One reason may be the common persistence of leiomyomas in their reproductive tracts which are usually subclinical but may cause interference or signify abnormalities with reproductive mechanisms.

In humans, the etiology and symptomatology of genital leiomyomas are still not fully understood but are believed to be related to

Acknowlegments:

The authors thank the veterinary staff at the following zoological parks for contributing epidemiological information from their elephant collections for this leiomyoma study: In Europe, Carl Hagenbecks Tierpark Hamburg, Tierpark Berlin Friedrichsfelde, and the Allwetter Zoo Monster. In North America, The Chaffee Zoo (Fresno, CA), Sacramento Zoo, San Diego Zoo, St. Louis Zoo, Zoo Atlanta, Brookfield Zoo, Detroit Zoo, Denver Zoo, Honolulu Zoo, Syracuse Zoo, Busch Gardens, Garden City Zoo, Bronx Zoo, Philadelphia Zoo, Prospect Park Zoo, Tulsa Zoo, Dickerson Park Zoo, Gladys Porter Zoo, Sedgewick County Zoo, King's Island, North Carolina Zoo and the Baltimore Zoo; and Drs. Linda Munson and Michael Leach for pathology work on some of the cases presented in this study. This work was supported in part by FONZ 97-3545B and the Smithsonian Research Opportunity Fund.

dysharmonia of the ovarian steroid hormones; consequently, clinical management of these tumors in women patients has not been consistent.

The purpose of this paper is to describe some newer aspects of transrectal ultrasonographic imaging techniques in female Asian elephants that were used to detect leiomyomas of their reproductive tracts by correlative pathological examinations. Recent surveys of genital tract leiomyomas in elephants kept in North American and European zoos are also described and compared with reported cases of genital tract leiomyomas in other species and in women, emphasizing the potential impact of these tumors on reproduction.

Material and Methods

Ultrasonographic Imaging work:

The reproductive health and aspects of the sexual cycle were assessed in 33 captive, non-sedated Asian Elephants from European Zoos by transrectal ultrasonographical examination of the uterine tracts. Ultrasonographic equipment used was an S 9100 Oculus, Ecoscan with a 3.5 MHz probe and a specially designed 7.5 Mhz ultrasound probe for transrectal use in elephants to enlarge the examiners field of exploration and to achieve better sonographic coupling. (HILDEBRANDT and GÖRITZ, 1995, GÖRITZ et al., 1995). Post mortem transrectal sonograms of the genital tracts were performed on 5 additional European Zoo Asian elephants that died or were euthanized for humane reasons unrelated to

Table 1 *Necropsy Prevalence of Leiomyomas from Mammal in Selected US and European Zoos (From HILDEBRANDT et al., 1995)*

Species	#of a with leiomyomas	# of a necropsied	Prevalence %	Distribution/Location of leiomyomas
Asian elephant, *Elephas maximus*	27	27	100	multiple / uterus
Indian Rhinoceros, *Rhinoceros unicornis*	4	5	80	multiple / uterus, cervix, vagina
Bactrian camel, *Camelus bactrianus*	1	23	4	solitary / vagina
Clouded leopard, *Panthera nebulosa*	4	23	17	multiple / uterus, cervix
Asian lion, *Panthera leo persica*	3	24	12	multiple / uterus, cervix
Leopard, *Panthera pardus*	4	9	44	multiple / uterus
Bengal tiger, *Panthera tigris tigris*	2	27	7	solitary / uterus, cervix
Lesser Grison, *Galictis cuja*	2	2	100	multiple / uterus
Giant panda, *Ailuropoda melanoleuca*	1	1	100	multiple / uterus
Maned wolf, *Chrysocyon brachyurus*	1	11	9	solitary / vagina
Opossum, *Monodelphis domestica*	2	25	8	solitary / uterus
Rock cavy, *Kerodon rupestris*	1	22	4	multiple / uterus
Pere David's deer, *Elaphurus davidianus*	1	31	3	multiple / uterus
Guinea pig, *Cavia porcellus*	1	48	2	solitary / Uterus

reproductive tract abnormalities. The sonograms were correlated with pathologic examinations of the elephants' reproductive tracts carried out right after the ultrasonography. Video recordings of all ultrasonographic examinations underwent detailed retrospective analysis.

Pathology studies:

Necropsy findings in the reproductive tracts of the 5 captive Asian elephants from Europe, together with pathology data from a survey obtained from North American Zoos (via co-author E. RAMSAY) from the reproductive tracts of 18 female Asian and 8 African elephants 20 years and older that died between 1985 and 1995 were analyzed, compared and collated. Specimens taken from selected genital tract masses described in many of the cases were routinely processed in paraffin and determined to be uterine leiomyomas by histopathological examination.

Results

Ultrasound studies:

Masses consistent with leiomyomas (as determined by the correlative post mortem ultrasonographic and necropsy studies in the 5 captive elephants from Europe) were evident within the uteruses of 10/33 Asian elephants examined by transrectal ultrasonography. The masses were characterized as spherical, circumscribed structures with hyperechogenic borders (Fig.1). Up to 20 masses were distributed throughout the uterus and ranged from 2 to 20 cm in diameter; in a few cases masses were limited to the body and posterior segment of the uterine horns. Changes of leiomyomas in size and echostructure attributed to the sexual cycle were also noted; during estrus tumors appeared larger and more hypoechogenic. Other abnormalities of the uterus by ultrasonography in elephants with the masses were not evident. The elephant cows with these uterine masses ranged in age from 21 to 46 years old. The 23 elephants without uterine masses were from 7 to 35 years old and with the exception of one cow with a cystic endometrium, other abnormalities were not evident. Parity and status of sexual cycle of each animal are noted in Table 2.

Pathological Studies:

As noted in Table 3 a total of 10 uterine leiomyoma cases (5 from European post mortem cases and 5 from North American survey) were identified from 23 necropsied Asian elephants. The necropsy prevalence of these benign genital tract neoplasms in female Asian elephants ranged from 100% (5/5 reproductive tracts examined) in the European cases, to 28% (5/18 reproductive tracts examined) in the North American Zoo survey for a combined necropsy prevalence of 43%. Leiomyomas were not found in any of the 8 African elephants from the North American survey. The tumors usually appeared as well circumscribed sessile masses within the wall of the uterine components. Tumors were often multicentric, ranged from 2 cm up to 25 cm in diameter and were distributed throughout the uterus (Fig. 2). In a few cases the tumor masses were limited to the body and posterior segment of the uterine horns. The cut surfaces of the tumor masses were yellowish-white and had a fibrous appearance. Some of the larger masses had liquid, hemorrhagic and/or necrotic centers. Histologically the tumors consisted of well-differentiated, smooth muscle-like cells that formed whorls and interlacing patterns. In a few of the leiomyoma cases, cystic changes in the ovaries and endometrium were also described and a malignant cervical tumor occurred in one of the Asian elephants without uterine leiomyomas.

Table 2 *Results of Transrectal Ultrasound Examinations in 33 Asian Elephant Cows from European Zoos with and without imaging evidence of uterine masses.*

ID	age (years)	reproductive status	sexual cycle	Leiomyomas		
				presence	severity	location
Asian elephant						
24Mü	~7	nulliparous	no	no	-	-
27Sp	~14	pregnant	no	no	-	-
12Ro	~15	pregnant	no	no	-	-
13Ro	~15	pregnant	no	no	-	-
1HH	~17	primiparous	yes	no	-	-
23Se	~16	nulliparous	yes	no	-	-
26Mü	~19	nulliparous	yes	no	-	-
22Se	~20	nulliparous	yes	no	-	-
20Wo	~20	nulliparous	yes	no	-	-
9	~20	nulliparous	?	no	-	-
11	~20	nulliparous	?	no	-	-
14DC	~20	primiparous	yes	no	-	-
32Sp	~22	nulliparous	yes	no	-	-
16St	~22	aborted	yes	no	-	-
17St	~22	nulliparous	irregular	no (cystic)	-	endometrium
28Sp	~22	nulliparous	no	no	-	-
15St	~25	primiparous	no	no	-	-
7	~28	primiparous	yes	no	-	-
8	~28	nulliparous	no	no	-	-
18Wo	~29	aborted	yes	no	-	-
31Sp	~31	pluriparous	yes	no	-	-
33Sp	~32	nulliparous	yes	no	-	-
6	~35	primiparous	yes	no	-	-
10	~21	nulliparous	?	yes	2+	uterine body
2	~27	nulliparous	yes	yes	2+	entire uterus
5	~28	nulliparous	yes	yes	3+	entire uterus
19Wo	~28	nulliparous	irregular	yes	3+	entire uterus
4	~30	nulliparous	yes	yes	3+	entire uterus
34Mü	~30	nulliparous	yes	yes	2+	uterine horns
21Se	~30	nulliparous	yes	yes	2+	uterine horns
3	~31	nulliparous	yes	yes	2+	entire uterus
29Sp	~32	nulliparous	yes	yes	2+	uterine horns
30Sp	~46	nulliparous	yes	yes	4+	entire uterus

Key		
grade	**#of tumors**	**diameter (cm)**
1+	≤5	≥3
2+	≥5 ≤10	4-6
3+	≥11 ≤20	7-12
4+	≥21	13-20
5+	≥21	21-25

Table 3 Combined post mortem findings of 10 Asian Elephant cows from European and US Zoos with Uterine Leiomyomas.

ID	age (years)	reproductive status	sexual cycle	Leiomyomas		
				presence	severity	location
Elephants from Europe						
P1	~60	primiparous	no	yes	1+	uterine bifurcation
P2	~34	nulliparous	yes	yes	4+	entire uterus
P3	~38	nulliparous	yes	yes	3+	entire uterus
P4	~34	nulliparous	yes	yes	5+	entire uterus
P5	~54	nulliparous	yes	yes	5+	entire uterus
Elephants from North America						
Suzie	~47	nulliparous	yes	yes	2+	entire uterus
Maya	~51	nulliparous	yes	yes	4+	entire uterus
Lucki	~50	?	yes	yes	3+	entire uterus
Marie	~42	nulliparous	yes	yes	4+	entire uterus
Pumi	~34	?	?	yes	present	entire uterus

(key same as for Table 2)

Discussion

As noted in the results, Asian elephants appear to have a high prevalence of uterine leiomyomas based on correlative post mortem findings and ultrasonographic examination. Masses observed post mortem by transrectal ultrasonographic imaging in the 5 elephants from European Zoos were compatible with the size and distribution of uterine leiomyomas identified later at necropsy in the same 5 elephants and were proven as benign tumors of smooth muscle origin by histopathological examination. As noted in Table 2, trends coincident with these leiomyomas in Asian elephants include increased tumor involvement and severity with aging, a functioning sexual cycle, and no consistent association with any other anatomical reproductive tract abnormalities.

This high prevalence in Asian elephants may exceed the 20-25% incidence of uterine leiomyomas (fibroids) in women of reproductive age, 40% of whom have a history of infertility (ADASHI et al., 1996). The exact mechanism of leiomyoma formation in women is unclear but is thought to be related to hyperestrogenic states either from abnormal physiologic responses to estrogen or increased estrogen receptor sites with hyperstimulation of the putative smooth muscle tumor cells. The role of estrogen as a causative factor has been experimentally supported by estrogen responsiveness and subsequent inhibition by tamoxifen (a nonsteroidal anti-estrogen) of an Eker rat leiomyoma-derived cell line *in vitro*, and a nude mouse xenograft of leiomyoma tumor cells *in vivo* (HOWE et al., 1995; EVERITT et al., 1995).

Use of synthetic gonadatropin releasing hormones (GnRH), e.g., leuprolide acetate, (Lupron Depot, TAP Pharmaceuticals, Inc., Deerfield, IL 60015) an analog of leutinizing hormone

releasing hormone (LHRH) apparently abolishes estrogen stimulation. Lupron has been used therapeutically in women to reverse uterine leiomyomas in order to improve fertility and to diminish injurious effects primarily by shrinkage of the large tumor masses. GnRH analogs have also been used in male elephants to control musth and one type, (Dekapeptyl®) has also been employed therapeutically in an Asian rhinoceros (*Rhinoceros unicornis*) with uterine leiomyomas with promising results (HILDEBRANDT and GÖRITZ, 1995). Therefore, GnRH analogs may be useful as a treatment for uterine leiomyomas after further research and a better understanding of this neoplastic condition in Asian elephants.

One other interesting comparative observation is the lack of any incidence of genital tract leiomyomas in African elephants in the North American survey and from other documented observations (HILDEBRANDT and GÖRITZ, 1995, GÖRITZ et al., 1995). Routine assays for monitoring the sexual cycle of both Asian and African elephants have indicated no major differences in the sex steroids and metabolites between the 2 species (Personal communication, 1997, Dr. Janine BROWN, National Zoological Park, Front Royal, VA USA). It may be a species difference perhaps based on genetic factors. However, more in depth endocrinological studies are required to address these differences between Asian and African elephant species.

A similar tumor relationship exists between Asian and African rhinoceroses in that some Asian rhinoceros species are prone to leiomyomas of the tubular genital tract but this does not appear to be a problem in the African species. Leiomyomas were previously reported in the vaginal, cervical and uterine segments of 4 older adult Indian rhinoceroses (*Rhinoceros unicornis*) to which infertility was attributed (MONTALI et al., 1982). Several small uterine masses visualized by transrectal ultrasonography were considered as possible leiomyomas in 2 Sumatran rhinoceroses (*Dicerorhinus sumatrensis*), (SCHAFFER et al., 1994); whereas, to our knowledge, incidences of such tumors by ultrasonography or at necropsy have not been identified in any African species of rhinoceroses.

