Determination of reproductive cyclicity and pregnancy in Asian Elephants (*Elephas Maximus*) by Rapid Radioimmunossay of serum progesterone. John H. Olsen, Chao-Ling Chen, Mona M. Boules, L. Scott Morris, and Betsy R. Coville,. (1994). *J. Zoo. Wildl. Med.* 25(3): 349-354.

The stages of the reproductive cycle and pregnancy of 15 Asian elephants (*Elephas maximus*) at Busch Gardens in Tampa, Florida were determined by measuring serum progesterone concentrations using a commercial radioimmunoassay (RIA) kit with a sensitivity of 40 pg/ml of serum. Blood samples were collected and analyzed weekly for a 45-mo period. Serum progesterone concentrations ranged from nondetectable (40 pg/ml) in nonpregnant elephants to 2,110 pg/ml in pregnant elephants. The highest concentration of progesterone during the estrous cycle was 1,490 pg/ml. The length of the estrous cycle ranged from 10 to 23 wk, with an average (+/-SEM) of 15.1 +/- 0.3 wk for 103 estrous cycles. One elephant had a consistently longer estrous cycle length (18.6 wk). The duration of the nonluteal phase was 4.6 +/- 0.2 wk, and the duration of the luteal phase was 10.5+/- 0.2 wk. The serum progesterone concentration during the estrous cycle was 214.0 +/- 5.2 pg/ml. The individual elephant average cycle length was used to estimate the time of estrus and ovulation. During the nonluteal phase, elephant cows were placed with a bull for mating. Two weeks after estrus began (based on rising progesterone at the end of the nonluteal phase), the cow was separated from the bull. Ovulation was predicted to occur during the first week after estrus began. Serum progesterone analyses were continued weekly to determine pregnancy or the next estrus. Eight elephants became pregnant, with a serum progesterone concentration of 554.6 +/- 16.4 pg/ml and a range of 40-2, 110 pg/ml. These results confirm that weekly measurement of serum progesterone by RIA can be used to characterize the luteal and nonluteal phases of the Asian elephant estrous cycle. Using this information, cows can be managed and placed with a bull at the appropriate time for breeding. Serum concentrations of progesterone also can be used to confirm pregnancy and to monitor and evaluate potential problems during pregnancy. The approximate date of parturition can then be predicted from average gestation length data, thus allowing staff to prepare facilities and be ready to assist with delivery.

Surgical Castration of the Elephant (*Elephas maximus* and *Loxodonta africana*). Joseph J. Foerner, Richard I. Houck, John F. Copeland, Michael J. Schmidt, H.T. Byron, and John H. Olsen. (1994). *J. Zoo. Wildl. Med.* 25(3): 355-359.

The purpose of this project was to develop a reliable, safe and efficient technique for surgical castration of the elephant (*Elephas maximus* and *Loxodonta africana*). To achieve this, there have been several modifications in surgical technique. Initially, sterilization by injecting caustic agents into the testicles via laparotomy was attempted, but results were unpredictable and had serious side effects. Castration of young males under 5 yr of age was relatively easy using a standard equine chain ecraseur through a single laparotomy incision. For larger males, most cases required two laparotomy incisions with several variations in techniques for removal of the testicles. Initially self-locking stainless steel bands were placed on the cord as ligatures, and the testicles were removed with an obstetrical wire saw. Because of technical difficulties, this method was abandoned, and an alternative technique was developed. The testicle was removed with an obstetrical wire saw, and then the artery was isolated by digital palpation. A Kelly forceps was secured on the vessel. The forceps was passed through a loop of an equine chain ecraseur and the chain positioned over the artery. The ecraseur was then closed, crushing the vessel. Currently, the most promising technique is the development of a large chain ecraseur that will allow removal of both testicles through a single laparotomy approach.

Asian Elephant (*Elephas maximus*) Milk Composition During the First 280 Days of Lactation. Susan A. Mainka, Robert M. Cooper, Sandra R. Black, and Ellen S. Dierenfeld. (1994). Zoo Biology, 13(4) : 389-393.

