Elephant References: Testosterone
Elephant Care International Database
www.elephantcare.org
Accessed 1 March 2018

Brown, J. L., M. Bush, D. E. Wildt, J. P. Raath, V. de Vos and J. G. Howard (1993). "**Effects of GnRH analogues on pituitary-testicular function in free-ranging African elephants (Loxodonta africana)**." Journal of Reproduction and Fertility **99**(2): 626-634.

In the first of 2 experiments, 6 free-living adult male elephants were given 4 or 12 mg GnRH antagonist (Detirelix) or saline intramuscularly on day 0. Animals were then recaptured about 48 h later and given 300 µg GnRH intravenously to assess the ability of the antagonist to block pituitary activity. Detirelix reduced (P<0.05) basal concentrations of serum LH and testosterone on day 2 compared with day 0, with no effect of dose. Similarly, LH and testosterone release induced by GnRH were also reduced (P<0.05) in the Detirelix-treated bulls (50-70% reduction in peak concentration). In the 2nd experiment, elephants were given 30 mg of a structurally similar GnRH antagonist (103-201-40; n = 6), 22.5 mg of a long-acting GnRH agonist (Lupron Depot; n = 4) or D-mannitol carrier (n = 4) intramuscularly on day 0. All bulls were recaptured and given GnRH on day 2 (103-201-40 treatment group) or on days 2 and 20 (Lupron Depot group) after the initial injection. In contrast to Detirelix, the antagonist 103-201-40 did not inhibit basal or GnRH-induced LH or testosterone secretion. Pituitary-testicular responses to Lupron Depot were initially stimulatory, as evidence by increased (P<0.05) LH and testosterone secretion on days 0 and 2. By day 20, basal LH concentrations had returned to baseline values and the response to GnRH was markedly reduced (P<0.05), indicating that the pituitary was at least partially desensitized. Basal testosterone concentrations had also returned to baseline values by day 20 after Lupron Depot treatment. However, despite the attenuated LH response to GnRH, subsequent testosterone secretion was increased (P<0.05) compared with controls, suggesting that the testes of agonist-treated bulls had become hyper-responsive to small increases in LH secretion. It is suggested that GnRH analogues can suppress the pituitary-gonadal axis in African elephants. However, longer treatment periods, more frequent injection intervals or higher doses are probably needed to completely inhibit testosterone secretion and, thus, musth.

Brown, J. L., F. Goritz, N. Pratt-Hawkes, R. Hermes, M. Galloway, L. H. Graham, C. Gray, S. L. Walker, A. Gomez, R. Moreland, S. Murray, D. L. Schmitt, J. Howard, J. Lehnhardt, B. Beck, A. Bellem, R. Montali and T. B. Hildebrandt (2004). "**Successful artificial insemination of an Asian elephant at the National Zoological Park**." Zoo Biology **23**(1): 45-63.

For decades, attempts to breed elephants using artificial insemination (AI) have failed despite considerable efforts and the use of various approaches. However, recent advances in equipment technology and endocrine-monitoring techniques have resulted in 12 elephants conceiving by AI within a 4-year period (19982002). The successful AT technique employs a unique endoscope-guided catheter and transrectal ultrasound to deliver semen into the anterior vagina or cervix, and uses the "double LH surge" (i.e., identifying the anovulatory LH (anLH) surge that predictably occurs 3 weeks before the ovulatory LH (ovLH) surge to time insemination. This study describes the 6-year collaboration between the National Zoological Park (NZP) and the Institute for Zoo Biology and Wildlife Research (IZW), Berlin, Germany, that led to the refinement of this AI technique and subsequent production of an Asian elephant calf. The NZP

female was the first elephant to be inseminated using the new AI approach, and was the fifth to conceive. A total of six AI trials were conducted beginning in 1995, and conception occurred in 2000. Semen was collected by manual rectal stimulation from several bulls in North America. Sperm quality among the bulls was variable and was thus a limiting factor for AI. For the successful AI, semen quality was good to excellent (75-90% motile sperm), and sperm was deposited into the anterior vagina on the day before and the day of the ovLH surge. Based on transrectal ultrasound, ovulation occurred the day after the ovLH surge. Pregnancy was monitored by serum and urinary progestagen, and serum prolactin analyses in samples collected weekly. Fetal development was assessed at 12, 20, and 28 weeks of gestation using transrectal ultrasound. Elevated testosterone measured in the maternal circulation after 36 weeks of gestation reliably predicted the calf was a male. Parturition was induced by administration of 40 IU oxytocin 3 days after serum progestagens dropped to undetectable baseline levels. We conclude that AI has potential as a supplement to natural breeding, and will be invaluable for improving the genetic management of elephants, provided that problems associated with inadequate numbers of trained personnel and semen donors are resolved.

Brown, J. L., M. Somerville, H. S. Riddle, M. Keele, C. K. Duer and E. W. Freeman (2007). "**Comparative endocrinology of testicular, adrenal and thyroid function in captive Asian and African elephant bulls**." General and Comparative Endocrinology **151**(2): 153-162.

Concentrations of serum testosterone, cortisol, thyroxine (free and total T4), triiodothyronine (free and total T3) and thyroid stimulating hormone (TSH) were measured to assess adrenal and thyroid function as they relate to testicular activity and musth in captive elephants. Blood samples were collected approximately weekly from Asian (n = 8) and African (n = 12) bulls at seven facilities for periods of 4 months to 9.5 years. Age ranges at study onset were 8-50 years for Asian and 10-21 years for African elephants. Based on keeper logs, seven Asian and three African bulls exhibited behavioral and/or physical (temporal gland secretion, TGS, or urine dribbling, UD) signs of musth, which lasted 2.8 ± 2.5 months in duration. Serum testosterone was elevated during musth, with concentrations often exceeding 100 ng/ml. Patterns of testosterone secretion and musth varied among bulls with no evidence of seasonality ($P > 0.05$). Only three bulls at one facility exhibited classic, well-defined yearly musth cycles. Others exhibited more irregular cycles, with musth symptoms often occurring more than once a year. A number of bulls (1 Asian, 9 African) had consistently low testosterone (<10 ng/ml) and never exhibited significant TGS or UD. At facilities with multiple bulls (n = 3), testosterone concentrations were highest in the oldest, most dominant male. There were positive correlations between testosterone and cortisol for six of seven Asian and all three African males that exhibited musth (range, $r = 0.23$-0.52; $P < 0.05$), but no significant correlations for bulls that did not ($P > 0.05$). For the three bulls that exhibited yearly musth cycles, TSH was positively correlated (range, $r = 0.22$-0.28; $P < 0.05$) and thyroid hormones (T3, T4) were negatively correlated (range, $r = -0.25$ to -0.47; $P < 0.05$) to testosterone secretion. In the remaining bulls, there were no clear relationships between thyroid activity and musth status. Overall mean testosterone and cortisol concentrations increased with age for all bulls combined, whereas thyroid activity declined. In summary, a number of bulls did not exhibit musth despite being of adequate physical maturity. Cortisol and testosterone were correlated in most bulls exhibiting musth, indicating a possible role for the adrenal gland in modulating or facilitating downstream responses. Data were generally inconclusive as to a role for thyroid hormones in male reproduction, but the finding of discrete patterns in bulls showing clear testosterone cycles suggests they may facilitate expression or control of musth in some individuals.

Buchanan, K. L. and A. R. Goldsmith (2004). "**Noninvasive endocrine data for behavioural studies: The importance of validation.**" Animal Behaviour **67**: 183-185.

There has been a substantial growth recently in the use of noninvasive methods to quantify hormone production, through the measurement of excreted hormones or hormone levels from saliva, sweat or hair (e.g.Wasser et al. 2000; Cook 2002; Pfeffer et al. 2002). These measures can quantify either current (e.g. Berg & Wynne-Edwards 2002; Maso et al. 2002) or past (e.g. Wasser et al. 2000; Ostner et al. 2002) levels of hormone production and the data can be used to determine the relations between a range of hormone levels and animal behaviour across taxa (Wasser et al. 2000). Such techniques have been used extensively to examine social stress (Goymann et al. 2001), the effects of environmental stress (Creel et al. 2002), reproductive cycles (Curtis et al. 2000) and social dominance (von Engelhardt et al. 2000; Langmore et al. 2002). They may have important applications in conservation science (Ishii 1999). There are several reasons why noninvasive methods of sampling are highly desirable. Importantly, animal suffering can potentially be reduced. In practical terms there are also several advantages: noninvasive methods allow samples to be obtained retrospectively, which represent average hormone production over a certain time frame, and the time spent handling the animal does not affect the levels obtained, which is advantageous for highly pulsatile hormones such as corticosteroids. In addition, the licensing constraints for noninvasive methods of sampling are less restrictive. However, such techniques also have disadvantages. In particular, faecal, hair or feather samples can indicate only average hormone levels over a considerable, and possibly unknown, period. Compared with plasma levels, noninvasive measures may result in a loss of sensitivity in any further analyses examining the relations between hormone levels and other variables (Shirtcliff et al. 2002). Furthermore, faecal samples in particular may not be available from known individuals a known amount of time after excretion, preventing reliable determination of individual hormone levels. It is also worth considering that while noninvasive sampling will not cause large increases in pulsatile 'stress' hormones as caused by capture and restraint, some increase may occur merely as a result of the presence of the sampler. In addition, there are a number of validation issues concerning the quantification of steroids from noninvasive samples which we outline below. Koren et al. (2002) documented a protocol for the extraction of testosterone and cortisol from hair obtained from the rock hyrax, Procavia capensis. They used this technique to quantify the levels of hormones contained in plucked hair samples, allowing hormone levels during the period of hair production to be determined, noninvasively. They found that the levels of testosterone extracted correlated positively with the dominance rank of male hyraxes. Although such methods are highly desirable, it is important to emphasize that all new methods of measuring levels of hormone production using hormone extracted from organic substrates should be appropriately validated, such that the limitations of the technique can be defined. This requires: (1) that the assay is validated for each new species and substrate and (2) that the extraction efficiency is determined for the target hormone in the species and substrate of interest. Although ready-made endocrine kits are provided with some data on the assay validation, the validation is relevant only for the species and substrate tested by the commercial supplier, generally in a limited range of biological media. It is essential to extend these validations for the species and substrate to which the kit is being applied. For example, a methanol extract of hair may contain substances that interfere with the assay procedure and thus would give misleading results.

Buss, I. O. and O. W. Johnson (1967). "**Relationships of leydig cell characteristics and intratesticular testosterone levels to sexual activity in the African elephant**." The Anatomical Record **157**(2): 191-196.

