SUPRESSION OF TESTICULAR FUNCTION IN A MALE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*) TREATED WITH GONADOTROPIN-RELEASING HORMONE VACCINES

Author(s): Imke Lueders, D.V.M., Dr. med. vet., Thomas Bernd Hildebrandt, D.V.M., Dr. med. vet., Prof., Charles Gray, Stephan Botha, M.Sc., Peter Rich, D.V.M., and Cheryl Niemuller, M.Sc., Ph.D.
Source: Journal of Zoo and Wildlife Medicine, 45(3):611-619. 2014.
Published By: American Association of Zoo Veterinarians
DOI: http://dx.doi.org/10.1638/2013-0233R.1
URL: http://www.bioone.org/doi/full/10.1638/2013-0233R.1

Journal of Zoo and Wildlife Medicine 45(3): 611–619, 2014

SUPRESSION OF TESTICULAR FUNCTION IN A MALE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*) TREATED WITH GONADOTROPIN-RELEASING HORMONE VACCINES

Imke Lueders, D.V.M., Dr. med. vet., Thomas Bernd Hildebrandt, D.V.M., Dr. med. vet., Prof., Charles Gray, Stephan Botha, M.Sc., Peter Rich, D.V.M., and Cheryl Niemuller, M.Sc., Ph.D.

Abstract: 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 immune-castration.

Key words: Anti-GnRH vaccination, bull, immunocontraception, musth, testosterone.

INTRODUCTION

Although Asian and African elephants are threatened in their range countries, and thus successful captive breeding programs are a high priority,[16] under some circumstances incapacitation of testicular cells may be warranted for elephant bulls, either as a contraception measure or to reduce testosterone-driven behaviors.[7] Due to overpopulation of African Savannah elephants (*Loxodonta africana africana*) in southern Africa,[34] several approaches for male (and female) contraception were tested in the past.[1,6] For example, males were provided hormones such as GnRH analogues in order to down-regulate testosterone.[3,8] For adult African elephant bulls, a field method for vasectomy has been successfully applied.[14,29] However, this intervention does not affect the testosterone production, as testicles remain intact. Surgical castration has been performed but only in young, captive males, and the risks and costs are high due to the intra-abdominally situated gonads.[17] An alternative method would be extremely valuable for elephant management purposes.

The need for a nonhormonal, noninvasive, and inexpensive method to prevent testicular development in domestic animals has driven the development of GnRH vaccines since they first underwent testing in the 1970s.[32] The commercial availability of this immunocontraceptive provided new possibilities for wildlife management.

GnRH vaccines interrupt the hypothalamic–hypophyseal–gonadal axis. Animals are exposed to a GnRH-protein conjugate which acts as an antigen, stimulating the immune system to produce anti-GnRH antibodies.[10] As a result, the endogenously produced GnRH is neutralized and cannot bind to its receptor in the hypophysis. Consequently, there is no triggered secretion of the gonadotrophins luteinizing hormone (LH) and follicle stimulating hormone (FSH). In males, the latter hormones are required to stimulate the Leydig cells in the testicles to produce testosterone as well as the Sertoli cells to develop fertile spermatozoa.[13] In contrast to hormonal contraceptives, the biologic risks appear lowered, as no

From GEOlifes (Animal Fertility and Reproductive Research), Frohmestr. 7, 22457 Hamburg, Germany (Lueders); the Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany (Hildebrandt); the African Lion Safari, 1386 Cooper Road, Cambridge, ON, N1R 5S2 Canada (Gray, Botha, Rich); and the Kingfisher International Inc., Conservation Biology Laboratories, 165 Mostar Street, Stouffville, ON, L4A 0Y2 Canada (Niemuller). Correspondence should be directed to Dr. Lueders (imke.lueders@geolifes.com).

exogenous hormones are applied. In addition, the GnRH vaccine may work in both sexes.[15]

This simple yet effective method has been developed mainly to suppress testicular development and boar taint in piglets.[9] Commercial vaccines are now available in America, Europe, and Australia. They are produced for different domestic species such as pigs (Improvac® Pfizer Animal Health Germany, 10785 Berlin, Germany; or Improvest®, Zoetis, New Jersey 07932, USA), cattle (Bopriva®, Zoetis New Zealand Limited, Auckland 1024, New Zealand), and horses (Equity®, Pfizer Animal Health Australia, West Ryde, New South Wales 2114, Australia). In wildlife, noncommercial, restricted-use GnRH vaccines (e.g. GonaCon™, USDA Pocatello Supply Depot, Pocatello, Idaho, USA) have been tested in various species, including white-tailed deer (*Odoicoileus virginianus*)[23] and bison (*Bison bison*),[24] to control overpopulation or pest animals.

Although the primary structure of GnRH is similar in most mammals, several different carrier molecules and adjuvants are used to induce an immunogenic effect in different species.[32] Furthermore, problems still exist in terms of contraceptive efficacy, with possible reasons including a low immune response due to being raised against a self-antigen, production of low-affinity antibodies, and induction of carrier protein tolerance.[11] The literature indicates there is a strong variation in the species-specific response to GnRH neutralization.[32] Therefore, GnRH vaccines have to be tested in each species. As the efficacy of the vaccine depends on the immune response, even within a species individual variations may occur.