Milk samples (n = 10) taken during the first 280 days of Lactation from one Asian elephant were examined for nutrient composition including total solids, protein, fat, ash, α tocopherol, and retinol levels. Total solids averaged 19.7 +/- 2.7% SD (range 15.0-23.3). Percent protein remained fairly stable throughout this portion of lactation and averaged 3.4 +/- 0.3% (range 3.0-4.0). Ash content averaged 0.54 +/- 0.03%. Milk fat and fat soluble vitamin levels varied considerably with a suggestion of a cyclic pattern. Fat content of milk averaged 7.6 +/- 2.6% (range 3.9-12.1): α - tocopheral levels averaged 0.33 +/- 0.12 ug/ml; and retinol levels averaged 0.46 +/- 0.1 ug/ml.

Relatedness Estimation of Captive Asian Elephants (*Elephas maximas*) by DNA Fingerprinting. Laura L. Bischof and Deborah A. Duffield. (1994). *Zoo Biology*, 13(1): 77-82

DNA from eighteen Asian elephants of known relatedness from three North American zoos was fingerprinted with the hypervariable probe M13. Paternity of a calf of known pedigree was verified. Paternity assignment of a calf having two proposed sires was suggested, but could not be conclusively determined due to a unique fragment in the calf and the unavailability of one potential sire for testing. It was concluded that DNA fingerprinting with M13 could provide a reasonable first estimator of relatedness for first degree relatives (mean S = 0.63 +/- .11) and for unrelated animals (mean S = 0.26 +/- .11), but would be unreliable

for the determination of intermediate degrees of relatedness. DNA fingerprinting can be efficiently used for paternity determination only when all potential sires can be tested.

Urinary Cortisol Analysis for Monitoring Adrenal Activity in Elephants. Janine L. Brown, Christen M. Wemmer, and John Lehnhardt . (1995). Zoo Biology , 14(6): 533-542.

Cortisol was measured in dichloromethane- extracted elephant urine using an ^{125}I solid-phase radioimmunoassay (RIA). The cortisol RIA was validated by demostrating 1) parallelism between dilutions of pooled urinary extracts and the standard curve, 2) significant recovery of exogenous cortisol added to elephant urine, and 3) a relationship between changes in peripheral and urinary cortisol after an adrenocorticotropin hormone (ACTH) challenge. One African (*Loxodonta africana*) and one Asian (*Elephas maximus*) elephant were given three injections of ACTH (1.25 mg) at 2 h intervals. Serum cortisol increased four to eightfold within 30 min after the first injection and peaked (nine to twelvefold increase) after the second injection. Serum concentrations began to decline 2-3 h after the last injection but were still approximately fourfold higher than baseline at the end of the collection period (hour 8). In the urine, cortisol concentrations were increased in the first sample postinjection(1.5 - 4 h) and peaked twenty - to fortyfold by 6 h. Urinary cortisol remained elevated at 8 h, but returned to baseline the following morning. Analysis of high performance liquid chromatography fractions of extracted urine revealed that immunoactivity was associated with free cortisol (90% of total immunoactivity) and a more polar, unidentified metabolite. A method for preserving urine was developed to allow storing unfrozen samples. One pool of urine from each of one African and two Asian elephants was divided into aliquots, placed in tubes containing absolute ethanol (10%), sodium azide (0.1%) or distilled water (control) and frozen after 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 weeks of storage at 25°C. In unpreserved samples, cortisol concentrations were reduced 46% by 2 weeks and 95% by 24 weeks. In contrast ethanol - and sodium azide-preserved samples retained 100 and 95 % of cortisol immunoactivity through 8 weeks and 93 and 85% of activity through 12 weeks, respectively. We infer from these data that changes in urinary cortisol excretion in the elephant reflect fluctuations in adrenal activity and may be a useful indicator of stress. Additionally urine samples can be collected and stored unfrozen for at least 2 months before any appreciable loss in cortisol immunoactivity occurs, a finding potentially useful to field application of this technique.