Histological characteristics of testis tissues from 25 African elephants (Loxodonta africana) collected in Uganda, showed no consistent relationships among the following variables: Leydig cell size, cytoplasmic characteristics, and abundance; testicular testosterone content; and age. From these findings, plus field observations of sexual behavior, emerges the hypothesis that individual cyclicity in Leydig cell function was inherent in the elephant population studied. Testosterone content of testes from 32 elephants (including the 25 studied histologically) suggested that lone bulls were not of a senile nature since they contained relatively large quantities of testosterone and were relatively young (from about 12 to 25 years of age). Also, lone bulls were observed searching out estrous females. Among bulls collected from family units and herds, testosterone levels and behavior differed conspicuously. Behavior appeared to be directly related to testosterone content in several instances. Nonaggressive behavior among members of bull herds, plus the high proportion of such aggressive behavior among members of bull herds, plus the high proportion of such individuals with low testosterone content, suggest that some of these animals were in a depressed phase of sexual activity whereas others were undergoing pubertal development. Copyright © 1967 Wiley-Liss, Inc.

Cheeran, J. V., K. Radhakrishnan and K. Chandrasekharan (2002). "**Musth**." Journal of Indian Veterinary Association Kerala **7**(3): 28-30.

Cooper, K. A., J. D. Harder, D. H. Clawson, D. L. Fredrick, G. A. Lodge, H. C. Peachey, T. J. Spellmire and D. P. Winstel (1990). "**Serum testosterone and musth in captive male African and Asian elephants**." Zoo Biol **9**: 297-306.

Testosterone concentrations in serum samples collected weekly over a 5-year period from a young adult male Asian elephant (*Elephas maximus*) and a young adult male African forest elephant (*Loxodonta africana cyclotis*) were measured by radioimmunoassay. Testosterone profiles during this maturational period were compared between the two species and related to the occurrence of *musth*, a recurring physiological and behavioral condition exhibited by most mature Asian, and some African, bull elephants. Musth is characterized by secretion from the bull's temporal glands, dribbling urine, and increased aggression. Serum testosterone concentrations in the Asian bull were elevated substantially between April and September each year, coincident with the presence of temporal gland secretion, urine dribbling, and aggressive behavior. Testosterone levels from April through September averaged (± SEM) 41.2 ± 2.8 ng/ml, compared to 7.6 ± 1.0 ng/ml during the rest of the year. In contrast, the testosterone profile of the African bull showed greater variability and lower levels overall, the only pattern being a tendency for levels to be lowest from November to February (avg. 6.8 ± 1.5 vs. 10.3 ± 0.8 ng/ml during the rest of the year). Temporal gland secretion and other signs of musth were first observed in this bull in 1988, at age 17. While his testosterone values did not show a pattern comparable to that in the Asian bull, average testosterone values were significantly greater in 1988 compared to previous years. The Asian bull showed sexual attention to preovulatory (estrous) cows whether in musth or not, and exposure to estrous cows did not appear to alter the highly consistent, annual pattern of musth as evidenced by temporal gland flow.

de Oliveira, C. A., G. D. West, R. Houck and M. Leblanc (2004). "**Control of musth in an Asian elephant bull (Elephas maximus) using leuprolide acetate
716**." J. Zoo. Wildl. Med **35**(1): 70-76.

The results of long-term administration of leuprolide acetate (LA) depot in a 52-yr-old Asian elephant bull (Elephas maximus) for control of musth are presented. Twelve injections were administered for 6 yr during our interpretation of early musth or "premusth." Intervals between musth periods during the study varied from 2 to 34 mo. Blood samples, drawn weekly, were assayed for serum testosterone concentrations; mean levels were 11.78 +/- 1.97 nmol/L throughout the first 26 mo of the study, 7.28 +/- 1.28 nmol/L during the following 21 mo, and 0.45 +/- 0.035 nmol/L in the last 34 mo of this study. Early musth signs ceased within 3 days of drug administration after 10 of 12 injections. The mean serum testosterone concentrations were significantly decreased by the last 34 mo of the study. The results suggest leuprolide is a suitable alternative for controlling or preventing (or both) musth in captive Asian elephants, although permanent reproductive effects may occur. Zoos and wildlife conservation institutions could benefit from the use of LA in Asian elephants to increase the male availability in captivity, consequently ensuring genetic diversity and the perpetuation of the species

De Oliveira, C. A., G. D. West, R. Houck and M. Leblanc (2004). "**Control of musth in an Asian elephant bull (Elephas maximus) using leuprolide acetate**." Journal of Zoo and Wildlife Medicine **35**(1): 70-76.

The results of long-term administration of leuprolide acetate (LA) depot in a 52-yr-old Asian elephant bull (Elephas maximus) for control of musth are presented. Twelve injections were administered for 6 yr during our interpretation of early musth or "premusth." Intervals between musth periods during the study varied from 2 to 34 mo. Blood samples, drawn weekly, were assayed for serum testosterone concentrations; mean levels were 11.78 ± 1.97 nmol/L throughout the first 26 mo of the study, 7.28 ± 1.28 nmol/L during the following 21 mo, and 0.45 ± 0.035 nmol/L in the last 34 mo of this study. Early musth signs ceased within 3 days of drug administration after 10 of 12 injections. The mean serum testosterone concentrations were significantly decreased by the last 34 mo of the study. The results suggest leuprolide is a suitable alternative for controlling or preventing (or both) musth in captive Asian elephants, although permanent reproductive effects may occur. Zoos and wildlife conservation institutions could benefit from the use of LA in Asian elephants to increase the male availability in captivity, consequently ensuring genetic diversity and the perpetuation of the species.

deOliveira, C. A., G. D. West, R. Houck and M. Leblanc (2004). "**Control of musth in an Asian elephant bull (*Elephas maximus)* using leuprolide acetate**." Journal of Zoo and Wildlife Medicine **35**(1): 70-76.

The results of long-term administration of leuprolide acetate (LA) depot in a 52-yr-old Asian elephant bull (*Elephas maximus)* for control of musth are presented. Twelve injections were administered for 6 yr during our interpretation of early musth or "premusth". Intervals between musth periods during the study varied from 2 to 34 mo. Blood samples, drawn weekly, were assayed for serum testosterone concentrations; mean levels were 11.78 +/- 1.97 nmol/L throughout the first 26 mo of the study. 7.28 +/- 1.28 nmol/L during the following 21 mo. and 0.45 +/- 0.035 nmol/L in the last 34 mo of this study. Early musth signs ceased within 3 days of drug administration after 10 of 12 injections. The mean serum testosterone concentrations were significantly decreased by the last 34 mo of the study. The results suggest leuprolide is a suitable alternative for controlling or preventing (or both) musth in captive Asian elephants, although permanent reproductive effects may occur. Zoos and wildlife conservation institutions could benefit from the use of LA in Asian elephants to increase the male availability in captivity, consequently ensuring genetic diversity and perpetuation of the species.

Diaz-Samayoa-de-Aguirre, L. (1990). **Sex hormones in blood plasma, urine and faeces from female and**

male Indian elephants. Hannover, Germany, Tierarztliche Hochschule**:** 1-145.

Dickerman, R. D., N. Y. Zachariah, M. Fouraker and W. J. McConathy (1997). "**Neuroendocrine-associated behavioral patterns in the male Asian elephant (Elephas maximus)**." Physiology and Behavior **61**(5): 771-773.

Steroid-responsive behaviors have been reported in various species; however, the reports thus far on the male Asian elephant (bull) during musth are few in number and most have been conducted on single captive animals for short time periods. The purpose of this investigation was to perform a longitudinal study on steroid-responsive behavior in 3 male Asian elephants from a captive herd of 11 male Asian elephants in Nepal. Male Asian elephants were 18, 25, and 43 years old. The animals had serum collected for 11 months and were observed on a daily basis for aggressive behavior according to the Species Survival Plan (SSP) collection protocol on SSP data sheets. Testosterone (T) and dihydrotestosterone (DHT) were measured in each animal by radioimmunoassay. Testosterone levels rose during musth 26-fold compared to nonmusth, and DHT was elevated 12-fold in musth. Maximal aggressive behavior episodes occurred during peak elevations of T and DHT, with correlation coefficients of 0.82 and 0.89, respectively. Therefore, we suggest that the aggressive episodes are dependent on elevated circulating androgens acting on androgen-responsive neural tissues.

Duer, C., M. Carden, D. Schmitt and T. Tomasi (2002). "**Utility of maternal serum total testosterone analysis for fetal gender determination in Asian elephants (Elephas maximus)**." Anim Reprod Sci **69**(1-2): 47-52.

It has been shown in some species that fetal testes produce testosterone early in gestation. This study investigated the possibility that fetal testosterone may be reflected in maternal serum levels in the Asian elephant (Elephas maximus). Weekly serum samples were collected from seventeen pregnant captive Asian elephants and analyzed via radioimmunoassay (RIA) for total testosterone levels. Nine of the cows carried male fetuses and eight carried female fetuses. A non-random pattern over time ($P<0.01$) was observed in cows carrying either a male or female fetus. Mean maternal serum total testosterone was significantly higher in cows carrying male versus female fetuses ($P<0.01$). Mean trimester values indicate that first trimester values are not significantly different among male versus female groups. The second and third trimester values of cows carrying male fetuses were higher than cows carrying female fetuses, ($P<0.01$ and <0.05, respectively). The results of this study show that it is possible via RIA of maternal serum for total testosterone to determine the gender of calves during gestation.

Duer, C., M. Carden and T. Tomasi (2007). "**Detection of fetal gender differences in maternal serum progesterone concentrations of Asian elephants (Elephas maximus) 486**." Anim Reprod. Sci **97**(3-4): 278-283.

Previous studies have analyzed total testosterone concentrations in maternal serum for a reliable method of fetal gender determination in Asian elephants (Elephas maximus). The present study investigated the possibility that progesterone concentrations in maternal serum may reflect these testosterone patterns. Weekly serum samples were collected from 17 pregnancies in captive Asian elephants and analyzed via radioimmunoassay (RIA) for progesterone concentrations. Nine and eight cows carried male and female calves, respectively. Mean progesterone concentrations in maternal serum of elephants carrying male calves were greater than in those carrying female calves ($P<0.01$). Mean progesterone concentrations (based on 5-week means) in maternal serum were greater at weeks 20-55 ($P<0.01$) and 60-65 ($P<0.05$)

for elephants carrying male calves

Fritsch, G., R. Hermes and J. Maltzan (2001). **New Aspects of Sexual Maturation in Male Elephants**. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Ganswindt, A., R. Palme, M. Heistermann, S. Borragan and J. K. Hodges (2003). "**Non-invasive assessment of adrenocortical function in the male African elephant (Loxodonta africana) and its relation to musth**." Gen Comp Endocrinol **134**(2): 156-166.