The high prevalence of genital tract leiomyomas in Asian elephants appears to be a very significant finding particularly in light of the negative effect these tumors have on fertility in women and other megavertebrates. Ultrasonographic studies have elucidated some very important preliminary findings in this study in Asian elephants. Further in depth work with ultrasonic imaging of the entire reproductive tract is required in elephants to correlate with endocrinological monitoring of the sexual cycle, breeding activities, and careful pathological examinations.

Summary

Ultrasonography and Pathology of Genital Tract Leiomyomas in Captive Asian Elephants: Implications for Reproductive Soundness.

Asian elephants in zoos from Europe and North America were found to have a high prevalence of uterine leiomyomas. This was based on ultrasonographic studies using a customized transrectal imaging instrument. Uterine masses were observed in 10/33 Asian elephants examined and correlated with post mortem sonograms and pathologic findings of leiomyomas in the reproductive tracts of 5 additional Asian elephants. Uterine leiomyomas were not found in any African elephant examined. As with women and female Asian rhinoceroses, the leiomyomas are considered detrimental to reproductive soundness in Asian elephants.

Literature

ADAMS, G.P., PLOTTKA, E.D., ASA, C.S. and O.J. GINTHER (1991): Feasibility characterizing reproductive events in large nondomestic species by transrectal ultrasonic imaging. J Zoo Biol 10, 247-259.

ADASHI, E.Y., ROCK, J.A. and Z. ROSENWAKS (1996): Reproductive Endocrinology, Surgery and Technology. Volume 2, Chapters 85 and 113. Lippincott-Raven, Philadelphia.;1656-1659 and 2160-2163.

du BOULAY, G.H. and O.L. WILSON (1988): Diagnosis of pregnancy and disease by ultrasound in exotic species. Symp Zool Soc Lond 60, 135-150

EVERITT, J.I., WOLF, D.C., HOWE, S.R., GOLDSWORTHY, T.L. and C. WALKER (1995): Rodent model of reproductive tract leiomyomata. Clinical and pathological features. American Journal of Pathology; 146, 1556-1567.

GÖRITZ, F., HILDEBRANDT, TH., QUANDT, S. und R. van AARDE (1995): Transrektale Ultraschalluntersuchung des Urogenitalsystems beim Afrikanischen Elefanten. Ultraschall, 16, Suppl., S 68.

HILDEBRANDT, TH. and F. GÖRITZ (1995): Ultrasonography as a tool in propagation of zoo animals. Proceedings Joint Conference AAZV/ WDA/AAWV, 356 - 364.

HILDEBRANDT, TH., GÖRITZ, F., QUANDT, S., PRATT, N.C., LEHNHARDT, J., MONTALI, R.J., und C. PITRA (1996): Ultrasonography as a tool to evaluate the reproductive tract in female Asian and African elephants. J. Ultrasound Med., 15, Suppl., S 59.

HILDEBRANDT, T.B., IPPEN, R., KAISER, H.E. and R.J. MONTALI (1995): Leiomyomas in the genital tract of captive exotic mammals. Anticancer Research, 15, 1754.

HODGES, J.K., van AARDE, R.J., HEISTERMANN, M. and H.O. HOPPEN (1994): Progestin content and biosynthetic potential of the corpus luteum of the African elephant (Loxodonta africana). J. Reprod. Fert. 102, 163-168.

HOWE, S.R., GOTTARDIS, M.M., EVERITT, J.I. and C. WALKER (1995): Estrogen stimulation and tamoxifen inhibition of leiomyoma cell growth in vitro and in vivo. Endocrinology;136, 4996-5003.

MONTALI, R.J., MANN, P.C., JONES, L.A., GRINER, G.R., KUEHN, E.N., NARUSHIMA, E. and M. BUSH (1982): Leiomyomas in the gentital tract of large zoo mammals. Proceedings of the 24th International Symposium of the Diseases of Zoo and Wild Animals, Akademie-Verlag, Berlin.; 117-122.

PLOTKA, E.D., SEAL, U:S., ZAREMBKA, F.R:, SIMMONS, L.G. TEARE, A., PHILLIPS, L.G., HINSHAW, K.C. and D.G. WOOD (1988): Ovarian function in the elephant: Luteinizing hormone and progesterone cycles in African and Asian elephants. Biol. Reprod. 38, 309-314.

SCHAFFER N.E., ZAINAL-ZAHARI Z., SURI M.S.M., JAINUDEEN M.R. and R.S. JEYENDRAN (1994): Ultrasonography of the reproductive anatomy in the Sumatran rhinoceros, Dicerorhinus sumatrensis. J. Zoo Wildl. Med.; 25, 337-348.

DYSTOCIA IN ZOO ELEPHANTS

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Dystocia in Zoo Elephants

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Introduction

During the last twenty years many zoos became active to start breeding elephants. As a result number of elephant births has been raised in captivity during the last decade. There are several reports on elephant births in captivity (Schmidt, 1999). However, as successful breeding of elephants in captivity is still rare in North America and Europe, the loss of elephant calves or even cows after 22 month of pregnancy because of dystocia is a tragic event. Therefore, methods to diagnose, treat and prevent dystocia are discussed in this paper.

The occurrence of dystocia seems to be more common in captive Asian elephants *(Elephas maximus)* than in African elephants *(Loxodonta africana)*. A total of 29 cases of dystocia were described in Europe since 1902, 28 in *Elephas maximus* and 1 in *Loxodonta africana*. There are 3 major causes of dystocia in elephants (Foerner, 1999): (1) problems in the uterus of the dam, (2) improper fetal positioning, and (3) complications resulting from fetal death.

Birth and diagnosis of dystocia

Duration of gestation is reported by Rüedi (1995) with 20-23 months. Schmidt (1999) reports a gestation length of 644 days for single pregnancies and 616 days for a twin pregnancy. Symptoms of the onset of the normal birth are playing with hay and throwing it against the abdomen or at the back, restlessness, intensive tail movement, increased frequency of urination and defecation, vaginal discharge (mucous in small amounts), touching the nipples with the trunk, stretching the hind legs, pressing with one hind leg at the wall or going down. The intensity of these symptoms usually increased during the course of the parturition, but there is no uniform pattern in behavior. Birth duration can vary from 25 minutes to more than 55 hours with a healthy born calf (Flügger et al., 2001). Schmidt (1999) reported the time of hard labour from 20 minutes to 4 hours, while Rüedi (1995) found 20 minutes to some hours as normal birth duration.

Diagnostic procedures of dystocia as described in other animals (e.g. rectal palpation of fetal movement; detection of fetal heart beat, ECG) are limited. Diagnosis of dystocia can be challenging because of the size, anatomy and physiology of the pregnant elephant. The following signs should lead to the suspicion of dystocia (Foerner, 1999) and to a further examination of the cow:

(1) Absence of labour 30 days past the known due date, (2) 2-4 weeks delay after a decrease in serum progesterone levels, (3) no delivery 24 hours after the appearance of fetal fluids, (4) no delivery several days after the appearance of a cervical plug or fetal membranes, and (5) continued non-productive labour.

As the elephant is able to cease labour voluntary

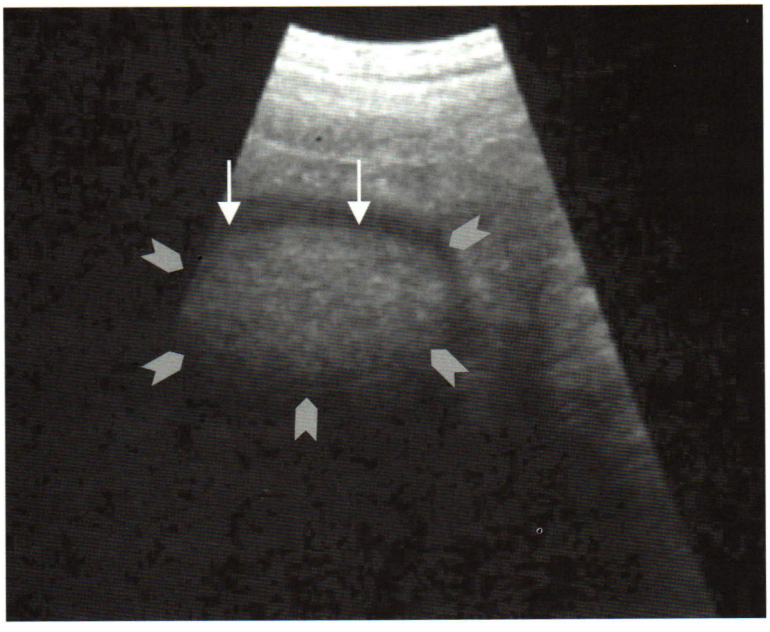

Fig. 1 *Ultrasonographic image (transrectal ultrasound, 3.5 MHz transducer) of a fetal front leg in cross section located in the vagina. The nails (arrow) appear as cresent-shaped hypoechoic areas in the periphery of the sole (arrowheads). Front legs and hind legs can be distinguished by the number of nails.*

to delay calving, it is difficult to distinguish this form of uterine inertia from pathological uterine insufficiency or delayed pregnancy.

Diagnosis of fetal position and posture by transrectal palpation is limited by the size and anatomy of the genital organs. If the limbs are pushed into the pelvic part of birth canal, the number and direction of the toes can be determined by transrectal ultrasound (Fig.1) and allows a guarded diagnosis of the positioning.

The vitality of the calf is difficult to determine by transrectal palpation either as movements of the cow, enteral contractions or labour can simulate movements of the fetus. Visualization of fetal blood vessels (fetal heart not reachable) and of the fetal fluids by transrectal ultrasonography allows conclusions on the calf's vitality. Absence of fetal blood flow (no velocity) in combination with thickened, hyperechogenic fetal fluid is strong indications of fetal death. In addition to ultrasound the birth canal can be evaluate by means of a 1.8-3 m flexible endoscope (colonoscope) in combination with a soft PVC pipe (length 1.2 m) as guide. The pipe is inserted into the vestibulum vaginae up to the brim of the pelvis, and then the endoscope is directed through the pipe and placed right in front of the os vaginale. The origin and condition of potentially visible fetal structures (membranes or limbs) can be determined. Final judgment of another diagnostic procedure is the episiotomy as it allows the direct visualization and palpation of the os vaginale and fetal structures. Furthermore, through the episiotomy a reduction of a malposition and extraction per vias naturalis might be initiated. If these attempts fail, the incision can be extended, and the extraction of the calf through episiotomy should be tried. Details for the performance of episiotomy are given below.

Treatment and prevention of dystocia

Birth assistance in elephants is a special problem because of the very long vestibulum vaginae. The length varies from 1.0 to 1.4 m (Hildebrandt et al., 2000). There is only one report on a manual extraction in an Asian elephant (Lang, 1963). As in nearly all cases of serious dystocia the fetus died in utero, efforts should concentrate on life support of the mother. Treat-

ment of dystocia can be medical, manipulative, or surgical. The successful use of oxytocin, acupuncture, extraction by vias naturalis, and episiotomy has been described, whereas **all performed cesarean sections ended with the dead of mother and calf!!!**

In accordance with our personal experience and in conclusion of other described cases of dystocia, the following recommendations can be given to prevent and to treat dystocia in elephants:

1. Pregnancy in elephants ≥ 20 years of age should be considered as critical.
2. Training and avoiding of adipositas during pregnancy.
3. Monitoring of pregnancy (serum progesterone levels, Ca, P, Mg, Se, ultrasonography, diagnosis of infectious diseases).
4. Preparation of an emergency plan considering staff and equipment for a surgical intervention.
5. Habituation of the cow to the environment of birth to avoid stress. Provided that the social structure is intact and experienced cows are present, the birth should take place in the group.
6. Only in the case of dystocia the cow should be separated in a prepared room. In this room, there have to be facilities for the fixation of each leg, belts, chains and ropes for the extraction of the calf. (Fig. 2). If dystocia is suspected, a gynecological examination should be performed as soon as possible. Ultrasonography and endoscopy if available are very helpful tools (Fig. 3, 4).
8. If the birth canal is not relaxed, it may be softened by the administration of estrogens over 24 hours. Oxytocin should only be administered if the calf is in a physiological position!

The dosages of oxytocin used in some of the 31 cases vary from 45 to 200 I.U. per elephant cow i.m. or i.v. (Flügger, 2001). Other authors injected 30 to 400 I.U. (Rüedi, 1995). The administration of oxytocin should not be extended until the mother is exhausted. In case of an extraction the mother needs a reserve of energy and the uterus must be reacting to oxytocin administration to push the calf into the pelvic canal! Rectal massage performed very gentle induces very strong labor activity! The muscle contractions are much more coordinated (real waves from cranial to caudal) than after administration of oxytocin. Manual stimulation of "special receptors" in the caudal part of birth canal may imitate "natural birth" is very effective to bring the calf out.