Immunocastration of elephant bulls would have several practical applications: In Africa it could serve as a population management tool for elephants; in Asia the GnRH vaccination may be used to suppress musth in captive bulls; and in western zoos it may be applied to manage permanent elephant bachelor groups.

Preliminary studies on the application of GnRH vaccines over a 1.5-yr period have been carried out already on both African and Asian elephant bulls. In Asian elephants, musth and testosterone levels were suppressed[26] and, in African elephants, it interrupted full musth and controlled aggressive, testosterone-driven behaviors in addition.[7] A single case study reported the interruption of estrus cycle activity and subsequent regression of a uterine leiomyoma in a female Asian elephant.[2] However, in a study involving a herd of wild African elephant females, fecal progesterone levels still showed occasional cycle patterns and thus insufficient suppression of ovarian activity was suggested.[34]

Currently, no published data exist documenting the effects of a GnRH vaccine on the reproductive tract and fertility of male elephants over an extended period. It is hypothesized that the vaccine may have an effect similar to surgical castration including: a decline in testosterone levels and subsequent maintenance at nonmusth concentrations; a reduction in testicular size; and cessation of spermatogenesis and the suppression of musth. Therefore, a young, GnRH-vaccinated Asian elephant bull and its 1.5-yr younger half-brother (as an untreated control) were closely monitored over 6 yr. Endocrine analysis, ultrasound examinations, and semen collection was used to document any changes. In addition, weights and behavioral and physical differences were recorded.

CASE REPORT

Captive born male Asian elephant bull 1 required castration due to medical-management reasons. An permanent urethral fistula, derived from surgery to resolve a urethral stone when bull 1 was 4 yr old, made this male unable to become a successful breeder. By the age of 7 yr, routine monitoring of testosterone indicated that levels were starting to rise. To keep bull 1 in a free contact (FC) situation and prevent musth in the future, management decided to start GnRH vaccination as a potential alternative to surgical castration. The treatment was initiated at the onset of puberty (testosterone levels spiked and healthy sperm cells appeared in the ejaculate). The half-brother (bull 2, same sire), born 1.5 yr later than bull 1 and raised under the same conditions, was simultaneously monitored but remained untreated. All adult elephants were kept together during the day, when keepers were present, and separate at night.

MATERIALS AND METHODS

For this study, two commercially produced vaccines, namely Equity (Pfizer Animal Health) and Improvac (Pfizer Animal Health) were applied. Both vaccines came from the same company and differed primarily in the concentration of the GnRH protein-conjugate (Equity: 200 μg/ml; Improvac: 150 μg/ml). Therefore, a larger volume of 3.0 ml was applied when using Improvac compared to 2.0 ml of Equity. Bull 1 was initially vaccinated on a monthly basis with 2.0 ml of

Equity in the neck region starting at 7 yr of age. After 6 mo, the monthly intervals were extended to boosters every 5–7 mo. When the more-economical piglet vaccine Improvac became available, bull 1 was then further treated with 3.0 ml Improvac and intervals were now extended to every 2 yr. Thus, the vaccine schedule was month 0, 1, 2, 3, 4, 5, 11, 17, 23, 29, and 53. Vaccinations were performed without difficulties by hand injection and with no visible adverse reactions (tissue swelling, itchiness).

In order to monitor the effect of the GnRH vaccination prior to and throughout this study blood was collected, ultrasound examinations were performed, and weights and behavioral changes were recorded for both bulls.

Blood was collected weekly from the ear vein. The blood was allowed to clot in serum separator tubes (Corvac™; Tyco Healthcare Group, Mansfield, Massachusetts 02048, USA), centrifuged, and pipetted into a capped and labeled plastic vials for storage at –20°C. For testosterone analysis, samples were defrosted and measured via enzyme immunoassay. All hormones and conjugates were prepared and supplied by Coralie Munro (University of California Davis, Davis, California 95616, USA). The testosterone working anti-body R 156 and the conjugate were both diluted 1 : 20,000 in standard assay buffer. Standard values ranged between 0.078 and 10 ng/ml. Sample volume was 100 µl per well and incubation lasted 2 hr. Assay sensitivity was 0.78 ng/ml and inter- and intra-assay coefficients of variation were 9.3% and 13.6%, respectively. Testosterone assay validation was determined via serial dilution of pooled elephant samples in order to determine the parallel displacement along the standard curve.

Transrectal ultrasound examinations were performed in bull 1 every 3 mo for the first 2 yr. Subsequently, exams were conducted at least once a year by the methods previously described.[17,18] A portable, 4D ultrasound machine (Voluson i, GE Canada, Mississauga, Ontario L5N 5P9, Canada) equipped with a 2–5 MHz volume probe was used. Twenty-second videos from the ampullae, seminal vesicles, and testicles were recorded for retrospective analysis. The width and height of the organs were measured at the largest diameter, and the mean of these values was used. In the paired organs, the combined measurements of the left and right sides were used in the text and figures.