German Primate Centre, Department of Reproductive Biology, Kellnerweg 4, 37077 Gottingen, Germany. ganswindt@www.dpz.gdwg.de

Adult male elephants periodically show the phenomenon of musth, a condition associated with increased aggressiveness, restlessness, significant weight reduction and markedly elevated androgen levels. It has been suggested that musth-related behaviours are costly and that therefore musth may represent a form of physiological stress. In order to provide data on this largely unanswered question, the first aim of this study was to evaluate different assays for non-invasive assessment of adrenocortical function in the male African elephant by (i) characterizing the metabolism and excretion of [3H]cortisol (3H-C) and [14C]testosterone (14C-T) and (ii) using this information to evaluate the specificity of four antibodies for determination of excreted cortisol metabolites, particularly with respect to possible cross-reactions with androgen metabolites, and to assess their biological validity using an ACTH challenge test. Based on the methodology established, the second objective was to provide data on fecal cortisol metabolite concentrations in bulls during the musth and non-musth condition. 3H-C (1 mCi) and 14C-T (100 microCi) were injected simultaneously into a 16 year old male and all urine and feces collected for 30 and 86 h, respectively. The majority (82%) of cortisol metabolites was excreted into the urine, whereas testosterone metabolites were mainly (57%) excreted into the feces. Almost all radioactive metabolites recovered from urine were conjugated (86% 3H-C and 97% 14C-T). In contrast, 86% and >99% of the 3H-C and 14C-T metabolites recovered from feces consisted of unconjugated forms. HPLC separations indicated the presence of various metabolites of cortisol in both urine and feces, with cortisol being abundant in hydrolysed urine, but virtually absent in feces. Although all antibodies measured substantial amounts of immunoreactivity after HPLC separation of peak radioactive samples and detected an increase in glucocorticoid output following the ACTH challenge, only two (in feces against 3alpha,11-oxo-cortisol metabolites, measured by an 11-oxo-etiocholanolone-EIA and in urine against cortisol, measured by a cortisol-EIA) did not show substantial cross-reactivity with excreted 14C-T metabolites and could provide an acceptable degree of specificity for reliable assessment of glucocorticoid output from urine and feces. Based on these findings, concentrations of immunoreactive 3alpha,11-oxo-cortisol metabolites were determined in weekly fecal samples collected from four adult bulls over periods of 11-20 months to examine whether musth is associated with increased adrenal activity. Results showed that in each male levels of these cortisol metabolites were not elevated during periods of musth, suggesting that in the African elephant musth is generally not associated with marked elevations in glucocorticoid output. Given the complex nature of musth and the variety of factors that are likely to influence its manifestation, it is clear, however, that further studies, particularly on free-ranging animals, are needed before a possible relationship between musth and adrenal function can be resolved. This study also clearly illustrates the potential problems associated

with cross-reacting metabolites of gonadal steroids in EIAs measuring glucocorticoid metabolites. This has to be taken into account when selecting assays and interpreting results of glucocorticoid metabolite analysis, not only for studies in the elephant but also in other species.

Ghosal, R., A. Ganswindt, P. B. Seshagiri and R. Sukumar (2013). "**Endocrine correlates of musth in free-ranging Asian elephants (Elephas maximus) determined by non-invasive faecal steroid hormone metabolite measurements**." PLoS ONE **8**(12).

The occurrence of musth, a period of elevated levels of androgens and heightened sexual activity, has been well documented for the male Asian elephant (Elephas maximus). However, the relationship between androgendependent musth and adrenocortical function in this species is unclear. The current study is the first assessment of testicular and adrenocortical function in free-ranging male Asian elephants by measuring levels of testosterone (androgen) and cortisol (glucocorticoid - a physiological indicator of stress) metabolites in faeces. During musth, males expectedly showed significant elevation in faecal testosterone metabolite levels. Interestingly, glucocorticoid metabolite concentrations remained unchanged between musth and non-musth periods. This observation is contrary to that observed with wild and captive African elephant bulls and captive Asian bull elephants. Our results show that musth may not necessarily represent a stressful condition in free-ranging male Asian elephants. © 2013 Ghosal et al.

Glickman, S. E., R. V. Short and M. B. Renfree (2005). "**Sexual differentiation in three unconventional mammals: spotted hyenas, elephants and tammar wallabies
566**." Horm. Behav **48**(4): 403-417.

The present review explores sexual differentiation in three non-conventional species: the spotted hyena, the elephant and the tammar wallaby, selected because of the natural challenges they present for contemporary understanding of sexual differentiation. According to the prevailing view of mammalian sexual differentiation, originally proposed by Alfred Jost, secretion of androgen and anti-Mullerian hormone (AMH) by the fetal testes during critical stages of development accounts for the full range of sexually dimorphic urogenital traits observed at birth. Jost's concept was subsequently expanded to encompass sexual differentiation of the brain and behavior. Although the central focus of this review involves urogenital development, we assume that the novel mechanisms described in this article have potentially significant implications for sexual differentiation of brain and behavior, a transposition with precedent in the history of this field. Contrary to the "specific" requirements of Jost's formulation, female spotted hyenas and elephants initially develop male-type external genitalia prior to gonadal differentiation. In addition, the administration of anti-androgens to pregnant female spotted hyenas does not prevent the formation of a scrotum, pseudoscrotum, penis or penile clitoris in the offspring of treated females, although it is not yet clear whether the creation of masculine genitalia involves other steroids or whether there is a genetic mechanism bypassing a hormonal mediator. Wallabies, where sexual differentiation occurs in the pouch after birth, provide the most conclusive evidence for direct genetic control of sexual dimorphism, with the scrotum developing only in males and the pouch and mammary glands only in females, before differentiation of the gonads. The development of the pouch and mammary gland in females and the scrotum in males is controlled by genes on the X chromosome. In keeping with the "expanded" version of Jost's formulation, secretion of androgens by the fetal testes provides the best current account of a broad array of sex differences in reproductive morphology and endocrinology of the spotted hyena, and androgens are essential for development of the prostate and penis of the wallaby. But the essential

circulating androgen in the male wallaby is 5alpha androstanediol, locally converted in target tissues to DHT, while in the pregnant female hyena, androstenedione, secreted by the maternal ovary, is converted by the placenta to testosterone (and estradiol) and transferred to the developing fetus. Testicular testosterone certainly seems to be responsible for the behavioral phenomenon of musth in male elephants. Both spotted hyenas and elephants display matrilineal social organization, and, in both species, female genital morphology requires feminine cooperation for successful copulation. We conclude that not all aspects of sexual differentiation have been delegated to testicular hormones in these mammals. In addition, we suggest that research on urogenital development in these non-traditional species directs attention to processes that may well be operating during the sexual differentiation of morphology and behavior in more common laboratory mammals, albeit in less dramatic fashion

Goymann, W. (2012). "**On the use of non-invasive hormone research in uncontrolled, natural environments: The problem with sex, diet, metabolic rate and the individual**." Methods in Ecology and Evolution **3**(4): 757-765.

1. Methods to measure metabolites of steroid hormones from faeces have become very popular in wildlife conservation and ecology, because they allow gathering physiological data without the necessity to capture the animals. However, this advantage comes at costs that are particularly relevant when studying free-living animals in their natural environments. Previous methodological reviews have stressed the importance of validations to prove that real metabolites of the hormone in question are measured, but the research community has largely ignored further caveats relating to sex, diet, metabolic rate and individual differences in hormone metabolite formation. 2. Often the sexes differ in how they metabolize hormones. As a consequence, one may not be able to compare hormone metabolite concentrations between males and females of one species. 3. Diet can alter the way hormones are metabolized, and different diets can change the amount of faecal bulk. Both phenomena can result in measurement artefacts that may seriously distort the estimation of hormone metabolite concentrations. As a consequence, comparisons of hormone metabolite concentrations, for example, between seasons or populations, may become problematic. 4. Changes in ambient temperature and food availability may trigger large fluctuations in metabolic rate of free-living animals. These fluctuations may then result in major distortions of faecal hormone metabolite concentrations without any change in bioactive hormone levels. 5. Bacteria metabolize hormones in the gut. Individual differences in bacterial composition can cause differences in how hormones are decomposed. Thus, individuals may differ with regard to what kind of hormone metabolites they form and with regard to the relative composition of these hormone metabolites. As only specific metabolites are measured, differences in metabolismmay distort the results. 6. In summary, non-invasive hormone research measures various end products of a hormone after its clearance from the circulation and extensive modification by bacteria. Not only does this increase random variance, it may also generate systematic noise, which may seriously distort the signal (i.e. the hormonal status of the individual) in a non-random manner. Thus, we still need to learn much more about whether this widely used technique reliably measures the physiological status of animals in uncontrolled environments.

Hall-Martin, A. J. (1987). "**The role of musth in the reproductive strategy of the African elephant (*Loxodonta africana*)**." S. Afr. J. Sci **83**: 616-620.

Behavioral and endocrinological data on African elephants (*Loxodonta africana*) are integrated

to provide a hypothesis of the adaptations reflected in the phenomenon of musth in bulls. Occupation of home ranges, movements, male dominance hierarchies and intra-specific agonistic behavior are reviewed. Bulls in musth leave their home range, travel far and fast, imitate more contacts with distant breeding herds, show aggression which overrides normal social male hierarchies, probably mate more frequently than non-musth bulls and then return to their home range. This behaviour is associated with elevated levels of serum testosterone and dihydrotestosterone. Elephants normally show a high degree of fidelity to sexually segregated adjoining home ranges, which results in regular contact between the same bulls and cows. This breeding strategy is applicable to older, dominant bulls within the locally resident hierarchy. The musth adaptation is a second strategy, whereby younger, lower ranking bulls (25-35 years) can ensure more contacts with cows and maximize their chances of breeding. Because musth bulls mate far from their normal ranges the strategy promotes gene flow and ensures outbreeding. In English with Afrikaans summary.

Hall-Martin, A. J. and L. A. van der Walt (1984). "**Plasma testosterone levels in relation to musth in the male African elephant**." Koedoe **27**: 147-149.

Heistermann, M. and J. P. Higham (2015). "**Urinary neopterin, a non-invasive marker of mammalian cellular immune activation, is highly stable under field conditions**." Scientific Reports **5**.