9. Episiotomy can be performed on the standing elephant under local anesthesia with or without

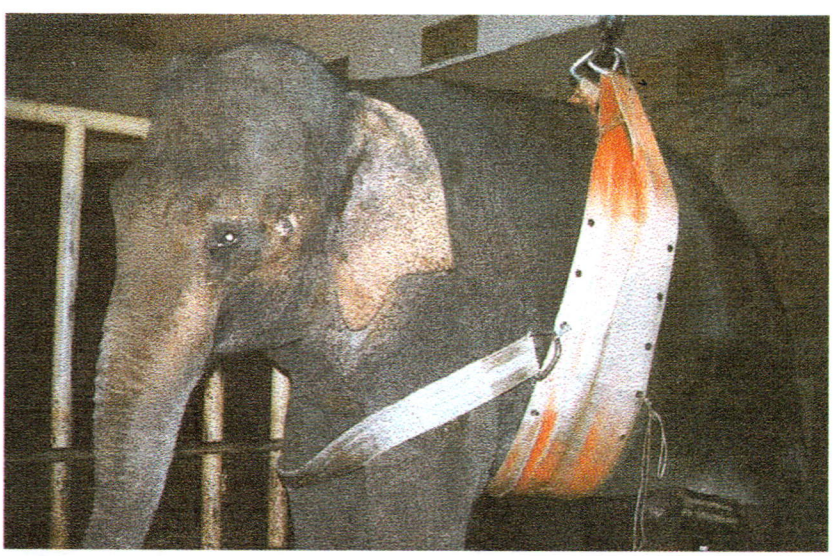

Fig. 2 *Preparation of a 25 years old female Asian elephant with dystocia for birth assistance. For this purpose the cow was separated from the herd and housed in a prepared room. The legs were chained. A belt system prevent laying down of the cow under sedation.*

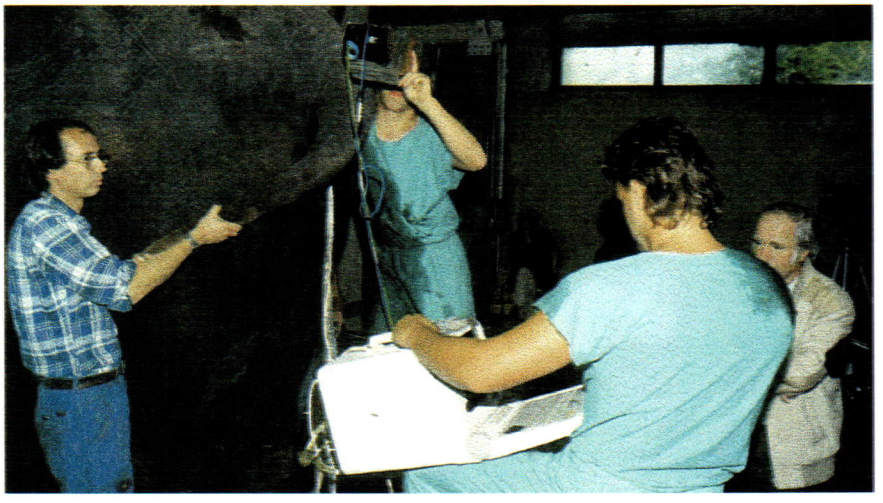

Fig. 3 *Transrectal ultrasound examination of the caudal part of genital tract during late pregnancy stage allows the determination of the position and vitality of the fetus.*

sedation. You should not wait too long to start surgery as the chance to deliver a vital calf as well as the general condition of the mother decrease. At first, a 10 cm vertical incision should be performed 2 handbreadths ventral of the M. sphincter ani externum to evaluate the position and condition of the calf. When incising the vestibulum, a fenestrated PVC pipe should be used to avoid a penetration of both sides of the vestibular wall (Fig. 5). Eventually, the calf can be directed into the vestibulum and extracted vias naturalis. If this is impossible, the incision has to be extended to 30-40 cm. Strong ropes are placed on both limbs, the birth canal is lubricated, and under protection of the perineum the calf is extracted (Fig. 6). Traction has to be directed in a 45° to the mother. As in all successful episiotomies the wound healing was disturbed with the result of a permanently fistula (Fig. 7 a-d), it might be better to suture only the vestibular wall and to leave subcutis and cutis unclosed for healing by second intention.

Fig. 4 *Endoscopical examination of the birth canal using a flexible videochip-colonoscope inserted into the vestibulum. The endoscopical image (insert) shows the Os vaginale with intact fetal membrane in the center.*

References

FLÜGGER, M., GÖRITZ, F., HILDEBRANDT, T.B., HERMES, R., E. ISENBÜGEL, E., KLARENBEEK, A. SCHAFTENAAR, W., SCHALLER,K. and STRAUSS, G. (2001):

Evaluation of physiological data and veterinary experiences in 31 Asian elephant birth in six European zoos. Verh.ber.Erkrg. Zootiere, in press.

FOERNER, J.J.(1999): Dystocia in the Elephant. In: M.E. Fowler and R.E.Miller (Eds.): Zoo and Wild Animal Medicine, Saunders, Philadelphia 522-525.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT, N.C., BROWN, J.L., MONTALI, R.J., SCHMITT, D.L., FRITSCH, G., and R. HERMES (2000): Ultrasonography of the Urogenital Tract in Elephants *(Loxodonta africana* and *Elephas maximus)*: An Important Tool for Assessing Female Reproductive Function. Zoo Biology 19, 321-332.

RÜEDI, D. (1995): Elefanten, in: GÖLTHENBOTH, R., and H. G. KLÖS (Hrsg.): Krankheiten der Zoo und Wildtiere, Blackwell, Berlin 1995, 156-189.

SCHMIDT, M.J.(1999): Calving Elephants (normal). In: FOWLER, M.E. and R.E. MILLER (Eds.): Zoo and Wild Animal Medicine, Saunders, Philadelphia 1998, 521-522.

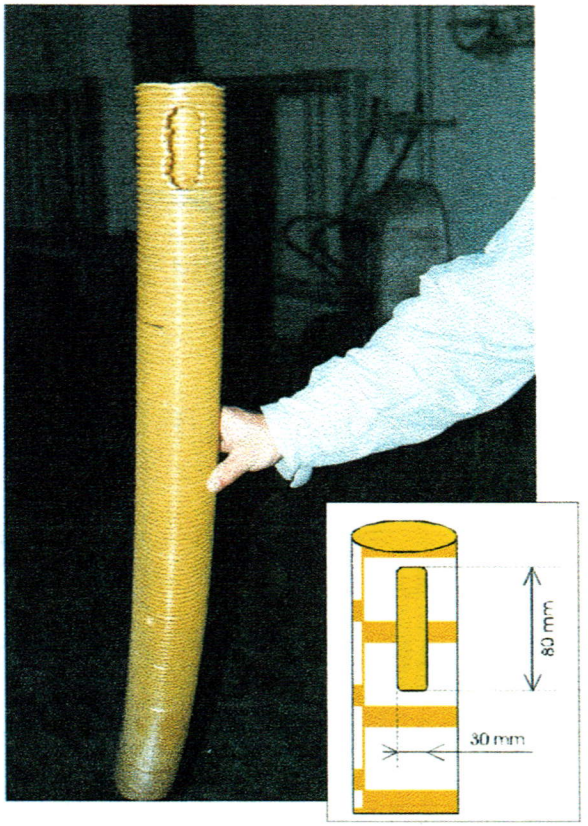

Fig. 5 *Plastic pipe with a fenestration on the tip (30 x 80 mm, insert) for insertion into the vestibulum prior episiotomy. Fenestration (could be palpate from outside!) indicate the location of incision and prevent penetration of the both sides of the vestibular wall.*

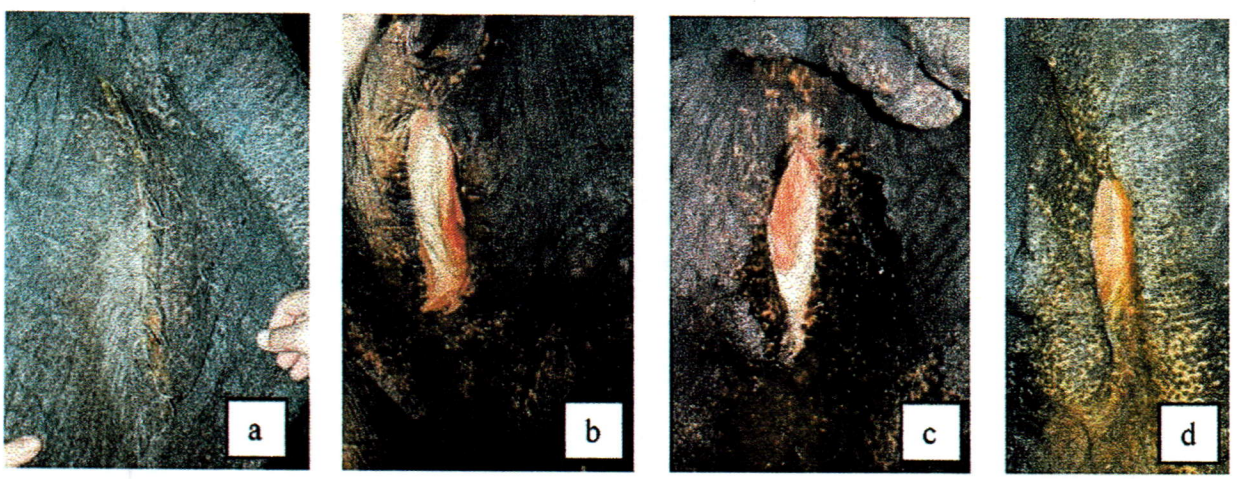

Fig. 7 *Wound healing after successful episiotomy and suturing of all layers of the vestibulum (a, 8 days; b, 15 day; c, 30 days and d, 2 month after surgery) with the result of a permanent fistula due to disturbed blood circulation. It might be better to suture the mucosa only and to leave subcutis and cutis unclosed.*

ARTIFICIAL INSEMINATION (AI) OF AN ASIAN ELEPHANT *(Elephas maximus)*

ARTIFICIAL INSEMINATION IN THE AFRICAN ELEPHANT "SABI" AT THE VIENNA ZOO - A TOUR BEHIND THE SCENES

SUCCESSFUL ARTIFICIAL INSEMINATION OF AFRICAN NULLIPAROUS ELEPHANTS AT THE INDIANAPOLIS ZOO*

Thomas Hildebrandt [1] et.al

*Verh.ber.Erkrg. Zootiere (1999), 39, 41-45.

[1]IZW, Berlin

Artificial Insemination (AI) of an Asian Elephant *(Elephas maximus)*

T. Hildebrandt

The development of assisted reproduction programmes in elephants will greatly enhance the potential for creating self sustaining populations in captivity. For elephants, it is critical that methods for evaluation of reproductive capacity be developed, including development and status of genital tract and integrity of the gonads. AI is one of the most effective methods for improving the breeding success of domestic species. But in over 25 years, different AI methods have never produced a confirmed elephant pregnancy. A new AI technology was developed at IZW in collaboration with the NZP, which incorporates ultrasonographical and endoscopical imaging techniques combined with a patented catheter system.

Potential AI candidates and semen donors were examined for a pre-selection by transrectal ultrasound. A B-mode scanning system first with a 3.5 MHz transducer (to visualize in female: vestibulum vaginae, urethra, urinary bladder, vagina, cervix, corpus uteri; in male: urethra, urinary bladder, prostate, seminal vesicles, ampullae, ductus deferentes) followed by a 7.5 MHz transducer (to visualize in female: uterine horns, ovaries; in male: epididymides, testes) was used. A 20-year-old, healthy tractable female Asian elephant with a history of successful natural breeding, pregnancy and parturition was chosen as the first candidate for the programme. Ear vein blood was sampled weekly, biweekly and finally daily as ovulation approached. Samples were centrifuged and stored frozen for analysis of P4 and LH. The AI was scheduled as soon as a 20.0 mm Graafian follicle was identified sonographically. Semen was collected from a 35-year-old bull at the Dickerson Park Zoo by rectal palpation of the accessory glands with manual penile stimulation. Ejaculate parameters were assessed at collection, the sample was extended in Test-Y, cooled to 4°c and flown to NZP. Thirty minutes prior to insemination, the semen was warmed to 36°c and parameters were reassessed. The AI procedure consisted of: catheterization of the vestibulum vaginae (1.3 m); endoscopic visualization of the vaginal and urethral openings (7.0 / 20.0 mm in diameter); catheterization of the vagina(1.5 m) and ultrasonographic verification; endoscopic visualization of the os cervix (20.0 mm in diameter); placement of the insemination catheter in the uterus (2.0 m) and ultrasonographic verification; ultrasonographically guided insemination. Ultrasonography was then performed weekly to look for evidence of embryogenesis and endometrial changes and to examine the health of the reproductive tract. Blood was sampled weekly to assess serum P4. This female showed no adverse behavioural, physical or physiological effects of this AI programme, and it was the first time uterine semen placement has been verified in an elephant AI.

Successful Artificial Insemination of African Nulliparous Elephants at the Indianapolis Zoo

T. B. Hildebrandt, F. Göritz, A. Schnorenberg, R. Hermes, D. Schmitt, D. Hagan, J. Peterson, Janine L. Brown, Naida Loskutoff, Nancy C. Pratt, J. Lehnhardt, G. Miller, R. J. Montali, Debbie Olson

Introduction

Successful captive elephant management is a priority among zoo and wildlife organisations worldwide. Captive populations have been maintained by collecting from the wild, transport of captive females on longterm breeding loans, and to a lesser extent, by management of males on-site, which can be unrealistic for many zoos. Conservation and safety concerns, along with the growing acknowledgement among elephant caretakers that removing females from their familiar social groupings for breeding loans can cause distress , have all contributed to the need for development of assisted reproductive tech-niques. Demonstration of a successful artificial insemination (AI) would open up many possibili-ties for captive elephant management, including the collection of genetic material from the wild for integration into captive populations once semen cryopreservation techniques have been perfected. A new technique involving the application of ultrasonography for reproductive assessment and AI has been implemented in the elephant management programme at the Indianapolis Zoo. The technology for this project was developed at the Institute for Zoo Biology and Wildlife Research in Berlin in co-operation with the company A. Schnorrenberg (HILDEBRANDT and SCHNORRENBERG, 1996). The AI component of this project involved simultaneous imaging by ultrasonography and endoscopy for verifiable semen placement. Both these compo-nents have never been accomplished together in an elephant AI. The insemination technique is noninvasive and has resulted in verifiable sperm deposition directly into the cervix.

