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EFFECTS OF A GONADOTROPIN-RELEASING HORMONE VACCINE ON OVARIAN CYCLICITY AND UTERINE MORPHOLOGY OF AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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Abstract: This report describes the successful use of a gonadotropin-releasing hormone (GnRH) vaccine to suppress ovarian steroidogenic activity and to treat hemorrhage and anemia associated with reproductive tract pathology in a 59-year-old Asian elephant (*Elephas maximus*). The Repro-BLOC® GnRH vaccine was administered subcutaneously as a series of 4 boosters of increasing dose from 3 to 30 mg of recombinant ovalbumin-GnRH fusion protein given at variable intervals after initial vaccination with 3 mg protein. Efficacy was confirmed over a year after initial vaccination based on complete ovarian cycle suppression determined by serum progestagen analyses. Estrous cycle suppression was associated with a significant increase in GnRH antibody binding and subsequent decrease in serum luteinizing hormone and follicle-stimulating hormone concentrations. Ultrasonographic examinations of the reproductive tract documented a reduction in uterine size and vascularity after immunization. The hematocrit level normalized soon after the initial intrauterine hemorrhage, and no recurrence of anemia has been detected. No substantive adverse effects were associated with GnRH vaccination. The results indicate that GnRH vaccination in elephants shows potential for contraception and management of uterine pathology in older elephants.

Key words: Contraception, elephant, *Elephas maximus,* gonadotropin-releasing, progestagen, vaccine.

INTRODUCTION

Ovarian function in elephants is similar to that in other mammals and is controlled by secretion of the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), presumably under the influence of hypothalamic gonadotropin-releasing hormone (GnRH).[4,5] In general, FSH is responsible for stimulating ovarian follicular growth and development and for the production of estrogens, whereas LH induces ovulation, formation of the corpus luteum, and production of progestagens.[6,25] Elephants are unique in having the longest documented mammalian estrous cycle, of 13–17 wk, and exhibiting two successive waves of follicular development that culminate in two distinct LH surges, with only the second inducing ovulation.[8,9,35]

One documented problem of older female elephants and other long-lived species in captivity is termed asymmetric reproductive aging.[24,28] In the wild, most adult female elephants are either pregnant or lactating; they experience comparatively few reproductive cycles in their lifetime. In captive Asian elephants, primarily nulliparous females over 30 yr of age or those that have not calved within a 10- to 15-yr period, numerous reproductive tract pathologies develop.[24,27–29,44] For example, older Asian elephants often develop endometrial hyperplasia and/or multiple leiomyomata (benign myometrial tumors).[1,27–29,44,45,57] Periodic mucohemorrhagic vaginal discharge can result from these pathologic alterations. These specific conditions have not been reported in wild Asian elephants. Because older cycling cows appear to be particularly susceptible, it has been speculated that continuous steroid hormone (estrogens and/or progestagens) exposure associated with ovarian cyclicity of nonbred females has a negative and cumulative effect on reproductive health and urogenital tract integrity.[1,24,28] To prevent these pathologies from developing, treatments to shut down ovarian activity should be considered if a female is not intended for breeding.

GnRH vaccines have been designed to stimulate the production of anti-GnRH antibodies that

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block binding of endogenous GnRH to gonadotrope receptors in the pituitary gland. This action inhibits the release of FSH and LH from the anterior pituitary, thereby causing the cessation of ovarian steroidogenic activity and reproductive cyclicity. Immunization against GnRH has been used successfully to suppress ovarian function in a number of domestic, laboratory, wildlife, and livestock species.[3,10,18,22,23,30,31,36,42,49,55] The primary structure of the decapeptide GnRH is largely conserved in mammals, but by itself is not immunogenic.[23,46] The vaccine in this study was developed by using a recombinant ovalbumin-GnRH fusion protein, marketed under the trade name Repro-BLOC® (Amplicon Vaccine, LLC, Pullman, Washington 99163, USA).[11,20,33,51,56] The adjuvant is a combination of Freund's incomplete adjuvant with a synthetic cytosine guanine oligodeoxynucleotide that mimics the immunostimulatory effects of bacterial deoxyribonucleic acid because of its relatively high content of unmethylated cytosine guanine dinucleotides.[10,11]

This case report describes the use of Repro-BLOC to suppress ovarian cyclicity to prevent hemorrhage and anemia associated with reproductive tract pathologies in an older female Asian elephant at the National Zoological Park. To date, to the authors' knowledge, there are no published reports on the use of GnRH vaccination to control reproductive activity in female elephants. Possible benefits of using this approach to address problems associated with asymmetric reproductive aging as well as for contraception in elephants are discussed.