Studying immunity and immune function in ecology and evolution requires field studies, but there has been a dearth of non-invasive markers of immune activation available for studying large wild mammals. Recently, we analytically and biologically validated the measurement of urinary neopterin (NEO), a biomarker of cellular immune activation, in captive macaques. However, applying this to free-ranging settings is complicated by issues involving sample collection, processing, storage, and transport. Here, we collected urine samples from captive macaques and undertook experiments simulating common field issues. We tested the effects on urinary NEO sample measurements following: dirt and faecal contamination; storage at room temperature; differences in processing and long-term storage methods (freezing, lyophilising, blotting onto filter paper); and freeze-thaw cycles. Our results show that concentrations of urinary NEO are highly stable - they are not affected by soil or faecal contamination, can be collected on filter paper and stored for many months frozen or lyophilised with minimal effect, and are resistant to multiple 24 hr freeze-thaws. With the addition of a biocidal preservative, concentrations are even stable at room temperature for long periods. Urinary NEO is remarkably resilient, and is highly suitable for non-invasive field studies of cellular immune responses in wild large mammals.

Hildebrandt, T. B., G. Fritsch, R. Hermes, K. Jewgenow, M. Rudolph, J. Maltzan, H. Wiesner, N. C. Pratt, D. L. Schmitt and F. Goritz (1999). **Ultrasound Monitoring of the Sexual Maturation in the Male Elephant**. 1999 Proceedings of the American Association of Zoo Veterinarians.

In general, the reproductive rate of elephants in captivity is low. This is partly because of logistic difficulties associated with transporting these large animals for breeding purposes and there may be physiologic problems which also contribute to this low reproductive rate. In context with a reproductive assessment of potential breeding bulls it appears that many adult bulls of both species (*Loxodonta africana* and *Elephas maximus*) are not producing viable sperms and/or sufficient ejaculate. Our current understanding of incomplete sexual maturation or temporary infertility in male elephants is at best fragmentary. The following study was performed for characterizing the physiologic sexual maturation process in young male elephants. Two

adolescent individuals of both species have been examined in order to investigate the time of their sexual maturity. The examination utilized transrectal ultrasonography of the urogenital tract, rectal stimulation for the collection of ejaculates as well as blood samples for plasma testosterone determination. The development of the testes, the accessory glands (especially the ampullae), the concentration of the testosterone, the body-height and the success of ejaculation after manual stimulation was documented and evaluated over a 3-yr period. The results were compared with data from other bull elephants which had ultrasonographic examinations or post mortem investigations. The findings of this study led to important conclusions about the characterization of the reproductive status of male elephants by means of ultrasonographic examinations. We established criteria for reproductive soundness in connection with the recruitment of potential semen donors for future artificial insemination projects. The ultrasonographic examination combined with the semen collection were appropriate methods for characterizing the exact state of sexual maturity or for identifying potential reproductive disorders in male elephants.

Howard, J., M. Bush, V. De Vos and D. E. Wildt (1984). "**Electroejaculation, semen characteristics and serum testosterone concentrations of free-ranging African elephants (Loxodonta africana)**." Journal of Reproduction and Fertility **72**(1): 187-195.

A regimented electroejaculation protocol (120 electrical stimulations; 10-30 V) was used to collect semen and characterize ejaculate quality from 9 adult, free-ranging African elephants under anaesthesia. Eight of the 9 ejaculates contained high concentrations of progressively motile spermatozoa. The overall mean ejaculate volume, sperm concentration/ml ejaculate, sperm motility, sperm status and ejaculate pH were 93.3 ml, 2408.6 x 106 spermatozoa/ml, 70%, 3.9 and 7.4, respectively. A high percentage (mean 77.5%) of spermatozoa within each ejaculate was morphologically normal. Of the aberrant spermatozoa, 72% had a cytoplasmic droplet defect. When sperm viability was tested in vitro at 37°C, sperm motility rating declined by at least half of the initial assessment within 3.5 h of semen collection. Generally, spermatozoa maintained motility in vitro for <6 h. Serum testosterone ranged from 1.4 to 8.2 ng/ml in 4 males evaluated in the morning (07:30-08:00 h). In 4 of the 5 bulls assessed in the afternoon (15:00-18:00 h), testosterone levels were <0.9 ng/ml. The remaining bull evaluated at 16:00 h, had exceptionally high testosterone concentrations (peak 25.6 ng/ml) and a preputial discharge potentially indicative of 'musth'. The present study demonstrates that high quality semen can be collected consistently from the African elephant and that striking differences exist in serum testosterone amongst free-ranging males which may be due, in part, to a diurnal rhythm.

Jainudeen, M. R., C. B. Katongole and R. V. Short (1972). "**Plasma testosterone levels in relation to musth and sexual activity in the male Asiatic elephant, *Elephas maximus***." J. Reprod. Fertil **29**(1): 99-103.

Testosterone was measured in the peripheral blood plasma of eleven male Asiatic elephants, using a competitive protein-binding assay. When the animals showed no signs of musth, the testosterone levels were low (<0.2 to 1.4 ng/ml); as they began to come into musth and the temporal glands started to enlarge, the testosterone levels rose (4.3 to 13.7 ng/ml), and when the animals were in full musth, with discharging temporal glands and an aggressive temperment, the levels were extremely high (29.6 to 65.4 ng/ml). Musth may therefore be comparable to the rutting behavior of some seasonally breeding mammals, although, in the elephant, there is some indication that it may be induced by sexual activity.

Kaewmanee, S., G. Watanabe, M. Keio, Y. Yamamoto, T. Yamamoto, M. Kishimoto, K. Nagaoka, E. Narushima, M. Katayanagi, R. Nakao, Y. Sakurai, S. Morikubo, M. Kaneko, M. Yoshihara, T. Yabe and K. Taya (2011). "**A surge-like increase in luteinizing hormone preceding musth in a captive bull African elephant (Loxodonta africana)**." J Vet Med Sci **73**(3): 379-383.

This study was conducted to determine the correlation between reproductive hormones and musth in a male African elephant. Changes in circulating luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and immunoreactive (ir-) inhibin and the degree of musth were evaluated for 4 years. LH increased 4 weeks before musth began. The highest concentrations of testosterone and ir-inhibin were observed from April to October. There were positive correlations among testosterone, ir-inhibin and musth behavior. These findings suggested that the surge-like LH in the pre-musth period might stimulate secretion of testosterone and ir-inhibin and thus initiate the musth behavior. This study also suggested that the high LH level before musth might be a useful biomarker for the beginning of the musth season.

Lincoln, G. A. and W. D. Ratnasooriya (1996). "**Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator**." Journal of Reproduction and Fertility **108**(1): 107-113.

Blood samples collected weekly over a 5-year period from 6 adult (19- to 40-year old) male Asian elephants (Elephas maximus maximus) living in captivity in Sri Lanka (7°N). Testosterone profiles were very variable within and between animals. Long-term phasic changes in blood concentrations of testosterone, associated with periods of musth (temporal gland secretion, drip urination and aggressive behaviour), occurred in 3 of the 6 elephants, the most pronounced cyclicity occurring in the oldest animal. Musth occurred annually after periods of high androgen secretion and the duration of musth was positively correlated with the mean concentration of testosterone during the previous 2 months. The time of musth, while consistent for an individual, varied between animals. In 4 bulls living in 1 social group, social rank was positively correlated with the mean concentration of testosterone over the 5-year period, and only the dominant animal showed periodic musth. Short-term changes in testosterone concentration occurred in blood samples collected every 15 min for 7 h, and after the injection of 20 µg GnRH, consistent with regulation through the pulsatile secretion of LH. The results support the view that fully mature male Asian elephants living near the equator express an asynchronous, cyclical, circannual pattern of gonadal activity. The periodic increase in testosterone secretion during the gonadal cycle induces the development of musth; however, androgen withdrawal following a period of hypersecretion may be the cause of some aspects of musth behaviour (aggression, unpredictability, disobedience) which make bull elephants very difficult to manage in captivity.

Lueders, I., T. B. Hildebrandt, C. Gray, S. Botha, P. Rich and C. Niemuller (2014). "**Supression of testicular function in a male asian elephant (Elephas maximus) treated with gonadotropin-releasing hormone vaccines**." Journal of Zoo and Wildlife Medicine **45**(3): 611-619.

The ability to control testosterone concentrations and sperm production is of great interest in both Asian (Elephas maximus) and African (Loxodonta africana) elephants. GnRH vaccination may pose an alternative to surgical castration. This is a case report of a male Asian elephant treated with two commercial GnRH vaccines (Equity® and Improvac®). Beginning at the age of 7 yr, the male was vaccinated monthly for 6 consecutive months, then every 6 mo and, finally, every 12 to 24 mo over a period of 6 yr. In order to evaluate the GnRH vaccine as a potential

method of immunologic castration, behavioral observations, testosterone level analysis, body weights, ultrasound examinations, and semen collection were part of the routine monitoring of this bull (no. 1) and a half-brother (bull 2) who remained untreated and served as control. The results showed a decrease in serum testosterone concentrations after the second booster. Levels stayed continuously below 5.0 ng/ml within the study period. The combined testicle diameter of 9.03 ± 0.3 cm prior to treatment had decreased to a size of 6.93 ± 0.19 cm ($P < 0.001$) when measured 2 yr later. Accessory sex gland fluid content disappeared and penile atrophy was observed. Semen collections yielded no spermatozoa 1 yr after the initial treatment. Bull 1 showed slowed weight gain as compared to bull 2 and, due to its friendly temperament and the absence of musth, remained in free contact. This report documents the GnRH vaccine as a possible noninvasive and inexpensive method for immunecastration. © 2014 American Association of Zoo Veterinarians.

Mcneilly, A. S., R. D. Martin, J. K. Hodges and G. L. Smuts (1983). "**Blood concentrations of gonadotropins, prolactin and gonadal steroids in males and in non-pregnant and pregnant female African elephants (*Loxodonta africana*)**." J. Reprod. Fertil **67**(1): 113-120.

No seasonal variation in any of the hormones measured was apparent in males or females. Testosterone levels in males increased around puberty (10-11 years) and remained significantly higher in adults than prepubertal males. This was not accompanied by any significant change in levels of LH, FSH or prolactin. In non-pregnant females there was no apparent difference in levels of LH, FSH or prolactin with age.There was a significant increase in progesterone around puberty (12 years) but there was considerable overlap in values between prepubertal and adult females. During pregnancy, progesterone levels were significantly higher than in non-pregnant females with maximum levels occurring at mid-pregnancy (9-12 months). However, there was considerable overlap in values between non-pregnancy and pregnancy. Concentrations of LH and FSH decreased significantly during mid-pregnancy while prolactin levels increased dramatically during pregnancy; after 7 months gestation until term levels were always at least 8ng/ml greater than in any non-pregnant female. It is suggested that this consistent increase in plasma/serum levels of prolactin can be used to diagnose pregnancy in the elephant.