Elephants and training

Ultrasoundguided AI has been attempted, in three nulliparous African cows at the Indianapolis Zoo, "Ivory" (16-year-old), "Tombi", (22-year-old), and "Kubwa" (22-year-old). All three females are wildborn.

The reproductive hormone levels (progesterone [P_4]; luteinizing hormone [LH]) have been monitored from blood samples taken from their ear veins regularly since 1996. The elephant management team at Indianapolis determined that all three were excellent candidates for this project for several reasons:

1. they are of prime breeding age,
2. they are extremely calm mannered, tractable and well trained,
3. they are in very good general and

Acknowledgements

The authors are grateful for the assistance from the elephant staff of the Indianapolis Zoo for training the female elephants to stand unrestrained for the AI procedure, the staff of the Kansas City Zoo collected semen from the male elephant Dale, and the help with the primary semen assessment protocol performed by Erin O'Brian from the Omaha Henry Doorly Zoo.

reproductive health,

4 they have been palpated both vestibulary and rectally on a routine basis, and have shown no signs of distress or injury during these procedures.

Ultrasonographic monitoring and timing of the AI

The technique for transrectal ultrasonography in elephants is performed in the standing or laying position without the use of tranquillizers, anaesthetics or restrictive devices. Faeces are removed manually with the use of ultrasound gel for lubrication. The rectum is then irrigated with lukewarm water. A real-time B-mode ultrasound scanning system is used. For visualizing the caudal component of the urogenital tract (vestibule, urethra, vagina, urinary bladder, cervix, caudal corpus uteri) a 3.5 MHz transducer is manually introduced into the rectum with ultrasound gel for coupling. To visualize the cranial component of the genital tract (cranial corpus uteri, uterine horns, ovaries, surrounding tissues) a 5.0 - 7.5 MHz transducer is attached to a specially adapted extension and guided manually into the rectum. Ultrasonography can provide valuable information on ovarian activity, uterine integrity and reproductive disease or dysfunction (HILDEBRANDT et al., 1997). It can be used to visualize structures of the entire reproductive tract. Specifically, it can detect evidence of endometrial cystic degeneration, and indicate the gestational capacity of the uterus. Ultrasound may also detect pathological changes in the oviduct. The ovaries can also be screened for pathological structures such as cysts and atrophic processes of the parenchyma.

Ultrasonographic examinations of the three AI candidates revealed that there were no indications of reproductive pathology in the urogenital tract or in the ovaries. Several small cysts were visualized, one in particular served as a landmark near the opening of the cervix. There was no evidence of injury or inflammation due to these ultrasound procedures. Both endocrine data and ultrasonographic images were used to determine the timing of AI trials. Each time, we attempted to perform the AI series as close to the predicted time of ovulation as possible, ideally inseminating at least one before and once after the actual day of ovulation. In preparation for the AI attempts, the females were monitored daily for circulating levels of P_4 and LH. Two LH peaks (KAPUSTIN et al., 1997), separated by 20 days, were detectable in their oestrus cycle, with the second peak being the ovulatory LH surge. Detection of the first peak provided us with a three week window to prepare for the inseminations. Transrectal ultrasonography was employed daily in this time period to identify morphological changes in the vagina and endometrium and to characterize developing ovarian structures during the follicular phase. Ultrasonography allowed the visualization of follicle growth and maturation and the development of Graafian follicles by the detection of cumulus oophorus. The ruptured ovulatory follicle and corpus hemorrhagicum could also be visualized. The visualization of these ovulatory events have never been possible before.

Semen collection and processing

The semen donor for the AIs in "Tombi" and "Kubwa" was a 20-year-old African bull named "Dale". He is owned by Jo-Don farms currently being housed at the Kansas City Zoo. The ejaculate for the insemination in "Ivory" was collected from a 16-year-old African bull with the name "McLean" at Disney's Animal Kingdom. The semen was collected by rectal palpation

(HILDEBRANDT et al., 1998; SCHMITT and HILDEBRANDT, 1998) of the accessory glands with manual penile stimulation. Ejaculate volume, concentration and pH, and sperm motility and viability were assessed at collection (LOSKUTOFF, 1997). The samples were fractionated, and each fraction was assessed separately before combining. The semen was diluted 1x1 with TL Hepes solution. A microbiological evaluation of the semen was performed to monitor the potential risk of pathogenicity caused by the procedure. Extended semen was transported by air at 4 °c in a refrigerated vessel. Preliminary trials had revealed that the semen maintained good motility for five or six days after collection. The sample was warmed to 37 °c prior to insemination. Samples used for AI trials ranged in motility status from 5-10% in Tombi's trial, 80-85% in Kubwa's trial and 65-70% in Ivory's trial.

AI procedure

All ultrasonographic evaluations and the AI were conducted in the Elephant House at Indianapolis Zoo. No sedation or physical restraint was used for the insemination protocol. Sterile technique was employed throughout the procedures. A balloon catheter was inserted in the vestibule (*Canalis urogenitalis*) to slightly distend the reproductive tract for optimal visualization and placement of endoscopes, and insemination catheter. Both endoscopic and ultrasonographic visualization were used to guide the placement of semen deep into the cervix if possible. The actual introduction of semen was monitored ultrasonographically to verify its placement. Insemination was performed in "Tombi" 3 times (16.-18.04.98), in "Kubwa" 2 times (24.-25.05.98) and "Ivory" 2 times (30.10., 01.11.98) once a day, depending on the availability of semen from the bulls. Additionally, "Ivory" was inseminated unsuccessfully in an initial trial ten days prior her ovulation time point (03.-06.07.98). The time needed for an AI procedure varied from 10 minutes to 2.0 hours at maximum. Olfactory cues, such as semen, urine, faeces and temporal gland secretions, were presented to the AI candidates before and after the AI series. They showed strong reactions to these stimuli, often rumbling and pelvic thrusting. They have shown no adverse behavioural, physical or physiological effects of this AI programme.

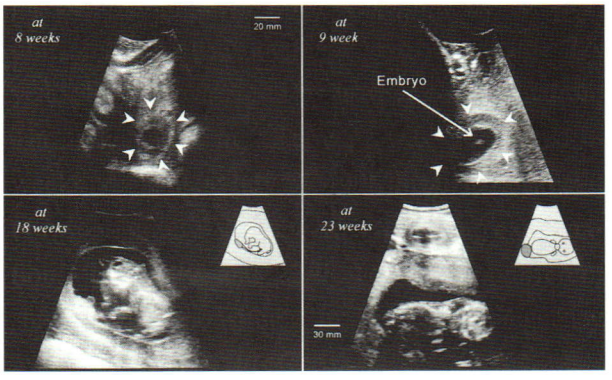

Fig. 1 *Ultrasonographic pregnancy diagnosis in the 22-year-old female African elephant Kubwa 8, 9, 18 and 23 weeks after the artificial insemination.*

Pregnancy detection

Following the AI procedures the females were monitored endocrinologically for P_4 on weekly basis. The first ultrasonographic examinations were performed in "Tombi" and "Kubwa" 8 weeks after the insemination. The 22-year-old female African elephant "Tombi" did not get pregnant. The explanation for this failure is clear the poor semen quality (motility 5-10%) used for the AI attempt. In contrast to Tombi, the 16-year-old female African elephant "Ivory" and the 22-year-old "Kubwa" bred by artificial insemination. The pregnancy in "Kubwa" was confirmed 8 weeks pregnant on July 24, 1998 through transrectal ultrasonography. There were 3 follow up examination (9^{th}, 18^{th} and 23^{th} week). The different stages are demonstrated in Fig1. The pregnancy

in "Ivory" is confirmed yet by the characteristic hormone levels 14 weeks after the AI.

Conclusion

Artificial insemination (AI) is one of the most effective methods for improving the breeding success of domestic species. But 35 years, different AI methods have never produced a confirmed elephant pregnancy until the year 1998. The introduction of AI in captive elephant breeding programmes is enormously important in terms of the ultimate viability of assisted reproduction for use in the entire captive population of elephants. Most of which may never have the opportunity for natural conception. The Dickerson Park Zoo, one of the collaborator in this project, announced the first successful insemination of a primiparous female Asian elephant in June of 1998 (SCHMITT, 1998). Last year a total of three elephants got pregnant by AI in North America. "Kubwa" and "Ivory" are the first virgin elephant cows to be successfully impregnated by artificial insemination. The combination of a reproductive assessment programme with a newly developed insemination technology resulted in this groundbreaking success. This project has been a team effort, requiring the co-operation and expertise of many individuals: curators, keepers, researchers, pathologists, volunteers, educators and public relations specialists.

Summary

Successful artificial insemination of African nulliparous elephants at the Indianapolis

Two virgin African elephants cows were successfully impregnated by artificial insemination at the Indianapolis Zoo. A total of two inseminations (two days) around the ovulation time point was efficient for both elephants to get pregnant. Each pregnancies is from a different semen donor. Both bulls didn't sire any pregnancy by natural breeding until now.

References

HILDEBRANDT, T.B. und A.SCHNORRENBERG (1996): Besteck zur künstlichen Besamung von Elefanten. Deutsches Patentamt, Offenlegungsschrift, DE 196 06 925 A1.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT N. C., SCHMITT, D.L., LEHNHARDT, J., HERMES R., QUANDT, S., RAATH, J., WEST, G. and R.J. MONTALI (1997): Assessment of health and reproductive status in African and Asian elephants by transrectal ultrasonography. Proc. Annu. Meet. Am. Assoc. Zoo. Vet. 207-213.

HILDEBRANDT, T.B. and F. GÖRITZ (1998): Use of ultrasonography in zoo animals. In: M. E. Fowler and R. E. Miller (eds.): Zoo and wild animal medicine. Current Therapy 4. W.B. Saunders Company, Philadelphia, 41-54.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT, N.C., SCHMITT, D.L., QUANDT, S., RAATH, J. and R.R. HOFMANN. (1998): Reproductive assessment of male elephants (*Loxodonta africana* and *Elephas maximus*) by ultrasonography. J. Zoo Wildl. Med. 29(2), 114-128.

KAPUSTIN, N., CRITSER, J.K., OLSON, D. and P.V. MALVEN (1996): Nonluteal estrous cycle of 3 week duration are initiated by anovulatory luteinizing hormone peaks in African elephants. Biol. Reprod., 55, 1147 - 1154.

LOSKUTOFF, N. (1997): Standardization of protocols for evaluating and processing African elephant semen. Proc. Eleph. Res. Symp., 1-5.

SCHMITT, D.L. and T.B. HILDEBRANDT (1998): Manual collection and characterisation of semen from the Asian elephant (*Elephas maximus*). Anim. Reprod. Sci., 53, 309 - 314.

Artificial insemination in the African elephant "Sabi" at the Vienna Zoo - a tour behind the scenes

Hildebrandt, T. B., Göritz, F., Fritsch, G., Rohleder, M., Schwarzenberger, F., Schwammer, H., Mohlin, F., Tropeano, A., Mitchell, T. and R. Hermes

The first successful artificial insemination (AI) in an African elephant in Europe has been accomplished at the Vienna Zoo in 1999. While scientists, the management of the Vienna Zoo and the Zoos' visitors start to focus on the elephant babys' birth in 2001, international interest increases in this new technique of modern reproduction management of elephants in captivity. To meet the growing demand for background information, different stages in the planning, the organization and the safe and successful artificial insemination of "Sabi" will be shortly summarized here.

The most important elements of an AI program were **1.** The ultrasound examination and identification of suitable artificial breeding candidates, **2.** The prediction of the ovulation by ultrasonographic and endocrine oestrous monitoring for the accurate timing of inseminations, **3.** The collection, assessment, processing and transportation of semen from a previously evaluated potential breeder from the Colchester Zoo, UK and **4.** The nonsurgical deposition of semen deep into the female genital tract.

1. Selection of "Sabi" as candidate for AI

Fundamental to the planning of an AI was the identification of "Sabi" as a suitable candidate for an artificial breeding attempt. The initial transrectal ultrasound examination (HILDEBRANDT, T.B. and F. GÖRITZ, 1998) of "Sabi" by Drs. Hildebrandt and Göritz from the Institute for Zoo Biology and Wildlife Research (IZW) in December 1998 evaluated "Sabi'" reproductive health status and put her individual characteristics on test. The state of development and activity of the internal genital organs vestibule, vagina, cervix, uterus and ovaries was first assessed by this ultrasound examination. The evaluation of "Sabi'" reproductive soundness and an accidentally documented imminent ovulation by ultrasound, retrospectively confirmed by hormone analysis, made her an ideal candidate for an AI program which was set up on March 1999.