CASE REPORT

The subject was a 59-yr-old, approximately 3,300 kg, female Asian elephant who had been at the Smithsonian Institution's National Zoological Park for 46 yr. She was housed with a 33-yr-old female Asian elephant and occasionally that female's 6-yr-old male offspring. This female had a relatively unremarkable medical history, with the exception of chronic dental malposition and a small chronic left elbow abscess. At the time of presentation, she was on long-term, low-dose ibuprofen (3,600 mg p.o. s.i.d.) for management of possible discomfort associated with mastication, and a glucosamine and chondroitin supplement (Cosequin® Equine Concentrated Powder, Nutramax Laboratories, Edgewood, Maryland, 21040 USA) intended for maintenance of joint health.

On 14 January 2007, keepers reported that the elephant had a decreased appetite and stool production, mild lethargy, and reluctance to train, particularly for behaviors that involved lying down. A complete blood cell count (CBC) and chemistry panel were unremarkable other than a hematocrit concentration of 30.8% and hemoglobin level of 10.1 g/dl, both at the low end of her historic range (hematocrit concentration, 30–43.7%, average 36%; hemoglobin level, 10.1–14.9 g/dl, average 12.02 g/dl), and an elevated fibrinogen level of 900 mg/dl (range, 200–700 mg/dl; average, 479 mg/dl). Urinalysis, fecal parasite check, fecal cytology, and fecal occult blood were unremarkable. Mild colic of unknown etiology was initially suspected, and clinical signs were rapidly responsive to a 2-day course of treatment with injectable flunixin meglumine (Banamine® 50 mg/ml, Merck Intervet/Schering Plough Animal Health, Summit, New Jersey 07901, USA; 1,000–3,000 mg i.m. s.i.d.). The hematocrit concentration remained relatively stable, at 30.1% on 16 January, and 31.9% on 22 January 2007.

On 29 January 2007, 15 days after initial presentation, the female's appetite and activity had improved, although it was not completely back to normal levels. However, bloodwork showed that the hematocrit concentration had dropped dramatically, to 19.3%, with a hemoglobin concentration of 6.4 g/dl. Repeated samples confirmed the severity of the anemia. There was no evidence of hemolysis and mean corpuscular volume (MCV) (128/fl), mean corpuscular hemoglobin (MCH) (42 pgm/cell), and mean corpuscular hemoglobin concentration (MCHC) (33 g/dl) remained within her normal ranges, but target cells were subjectively increased. Fibrinogen level had returned to within her normal range, at 500 mg/dl. Fecal parasite check and fecal occult blood tests were negative. Urinalysis, fecal cytology, iron panel, mineral panel, estrogen level, and coagulation panel results were within acceptable limits. Based on the diagnostic test results and clinical signs, a recent episode of acute internal hemorrhage was suspected. Empiric treatment with ceftiofur (Naxcel®, Pfizer Animal Health, New York, New York 10017, USA) was administered at 6–8 g i.m. s.i.d. beginning on 29 January 2007, for a total of 23 days.

By 7 February 2007, 24 days after initial presentation, the elephant's appetite and activity had returned to normal baseline levels and s.i.d. to every other day blood samples showed a gradual increase in the hematocrit concentration, to 28.7%, and in the hemoglobin level, to 9.1 mg/dl. At that time, the elephant was given 30 mg detomidine (Dormosedan® 10 mg/ml; Pfizer Animal Health, New York, New York, 10017,