Semen samples were collected in both bulls by rectal prostate massage.[28] Bull 1 was collected 6 times during the first year until spermatozoa were no longer found. Semen was caught in 50-ml plastic tubes. A drop of native semen was immediately analyzed microscopically on a pre-warmed slide.

Both bulls were weighed weekly on a portable electronic scale (ZooScale-10k, Gagetek, Folsom, California 95630, USA). Elephants were observed by at least one keeper during the entire day. Special behavioral observations for both males such as sparring, fighting, sexual interest, or mating, as well as physical changes such as temporal gland draining, were recorded into the daily log book. The books were retrospectively analyzed for the number of events during each year.

Values are reported as means ± standard deviation (SD). A student's *t*-test was performed on pre- and postvaccination values of testicular size, testosterone levels, ampullae size, and seminal vesicles size, as well as on all comparative measurement between the two bulls, in order to determine if they differed significantly. The data for weight were not normally distributed and had unequal variance; therefore; Mann–Whitney rank sum tests were preformed to test for differences.

RESULTS

Bull 1 had already shown occasional serum testosterone spikes up to 15.0 ng/ml prior to treatment (Fig. 1A). After two injections, testosterone levels remained below 5.0 ng/ml (Fig. 1A). After 1 yr of treatment with a total of 7 booster injections following the initial inoculation, serum testosterone remained at baseline concentrations. The baseline was defined as values below the postvaccination average plus one SD (1.6 ng/ml). The postvaccination average level was 0.7 ± 0.9 ng/ml, which was significantly lower than the prevaccination average of 3.1 ± 3.6 ng/ml ($P < 0.001$, df = 197). Even as the vaccination intervals increased from 12 and 24 mo, no rise in hormone concentrations for bull 1 was seen. In contrast, they rose sharply in the untreated bull 2, reaching peaks above 20 ng/ml (Fig. 1B). This is considered premusth or musth level, if elevated for extended periods.[22,25]

Ultrasonography in bull 1 showed that the initially round testicles significantly decreased from a combined diameter of 9.0 ± 0.7 cm to 6.9 ± 0.6 cm ($P < 0.001$, df = 14) 2 yr after the start of treatment (Figs. 2, 3). They also became amorphous in shape (Fig. 3). The fluid filling within the small ampullae of the vas deferens and the seminal vesicles disappeared completely, resulting also in a reduction of overall size from 2.7 ±

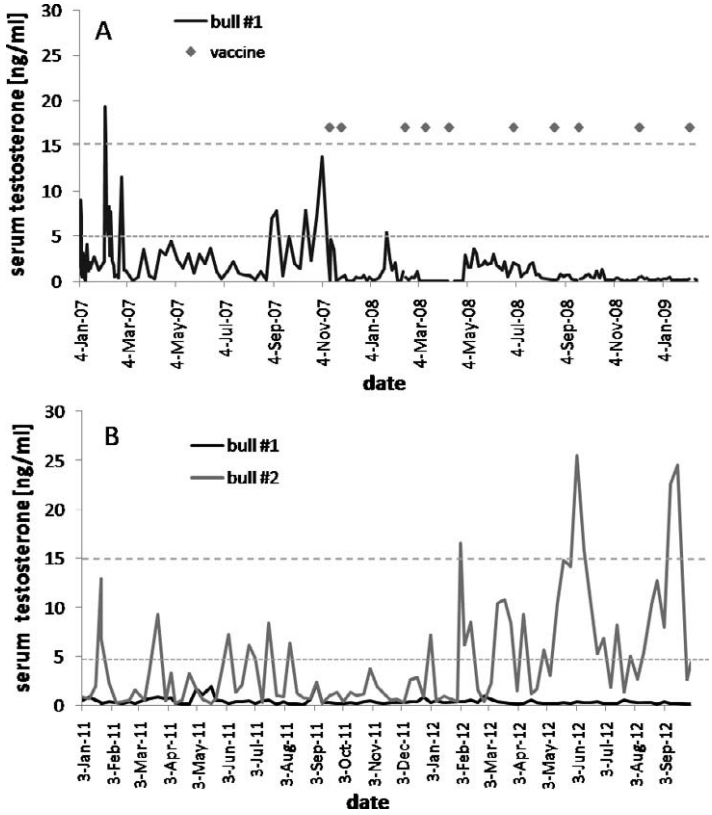

Figure 1. Serum testosterone concentrations of two elephant bulls: **A.** Bull 1: Prior to GnRH vaccination, testosterone spikes occurred; however, the hormone level remained below 5 ng/ml (dotted line) after the booster vaccine in 2007. Diamonds indicate each vaccination. **B.** Direct comparison of testosterone levels in bull 1 (black line, GnRH vaccinated) compared to its half-brother bull 2 (gray line, untreated) during 2011–2012 (more than 3 yr after GnRH vaccination began).