2. Prediction of Ovulation

Monitoring the oestrous cycle required weekly blood and faecal samples which were analysed at the veterinary faculty of the Vienna university and at the IZW in Berlin. Especially the blood sampling from the ear or hind leg was part of the weekly routine and was taken on a fixed day at a certain time. The hormone analysis of progesterone metabolite concentrations in the faeces and blood gave basic information on "Sabi" oestrous cycle length. During the follicular phase of the oestrous cycle progesterone concentration is at baseline, indicating the development of follicles on the ovaries at this stage of the cycle. The development, maturation and ovulation of a Graafian follicle and subsequent development of a corpus luteum marks the end of the 5 - 7 week follicular phase. The corpus luteum which derives from ovulation in association with accessory corpora lutea is responsible for the synthesis of

progesterone and the steep increase of progesterone concentration in the serum or faeces after ovulation. However, the determination of ovulation by faecal or serum progesterone analysis is retrospective and therefore not appropriate for an ovulation prediction and precise insemination timing.

The only method to predict the ovulation in elephants precisely, besides a serum hormone analysis of the luteinising hormone (LH) (KAPUSTIN et al., 1996), not yet available in Europe, was a daily ultrasound examination and evaluation of the ovaries. Therefore, Dr. Hermes and Fritsch from the IZW performed serial ultrasound examinations since mid February 1999 to precondition the AI candidate and more importantly to monitor the follicular development on the ovary for accurate prediction of ovulation (HERMES et al., im Druck). Misinterpretation of a cystic structure near "Sabi" right ovary during the time of suspected ovulation led to an incorrect reading of the ovarian dynamic during the first AI attempt in March 1999. Taking the identified cystic structure under close consideration, the second monitoring of a follicular phase in July 1999 resulted in an exact prediction of ovulation and subsequently to a successful insemination.

3. Semen collection, assessment, processing and transport

A potential male breeding candidate suitable for an AI program was identified in the Colchester Zoo, UK. Similar to "Sabi", the reproductive health status of the 18 year old male "Tembo" had to be evaluated prior to the start of the project. During two visits to the Colchester Zoo in January and February 1999 multiple ultrasound examinations and semen collections by Drs. Hildebrandt and Hermes (HILDEBRANDT et al.,1998, SCHMITT, and HILDEBRANDT, 1998) assessed "Tembo" breeding potentials. The ejaculation in elephants is triggered by a manual rectal massage of the pelvic part of the urethra and the accessory sex glands located in that region. The collected ejaculatory fractions were immediately examined for motility, progressive motility, concentration, acrosome integrity and morphology of the spermatozoa. Excellent spermatological results from these preliminary semen collections (e.g. 85-90% semen motility) recommended "Tembo" as a reliable semen donor and potential yet not proven breeder.

Main organizing obstacle during the AI, apart from the prediction of ovulation, was the still limited ability to preserve elephant semen over a longer period of time. Different from domestic species such as cattle, where semen can be collected, cryopreserved, transported and thawed at any given day, elephant semen has to be collected, cooled, transported and inseminated at the same day (LOSKUTOFF, 1997). During both AI trials in "Sabi" three inseminations were scheduled on three successive days. Therefore, semen needed to be collected from "Tembo" also on three successive days. "Tembo" collected semen sample was assessed, extended with a specific nutritional solution and transported from Colchester, UK to Vienna, Austria at 4°c in a special thermo-regulated cooling container daily by the IZW team. To underline the aspect of the insemination of a female never mated before, Virgin Airlines was in part carrier of the precious shipment. Herr Lienhart form the Vienna Zoo was responsible for the outstanding co-ordination of flights during both AI trials.

4. Artificial insemination

The most important factor to the successful insemination was the tolerance of the AI candidate herself, "Sabi". The non surgical

insemination technique, which had been developed and patented at the IZW, required 1/2 - 2 hours steady standing on four specially made AI stands. The fact that the insemination procedure was tolerated by "Sabi" unchained and non sedated, rewarded long term training efforts of both the animal and the elephant handlers. This teamwork enabled "Sabi" to support the insemination process by reflectory contractions of the uterus at the moment of semen deposition, transporting the semen actively deep into her genital tract. For the semen deposition in nulliparous elephants close to the cervix a distance of 1.5-1.8 m from the genital tract opening had to be covered. A specially designed balloon catheter was first inserted into the vestibule and guided over the pelvic rim. A video-chip-endoscope, placed in the lumen of the balloon catheter by Dr. Göritz, visualised the hymenal structure and vaginal os. This tiny vaginal os, which is exclusively observed in nulliparous females, represented the greatest anatomical obstacle to overcome during the AI in the nulliparous female. Through this 0.5 cm wide vaginal os, 1.3 m from the genital opening an insemination catheter 0.3 cm in diameter was inserted into the vagina and placed close the cervical opening. Simultaneous transrectal ultrasound by Dr. Hildebrandt verified the exact positioning of the insemination catheter, before the warmed semen was injected slowly (HILDEBRANDT et al., 1999). Olfactory stimulation of "Sabi" following the insemination was achieved by urine, faeces and non-usable semen fractions from "Tembo". So far, four ultrasonographic pregnancy examinations were performed in "Sabi" to determine the embryonic and foetal development in vivo. Foetal heart activity as well as characteristic features such as the trunk were already distinguished in the 3-D-ultrasound.

Literature

HERMES, R., OLSON, D., GÖRITZ, F., BROWN, J. L., SCHMITT, D. L., HAGAN, D., PETERSON, J. S., FRITSCH, G., HILDEBRANDT T. B. (1999): Ultrasonography of the sexual cycle in female African elephants (*Loxodonta africana*). Zoo Biology (angenommen).

HILDEBRANDT, T. B., GÖRITZ, F., HERMES, R., SCHMITT, D. L., BROWN, J. L. SCHWAMMER, H., LOSKUTOFF, N., PRATT, N. C., LEHNHARDT, J. L., MONTALI, R. J., OLSON, D. (1999): Artifical insemination in the African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. Proc. Am. Assoc. Zoo. Vet., 83-86.

HILDEBRANDT, T.B. and F. GÖRITZ (1998): Use of ultrasonography in zoo animals. In: M. E. Fowler and R. E. Miller (eds.):Zoo and Wild Animal Medicine. Current Therapy 4. W.B. Saunders Company, Philadelphia, 41-54.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT N. C., SCHMITT, D. L., QUANDT, S., RAATH, J. AND R.R. HOFMANN. (1998): Reproductive assessment of male elephants (*Loxodonta africana* and *Elephas maximus*) by ultrasonography. J. Zoo Wildl. Med. 29(2), 114-128.

KAPUSTIN, N., CRITSER, J.K., OLSON, D. and P.V. MALVEN (1996): Nonluteal estrous cycle of 3 week duration are initiated by anovulatory luteinizing hormone peaks in African elephants. Biol. Reprod., 55, 1147 - 1154.

LOSKUTOFF, N. (1997): Standardization of protocols for evaluating and processing African elephant semen. Proc. Eleph. Res. Symp., 1-5.

SCHMITT, D.L. and T.B. HILDEBRANDT (1998): Manual collection and characterization of semen from the Asian elephant (*Elephas maximus*). Anim. Reprod. Sci., 53, 309 - 314.

ANNEX

THE TROUBLE WITH ENDOTHELIOTROPIC ELEPHANT HERPES VIRUS (EEHV)

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The Trouble with the Endotheliotropic Elephant Herpes Virus (EEHV)

Joerns Fickel

A. Introduction

Both species of elephants, the Asian elephant (*Elephas maximus*) and its relative, the African elephant (*Loxodonta africana*) are both listed in Appendix I of CITES as endangered species (African elephants living in Zimbabwe, Botswana and Namibia are listed in Appendix II) and great conservational efforts are required to prevent reduction of genetic diversity or even extinction for both species. That by itself means that free living populations are no longer accessible to maintain zoo and other captive populations. This fact is even more aggravated by the recent reports on the phylogenetic structures within the Asian and African elephant populations. But not only are the free living populations at risk, the captive populations too share the same fate since they will become reproductively unfit within the next three decades. This is mainly due to their very low reproductive success, combined with an offspring survival rate lower than in free living populations. Management and welfare issues, particularily in terms of health care have therefore gained additional importance for elephant populations both in captivity and in the wild, since diseases counteract all efforts to increase the reproductive success of elephant populations. The recently discovered and described new endotheliotropic elephant herpes virus (EEHV) falls into that scope of diseases. Carried by otherwise healthy African elephants it can be lethal in Asian elephants if left untreated. Since awareness for modes of EEHV transmission had not been established until recently and with the stability of the virus outside its host yet unknown, viral transmission could unwaringly have taken place. Since some of the outbreaks were linked to stressfull events, stress might play a role in causing incompetence of the immune system thereby triggering the onset of viral replication. As of now the only remedy for an animal with an (potentially lethal) EEHV episode is to treat it with Famciclovir®. We therefore urge all elephant keepers and caretakers to improve elephant management particularily in terms of the neccessity of stress avoidance such as translocation, integration of new group members, changes in management regimens etc.

A very particular situation has emerged in Thailand. Besides the question whether or not the native Asian elephant population carries an "Asian version" of the EEHV (that could become a threat to African elephants in Zoos), the African elephants recently introduced into Thailand might also pose a threat to the native Asian elephant populations since they might be carrier of the EEHV.

Further reading:

1. Barriel V, Thuet E, Passy P, 1999. Molecular phylogeny of Elephantidae. Extreme divergence of the extant forest African elephant. C.R.Acad. Sci.Paris/ Life Sciences, 322: 447-454.
2. Daszak P, Cunningham AA, Hyatt AD, 2000. Emerging indectious diseases of wildlife - threats to biodiversity and human health. Science 287: 443-449.
3. Fernando P, Pfrender ME, Encalada SE, Lande R, 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. Heredity 84:362-372.
4. McCully RM, Basson PA, Pienaar JG, Erasmus BJ, Young E, 1971. Herpes nodules in the lung of the African elephant (Loxodonta africana [Blumenbach, 1797]). Onderstepoort J.Vet.Res. 38, 225-236.
5. Ossent P, Guscetti F, Metzler AE, Lang EM, Rübel A, Hauser B, 1990. Acute and fatal herpesvirus infection in a young Asian elephant (*Elephas maximus*). Vet. Pathol. 27, 131-133.
6. Pilaski J, Rosenbruch M, Gelderblom H, Olberding P, Hagenbeck C, 1987. Herpesvirus infection in an Asian elephant (*Elephas maximus*). Verh.ber.Erkrg. Zootiere 29, 179-184.
7. Richman LK, Montali RJ, Garber RL, Kennedy MA, Lehnhardt J, Hildebrandt T, Schmitt D, Hardy D, Alcendor DJ, Hayward GJ, 1999. Novel endotheliotropic herpesviruses fatal for Asian and African elephants. Science 283, 1171-1176.
8. Richman L, Montali RJ, Hildebrandt TB, Fickel J, Schmitt DL, Hayward GS, 1999. Status of a new, fatal herpesvirus disease of elephants in North America and Europe. Verh.ber.Erkrg. Zootiere 39, 17-21.
9. Richman LK, Montali RJ, Cambre RC, Schmitt D, Hardy D, Garber RL, Hildebrandt T, Fickel J, Schaftenaar W, Hayward GS, 1999. Clinical and pathological aspects of a fatal herpesvirus disease in Asian (*Elephas maximus*) and African Elephants (*Loxodonta africana*). Proc.Amer. Assoc.ZooVet. pp.

263-265.
10. Rietschel, W, Heckel, J.O, and C.P, Cerny 1997. Symptomatik, Diagnostik und Therapie einer Herpes- Infektion bei einer Asiatischen Elefantenkuh (Symptoms, diagnostic and therapy of a herpes-infection in a female Asian elephant). Proc. 17th Meeting germanspeak. Zoo.Vet., pp. 89-91.
11. Schmitt DL, Hardy DG, 1998. Use of famciclovir for the treatment of herpesvirus in an Asian elephant. J. Elephant Manager Assoc. JEMA 9, 2, 103-104.
12. VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM, 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. J.Clin. Microbiol. 34, 1666-1671.

B. Sampling of material (Collecting and storage)

There are two basic forms of samples that are suitable for the investigation: fresh samples and preserved samples.

fresh samples

- blood: drawn either into a heparin- or an EDTA-tube (store in a refrigerator if processing will happen within the next two days, otherwise freeze - the colder the better).
- biopsies: from suspicious regions (warts, skin lesions, petechia, ...), transfer material into 1.5ml reaction tube, add 50-100µl 1x TE (see buffer-section below), storage similar to blood samples.
- fresh tissue from anesthetized animals: store as cold as possible (-20°C, -80°C, liquid N_2).
- fresh tissue from deceased animals: if possible, try to get blood vessel tissue from heart, liver, spleen, lymphnodes, bone marrow, brain.

preserved samples

- formalin fixed (and paraffin embedded) samples (tissues similar to fresh samples-section).
- samples in alcohol: use clean ethanol only (no additives), sample has to be small (<0.5g), use 10-fold volume ethanol, sample can be kept at room temperature.
- dried samples: works good for body fluids like blood, tears, exudates,...: there are special filter papers out there, but normal filter paper (new) will do just fine, let a drop of fluid air-dry on the paper and keep it cool and **dry!** (cool is not as important as dry! If using a refrigerator, seal the sample first, fridges have high humidity!).

C. DNA extraction using organic solvents

(DNA can also be yielded by use of commercially available DNA extraction kits, which will lead to quicker results but will consequently also lead to increased costs, another option are methods described in numerous manuals such as **Maniatis et al.** We here describe the method that we use.).