Mouttham, L. L., M. Buhr, E. W. Freeman, T. M. Widowski, L. H. Graham and J. L. Brown (2011). "**Interrelationship of serum testosterone, dominance and ovarian cyclicity status in female African elephants**." Animal Reproduction Science **126**(1-2): 115-121.

The African elephant population in North American zoos is not self-sustaining, in part due to the prevalence of ovarian acyclicity. While little is known about the cause of this condition, earlier research has shown that females without cyclic corpus luteum (CL) function rank higher in the dominance hierarchy than females with cyclic CL function. The goal of this study was to measure longitudinal serum testosterone concentrations in captive female African elephants to determine if there is a relationship among serum testosterone concentrations, social dominance rank and ovarian cyclicity status. Weekly blood samples from 49 female African elephants (24 having and 25 not having cyclic CL function at 22 facilities) were collected over a 12-month period and analyzed for serum testosterone using an enzymeimmunoassay. A progesterone radioimmunoassay was used to quantify serum progestagen concentrations and categorize ovarian cyclicity status. The dominance hierarchy of individual elephants within each herd was assessed by a written temperament survey, which identified 19 dominant, 15 middle and 15 subordinate females. No clear patterns of serum testosterone secretion were observed in females with and without cyclic CL function. Furthermore, no significant relationships were

found among serum testosterone concentrations, dominance rank, and ovarian cyclicity status. These data suggest that increased circulating testosterone concentrations are not associated with greater rates of ovarian acyclicity or dominance status in captive female African elephants. © 2011.

Niemuller, C., P. A. Gentry and R. M. Liptrap (1990). "**Longitudinal study of haematological and biochemical constituents in blood of the Asian elephant (*Elephas maximus*)**." Comp. Biochem. Physiol. [A] **96**(1): 131-134.

1. Haematological parameters and biochemical analyses were determined in four elephants over a period of one year. 2. The haematological profile remained constant over time and was similar between animals. 3. Values for biochemical analyses were stable except for alkaline phosphatase, gamma glutamyl transferase and creatinine which rose during musth in male elephants. 4. The association of elevated enzyme levels and increased testosterone concentration is discussed.

Niemuller, C. and R. M. Liptrap (1991). "**Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (*Elephas maximus*)**." J. Reprod. Fertil **91**(1): 139-146.

Greater concentrations of androstenedione than testosterone were usually present during periods of non-musth in plasma collected weekly for periods up to 2 years in 8 male Asian elephants (4-35 years of age). For the 6 males that exhibited musth the androstenedione/testosterone ratio shifted greatly in favour of testosterone. The severity of musth was assessed weekly using a scale of 1 to 5 for each of 8 behavioural traits including urine dribbling, temporal gland secretion and aggression. Brief shifts in the ratio of the two androgens when testosterone predominated (n=106) were seen during the non-musth period in 3 of the males studied continuously for 2 years. In 82% of these instances, stimuli of a sexual or aggressive nature had occurred in the preceding 48 h (x^2, $p < 0.01$). A heterologous bovine assay was used to measure LH values in plasma collected every 15 minutes for 12h. Increases in testosterone concentrations followed pulsatile increases in plasma LH concentrations during 7 non-musth periods in 4 animals. Apart from pulse frequency, increases in the variables describing pulsatile LH secretion were seen in 2 strong musth and 2 mild musth episodes compared to non-musth values. A strong musth, however, was characterized by a much greater increase in pulsatile testosterone secretion than was a mild musth and which may be a function of the duration of musth.

Niemuller, C. A. and R. M. Liptrap (1991). "**Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (Elephas maximus)**." Journal of Reproduction and Fertility **91**(1): 139-146.

Greater concentrations of androstenedione than testosterone were usually present during periods of non-musth in plasma collected weekly for various periods up to 2 years in 8 ma1e Asian elephants (4-35 years of age). For the 6 males that exhibited musth the androstenedione/testosterone ratio shifted greatly in favour of testosterone. The severity of musth was assessed weekly using a scale of 1 to 5 for each of 8 behavioural traits including urine dribbling, temporal gland secretion and aggression. A significant correlation (P < 0.05) was noted between plasma testosterone concentrations and the musth score value in 5 of 6 musth episodes. Brief shifts in the ratio of the two androgens when testosterone predominated (n = 106) were seen during the non-musth period in 3 of the males studied continuously for 2 years.

In 82% of these instances, stimuli of a sexual or aggressive nature had occurred in the preceding 48 h (χ2, P < 0.01). A heterologous bovine assay was used to measure LH values in plasma collected every 15 min for 12 h. Increases in testosterone concentrations followed pulsatile increases in plasma LH concentrations during 7 non-musth periods in 4 animals. Apart from pulse frequency, increases in the variables ribing pulsatile LH secretion were seen in 2 strong musth and 2 mild musth episodes compared to non-musth values. A strong musth, however, was characterized by a much greater increase in pulsatile testosterone secretion than was a mild musth and which may be a function of the duration of musth.

Niemuller-Hare, C., C. Gray and R. Liptrap (1988). **A preliminary report on musth in male Asian elephants**. Proc.Ann.Elephant Workshop 9.

Poole, J. H., L. H. Kasman, E. C. Ramsay and B. L. Lasley (1984). "**Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*)**." J. Reprod. Fertil **70**(1): 255-260.

Urine samples were obtained from free-ranging African elephants that were considered to be in and out of musth. Testosterone concentrations, measured by radioimmunoassay were significantly greater in males that were in or around the time of behavioral musth. This study supports a correlation between the observed behavioral characteristics of musth and urinary testosterone levels.

Rajaram, A. and V. Krishnamurthy (2003). "**Elephant temporal gland ultrastructure and androgen secretion during musth**." Current Science **85**(10): 1467-1471.

We have investigated the ultrastructure of the temporal gland of the Asian elephant (Elephas maximus) in the musth condition. We find that the organelles are highly evolved for the production of the androgen, testosterone which is reported to be very high in the Asian male elephant in full musth. The mitochondria bear cristae which are profuse and tubular, and occur along with many Golgi bodies. There is hypertrophy of smooth endoplasmic reticulum. All the structures involved in the production of androgen, as in the Leydig cell or the cells of the adrenal cortex, are thus found in abundance. Cellular structures also seem singularly evolved for the secretion of androgen and its degradation products.

Rasmussen, L. E. L., D. L. Hess and A. Hall-Martin (1990). "**Chemical profiles of temporal gland secretions from captive Asian bull elephants during musth and from African bull elephants living in wild but crowded conditions**." Chemical Senses **15**: 628.

Full-text: This study compares the volatile components of the temporal gland secretions of captive Asian bull elephants in musth and a distinctive group of wild African bull elephants, confined to a national park. The captive Asian population has been well studied (Rasmussen *et al.*, 1984; Rasmussen, 1988). Serum testosterone was elevated at specific times; aggressive behaviors occurred concomitantly with temporal gland secretions, although aggression and elevated serum testosterone were not always related. Selected volatiles among the 23 compounds identified demonstrated concentration changes during the progression of musth, at times simultaneously with alterations in testosterone levels (Rasmussen *et al.*, in press). The African bull elephants have been monitored, behaviorally and physiologically, by radiocontrolled tracking and monthly sampling during the past 5 years. Aggressive behaviors similar to those of Asian bull elephants have been documented; serum and temporal gland testosterone were elevated concomitantly in a cyclical fashion similar to musth in Asian elephants. Chemical characterization of the volatiles of the temporal gland secretions from these bulls revealed

several similarities to the compounds described in Asian bulls, including several compounds not previously described in African temporal gland secretions. It is suggested that these chemicals, or other, more ephemeral compounds, may chemically inform other bulls and cows of the musth-like state of these bulls.

Rasmussen, L. E. L. (1988). "**Chemosensory responses in two species of elephants to constituents of temporal gland secretion and musth urine**." J. Chem. Ecol **14**(8): 1687-1711.

This report discusses three areas of investigation: (1) The chemical components in the temporal gland secretion (TGS) of Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants were characterized by radioimmunoassay (RIA) for testosterone (T) and dihydrotestosterone(DHT) levels and by on-column capillary column gas chromatographic analysis of volatiles. An inverse relationship between TGS testosterone levels and (E)-farnesol levels was observed. (2) African elephants responded preferentially toward a particular constituent of African elephant (TGS). (3) Urine from Asian bull elephants in musth was partially fractionated by high-performance liquid chromatography. Specific chromatographic regions elicited dramatic avoidance responses from female African elephants. These results support the suggestion that the TGS plays multiple chemocommunicative roles.

Rasmussen, L. E. L. and T. E. Perrin (1999). "**Physiological correlates of musth: lipid metabolites and chemical composition of exudates**." Physiology and Behavior **67**(4): 539-549.

Physiological changes related to lipid metabolism, behaviour and chemicals released in body exudates were studied during musth in the Asian elephant (Elephas maximus) as a case study. During musth, changes in serum testosterone and triglyceride concentrations followed similar patterns, with the former increasing sooner than the latter. Deviant behaviour increased during changing androgen levels. The observed high concentrations of testosterone were positively and significantly correlated with increased triglycerides. Lipase activity elevated significantly immediately before and after musth. Blood pH increased significantly in alkalinity. Urine and temporal gland secretions released variable amounts of compounds, some of which may be chemical signals. During musth, temporal gland and urinary exudates demonstrated increased acetone and other ketones indicative of lipid metabolic alterations. Large quantities of nonmethane hydrocarbons, especially 2-butanone, were released from the seemingly dry orifice of the temporal gland before the start of over musth and before maximum blood elevations were observed; isoprene release was similar. However, maximal acetone levels occurred simultaneously in blood, temporal gland secretions, and urine. Metabolically, musth is a series of interwoven, changing stages of increasing and decreasing hormones and lipid-related constituents. Released chemicals can be quantitatively related to these internal physiological events; some observed behaviours appear to result from altered chemical signals.

Rasmussen, L. E. L. and B. A. Schulte (1998). "**Chemical signals in the reproduction of Asian (Elephas maximus) and African (Loxodonta africana) elephants**." Animal Reproduction Science **53**(1-4): 19-34.