1.3 to 0.7 ± 0.1 and 3.7 ± 0.3 to 2.5 ± 0.3 ($P < 0.001$, df = 9), respectively (Fig. 2). There was a marked increase in seminal vesicle wall thickness (Fig. 2). Furthermore, the penis stopped developing and, by 12 yr of age, the growth retardation became extremely apparent compared to the younger half brother, bull 2 (Fig. 4). All reproductive organs developed normally in bull 2, and a marked increase in testicle size was recorded over the 6-yr monitoring period. The accessory sex glands and testicles in bull 2 were larger as compared to bull 1 at the age of 11 and 12 yr, respectively (Fig. 5).

Semen collection in bull 1 resulted in 5- to 12-ml ejaculates and yielded few viable sperm prior to vaccination. It appeared that this male was just starting to become reproductively mature when the first injection was scheduled. At approximately 8 mo after the start of treatment, only seminal plasma and structurally immature spermatozoa were retrieved. After 1 yr no spermatozoa were found and the volume of ejaculatory fluids collected had greatly decreased, reflecting an ultrasonographically visualized absence of seminal vesicle and ampullae content. In contrast, bull 2 had an increase in semen quality and quantity over the course of the study, reflecting maturation. Bull 2 produced ejaculates of 5–25 ml, yielding 65–90% motility during semen collection, and sired the first pregnancy at the age of 11 yr.

The monthly weighing of both bulls showed that bull 1 did not gain weight at the same rate as compared to prior to vaccination (Fig. 6). This effect commenced 2 mo after the first booster vaccine. Although it was not statistically significant at this stage, it became obvious that the half-brother, bull 2, gained weight more rapidly despite the 1.5-yr age gap. Bull 1 did not increase muscle mass as much (neck, shoulder) and appeared less masculine compared to bull 2.

Bull 2 presented with increased temporal draining and dominant behavior and, thus, was turned

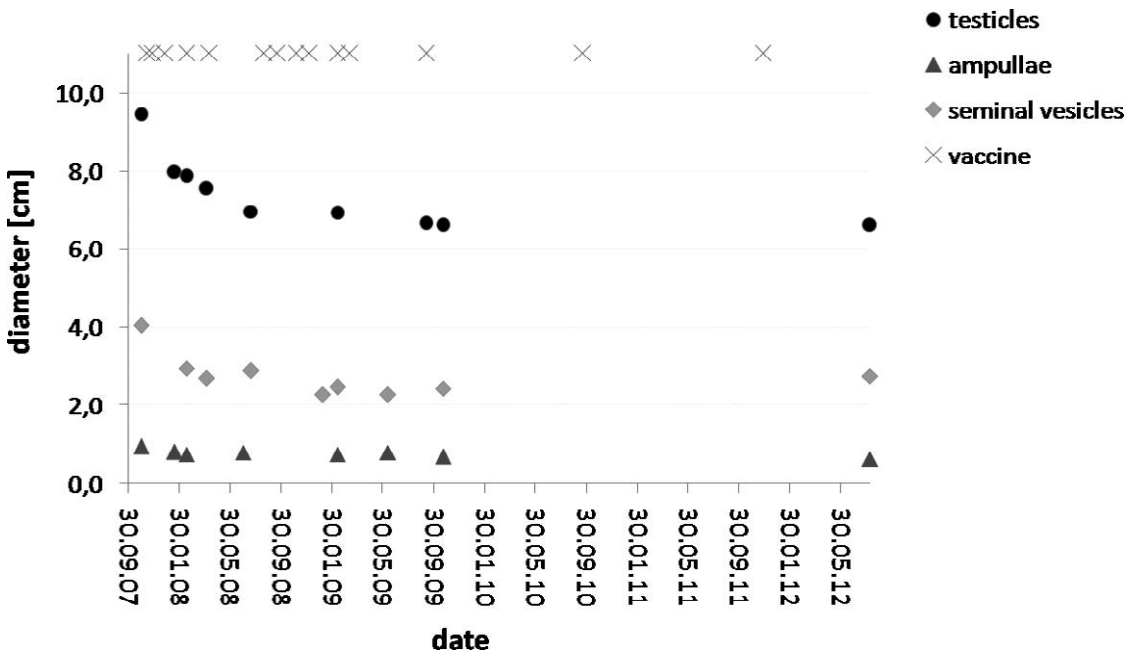

Figure 2. Measurements of testicle and accessory sex gland diameter (ampullae and seminal vesicles) in bull 1. After the first two vaccinations, the decrease in testicular size commenced immediately. The effect on seminal vesicles and ampullae size was less dramatic but apparent. X indicates vaccination dates.

into protected contact (PC; working only through a barrier with the elephant, the common management system for mature bulls in western zoos) at the age of 11 yr. Bull 1 remained in FC due to its manageable temperament. Throughout the study, bull 1 showed no signs of musth and no sexual interest in the females, despite being kept with fertile cows. Both males never showed any health issues for the period of the study.