Basics:

Take very good care of your lab environment. PCR-fragment carry-over is a serious problem as well as an otherwise contaminated lab! Wipe your bench with 10% bleach or disinfectant and put a fresh clean sheet of paper on your bench to work on. Always work with gloves. Use only sterilized tubes, tips, water. Make sure you use filter-containing pipette-tips. Forceps and other tools should always be cleaned thouroughly after use.

1. Lysis

- transfer 100-200µl whole blood (Heparin or EDTA) or leucocytes pellet or 0.2g tissue into 1.5 ml Eppendorf tube
- add 300µl TNE-buffer with DTT
- add 15µl 20 mg/ml proteinase K
- short vortex (slow)
- incubate at least for 2 hrs (better overnight) at 40°C (tissue at 50°C)

2. DNA extraction

- shortly spin the Eppendorf tube
- add 300µl Phenol (equilibrated in 1x TE, pH 7.5-8.0)
- slowly shake 5-10 min on a rotary (solution should look opaque)
- spin at 13,000x g for 5 min
- yield upper phase and transfer it into new tube (avoid touching the interphase)
- add equal volume of Phenol/Chloroform/Isoamylalcohol (25:24:1)
- slowly shake for 10 min on a rotary
- spin at 13,000x g for 5 min
- again yield upper phase and transfer it into new tube (avoid touching the interphase)
- add equal volume of Chloroform/Isoamylalcohol (24:1)
- slowly shake for 10 min on a rotary
- spin at 13,000x g for 5 min
- again yield upper phase and transfer it into new tube (avoid touching the interphase)

3. DNA-precipitation

- add 30ml 4M LiCl
- add 1µl Glycogen-solution
- add 600µl icecold ethanol
- mix quickly
- DNA should become visible as white thread (if not, put tube at -20°C for 2 hrs)

- spin for 20 min at 8 °C and 13,000x g
- carefully remove liquid.
- wash DNA-pellet with 70% icecold ethanol.
- spin for 20 min at 8 °C and 13,000x g.
- carefully remove liquid.
- dry pellet on air.
- redissolve pellet in either water or 1x TE (approx. 20µl).
- aliquote sample (2x 10µl).
- sample in use should be stored in refrigerator, store other aliquote at -20 °C or -80 °C if available.

Buffers (store all at room temperature)

- TE buffer : 10mM Tris, 1mM EDTA, pH 7.5-8.0
- TNE-buffer : 10mM Tris, 100mM NaCl, 1mM EDTA, 2% SDS (Sodium dodecyl sulfate = sodium lauryl sulfate).
- TNE-buffer with DTT (Dithiothreitol) : TNE with 39mM DTT (add just prior use), buffer should be discarded after two weeks.

Solutions

- Phenol/ Chloroform/ Isoamylalcohol (25:24:1) stored at 8 °C: Mix 25 parts Phenol (see above) with 24 parts Chloroform (Trichloromethane) and 1 part Isoamylalcohol (3-methyl-1-butanol).
- Chloroform/ Isoamylalcohol (24:1) stored at 8 °C: Mix 24 parts Chloroform (Trichloromethane) and 1 part Isoamylalcohol (3-methyl-1-butanol).
- 4M LiCl stored at 8 °C.
- Glycogen-Solution stored at -20 °C: 20mg/ml Glycogen.
- Ethanol stored at -20 °C: Use pure ethanol (at least 96%, with no additives!).
- 70% ethanol stored at -20 °C.

Equipments

- 1.5ml or 2ml reaction tubes (all autoclaved!) and fitting racks.
- centrifuge with integrated cooling system.
- pipettes and autoclaved tips.
- sterile water (Milli Q or equivalent).
- autoclave (also for disposals).
- vortex mixer.
- thermomixer or incubator or waterbath.
- sheets of filter paper.
- gloves, labcoat, timer.
- laminar flow cabinet with UV.
- bleach, disinfectant.

D. PCR

IMPORTANT NOTICE!

ALL PCRS SHOULD BE CARRIED OUT IN A ROOM PHYSICALLY SEPARATED FROM THE DNA ISOLATION ROOM TO PREVENT DNA CARRY-OVER! USE A BIOSAFETY-BOX (LAMINARY FLOW) THAT HAS A UV-LAMP IF POSSIBLE. USE STUFFED TIPS (TIPS THAT HAVE AN INCORPORATED FILTER)! USE YOUR PCR-PIPETTES FOR PCR ONLY! CLEAN THEM REGULARLY (FOLLOW MANUFACTURER'S INSTRUCTIONS).

Polymerase chain reaction

Decide on how to label your tubes, avoid confusion with later PCRs! We label all tubes first with the PCR number (e.g. E1, with E standing for EEHV) and then each tube with its individual number: E1/1, E1/2,....E1/20.) Proper documentation is of the essence!

Primers needed to amplify a fragment of the viral terminase gene are from *Richman et al.* (Science **283:** 1171-1176, 1999). This primer pair will yield a fragment of ~340 bp in length. The use of a proof reading thermostabile polymerase is recommended. Regarding the experimental conditions (PCR setup) follow the instructions of the polymerase's vendor.

Beware that a positive signal (presumable EEHV-PCR fragment) needs to be verified (either by restriction analysis or sequencing) and that a lack of signal does not necessarily mean that the animal the sample is from has no EEHV.

Sequencing

Sequencing should be performed for both strands. All standard sequencing techniques are applicable. We use the BigDye™ terminator mix (Applied Biosystems = Perkin Elmer, Weiterstadt, Germany) in a cycle sequencing PCR according to the dideoxy method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA **74**:5463-5467). An ABI PRISM Sequence Analyzer 310 (Applied Biosystems) is used to analyze the sequence. The obtained sequence can be identified by comparing it with the sequence of the Herpes virus isolated from case 1 (see Science **283**, p. 1173, Fig. A, which also contains the database accession numbers). For the sequence alignment we use the ClustalW (DOS) or ClustalV (Windows) routine (downloadable via Internet, reference: Higgins DG, Bleasby AJ and Fuchs R (1991) CLUSTAL V: improved software for multiple sequence alignment. CABIOS, vol .8, 189-191.).

E. Good luck

ASPECTS AND METHODS OF ELEPHANT FOOT CARE IN ZOOS AND ELEPHANT CAMPS

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Aspects and Methods of Elephant Foot Care in Zoos and Elephant Camps

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Introduction

Zoo visitors consistently rank elephants as number one on their list of the most fascinating and popular animals.

In the wild, the Asian elephant is just before extinction, the African elephant is endangered, but in captivity the breeding results are still far from being sufficient to maintain the zoo population. There is still a certain number of elephants in captivity dying every year because of results from foot problems, and often because people have no experience or even do not care about very much.

Foot care

Foot problems occur in the wild and in captivity and seems to have multifunctional sources. The types of problems seen in domestic and non-domestic ungulates may also be seen in elephants. There include penetrating injuries, sole cracks, nail cracks, overgrowth and infections. In zoos wet conditions and inadequate exercise are predisposing factors.

There should be always under consideration that there are differences between African and Asian elephants. African elephants seem to have fewer foot problems, but the reasons are unknown. This causes also different philosophies about using floor heating for both species. Some zoos do not use floor warming for Asian elephants, because they are afraid of disturbing the sweat glands between the toes, causing further foot problems. Others are afraid of using concrete on the floor inside the boxes.

There are mostly personally discussions, but not enough proofed datas for verifying these hypothesis.

Foot control and care

Daily control of every individual is an important part of the daily routines. In order to have continuous control over the foot-condition, there is the need of daily work and care to avoid problems, and not to react if they already exist.

Basic element is the daily training of the elephant for foot care, because only if the procedure is possible in an easy way, it is done regularly.

We started our program only one and a half years ago, but now even the cracks have diminished and the common condition is quite well.

A case of foot abscess of an 39 years old African elephant (Vienna)

This individual, additional having arthritic problems, showed in 1998 first signs of an abscess on the right foreleg. It reached from the coronary border of the toe nail to the sole. The abscess opened spontaneously at the top of the

toe nail, additionally an opening was cut at the sole. It was treated by daily flushing with "Betadine" solution in the first two months.

The drainage and medical treatment showed some positive results. During the hot summer we decided at least, to allow the female to go outside and move in the sun-heated dry sand. And this treatment succeeded most effective, because the hot sand seemed to dry out the abscess. At least it healed up completely within six months.

The experiences in Vienna and different cases show the needs for prevention and proper foot care.

International exchange of experiences is very important. Practical courses are organized on the Riddle's Elephant Farm (Arkansas). Special training courses and workshops (Training elephants for medical care, treatment and research) are offered at the Vienna Zoo (Austria) regularly.

Prevention of foot problem is better than every medical treatment:

Daily training and working routines contribute to a proper body condition.

Daily washing routines (showering once or even twice) enables perfect skin and foot control, because the animals are clean, then.

In regions with cold winters it is necessary to offer the elephants an inside area, where the animals can move freely day and night. Every effort to dry out and eliminate dirty floors should be made.

An important point of discussion is the quality of food, offered to the animals. Comparable like in domestic ungulates, high energy food comes overgrowing of hoofs and toe nails and it is less a question of mechanical wearing down by walking on hard ground, as often assumed.

In many zoos and facilities prevention is not succeeding, therefore active foot care is basic program, still.

In case of problems the keeping system should be checked: nutrition, housing, behavioral activities, physical exercise, and last not least skill of elephant handlers and training level of the elephants. The cooperation between elephant handlers, veterinarians and curators is basic need in order to succeed in prevention of foot problems and in case of performing foot care and treatment.

All equipments for foot care have to be kept clean and in good condition, in order to avoid additional, secondary infections and problems.

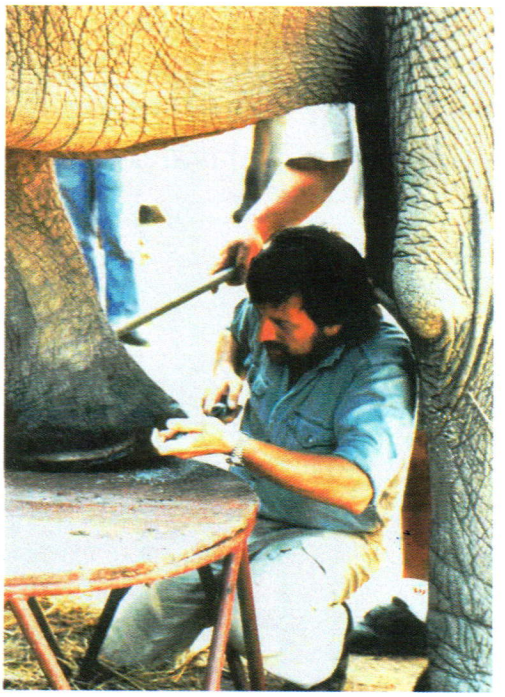
In cause of wounds and abscesses it seems more successful to dry it up and not using shoes or sandals. After disinfection the animals were allowed to go outside, because warming sun and fresh air is contributing to the healing process.

The body and health condition of the elephants, today used nearly for tourist riding and trekking, is different from camp to camp. Usually no foot care is made and a lot of elephants have real foot problems. Often elephants are not trained

for such procedures and even are not used to laying down.

There are camp owners, who really care for their animals. In those camps the mahouts have e.g. perfect saddles and shower the elephants between waiting times in the sun, etc. Elephants are used for tourist riding about some hours, afterwards the mahouts are riding them to the woods for eating and rivers for cooling. Especially those elephants have good foot conditions and do not need much additional foot care. But those coming from wood logging camps are often in very bad condition, sometimes because of mechanical damages.

As there is also a lack of information and experience of the people which are responsible for the elephants, special training workshops will be organized concerning this topic and problems. During the practical part of this workshop we will be able to show introduction to some methods of toenail cutting, pad trimming and general foot care.



An elephants's feet frequently require curing and/or examination to remove foreign objects, to check for excessive wear of the footpads, or to trim nails or hangnails. If toenails are rasped frequently there is no need to cut them too short. Elephants should be taught to raise a foot on command and holt in the air for examination, and rest gently on a pedestal for treatment.

ACKNOWLEDGEMENTS

The editor would like to express sincerely thank to the following persons for their great contribution to the workshop, survey and conference.

1. Dr. Nareerat Visesahakul, Faculty of Venterinary Science, Mahidol University, Salaya, Nakornpathom, Thailand.

2. Dr. Pingpol Charoonrat, Faculty of Veterinary Science, Mahidol University, Salaya, Nakornpathom, Thailand.

3. Dr. Ronachit Roongsri, Asian Elephant Foundation of Thailand.

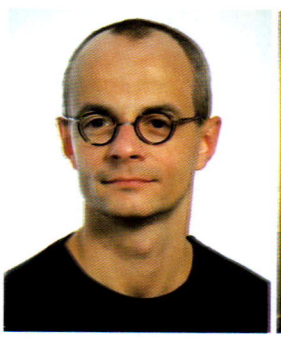
4. Miss Samang Pandum, Secretariat office, Asian Elephant foundation of Thailand.