Serum and Urinary Hormones during Pregnancy and the Peri- and Postpartum period in an Asian Elephant. (*Elephas maximus*). Janine L. Brown and John Lehnhardt. (1995). Zoo Biology, 14(6): 555-564.

Blood and urine samples were collected weekly from an Asian elephant (*Elephas maximus*) for 10 months before conception, throughout pregnancy, and for 10 months after parturition. Additional daily samples were collected for 41 days before through 10 days after parturition to define endocrine events during the peripartum period. During gestation, serum progesterone concentrations increased gradually and, after 13 weeks, were higher ($P < 0.05$) than those observed during the nonpregnant luteal phase. Concentrations peaked at 12 months of gestation, gradually declined during the last month, and then decreased sharply to nondetectable levels 2 days before parturition. A 12 week lactational anestrus was observed before cyclicity resumed. The urinary profile of progestagen excretion paralleled that of circulating progesterone ($r = 0.79$, $P < 0.05$); however radioimmunoassay of HPLC-separated fractions of urinary eluates indicated that this immunoactivity was not associated with native progesterone. After remaining basal through the first 16 weeks of gestation, serum prolactin concentrations increased to 100 fold about midterm and remained elevated until after parturition. Neither serum nor urinary cortisol concentrations were altered during pregnancy, but both increased markedly the day after parturition and remained elevated above prepartum levels for several weeks thereafter. These data indicate that analysis of serum prolactin can confirm pregnancy in the Asian elephant after 4 months of gestation and that daily monitoring of serum or urinary progestagens is useful for predicting parturition.

Estrogen Metabolism in the Asian Elephant (*Elephas maximus*) Czekala, N. M., Roocroft, A., Bates, M., Allen, J. and Lasley, B. L. (1992) *Zoo Biology* 11: 75-80.

A study of estrogen metabolism in two female Asian elephants revealed a rapid clearance of circulating free estradiol and identified a major metabolite in the serum and urine as estradiol conjugate. The urinary estrogen metabolites were in the conjugated form with an estradiol: estrone ratio of 60:40.

The Evolution of Elephant Husbandry from Free Contact to Protected contact a Veterinarians Perspective. James, E. Oosterhuis. (1995). Proceeding Joint Conference - American Association of Zoo Veterinarians , Wildlife Disease Association and American Association of Wildlife Veterinarians, East Lansing, Michigan August 12-17,1995. Pp. 250.

In North America there is an increasing movement towards protected contact management techniques for elephants kept in zoos. This means that elephants are managed with a protective barrier between the handler and the elephant and that all behaviours are rewarded by positive reinforcement.

This change has been brought about by an increased desire for employee safety and to avoid animal discipline.

Implementation requires facility modifications with the installation of an elephant restraint device being one of the biggest additions needed. The change also requires an extensive training program for both handlers and elephants.

The veterianarian has to learn to work around the protective barriers and to be aware that short painful procedures such as injections or foot abscess examinations will now take longer and require a lot of patience.

The most important concerns however, after human safety, must be for the elephants. In the short term their welfare, both mental and physical must be considered and in the long term their future offspring and their needs must be met.

Surgical Removal of Infected Phalanges from Asian Elephant (*Elephas maximus*) Laurie J. Gage, David Blasko, Murray E. Fowler and John Pascoe. (1995). Proceeding Joint Conference - American Association of Zoo Veterinarians , Wildlife Disease Association and American Association of Wildlife Veterinarians, East Lansing, Michigan August 12-17,1995. Pp. 309.