DISCUSSION

This study followed two Asian elephant males growing up under the same conditions, with bull 1 treated with a vaccine against endogenous GnRH

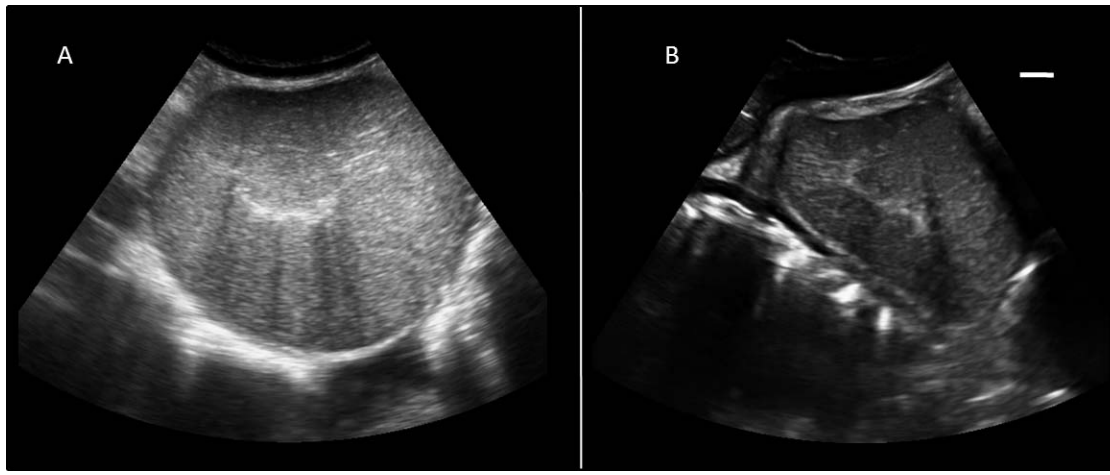

Figure 3. Ultrasound image of the right testicle of bull 1. **A.** Prior to GnRH vaccination, the testicle appeared round and firm and measured 8.5 × 10.2 cm. **B.** Two years after treatment, the right testicle was markedly smaller (5.3 × 7.5 cm) and softened, indicated by the amorphous shape when pushed against intestinal loops. Bar = 1 cm.

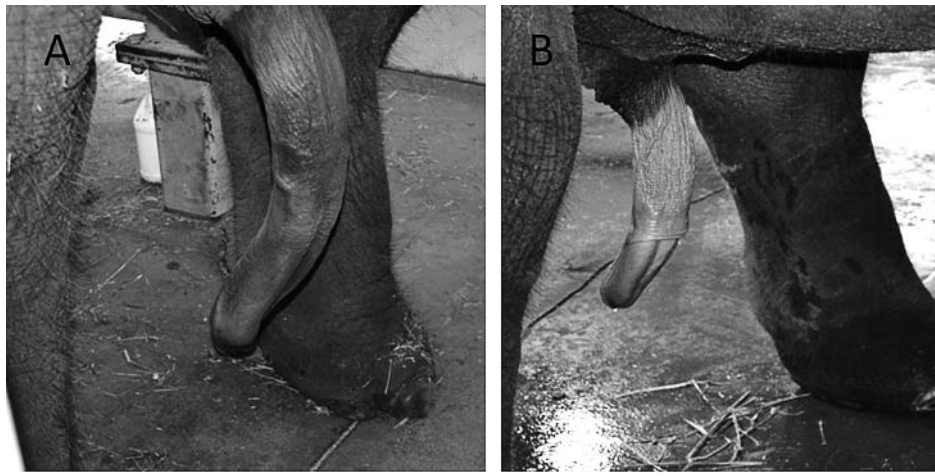

Figure 4. Photographs of the penis of nontreated bull 2 (**A**) and GnRH-vaccinated bull 1 (**B**) as seen shortly after urination. The size difference is apparent. Note the unpigmented skin in bull 1.

starting at the age of 7 yr whilst the related, 1.5 yr-younger bull 2 remained untreated.

The results show that there is a strong effect after vaccination on reproductive organs, testosterone concentration, sperm production, and body weight. Vaccination concentrations and treatment intervals were lower and more frequent in the beginning, as dosage and reaction were not known. As the study progressed and data were analyzed, the treatment regime was revised accordingly.

As shown in previous studies,[7,26] this report confirms the suppression of testosterone levels after two injections. Serum testosterone concentrations remained at levels comparable to those of castrated animals for the entire study. Thus, no signs of musth or musth-like phases occurred.

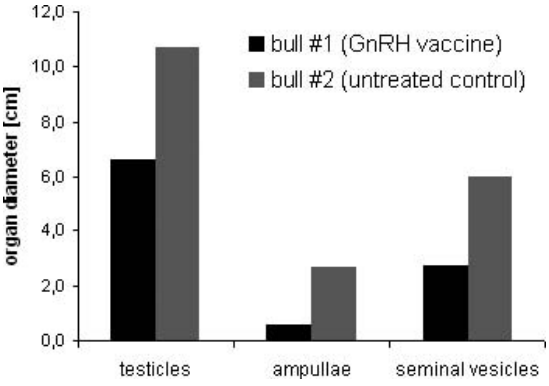

Figure 5. Direct comparison of testicle, ampullae, and seminal vesicle diameter of GnRH-vaccinated bull 1 and untreated control bull 2 when measured in August 2012 (6 yr after start of vaccinations).