Asian (Elephas maximus) and African (Loxodonta africana) elephants exhibit polygynous mating that involves female choice of mates and male-male competition for access to females. Chemical signals mediate intersexual and intrasexual interactions associated with reproduction. The need for reliable and honest signals is accentuated by the markedly different social structure of adult males and females. Adult female elephants live in matriarchal herds consisting of a dominant female and several generations of offspring. Adult males are solitary or travel with other males except during breeding periods. Because females have a long 16-week

oestrous cycle with a brief 1-week receptive period and a 4-5 year interval between births, a sexually active female is a limited resource. Asian elephant females advertise a forthcoming ovulation by releasing (Z)-7-dodecen-1-yl acetate in their urine during the preovulatory period. African elephants probably produce a sex pheromone as well. Females regularly contact the ano-genital region of other females and show heightened chemosensory responsiveness to urine during the follicular phase. The physiological impacts of this ability to detect reproductive condition (e.g. possible synchronizing or suppressing of oestrus) are uncertain. Males experience an annual period of heightened aggressiveness and highly elevated testosterone concentrations known as musth. Males secrete fluid copiously from their temporal gland and dribble strongly odoriferous urine during musth. Females appear to prefer musth males as mates, and captive Asian females exhibit greater chemosensory responses to urine from males in musth than not. Males in musth are competitively dominant to all other males, even those larger than themselves. Nonmusth males avoid males in musth, and captive Asian bulls show greater interest in musth than nonmusth urine. In captivity subordinate Asian females back away from musth secretions, and females with calves sometimes display protective behaviour. Clearly, chemical signals play an important role in communication by elephants between and within the sexes. Further work is needed to identify more of these chemical messengers and to understand their complete function in mediating reproductive interactions in the elephant social system.

Rasmussen, L. E. L., A. J. Hall-Martin and D. L. Hess (1996). "**Chemical profiles of African bull elephants, *Loxodonta africana*: physiological and ecological implications**." Journal of Mammalogy **77**(2): 422-439.

This study reports concentrations of testosterone and dihydrotestosterone in both serum and temporal-gland secretion of male African elephant (Loxodonta africana), including radiocollared elephants, and identifies a spectrum of volatile components in the temporal-gland secretions. Androgens in the serum (testosterone and dihydrotestosterone) were measured in 111 adult male African elephants, ages 21-40 years, from two national parks in South Africa during several years and seasons. About one-fifth (18.6%) of these mature, male, African elephants exhibited dramatically increased concentrations of testosterone in serum characteristic of male Asian elephants during musth. In Krueger National Park, six radiocollared male African elephants, ages 25-35 years, were tracked and serially samples for both serum and temporal-gland secretions during a 5-year period. Concentrations of testosterone in serum and temporal gland secretions were elevated cyclically at times when typical musth behaviors, including aggression, were observed. This study reports the first chemical characterization of the volatile compounds of the temporal gland secretions from male African elephants in musth. It reveals many similarities between the chemical constituents of the temporal-gland secretions of these male African elephants and the compounds identified in male Asian elephants. In addition, several compounds, not previously identified in temporal-gland secretions of African elephants, are described. Such chemical data support the behavioral observations by ourselves and other researchers that male African elephants experience musth. Especially convincing are the concurrent hormonal and chemical data from the radiocollared males during episodic periods of behavioral musth. Implications of the incidence of musth in the past and present ecology of African elephants are discussed in view of the increasing compression within national parks.

Rasmussen, L. E. L., D. L. Hess and J. D. Haight (1990). "**Chemical analysis of temporal gland secretions collected from an Asian bull elephant during a four-month musth episode**." Journal of Chemical Ecology **16**(7): 2167-2181.

The temporal glands, modified facial apocrine sweat glands unique to elephants, release

collectable secretions during an unusual physiological state termed "musth" in the Asian bull elephant (Elephas maximus). Recently we began the characterization of the chemical components of musth, especially in the temporal gland secretions (TGS), and the examination of the role of such secretions as agents for chemical communication between elephants. The present study focuses on possible correlations between testosterone levels in the serum and temporal gland secretions. We were especially interested in possible qualitative and/or quantitative changes in volatile compounds as the testosterone levels varied during a discrete musth period. Quantitative changes in TGS and serum testosterone were determined by radioimmunoassay. Qualitative and semiquantitative changes occurring in volatile composition were studied by high-resolution gas chromatography (fused silica capillary column, on column injection). Compound identification was by nuclear magnetic resonance, gas chromatography-mass spectrometry, and gas chromatography internal standards. Twenty-three major compounds and a number of minor components were identified. Androgen concentrations were correlated with TGS-specific volatiles including benzoic acid, 2-nonanone, 5-nonanol, tetradecanoic acid, and decanoic acid. The latter two compounds and (E)-farnesol, a major component of African TGS, demonstrated an inverse relationship to T levels. © 1990 Plenum Publishing Corporation.

Rasmussen, L. E., I. O. Buss, D. L. Hess and M. J. Schmidt (1984). "**Testosterone and dihydrotestosterone concentrations in elephant serum and temporal gland secretions**." Biology of Reproduction **30**(2): 352-362.

Serum and temporal gland secretions (TGS) were obtained from mature wild African (Loxodonta africana) and captive Asian elephants (Elephas maximus). Samples were obtained from five cows and eight bulls culled for management purposes in Kruger National Park, South Africa, and from four females and two males residing at the Washington Park Zoo, Portland, Oregon. Our purpose was to describe the levels of the androgens, testosterone (T), and dihydrotestosterone (DHT), and to correlate these observations with sex, species and behavioral status. Male-female differences in serum T were pronounced in the Asian species, whereas male and female concentrations overlapped in the African elephant serum. Serum T concentrations in African females were greater than in Asian females. Serum DHT reflected T levels, except that the striking elevation of testosterone in Asian bulls during musth was not paralleled by equal increases in DHT levels. A species difference observed among males was higher serum T levels in nonmusth Asian bulls (1.84-5.35 ng/ml) compared to the levels in African bulls (0.38-0.68 ng/ml), except for one dominant African bull (6.64 ng/ml). This single African value was still considerably lower than the serum T values of the Asian males during musth. These musth values were the highest serum androgen concentrations: T was between 19 and 40 ng/ml (average 26.10 ng/ml). The TSG values of T and DHT were much higher than serum levels except in the Asian female. T/DHT ratios in TGS were more similar than in serum. One dominant African bull had a T TGS value of 78 ng/ml, which was much higher than the rest of the African males or females, but considerably lower than as Asian bull in musth (547 ng/ml). It seems apparent that a change in androgen status as reflected in serum and TGS levels of T and DHT precedes or is concomitant with overt alteration in behavior in the Asian male. The temporal gland appears to actively concentrate androgens in both African males and females, but in the Asian male the gland secretes only during musth when the greatest concentration of both T and DHT were observed. The apparent difference in the degree of temporal gland secretory activity between the two species suggests a more specific communicative function within the Asian male.

Ratnasooriya, W. D., S. B. U. Fernando and A. N. V. R. Manatunga (1992). "**Serum testosterone levels of Sri Lankan female elephants (*Elephas maximus maximus*)**." Med. Sci. Res **20**(2): 79-80.

Schulte, B. A. and L. E. Rasmussen (1999). "**Signal-receiver interplay in the communication of male condition by Asian elephants**." Animal Behaviour **57**(6): 1265-1274.

Signal design and meaning are dependent on the condition of the sender and receiver as well as the response of the receiver. This study examined (1) whether female Asian elephants, Elephas maximus, can distinguish between a conspecific male in musth and nonmusth states using urinary signals, (2) how the oestrous condition of the female affects discrimination, and (3) correlation of female responses with the testosterone level of the male. Musth is a rut-like state displayed by healthy adult male elephants. Males in musth dominate nonmusth males and may be preferred by females as mates. Urine was collected from two captive male Asian elephants during nonmusth periods and from one of these males during times of musth. Samples of musth and nonmusth urine and control liquids were placed in an elephant enclosure weekly for 16 weeks, the length of a female oestrous cycle. Primary response behaviours were approach and four trunk-tip motions, namely sniff, check, place and flehmen. Musth urine consistently elicited greater responses than nonmusth and control samples. Females were more responsive during their follicular (sexually receptive) than luteal (unreceptive) stages of oestrus. Furthermore, females appeared to be sensitive to the degree of musth as responses increased with rising serum testosterone levels of the male donor. Chemical signals from males are a likely source of honest signals related to status and reproductive condition. Female elephants appear capable of detecting differences in a male based upon urinary chemosignals.

Schulte, B. A. and L. E. L. Rasmussen (1999). Musth, sexual selection, testosterone and metabolites. **Advances in Chemical Communication in Vertebrates 8**. R. E. Johnston, D. Muller-Schwarze and P. W. Sorenson, Kluwer Academic/Plenum Press**:** 383-397.

Musth is an annual, yet asynchronous, rut-like condition that is experienced by many adult African and Asian male elephants. Behaviorally, musth is characterized by heightened aggression, decreased feeding, urine dribbling, temporal gland secretion and enhanced sexual activity. Musth improves the access of a male to reproductively active females through increased mobility and a higher dominance ranking (intrasexual competition). Whether females prefer males in musth as mates is as yet uncertain (intersexual choice). Females can distinguish among the odors of males in musth and nonmusth. Although behavioral musth has been associated with greatly elevated plasma testosterone levels, a recent study in Sri Lanka shows that intensified aggressiveness follows maximal testosterone secretion and proposes that behavioral musth is a consequence of declining androgen levels. Our data from an Asian male elephant in North America suggest that either declining or rising serum testosterone may be related to "musth behaviors." Our report demonstrates that certain aspects of body physiology are greatly altered during musth. Rather than a single state, our data suggest that musth is an ever-changing condition with some typical stages. Specific chemical compounds released at different stages of musth may serve individually or in combination as honest signals of male condition.

Schulte, B. A. (2001). **Examining Ideas on the Evolution of Musth**. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Short, R. V., T. Mann and M. F. Hay (1967). "**Male reproductive organs of the African elephant, *Loxodonta africana***." J. Reprod. Fertil **13**(3): 517-536.

Smith, D. A., R. Nadaraja, B. Beck, N. Honhold, D. Hale, D. C. Knottenbelt and F. W. G. Hill (1987). "**Serum testosterone levels in male African elephants, *Loxodonta africana*, in Hwange National Park, Zimbabwe**." Zimbabwe Vet. J **18**: 58-63.