A forty year old female Asian elephant (*Elephas maximus*) developed a draining tract behind the lateral nail of her left front foot. There had been an infection in the pad in this area several months previously that had resolved. There had been a crack in that nail that had been present for several years. The day after the discovery of purulent material coming from the lesion in the pad, the left front limb became swollen. The cuticle was swollen around the left lateral nail. The foot was soaked in disinfectant solutions and epsom salts. An incision was made into the cuticle at the proximal most portion of the nail, and a tract was found that measured 10 cm and extended distally and slightly medially. The distal region of the cracked nailbed was blocked with 2% lidocaine and a 6 cm diameter hole was cut. A tract was found that communicated with the previously discovered tract. The lesion was treated by aggressive irrigation using a variety of standard disinfectant solutions. The elephant was placed on 100 cc benzathine penicillin i.m. s.i.d. x 5 days, then 25 grams ampicillin i.m.s.i.d x 10 days. Radiographs were taken and degeneration was evident in the third phalange (P-3) of the fifth digit, and there was evidence of osteomyelitis in P-2. The tract was flushed with a variety of disinfectant solutions for four months, however, radiographs indicated the infection was progressing. The infected portions of P-2 and P-3 were removed surgically.

Six months after surgery the incision had healed, but a fistulous tract remained behind the nailbed, exiting in the pad tissue below the lateral nail. There was radiographic evidence of osteomyelitis that had progressed to the distal portion of P-1 Aggressive irrigation and antibiotic therapy did not resolve the problem. A second surgery was performed, during which the remainder of P-2, and the distal portion of P-1 were removed. Aggressive aftercare included 16 grams gentomycin diluted in one liter lactated ringers i.v. s.i.d. x 10 days, sterile wrap changes s.i.d. x 13 days, and a 34-day around the clock training staff member present to ensure the elephant did not remove the wraps. The elephant was maintained on 67 grams trimethoprim-sulfa p.o.s.i.d. for two weeks after the i.v. gentomycin treatment was discontinued. Once a healthy layer of granulation tissue was covering the remainder of P-1, the night watch was discontinued. *Pseudomonas* sp. was cultured from the lesion two weeks post-surgery, and the lesion was then packed with sterile gentocin soaked gauze sponges each day when the bandage was changed. Three weeks after this treatment had started, the cultures were negative for *Pseudomonas*, however, this treatment was continued for a total of 12 weeks. The foot bandage was changed daily for a total of three months post-surgery, the elephant would occasionally remove the wraps when the elephant personnel were not present, however, the foot continued to heal without incident, and was completely healed four months post-surgery.

Concentrations of Progesterone, Testosterone and Estradiol-17β in the Serum During the Estrous Cycle of Asian Elephants (*Elephas maximus*). K. Taya, H. Komura, M. Kondoh, Y. Ogawa, K. Nakada, G. Watanabe, S. Sasamoto, K. Tanabe, K. Saito, H. Tajima and E. Narushima. (1991). *Zoo Biology,* 10(4): 299

The levels of progresterone, testosterone and estradiol-17β in serum samples from two female Asian elephants were measured for the period of 32 months from February 1987 to September 1989. Serum samples were collected weekly from unanesthetized elephants. Each elephant showed eight ovarian cycles in 32 months. Ovarian cycles, characterized by changes in concentrations of serum progesterone, averaged 16.8 (+/-) 0.6 (mean (+/-) SEM, n = 14) weeks in length. The changes in concentrations of testosterone in the serum showed a similar pattern to those of progesterone with a striking increase noted during the luteal phase. The highest levels of serum estradiol-17β were noted when progesterone levels showed low basal values. These results suggest that estradiol-17β may be an index of follicular maturation during the estrous cycle in Asian elephants, and that the ovaries of Asian elephants may produce testoserone in the luteal phase.

Salivary Cortisol Assessment for Stress Detection in the Asian Elephant (*Elephas maximus*): A Pilot Study Holger H. Dathe, Bernd Kuckelkorn and Dieter Minnemann. (1992). *Zoo Biology,* 11(4): 285

Effects of introducing an unfamiliar female into an Asian elephant herd at Tierpark, Berlin were monitored by means of salivary cortisol assessment. Saliva samples were obtained from a second female for comparative purposes. The period of familiarization was characterized by an enhanced cortisol level in both animals, with a maximum on the second day after joining. Cortisol returned to normal on the following day. Manipulations of the keepers caused a transitory increse on two other days. Possibilities for the use of this noninvasive method of stress monitoring in various management situations are indicated.