When vaccination intervals were increased, 1.5 yr after the first GnRH injection, serum testosterone concentrations still remained around 1.0 ng/ml. This indicates that vaccinations may be spaced out to every 2 yr or more. Previous studies also suggest that larger vaccination intervals are effective in suppressing testosterone levels and musth.[7,26] During the course of the study, at 11 yr of age the nontreated bull 2 was switched from FC to PC, when it showed signs of increasing testosterone levels and temporal gland draining. Bull 1 in this study remained manageable in FC, an effect previously described for vaccinated African elephant bulls.[7] This result is invaluable for elephant (musth) bull management in captivity and may also impact handling practices of adult, musth bulls in Asian range countries. Here, captive elephants represent 20% of the entire Asian elephant population.[31] Traditionally, the larger and more impressive bulls are preferred as working or ceremonial animals.[30] Most temple, working, or privately owned bulls are seldom, if ever, used for breeding but undergo an annual musth phase of 2–3 mo or longer in duration.[22,25] During musth, elephant bulls are more aggressive and less attentive to their mahouts, which may threaten the health and lives of mahouts, other people, or livestock.[22] Currently, musth bulls are restricted in movements (hobbles, short chains) for extended periods; water and food are reduced and tranquillizers may even be applied,[22] all of which present significant welfare issues.

Studies to suppress musth have been carried out using GnRH analogue injections (leuprolide acetate) in an Asian elephant[8] or depots (Depot

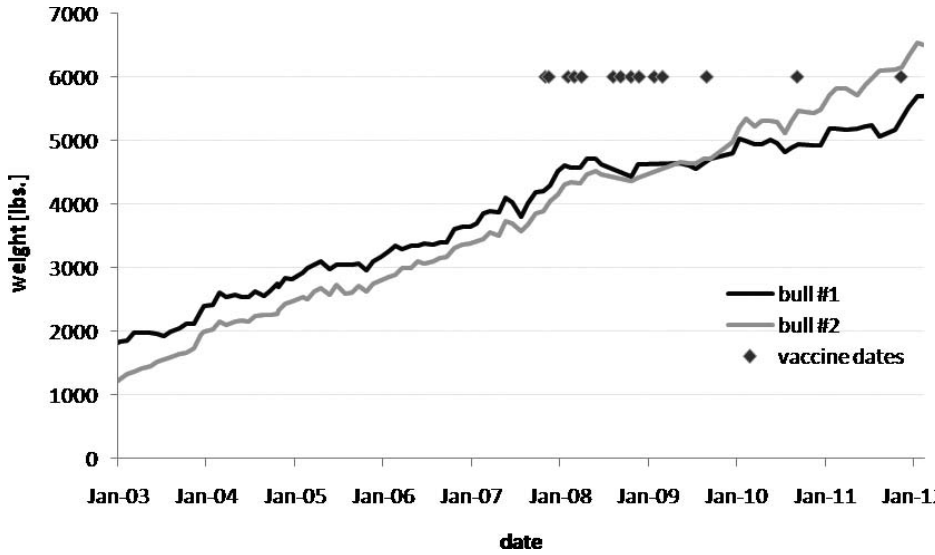

Figure 6. Monthly weights (lbs.) of bull 1 and bull 2, which was born 1.5 yrs later. While bull 2 was initially lighter than bull 1, this changed when the GnRH vaccination started (indicated by diamonds) in 2007.

Lupron) in wild African elephant bulls.[3] Here, the synthetic hormones bind to the GnRH receptors and induce GnRH release. While concentrations are rising initially, the body reacts with a negative-feedback and subsequently arrests GnRH secretion as long as exogeneous GnRH is circulating. A GnRH vaccine has the advantage of inducing its effects through an immune response rather than through exogenous hormone administration. Furthermore, it is more cost effective and may require fewer injections, with a longer-lasting effect compared to GnRH agonist applications.

While the vaccine may lower aggression or destructive behaviors caused by increased testosterone levels, it likely cannot change learned habits or individual personality characteristics that are not related to hormones. Mitigation of testosterone-driven traits through GnRH vaccination may be particularly achieved if applied at a prepubertal age so that mature bull habits do not develop. Male elephants in Africa were shown to be more likely to raid crops and break fences.[4] GnRH vaccination and subsequent testosterone suppression may positively affect behavior of these "problem" bulls.

In Europe and North America, more male than female calves are born.[27] The housing and management of these excess males as they mature is a rapidly looming problem. Typically, no more than one mature bull per elephant breeding facility is warranted. Keeping males in stable bachelor groups may be one possible management solution for managing these surplus males. Regular vaccinations may help to form stable groups of multiple males by removing testosterone-driven behavior. Because volumes are between 2–3 ml, the GnRH vaccine can also be safely applied via dart gun.