Age and serum testosterone levels were determined for 44 male African elephants (*Loxodonta africana*) from Hwange National Park. Testicular weight was measured in 26 animals. Age and testicular weight were found to be highly correlated (r=0.94), while serum testosterone levels were correlated to both age (r=0.54) and testicular weight (r=0.43). Although the range of serum testosterone levels and maximum value increased with age, the minimum value did not.

Somgird, C., S. Sripiboon, S. Mahasawangkul, K. Boonprasert, J. L. Brown, T. A. Stout, B. Colenbrander and C. Thitaram (2016). "**Differential testosterone response to GnRH-induced LH release before and after musth in adult Asian elephant (Elephas maximus) bulls**." Theriogenology **85**(7): 1225-1232.

Bull elephants exhibit marked increases in testosterone secretion during musth, and studies have shown a heightened sensitivity of the testis to GnRH-stimulated testosterone production in musth compared to nonmusth males. However, activity of the hypothalamo-pituitary-gonadal axis before or soon after musth has not been studied in detail. The aim of this study was to evaluate LH and testosterone responses to GnRH challenge in nine adult Asian elephant (Elephas maximus) bulls during three periods relative to musth: premusth, postmusth, and nonmusth. Bulls were administered 80 mug of a GnRH agonist, and blood was collected before and after injection to monitor serum hormone concentrations. The same bulls were injected with saline 2 weeks before each GnRH challenge and monitored using the same blood collection protocol. All bulls responded to GnRH, but not saline, with an increase in LH and testosterone during all three periods. The mean peak LH (1.76 +/- 0.19 ng/mL; P < 0.001) and testosterone (6.71 +/- 1.62 ng/mL; P = 0.019) concentrations after GnRH were higher than the respective baselines (0.57 +/- 0.07 ng/mL, 3.05 +/- 0.60 ng/mL). Although basal- and GnRH-induced LH secretion were similar across the stages, evaluation of the area under the curve in GnRH-treated bulls indicated that the testosterone response was greatest during premusth (2.84 +/- 0.76 area units; P = 0.019) compared to postmusth (2.02 +/- 0.63 area units), and nonmusth (2.01 +/- 0.46 area units). This confirms earlier reports that GnRH stimulates LH release and subsequent testosterone production in bull elephants. Furthermore, although the hypothalamo-pituitary-gonadal axis is active throughout the year, the testis appears to be more responsive to LH in terms of testosterone production in the period leading up to musth, compared to the nonmusth and postmusth periods. This heightened sensitivity, perhaps as a result of LH receptor up-regulation, may prime the testis for maximal testosterone production, leading to the physiological and behavioral changes associated with musth.

Somgird, C., S. Sripiboon, S. Mahasawangkul, K. Boonprasert, J. L. Brown, T. A. E. Stout, B. Colenbrander and C. Thitaram (2016). "**Differential testosterone response to GnRH-induced LH release before and after musth in adult Asian elephant (Elephas maximus) bulls**." Theriogenology **85**(7): 1225-1232.

Bull elephants exhibit marked increases in testosterone secretion during musth, and studies have shown a heightened sensitivity of the testis to GnRH-stimulated testosterone production in musth compared to nonmusth males. However, activity of the hypothalamo-pituitary-gonadal axis before or soon after musth has not been studied in detail. The aim of this study was to evaluate LH and testosterone responses to GnRH challenge in nine adult Asian elephant (Elephas

maximus) bulls during three periods relative to musth: premusth, postmusth, and nonmusth. Bulls were administered 80 μg of a GnRH agonist, and blood was collected before and after injection to monitor serum hormone concentrations. The same bulls were injected with saline 2 weeks before each GnRH challenge and monitored using the same blood collection protocol. All bulls responded to GnRH, but not saline, with an increase in LH and testosterone during all three periods. The mean peak LH (1.76 ± 0.19 ng/mL; $P < 0.001$) and testosterone (6.71 ± 1.62 ng/mL; $P = 0.019$) concentrations after GnRH were higher than the respective baselines (0.57 ± 0.07 ng/mL, 3.05 ± 0.60 ng/mL). Although basal- and GnRH-induced LH secretion were similar across the stages, evaluation of the area under the curve in GnRH-treated bulls indicated that the testosterone response was greatest during premusth (2.84 ± 0.76 area units; $P = 0.019$) compared to postmusth (2.02 ± 0.63 area units), and nonmusth (2.01 ± 0.46 area units). This confirms earlier reports that GnRH stimulates LH release and subsequent testosterone production in bull elephants. Furthermore, although the hypothalamo-pituitary-gonadal axis is active throughout the year, the testis appears to be more responsive to LH in terms of testosterone production in the period leading up to musth, compared to the nonmusth and postmusth periods. This heightened sensitivity, perhaps as a result of LH receptor up-regulation, may prime the testis for maximal testosterone production, leading to the physiological and behavioral changes associated with musth. © 2016 Elsevier Inc..

Somgird, C., P. Homkong, S. Sripiboon, J. L. Brown, T. A. E. Stout, B. Colenbrander, S. Mahasawangkul and C. Thitaram (2016). "**Potential of a gonadotropin-releasing hormone vaccine to suppress musth in captive male Asian elephants (Elephas maximus)**." Animal Reproduction Science **164**: 111-120.

Musth in adult bull elephants is a period of increased androgen concentrations ranging from a few weeks to several months. For captive elephant bull management, musth presents a serious challenge because of the aggressive behavior of musth bulls toward people and other elephants. Commercially available GnRH vaccines have been shown to suppress testicular function by interrupting the hypothalamo-pituitary-gonadal (HPG) axis in many species. The aim of this study was to test the efficacy of a GnRH vaccine in elephant bulls for suppressing the HPG axis and mitigating musth-related aggressive behavior. Five adult Asian elephant bulls (22-55 years old) were immunized with a GnRH vaccine starting with an initial injection 2-4 months before the predicted musth period, and followed by three boosters at approximately 4-week intervals. Blood samples were collected twice weekly for hormone and antibody titer analysis. An increase in GnRH antibody titers was observed in all bulls after the second or third booster, and titers remained elevated for 2-3 months after the final booster. Musth was attenuated and shortened in three bulls and postponed completely in two. We conclude that GnRH vaccination is capable of suppressing symptoms of musth in adult bull elephants. With appropriate timing, GnRH vaccination could be used to control or manage musth and aggressive behavior in captive elephant bulls. However, more work is needed to identify an optimal dose, booster interval, and vaccination schedule for complete suppression of testicular steroidogenesis. © 2015 Elsevier B.V.

Taya, K., H. Komura, M. Kondoh, Y. Ogawa, K. Nakada, G. Watanabe, S. Sasamoto, K. Tanabe, K. Saito, H. Tajima and E. Narushima (1991). "**Concentrations of progesterone, testosterone and estradiol-17B in the serum during the estrous cycle of Asian elephants (*Elephas maximus*)**." Zoo Biol **10**: 299-307.

Taya, K. (1993). "**The reproductive physiology of the elephant**." Journal of Reproduction and Development **39**(6): 77-91.

A discussion. In the females the vagina opens ventrally, ovarian cycles average 15-17 weeks in length, the gestation period is 22 months, and the ovary has a number of corpora lutea, although elephants are monovular. In males, there are permanent intra-abdominal testes, there is no distinct epididymis, but instead there is an extremely tortuous and convoluted duct which connects the testes to the openings of the seminal vesicles, the accessory organs are extremely well developed, especially the seminal vesicles and the bulbo-urethral glands. During musth, adult bulls become disobedient, aggressive and extremely dangerous, often attempting to kill their mahouts, or anybody else who comes within range. Bulls in musth have high testosterone levels and show urine dribbling and swollen temporal glands.

Thongtip, N., J. Saikhun, S. Mahasawangkul, K. Kornkaewrat, P. Pongsopavijitr, N. Songsasen and A. Pinyopummin (2008). "**Potential factors affecting semen quality in the Asian elephant (Elephas maximus)**." Reproductive Biology and Endocrinology **6**.

Background: One of the major obstacles in using artificial insemination to manage genetics of elephant population in captivity is the large variations in semen quality among ejaculates within the same and among individuals. The objectives of this study were to determine the influences of (1) age (2) seasonality (3) and circulating testosterone (SrTest), triiodothyronine (SrT3) and tetraiodothyronine (SrT4), as well as seminal (4) testosterone (SpTest), zinc (SpZn) and protein (SpTP) on semen quality in the Asian elephant Methods: Analyses, including motility, viability and morphology were performed in semen samples collected twice monthly from 13 elephant bulls (age range, 10-to 72-years) by manual stimulation between July 2004 and June 2005. Serum samples obtained monthly were assessed for SrTest, SrT3, SrT4, and seminal plasma samples were evaluated for, SpTest, SpZn and SpTP. Results: The highest semen quality was observed at age 23 to 43 years. Percentages of progressive motility and viable sperm were lowest at age 51 to 70 years ($P < 0.05$); on the other hand, sperm concentration was lowest at age 10 to 19 years ($P < 0.05$). Percentage of sperm with normal morphology was highest at age 23 to 43 years. The levels of SrT3, SrTest, SpTest and SpZn were lowest at age 51 to 70 years, whereas SrT4 was lowest at age 23 to 43 years. Seasonality significantly affected semen characteristics in which percentage of viable sperm and cell concentration were highest during rainy season and lowest during summer months ($P < 0.05$). However, percentage of sperm with normal morphology was highest in summer and lowest in rainy season ($P < 0.05$). Seasonality significantly influenced SrTest with elevated concentrations observed in rainy season and winter ($P < 0.05$). Conclusion: This study indicates that age and seasonality had influence on semen characteristics in the Asian elephant. The knowledge obtained in this study will improve our understanding of the reproductive biology of this species.

Turczynski, C. J. (1993). **The endocrinology of musth in the male Asiatic elephant (*Elephas maximus*): Serum estradiol, serum LH and serum, fecal and urinary testosterone**. College Station, TX. USA, Texas A&M University. **PhD**.

Wilson, J. D., M. W. Leihy, G. Shaw and M. B. Renfree (2003). "**Androgen physiology: unsolved problems at the millennium**." Molecular and Cellular Endocrinology **198**(1-2): 1-5.