5. All support staffs, Faculty of Venterinary Science, Mahidol University, Salaya, Nakornpathom, Thailand.

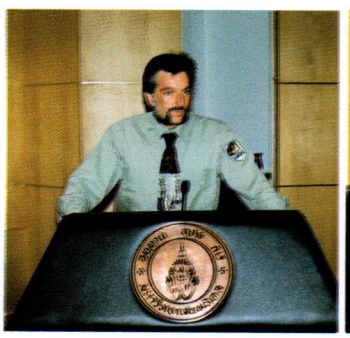
Dr. Thomas Hildebrandt

Dr. Frank Göritz

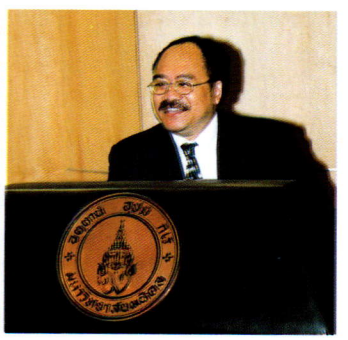
Dr. Wolfram Rietschel



Dr. Joerns Fickel



Dr. Harald Schwammer



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Education

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Veterinary registration	Feb. 1992

Employment

Head, Dept. Reproduction Management	March 1997 - present, Institute for Zoo Biology and Wildlife Research (IZW), Berlin
Head, Research Group Ultrasound	Jan. 1995 - Feb. 1997, IZW
Scinetific researcher	Feb. 1992-1994, IZW
Pathology assistant	1985-1986, veterinary pathology of the faculty of veterinary medicine, Humboldt-University Berlin

Thomas Hildebrandt has worked at the Institute for Biology and Wildlife Research in Berlin since 1987. As head of the Reproduction Management Research Group, he is responsible for the development of transrectal ultrasonography tecniques in species as diverse as Komodo dragons and elephants. His expertise in reproduction biology and pathology in elephants and rhinoceros is recognized worldwide. His development of a non-surgical AI technique in elephants was a key innovation that made artificial insemination successful in the species. His work culminated in the successful insemination of three nulliparous African elephants 1998/1999.

Scientific Awards

Tony Bubenik Memorial Award, International Deer Biology Congress
3. Poster - Award, Intern. Symp. Physiol. Ethol. Wild and Zoo Anim
Honourablae Award, Short - Term Visitor Award at the Smithsonian Institution's National Zoological Park
Award of Excellence for the poster presentation, 28th Congress for Pysiology and Pathology of Reproduction
Award of Excellence for the presentation, Swiss Society for Ultrasound and Biology

Publications : (selected from 34 peer-reviewed manuscripts)

HILDEBRANDT, T.B. and F.GÖRITZ (1995): Ultrasonography as a tool in propagation of zoo animals and wildlife. Proc. Annu. Meet. Am. Assoc. Zoo. Vet. 356-364.

HILDEBRANDT, T.B., GÖRITZ, F., BOSCH, H., SEIDEL, B. and C. PITRA (1996): Ultrasonographic sexing and reproductive assessment of penguins. Penguin Conversation, 4; 6-12.

HILDEBRANDT, T.B., GÖRITZ, F., PITRA, C., SPELMAN, L. H., WALSH, T.A., ROSSCOE, R. AND N.C. PATT (1996): Sonomorphological sex determination in subadult komodo dragons. Proc. Annu. Meet. Am. Assoc. Zoo. Vet., Pp. 251-254.

MONTALI, R. J., HILDEBRANDT, T., GÖRITZ., HERMES, R., IPPEN, R. and E. RAMSAY (1997): Ultrasonography and pathology of genital tract leiomyomas in captive Asian elephants: Implications for eproductive soundness. Verh. ber. Erkrg. Zootiere, 38, 253-258.

HILDEBRANDT, T.B., GÖRITZ, F., STETTER, M.D., HERMES, R. and R.R. HOFMANN (1998): Applications of sonography in vertebrates. Zoology, 101, 64-73.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT, N.C., SCHMITT, D.L., QUANDT, S., RAATH, J. and R. R. HOFMANN (1998): Reproductive assessment of male elephants *(Loxodonta africana* and *Elephas maximus)* by ultrasonography. J.Zoo Wildl. Med. 29, 114-128.

SCHMITT, D.L. and T.B. HILDEBRANDT (1998): Manual coolection and characterization of semen from Asian elephants *(Elephas maximus)*. Anim. Reprod. Sci. 53, 309-314.

HILDEBRANDT, T.B., GÖRITZ, F. SCHNORRENBERG, A., HERMES,R., SCHMITT, D.L., HAGAN, D., OETERSON, J., BROWN, J., LOSKUTOFF, N., PRATT, N. C., LEHNHARDT. J. L., MILLER, G., MONTALI, R.J. and D. OLSON (1999): Successful artificial insemination of African nulliparous elephants at the Indianapolis Zoo. Verh. ber. Erkrg. Zootiere, 39,41-46.

HILDEBRANDT, T.B. FRITSCH, G., HERMES, R., JEWGENOW, K., ROHLEDER, M., RUDOLPH, M., MALTZAN, J., WIESNER, H., PRATT, N. C., NEIFFER, D. und F. GÖRITZ (1999): Charakterisierung der Pubertät männlicher Elefanten mit Hilfe ultrasonographischer, morphologischer, endokrinologischer, morphologischer, endokrinologischer und spermatologischer Untersuchungsmethoden. Verh. ber. Erkrg. Zootiere, 39, .27-34.

HERMES, R. OLSON, D., GÖRITZ,F., SCHMITT, D.L., HAGAN, D., PETERSON, J., BROWN, J., FRITSCH, G. A and T.b. HILDEBRANDT (1999) : Ultrasonographical characterisation of the sexual cycle in female African elephants *(Loxodonta africana)*. Verh. ber. Erkrg. Zootiere, 39, 35-38.

RICHMAN, L.K., MONTALI, R. J., GARBER, R, L., KENNEDY, M. A., LEHMHARDT, J., HILDEBRANDT, T.B., SCHMITT, D.L., HARDY, D., ALCENDOR, D. J. and G.S. HAYWARD (1999): Novel endotheliotropic herpesviruses fatal for Asian and African elephants. Science, 283. 1171-1176.

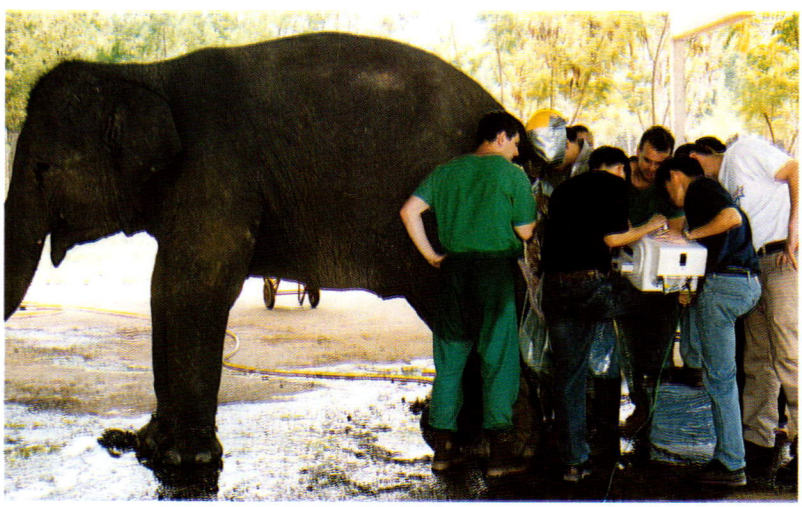
RICHMAN, L. K., MONTALI, R. J., HILDEBRANDT, T.B. FICKEL, J., SCHMITT, D.L., and G.S. HAYWARD (1999): Status of a new, fatal herpesvirus disease of elephants in North America and Europe. Verh. ber. Erkrg. Zootiere, 39, 17-22.

ZWART, P., IPPEN, R., FLÜGGER, M., GÖRITZ, F. and T.B. HILDEBRANDT (1999): Post mortem findings in a 60-year-old Sri lankan elephant *(Elephas maximus maximus)*. Verh. ber. Erkrg. Zootiere, 39, 143-148.

Book chapter (selected)

HILDEBRANDT, T.B. and F. GÖRITZ (1996): Ultrasonography as a tool in zoo management. In: Gansloßer U., Hodges J. K., Kaumanns W. (eds.): Research and captive propagation. Filander Verlag GmbH. Fürth, 51-58.

HILDEBRANDT, T.B. and F. GÖRITZ (1998): Use of ultrasonography in zoo animals. In: M. E. Fowler and R. E. Miller (eds.) Zoo and wild animal medicine. Current Therapy 4. W.B. Saunders Company, Philadelphia, 41-54.



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Borad certification for zoo and wildlife medicine — June 1997.

2nd Degree in Veterinary Medicine: — Free University Berlin, Department of Veterinary Medicine with a dissertation entitled: "Ulttasonography in zoo and wild animals.", FU Berlin, 1996.

Veterinary registration — Feb. 1992.

Employment

Senior veterination of IZW — Jan. 1997 -present, Institute for Zoo Biology and Wildlife Research, Berlin (IZW).

Scientific co-worker — Feb. 1992 -present, Institue for Biology and Wildlife Research, Berlin (IZW), Dept. Reproduction Management.

Scientific Awards:

Heinrich Lüßen Award 1997 German Society of friends and sponsors of veterinary medicine.
Tony Bubenik Memorial Award 1998, International Deer Biology Congress.
3. Poster-Award Intern. Symp. Physiol. Ethol. Wild and Zoo Anim.
Award of Excellence for the presentation, Swiss Society for Ultrasound and Biology.
Award of Exellence for the poster presentation Congress for Physicology and Pathology of Reproduction.

Publications: (selected from 27 peer-reviewed manuscripts)

GÖRITZ, F., HLIDEBRANDT, T.B., NÖTZOLD, G., EULENBERGER, K., GOTTSCHALK, J., FRANK, S. und H. CORREIA (1994): Untersuchungen zum reproduktiven Status und Zyklusgeschehen beim Anoa *(Bunalus depressicornis)* mittels transrektaler Ultrasonographie. Verh. ber. Erkrg. Zootiere 36, 107-119.

GÖRITZ, F. (1996): Ultrasonography in zoo and wild animals. Vet. med. Diss. Freie Univ. Berlin.

GÖRITZ, F.; JEWGWNOW, K.; MEYER H. H. D. (1996): Epidermal growth factor and epidermal growth factor receptor in the ovary of the domestic cat *(Felis catus)*. J. Reprod. Fert. 106: 117-124.

GÖRITZ, F., HILDEBRANDT T.B., JEWGENOW K., WAGNER N., HERMES R., STRAUß G. and MEYER H.H.D. (1997): Transrectal ultrasonographic examination of the female urogenital tract in nonpregnant and pregnant captive bears (Ursidae). J. Reprod. Fert. Suppl 51. 303-312.

GÖRITZ, F., HILDEBRANDT T., HERMES R., THIELEBEIN J., SPRETTKE T., GÖLTENBOTH R. und B. SEIDEL (1997) : Immobilisation und transintestinale Ultrasonographie bei Kasuren. Verh. ber. Erkrg. Zootiere 38, 181-186.

HILDEBRANDT, T.B., GÖRITZ, F., STETTER, M.D., HERMES, R. and R.R. HOFMANN (1998): Applications of sonography in vertebrates. Zoology 101(3), 64-73.

HILDEBRANDT, T.B., GÖRITZ, F., PRATT N.C., SCHMITT, D.L., QUANDT, S., RAATH, J. and R.R. HOFMANN (1998): Reproductive assesment of male elephants *(Loxodonta africana* and *Elephas maximus)* by ultrasonography. J. Zoo Wildl. Med. 29(2), 114-128.

GÖRITZ, F., M. QUEST, T.B. HILDEBRANDT, J. MALTAZAN, H. WIESNER, H.H.D. MEYER, M. ROHLEDER, L. KOLTER, K. JEWGENOW (1999): Anitigestagens - a new approach to Control Reproduction in captive bears (Ursidae). Verh. ber. Erkrg. Zootiere 38, 295-296.

GÖRITZ, F., T.B. HILDEBRANDT, R. HERMES, S.QUANDT, D. GROBLER, K. JEWGENOW, M. ROHLDER, H.H.D. MEYER, R.R. HOFMANN (1999): Results of hormonal contraception programme in free ranging African elephants at the Kruger National Park. Verh. ber. Erkrg. Zootiere 38, 39-40.

Book chapter

HILDEBRANDT, T.B. and F. GÖRITZ(1996): Ultrasonography as a tool in zoo management. In: Ganslοßer U., Hodges J. K., Kaumanns W. (eds.): Research and captive propagation. Filander Verlag GmbH, Fürth, 51-58.

HILDEBRANDT, T.B. and F. GÖRITZ(1998): Use of ultrasonography in zoo animals. In: M.E. Fowler and R.E. Miller (eds.) Zoo and wild animal medicine. Current Therapy 4. W.B. Saunders Company, Philadelphia, 41-54.

Curriculum Vitae: Dr. Wolfram Rietschel Dr. Med. Vet.