Finally, this study has provided the initial indications that this vaccine has potential as a male contraceptive. Along with arrest of sperm production, we found a significant decrease in penis (Fig. 4) and testicle size as well as in accessory sex glands (Figs. 2,3,5). The accessory sex glands, such as seminal vesicles, ampullae, ductus deferentes, and prostate, have already been shown to be markers for predicting fertility–breeding capability in male elephants.[18] Reduction in testicular size has been described in several GnRH vaccine studies on various species.[12,19,20] It appears to be a useful indicator for the successful application in elephants because we noted a diameter reduction of about 40% (Fig. 2). This is consistent with a 70% testicle weight reduction in pigs,[20] 40–80% in ram lambs,[21] and about 50% in cattle bulls.[5]

The reversibility of this vaccination regime still needs to be tested. The loss of testosterone in bull 1 was apparent in the small, empty ampullae and seminal vesicles as well as in the retarded growth of the penis, which remained small and pink and similar in appearance to juvenile elephants. Bull 1 was lighter, less muscular, and physically unable to breed a female properly. It seems unlikely that the treatment is reversible in this case. Extended investigations on long-term effects on the revers-

ibility of a GnRH vaccine in adult bulls are warranted. For antifertility peptide vaccines, further work is needed to examine the processes of immunity in different species because spermatogenesis is not always completely arrested.[12] The absence of spermatozoa when semen collection was performed and the low testosterone levels, together with a lack of sexual interest, suggest the castration-like impact of the GnRH vaccination in elephants.

Interestingly, while the weight gain was slowed down in bull 1, its growth (height) was undisturbed. This was also reported in ram lambs.[21] In other species (mice, rats;[12] pigs;[20] beef cattle bulls[19]), no or only little effect was observed, although an increased weight gain has been observed in some pigs.[9] In the latter case, less-aggressive interactions and physical activity were observed and regarded as the main reason for better weight gain.[9] In the case of bull 1, the missing anabolic effect of testosterone most likely resulted in a slower muscle mass gain and, therefore, reduced masculinity. In contrast, bull 2 had normal weight gain and muscle development as testosterone levels started to spike (Fig. 1), resulting in increased masculinity.

This case study describes the successful application of a GnRH vaccination in one male Asian elephant. The 6-yr treatment was an effective method to suppress testosterone-associated traits. More research needs to be conducted in order to determine treatment intervals, blood antibody titers, reversibility of the treatment, the application in adult elephant bulls, the effect on social and reproductive behaviors, and the potential long-term side effects.

LITERATURE CITED

1. Bertschinger K, Delsink AK. 2008. Reproductive control of elephants. In: Scholes RJ, Mennell KG (eds). Elephant management: a scientific assessment of South Africa. Johannesburg, South Africa: Witwatersrand University Press; 2008. p. 85–97.

2. Boedeker NC, Hayek L-AC, Murray S, de Avila DM, Brown JL. Effect of a gonadotropin-releasing hormone vaccine on ovarian cyclicity and uterine morphology of an Asian elephant (*Elephas maximus*). J Zoo Wildl Med. 2012;43:603–614.

3. Brown JL, Bush M, Wildt DE, Raath JR, de Vos V, and Howard JG. Effects of GnRH analogues on pituitary-testicular function in free-ranging African elephants (*Loxodonta africana*). J Reprod Fertil. 1993; 99:627–634.

4. Chiyo PI, Moss CM, Alberts SC. The influence of life history milestones and association networks on crop-raiding behavior in male African elephants. PLoS ONE 2012;7(2):e31382. doi:10.1371/journal.pone.0031382.

5. Cook RB, Popp JD, Kastelic JP, Robbins S, Harland R. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J Anim Sci. 2000;78:2778–2783.

6. Delsink AK, van Altena JJ, Kirkpatrick J, Grobler D, Fayrer-Hosken RA. Field applications of immunocontraception in African elephants (*Loxodonta africana*). Reprod Suppl. 2002;60:117–124.

7. De Nys HM, Bertschinger HJ, Turkstra JA, Colenbrander D, Palme R, Human AM. Vaccination against GnRH may suppress aggressive behaviour and musth in African elephant (*Loxodonta africana*) bulls—a pilot study. J S African Vet Assoc. 2010;81:8–15.

8. de Oliveira CA, West GD, Houck R, Leblanc M. Control of musth in an Asian elephant bull (*Elephas maximus*) using leuprolide acetate. J Zoo Wildl Med. 2004;35:70–76.

9. Dunshea FR, Colantoni C, Howard K, McCauley I, Jackson P, Long KA, Lopaticki S, Nugent EA, Simons JA, Walker J, Hennessy DP. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. J Anim Sci. 2001;79:2524–2535.

10. Einarsson S. Vaccination against GnRH: pros and cons. Acta Vet Scandinavica. 2006;48(Suppl. 1): S10.

11. Ferro VA. Current advances in anti-fertility vaccines for fertility control and non-contraceptive application. Exp Rev Vaccine 2002;1:443–452.