Androgen physiology differs from that of other steroid hormones in two major regards. First, testosterone, the predominant circulating testicular androgen, is both an active hormone and a prohormone for the formation of a more active androgen, the 5alpha-reduced steroid dihydrotestosterone. Genetic evidence indicates that testosterone and dihydrotestosterone

work via a common intracellular receptor, and studies involving in vitro reporter gene assays and intact mice in which both steroid 5alpha-reductase isoenzymes have been disrupted by homologous recombination indicate that dihydrotestosterone acts during embryonic life to amplify hormonal signals that can be mediated by testosterone at higher concentrations. However, in post-embryonic life dihydrotestosterone plays unique roles that have not been elucidated. Studies of other 5alpha-reduced steroids, including the plant hormone brassinolide, the hog pheromones androstanol and androstenol, and 5alpha-dihydroprogesterone (in horses and elephants) indicate that this reaction serves different functions in different systems. Second, during embryonic life androgen causes the formation of the male urogenital tract and hence is responsible for development of the tissues that serve as the major sites of androgen action in postnatal life. It has been generally assumed that androgens virilize the male fetus by the same mechanisms as in the adult, namely by the conversion of circulating testosterone to dihydrotestosterone in target tissues. However, in marsupial mammals there is no sexual dimorphism in the levels of testosterone or dihydrotestosterone at the time the male phenotype forms, and in the pouch young of one marsupial, the tammar wallaby, the testes secrete another 5alpha-reduced steroid, 5alpha-androstane-3alpha, 17beta-diol (5alpha-adiol), into plasma. The administration of 5alpha-adiol to female pouch young causes profound virilization of the urogenital sinus and external genitalia, but within target tissues 5alpha-adiol appears to work after oxidation to dihydrotestosterone. Thus, two separate mechanisms evolved for the formation of dihydrotestosterone in target tissues. 5alpha-adiol is the predominant androgen in neonatal testes in several placental mammals, but it is unclear whether it plays a similar role in other mammalian species.

Wingate, L. and B. Lasley (2001). **Is Musth a Reproductive Event: An Examination of Arguments For and Against this View**. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Wyse, J. M., I. C. W. Hardy, L. Yon and M. Mesterton-Gibbons (2017). "**The impact of competition on elephant musth strategies: A game–theoretic model**." Journal of Theoretical Biology **417**: 109-130.

Mature male African Savannah elephants are known to periodically enter a temporary state of heightened aggression called “musth”, often linked with increased androgens, particularly testosterone. Sexually mature males are capable of entering musth at any time of year, and will often travel long distances to find estrous females. When two musth bulls or two non-musth bulls encounter one another, the agonistic interaction is usually won by the larger male. However, when a smaller musth bull encounters a larger non-musth bull, the smaller musth male can win. The relative mating success of musth males is due partly to this fighting advantage, and partly to estrous females' general preference for musth males. Though musth behavior has long been observed and documented, the evolutionary advantages of musth remain poorly understood. Here we develop a game–theoretic model of male musth behavior which assumes musth duration as a parameter, and distributions of small, medium and large musth males are predicted in both time and space. The predicted results are similar to the musth timing behavior observed in the Amboseli National Park elephant population, and further results are generated with relevance to Samburu National Park. We discuss small male musth behavior, the effects of estrous female spatial heterogeneity on musth timing, conservation applications, and the assumptions underpinning the model. © 2017 Elsevier Ltd

Yon, L., J. Chen, P. Moran and B. Lasley (2007). "**An analysis of the androgens of musth in the Asian bull**

elephant (Elephas maximus)." Gen Comp Endocrinol **Mar 24; [Epub ahead of print]**.

During musth in bull elephants, the androgens testosterone (T), dihydrotestosterone (DHT), and androstenedione all increase significantly. Given the unusual endocrine physiology that has been discovered in female elephants, it is also possible that bull elephants produce some unusual androgens. A cell-based androgen receptor assay was used to explore this possibility using two different methods. The first method compared the level of T measured by radioimmunoassay (RIA) with the level of androgen receptor (AR) activity measured in the serum of eight bull elephants during musth and non-musth periods. A ratio was calculated for T/AR activity for non-musth and musth, to determine if there was a change in the ratio between these two states. The second method used HPLC to separate two pooled serum samples (one non-musth and one musth) into fractions using a protocol which separates known androgens into specific, previously identified fractions. Each fraction was then tested with the AR assay to determine the androgenicity of any compounds present. This was done to determine if there were any fractions which had androgenic activity but did not contain any previously identified androgens. Results from the first analysis indicated no change in the T/AR ratio between non-musth and musth states. Clearly whatever active androgens are present during musth, they increase proportionately with T. Findings from the second analysis suggested that the only bioactive androgen present in the serum of non-musth Asian bulls is a low level of T. During musth, the only bioactive androgens detected were T and DHT; of these, T was by far the predominant active androgen present. Taken together, these two analyses suggest that T is by far the predominant active androgen present during musth in Asian bull elephants, and that no previously unidentified bioactive androgen is present.

Yon, L., S. Kanchanapangka, N. Chaiyabutr, S. Meepan, F. Z. Stanczyk, N. Dahl and B. Lasley (2007). "**A longitudinal study of LH, gonadal and adrenal steroids in four intact Asian bull elephants (Elephas maximus) and one castrate African bull (Loxodonta africana) during musth and non-musth periods**." Gen. Comp Endocrinol **151**(3): 241-245.

During their annual musth cycle, adult African and Asian bull elephants have increased gonadal androgens (testosterone [T], dihydrotestosterone [DHT], androstenedione [A4]). Because musth is a physiologically and psychologically stressful time, this study was conducted to investigate whether the adrenal glands (stimulated by stress) increase production of both glucocorticoids and androgens during musth. Weekly serum samples were taken for 11-15 months from four intact adult Asian bull elephants, and from a castrate African bull elephant who exhibits musth. Testosterone, androstenediol (A5), A4, luteinizing hormone (LH), cortisol, and dehydroepiandrosterone (DHEA) were measured in each sample. In three of the four intact bulls, all hormones measured increased during musth. Adrenal androgens were strongly correlated with LH and testicular androgens, though not to cortisol. None of the hormones measured in the castrate bull increased during his musth cycles. While the significance of adrenal activity in the elephant during musth has yet to be determined, this study provides evidence that the adrenal gland actively produces both glucocorticoids and androgens during musth in the Asian elephant

Yon, L., S. Kanchanapangka, N. Chaiyabutr, F. Stanczyk, S. Meepan and B. Lasley (2007). "**ACTH stimulation in four Asian bull elephants (Elephas maximus): an investigation of androgen sources in bull elephants**." Gen. Comp Endocrinol **151**(3): 246-251.

The phenomenon of musth is a very stressful event, both behaviorally and physiologically. An ACTH stimulation test was conducted in four adult Asian bull elephants to investigate the

possibility that the classical hypothalamic-pituitary-adrenal (HPA) axis is active during musth, resulting in an increase in adrenally produced steroids. Serum cortisol, testosterone (T), androstenedione (A4), androstenediol (A5), and dehydroepiandrosterone (DHEA) were measured. Cortisol increased 3-10 times above baseline in response to ACTH stimulation, and DHEA doubled. A4 and A5 were erratic, while testosterone decreased significantly in all bulls. The pattern of results suggests that the adrenal steroid increase which occurs during musth results from some mechanism other than the classical HPA axis

Yon, L., B. Faulkner, S. Kanchanapangka, N. Chaiyabutr, S. Meepan and B. Lasley (2010). "**A safer method for studying hormone metabolism in an Asian elephant (Elephas maximus): Accelerator mass spectrometry**." Zoo Biology **29**(6): 760-766.

Noninvasive hormone assays provide a way to determine an animal's health or reproductive status without the need for physical or chemical restraint, both of which create unnecessary stress for the animal, and can potentially alter the hormones being measured. Because hormone metabolism is highly species-specific, each assay must be validated for use in the species of interest. Validation of noninvasive steroid hormone assays has traditionally required the administration of relatively high doses of radiolabelled compounds (100 μCi or more of 14C labeled hormone) to permit subsequent detection of the excreted metabolites in the urine and feces. Accelerator mass spectrometry (AMS) is sensitive to extremely low levels of rare isotopes such as 14C, and provides a way to validate hormone assays using much lower levels of radioactivity than those traditionally employed. A captive Asian bull elephant was given 1 μCi of 14C-testosterone intravenously, and an opportunistic urine sample was collected 2 hr after the injection. The sample was separated by HPLC and the 14C in the fractions was detected by AMS to characterize the metabolites present in the urine. A previously established HPLC protocol was used, which permitted the identification of fractions into which testosterone sulfate, testosterone glucuronide, and the parent compound testosterone elute. Results from this study indicate that the majority of testosterone excreted in the urine of the Asian bull elephant is in the form of testosterone sulfate. A small amount of testosterone glucuronide is also excreted, but there is no parent compound present in the urine at all. These results underscore the need for enzymatic hydrolysis to prepare urine samples for hormone assay measurement. Furthermore, they highlight the importance of proper hormone assay validation in order to ensure accurate measurement of the desired hormone. Although this study demonstrated the utility of AMS for safer validation of noninvasive hormone assays in nondomestic species, this methodology could also be applied to studies of nutrient metabolism and drug pharmakokinetics, both areas in great need of further study in wildlife species. © 2010 Wiley-Liss, Inc.

Yon, L., J. Chen, P. Moran and B. Lasley (2008). "**An analysis of the androgens of musth in the Asian bull elephant (Elephas maximus)**." General and Comparative Endocrinology **155**(1): 109-115.

During musth in bull elephants, the androgens testosterone (T), dihydrotestosterone (DHT), and androstenedione all increase significantly. Given the unusual endocrine physiology that has been discovered in female elephants, it is also possible that bull elephants produce some unusual androgens. A cell-based androgen receptor assay was used to explore this possibility using two different methods. The first method compared the level of T measured by radioimmunoassay (RIA) with the level of androgen receptor (AR) activity measured in the serum of eight bull elephants during musth and non-musth periods. A ratio was calculated for T/AR activity for non-musth and musth, to determine if there was a change in the ratio between

these two states. The second method used HPLC to separate two pooled serum samples (one non-musth and one musth) into fractions using a protocol which separates known androgens into specific, previously identified fractions. Each fraction was then tested with the AR assay to determine the androgenicity of any compounds present. This was done to determine if there were any fractions which had androgenic activity but did not contain any previously identified androgens. Results from the first analysis indicated no change in the T/AR ratio between non-musth and musth states. Clearly whatever active androgens are present during musth, they increase proportionately with T. Findings from the second analysis suggested that the only bioactive androgen present in the serum of non-musth Asian bulls is a low level of T. During musth, the only bioactive androgens detected were T and DHT; of these, T was by far the predominant active androgen present. Taken together, these two analyses suggest that T is by far the predominant active androgen present during musth in Asian bull elephants, and that no previously unidentified bioactive androgen is present.