09.05.1945	Born as third son of the biologist Prof.Dr. Peter Rietschel in GieBen, Germany
01.04.1965	Qualification for admission to an university at Goethe-Gymnasium, Frankfurt
1967-1972	Study of Veterinary Medicine and Biology at the GieBen University
20.12.1973	Graduation Dr.med. vet. at the Faculty of Veterinary medicine of the University of GieBen
01.10.1973-31.03.1974	Assistant lecturer at the Institute of Tropical Veterinary Medicine in GieBen.
01.04.1974-31.01.1976	Associated Expert at the FAO Animal Health Project, Kabul, Afghanistan. Veterinarian at Kabul Zoo.
1.02.1976-30.06.1981	Veterinary expert at the Thai German Dairy Project in Chiangmai, North Thailand and at the Regional Animal Health Project in Khon Kaen, Northeast Thailand (GTZ).
16.11.1976	Special degree: "Fachtierarzt fur Tropische Veterinamedizin"
02.02.1979	Special degree: "Fachtierarzt fur Zoo- und Wildtiere"
02.07.1981-31.05.1984	Zoo Veterinarian at the "Munchener Tierpark Hellabrunn", Munich, Germany.
01.06.1984-30.11.1984	Veterinarian at the German Primate Centre (DPZ) in Gottingen, Germany.
10.06.1984-present	Zoo veterinarian and vice general curator at the Zoological and Present Botanical Garden Wilhelma in Stuttgart.
1999	Award of the Nieberle Medal by the Vererinary Chamber of Beden-Wurttemberg in recognition of merits for the veterinary profession.
1972-2000	Projects and professional activities in Zoo and Wildlife Veterinary Medicine in Brazil(implementation of a zoo), Turkey (Brown bear workshop), French Guayana (Leather backed turtle project), Burundi (Vererinary field service), Pakistan (Verterinary training course for Afghan veterinarians), Vietnam (Cuc Phuong primate rescue centre), Kenia, Namibia, Botswana, Tanzania and South Africa (National Parks) and Thailand (Lecturer at the 1st International Conference on Regional Workshop on Asian Elephants Health Care, Survey of Herpesvirus in Asian Elephants, Reproduction Biology in Asian Elephants, treatment of ectoparasites in Asian elephants). Lectures at veterinary and agricultural faculties in Germany and other countries. Zoonoses research in primate collections in zoological gardens.

Curriculum Vitae: Dr. Joerns D. Fickel Ph.D.

INSTITUTE FOR ZOO BIOLOGY AND RESEARCH, Dept. Evolutionary Genetics.

Date of Birth: October 22nd, 1961, Germany.

Education:

B.Sc., 1998 (Biochemistry), Ph.D., 1991 (Genetics & Molecularbiology) Ernst-Moritz-Arndt-University of Greifswald (Germany).

Academic History

1983-1990:	Undergraduate and graduate student, Microbiology, Ernst-Mortiz-Arndt-University
1990-1993:	Researcher, Research Institute for Molecular Pharmacology, Dept. of Cellular Biology
1993-1995:	Postdoc, University of Michigan, School of Medicine, MHRI,
1996:	Research Institute of Molecular Pharmacology, Dept. Cell. & biochem. Phamacology
Since 1997:	Institute for Zoo Biology and Wildlife Research, Dept. Evolutionary Genetics

Publication

Theses: B.Sc.: Transformation of the imperfect Yeast *Canadida maltosa* with the *Escherichia coli - Saccharomyces cervisae* shuttle vector pC516. Ph.D.: Isolation and characterization of the ILV1 (Threonine dehydratase) gene of the imperfect Yeast *Canadida maltosa*.

Selection of papers:

J.Fickel, S.Savoly, U.Vogel, J.Furkert, M.Melzig, The proopiomelanocortin (POMC) gene expression of AtT20 mouse pituitary cells is dependent on cell culture conditions. Cell. Mol. Biol. (1994) 40:201-209.

J.Fickel, D.bagnol, St.Watson and H.Akil. Opioid receptor expression in the gastrointestinal tract of the rat: A quantitative study with comparison to the brain. Mol.Brain Res. (1997) 46:1-8.

J.Fickel, F.Göritz, B.A.Joest, Th.Hildebrandt, R.R.Hofmann. Analysis of parotid and mixed saliva in roe deer (*Capreolus Capreolus*, L.). J. Comp. Physiol. B (1998) 168:257-264.

J.Fickel, C.Pitra, B.A.Joest, R.R.Hofmann. A novel method to evaluate the relative tanninbinding capacities of salivary proteins. Comp. Biochem. Physiol. C (1996) 122:225-229.

J.Fickel, D.Lieckfeld, C.Pitra. Analysis of genetic diversity and structure in neighboring populations of the European brown hare (*Lepus europaeus*, Pallas 1778). Z. Jagdwiss (1999) 45:230-237.

J.Fickel, A.Reinsch. Microsatellite markers for the European Roe deer (*C. capreolus*). Mol. Ecol. (2000), 9, in press.

A.Winkler, J.Fickel, M.Melzig. Efficient remova of hybridization signal from RNA dot blots probed with digoxygenin labeled cRNA. BioTechniques (1994) 16(1):61-62.

I.Putscher, H.Haber, A.Winkler, J.Fickel and M.F.Melzig. Effects of S(-)- and R(+)-salaolinol on the POMC gene expression and ACTH release of an anterior pituitary cell line. Alcohol (1995) 12:447-452.

A.Winkler, G.Papsdorf, J.Odarjuk, W.-E.Siems, J.Fickel and M.F,melzig. Expression and characterization of the substance P (NK1) receptor in the rat pituitary and AtT20 mouse pituitary tumor cells. Eur. J. Pharmacol. (1995) 291 (1):51-55.

C.Pitra, W.J.Streich, A.Reinsch, J.Fickel and W.Mann. The Population of the Samali Wilddonkey (Equus africanus somalicus Sclater) under human care: Demographic and genetic aspects. Zool. Gar-ten N.F. (1995) 65:245-257.

A.Winkler, I.Roske, J.Furert, J.Fickel and M.F.Melzig. Effects of voluntary ethanol ingestion on the POMC gene expression in the rat pituitary and on the plasma b-endorphin content. Alcohol & Alcoholism (1995) 30:231-238.

K.Jewgenow, J.Fickel. Sequential expression of zona pellucida protein genes during the oogenesis of domestic cats. Biol. Reprod. (1999) 60:522-526.

L.K.Richman, R.J.Montali, T.Hildebrandt, J.Fikel, D.Schmitt and G.S.Hayward. Status of a new, fatal herpesvirus disease of elephants in North America and Europe. Verh.ber. Erkrg. Zootiere (1999) 39:17-21.

Richman, L., Montali, R., Hildebrandt, Fickel, J., Hayward, G. Une pathologie nouvellement identifiee chez l'elephant associee a des herpesvirus endotheliotropes. Virologie (1999) 3:483-485.

K.Frolich, O.Czupalla, L.Haas, J.Hentschke, J.Dudek, J.Fickel,. Epizootiological investigation about canine distemper in free-ranging carnivores from Germany. Vet. Microbiol. (2000) 74(4):283-292.

Selection of proceedings

J.Fickel, L.K.Richman, A.Reinsch, R.Montali, W.Schaftenaar, F.Göritz, T.B.Hildebrandt. Survey on the occurrence of the endotheliotropic elephant herpesvirus (EEHV) in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants in European Zoos. Proceedings of the 3rd Scientific Meeting of the European Association of Zoo and Wildlife Veterinarians (EAZWV), May 31st - June 4th, 2000, Paris, France, pp. 175-178.

A.Wagener, S.Blottner, R.Einspanier and J.Fickel. Investigation of growth factors gene expression in the testis of ruminants. 2nd International Symposium on Physiology and Ethology of Wild and Zoo Animals, 7-10 October, 1998, Berlin, Germany, Advances in Ethology (Suppl. to Ethology) 33:75.

K.Jewgenow, I.Wegner and J.Fickel. Sequential expression of zona pellucida proteins during fol liculogenesis of domestic cats. Proceedings of the 4th International Conference on Animal Reproduction (ICAR) "Gametes: Development and Function", September 14-16, 1998, Milano, Italy, In: Gametes: Development and Function. Eds. A.Lauria, F.Gandolfi, G.Enne, L.Gianoroli, Serano Symposia, 1998, Rom, p.550.

L.K.Richman, R.J.Montali, R.C.Cambre, D.Schmitt, D.Hardy, R.L.Garber, T.Hildebrandt, J.Fickel, W.Schaftenaar, G.S.Hayward. Clinical and pathological aspects of a fatal herpesvirus disease in Asian (*Elephas maximus*) and African Elephants (*Loxodonta africana*). 1999. Proceedings of the American Association of Zoo Veterinarians (AAZV), October 9-14, Columbus, Ohio, USA.

Curriculum: Dr.Harald Schwammer Ph.D.

Born: Sept 12, 1995, Wiener Neustadt (AUSTRIA).
Degree: Zoology, Botanic, Human Biology, Earth Science.
Profession: Zoologist.
Since 1986 juridical accredited expert for nature conversation, protection, keeping and dealing with animals.
1977-1992 employed on the University for Zoology in Vienna.
1993-1995 Curator for Science, Technique and Transport at the Vienna Zoo. Responsible fir the construction of various animal enclosures and buildings: Big cats, elephants, wofts, lynx, owls, mandrill, aquarium and terrarium and also for the renovation of enclosures and additional the new construction of a tirolyan farm house and the creation of a new masterplan for the zoo.
1995-1998 additional responsible as Curator for the terrarium. Construction of a new enclosure for iguna, basically planning for the refurbishment of the aquarium and terraium, which could be opened on April 6, 2000 in the Vienna Zoo.
Since 1998 Curator for mammals (elephants, hippos, big cats, apes, bears, wolfs, lynx, owls, animals of the African enclosure, penguins, and casuar). At the moment additional planning work of the rainforest house, refurbishment of the polarium and renovation of the hippo house.
Since 1999 Vice Director at the Vienna Zoo.

Consulation work for various projects for the keeping of wild animals, also for the conception of two new aquarims and a zoo.

Lectureship on the University of Vienna for:
Zoological preparation and museum methods,
Zoobiology: methods for the keeping of wild animals in modern zoos.
Ecology sub- and eu-mediterranean islands (Krk, Croatia).
Numerous scientific publications in ethnological, ecological fields of study, concerning various species - from apes to birds of pray, seagulls, reptiles, spiders, fishes, sea urchin, corals, African elephants, but also rare farm animals such as Norier houses.

Writing of guidelines and books (author and co-author):
Poisonous and dangerous animals of the sea
Guidelines and minimum standards for the keeping of reptiles
Guidelines and minimum standards for turtles
Guidelines for the keeping of wild animals in circueses
Ferrets as pets

Various scientific projects (elephants, tigers, eland antelopes, zebras, reptiles, oryx antelopes, etc.)

President of the EEKMA (European elephant keeper and manager association).
President of the Zoo friends organization of the Vienna Zoo.

Curriculum: Dr.Parntep Ratanakorn B.Sc., DVM., MSc.

Born: 22 September 1954, Bangkok, Thailand
Nationality: Thai
Office Address: Faculty of Veterinary Science
Mahifol University, Salaya Campus
Nakornpathom 73170, Thailand
Tel. 662-4410931-2 Fax. 662-4410937
e-mail: vsprt@mahidol.ac.th

Education: D.V.M., Kasetsart University and M.Sc.(Pathobiology), Mahidol University
Position: Assistant Professor / Dean

Award/Grant :

- "Outstanding Veterinarian of the year" Award from the Veterinary Practitioner Association of Thailand (VPAT), 1993
- "Survey of wild crocodilians in Thailand" in 1992, funding by the Asian Conservation and Sustainable Use Group. (ACSUG)
- "Database for the Management of Captive Asian Elephants in Thailand" in 1998, funding by International Foundation for the Conservation of Wildlife.

Professional Affiliation :

- President of the Crocodile Management Association of Thailand (CMAT)
- Secretary General of the Asian Elephant Foundation of Thailand (AEFT)
- Pass President of the Veterinary Practitioner Association of Thailand (VPAT)
- Pass Vice Chairman of the Asian Conservation and Sustainable Use Group (ACSUG)
- Executive Board Member of the Hornbill Research Foundation (HRF)
- Director of "Center for Animal Welfare Study" (CAWS)

Research Activities :

- Biodiversity of small mamals in Huey Kha Khaeng wildlife sanctuary.
- Effect of alien spicies on endemic fauna in Thailand.
- Sustainable utilization of wildlife in Thailand; crocodile, python, pheasant, deer and etc.
- Diseases of wildlife and their conservation.

Publications :

- Ratanakorn, P. (1988). Captive breeding of clouded leopard *(Neofelis nebulosa)* in Thailand. Proceeding of the 5th world conference on breeding endangered species in captivtty, Cincinnati, OH, 640-645.
- Ratanakorn, P. (1990). Captive breeding of douc langur *(Pygathrix nemaeus)* in Thailand. Proceeding of the 7th Federation of Asian Veterinarian Association Congress, Pattaya, 595-605.
- Ratanakorn, P. (1993). Conversation, Management and Farming of Crocodiles in Thailand. Proceedings of the 2nd Regional (Eastern Asia, Oceania, Australasia) Meeting of the Crocodile Specialist Group, 12-19 March 1993, Darwin, Australia, 1-30.
- Ratanakorn, P., B. Amget and B. Ottley, (1994). Preliminary Surveys of Crocodiles in Thailand. Proceedings of the 12th Working Meeting of the Crocodile Specialist Group of the Species Survival Commission of the IUCN The World Conservation Union, Volume 1, 2-6 May 1994, Pattaya, Thailand, 33-56.
- Ratanakorn, P. (1996). Manual for Handling of Elephant in Musth. Second Edition, The Prime Ministers Office. 89 pages.
- Ratanakorn, P. (1998). Database for the Management of Captive Asian Elephants in Thailand. PNEP Newsletter, Vol. 10, No.46, 8-9.
- Ratanakorn, P. (1999). Minimum Requirement for Health Status and Management of Asian Elephants in South East Asia. Proceedings of the First Regional Workshop on Asian Elephant Health Care. Chiang Mai. The National Identity Office, Secretariat of the Prime Minister, 200 pages.

The National Identity Board

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