12. Ferro VA., Khan MAH, McAdam D, Colston A, Aughey E, Mullen AB, Waterston MM, Harvey MJA. Efficacy of an anti-fertility vaccine based on mammalian gonadotrophin releasing hormone (GnRH-I)—a histological comparison in male animals. Vet Immunol Immunopathol. 2004;101:73–86.

13. Fink G. Gonadotropin secretion and its control. In: Knobil E, Neill JD. (eds). The physiology of reproduction. New York, New York: Raven Press; 1988. p. 1349–1377.

14. Hendrickson DA, Stetter, Zuba. Development of a laparoscopic approach for vasectomies in free ranging African elephants. Proc An Amer College Vet Surgeons Symp, San Diego, California; 2008.

15. Herbert CA, Trigg TE. Applications of GnRH in the control and management of fertility in female animals. Anim Reprod Sci. 2005;88:141–153.

16. Hildebrandt TB, Göritz F, Hermes R, Reid C, Dehnhard M, Brown JL, Aspects of the reproductive biology and breeding management of Asian and African elephants. Int Zoo Yrbk. 2006;40:20–40.

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

18. Hildebrandt TB, Hermes R, Pratt NC, Fritsch G, Blottner S, Schmitt DL, Ratanakorn P, Brown JL, Rietschel W, Göritz F. Ultrasonography of the urogenital tract in elephants (*Loxodonta africana* and *Elephas maximus*): an important tool for assessing male reproductive function. Zoo Biol. 2000;19:333–345.

19. Janett F, Stump R, Burger D, and Thun R. Suppression of testicular function and sexual behaviour by vaccination with GnRH (Equity™) in the adult stallion. Anim Reprod Sci. 2009;115:88–102.

20. Jaros P, Bürgi, Stärk, Claus, Hennessy, Thun. Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. Livestock Prod Sci. 2005;92:31–38.

21. Kiyma Z, Adams TE, Hess BW, Riley ML, Murdoch WJ, Moss GE. Gonadal function, sexual behavior, feedlot performance, and carcass traits of ram lambs actively immunized against GnRH. J Anim Sci. 2000;78:2237–2243.

22. Lincoln GA, Ratnasooriya WD. Testosterone secretion, musth behaviour and social dominance in captive male Asian elephants living near the equator. J Reprod Fert. 1996;108:107–113.

23. Miller LA, Johns BE, Killian GJ. Immunocontraception of white-tailed deer with GnRH vaccine. Americ J Reprod Immunol. 2000;44:266–274.

24. Miller LA, Rhyan JC, Drew M. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J Wildl Dis. 2004;40:725–730.

25. Niemuller CA, Liptrap RM. Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (*Elephas maximus*). J Reprod Fertil. 1991;91:139–146.

26. Rajapaska RC, Dissanayaka IPGHU, Somgird C, Thitaram C, Sirimalaisuwan A, Pushpakumara PGA, Colenbrander B, Brown JL, Perera BMAO, Stout TAE. Efficacy of GnRH vaccination for suppressing musth and aggressive behavior in male Asian elephants. EU–Asia Link Project Symposium "Managing the Health and Reproduction of Elephant Populations in Asia" May 2010. Chieng May, Thailand: Faculty of Veterinary Medicine, University Chieng MaY; 2010. p. 114–116.

27. Saragusty J, Hermes R, Göritz F, Schmitt DL, and Hildebrandt TB. Skewed birth sex ratio and premature mortality in elephants. Anim Reprod Sci. 209;115:247–254.

28. Schmitt DL, Hildebrandt TB. Manual collection and characterization of semen from Asian elephants (*Elephas maximus*). Anim Reprod Sci. 1998;53:309–314.

29. Stetter M, Hendrickson D, Zuba J, Stretter K, Grobler D, van Altena JJ, Small L-A. Laparoscopic vasectomy as a potential population control method in free ranging African elephants (*Loxodonta africana*). In: Proc Intern Elephant Conserv Res Symp; 2006. p. 177.

30. Sukumar R. Elephant days and nights. Ten years with the Indian elephant. Delhi, India: R-Oxford University Press; 1995.

31. Sukumar R. The living elephants: evolutionary ecology, behaviour and conservation. Oxford, United Kingdom: Oxford University Press; 2003.

32. Thompson DL. Immunization against GnRH in male species (comparative aspects). Anim Reprod Sci. 2000;60–61:459–469.

33. Valades GB, Ganswindt A, Annandale H, Schulman ML, Bertschinger HJ. Non-invasive assessment of the reproductive cycle in free-ranging female African elephants (*Loxodonta africana*) treated with a gonadotropin-releasing hormone (GnRH) vaccine for inducing anoestrus. Reprod Biol Endocrinol. 2012;10:63.

34. van Aarde R., Whyte I, Pimm S. Culling, and the dynamics of the Kruger National Park African elephant population. Anim Conserv. 1999;2:287–294.

Received for publication 26 September 2013