USA) and 30 mg butorphanol i.m. (Torbugesic® 10 mg/ml; Pfizer) for standing sedation in an elephant restraint device to allow for rectal and transabdominal ultrasound evaluation by an experienced elephant ultrasonographer. An additional 10 mg detomidine and 10 mg butorphanol were administered i.m. as a supplement to deepen sedation at approximately 60 min into the procedure. Transabdominal ultrasound was performed on the left side; no evidence of free fluid was identified, and the spleen, part of the caudal liver lobe, and intestinal loops appeared normal. Transrectal examination of the caudal abdomen was performed by using a 3.5 MHz convex transducer to scan the caudal region of the genital tract and by using a 5.0–7.5 MHz transducer attached to a specially designed probe extension to scan the cranial region. The examination revealed a large mass consistent with a blood clot, estimated at more than 18 L, which expanded the vagina and extended into the uterus (Fig. 1A). The uterus was drawn ventrally into the abdominal cavity and could not be examined in its entirety. Visibly enlarged blood vessels were identified on the dorsal surface of the reproductive tract near the cervix (Fig. 1B). Although these vessels were considered likely to be associated with a vascular tumor, no masses consistent with a tumor could be identified, possibly due to the limited visualization of the ventrally displaced uterus. The caudal lobe of the left kidney appeared normal, and no fluid was noted in the caudal pelvic flexure.

The most likely cause for this elephant's episode of colic followed by severe anemia was considered to be hemorrhage into the vagina and uterus. Underlying reproductive tract pathology was strongly suspected. Differentials included leiomyoma, cystic endometrial hyperplasia, uterine artery rupture, neoplasia, or infection, but the etiology could not be definitively determined due to limited visualization of the reproductive tract, which was expanded and weighed down by the suspected blood clot. The decision was made to resolve the reproductive tract pathologies by using a GnRH vaccine (Repro-BLOC).

MATERIALS AND METHODS

The elephant had been conditioned to venipuncture without sedation, as part of the normal management routine. Blood was collected from an ear vein approximately weekly, generally in the morning. The blood sample was maintained at approximately 4°C, allowed to clot at room temperature, then centrifuged (approximately 1,500 *g*) and frozen. Serum was stored at –20°C or colder until analysis.

The Repro-BLOC vaccine was first administered on 27 March 2007 at a dose and dose interval proven effective to sterilize heifers: 3 mg s.c., with a booster vaccination of 3 mg s.c. given approximately 14 wk later, on 3 July 2007.[11] Based on serial evaluation of serum progestagen concentrations and GnRH antibody titers, 3 additional boosters of increasing dosage (7.5 mg on 29 November 2007, 8 mo after initial injection; 12.5 mg on 27 March 2008, 12 mo after the initial injection; 30 mg on 20 November 2008, 20 mo after the initial injection) were given. The total injection volume increased from 4.5 to 22 ml, with increases in dosage; the volume of each dose was divided in two and administered s.c. by hand injection at two sites on the neck.

Management changes during the study interval included no longer asking the elephant to lie down or perform activities that were thought likely to disrupt the blood clot in her reproductive tract and potentiate further hemorrhage. Ibuprofen and Cosequin treatments were discontinued in case they were contributing to any tendency to bleed; no negative clinical effect of this change was observed. Monitoring by keepers included daily assessment of appetite, attitude, and activity level, and for vaccine site reactions, and for any indication of hemorrhage.

CBC and chemistry panels were obtained at least weekly in 2007, at least twice a month in 2008, and at least monthly with twice monthly hematocrit concentration assessments in 2009. Urine was submitted for urinalysis at least twice monthly throughout the years 2007 to 2009, primarily to monitor for hematuria. Follow-up rectal ultrasound examinations under standing sedation as previously described were performed in April of 2007 and April of 2009.

Anti-GnRH antibody titers were determined in sera at a 1:100 dilution by using a ^{125}I-GnRH radioimmunoassay.[33] Titer results were obtained from samples collected approximately weekly from 19 March 2007 (just before the initial vaccination) through 6 February 2008 and then approximately every 2 wk through 6 January 2010. Serum progestagens were analyzed by using a solid-phase ^{125}I-progesterone radioimmunoassay (Coat-a-Count®, Siemens Diagnostic Products Corporation, Costa Mesa, California 92626, USA) validated for elephants.[7] Serum FSH and LH concentrations were quantified by using double antibody ^{125}I radioimmunoassays validated for elephants.[6] Hormone results were analyzed

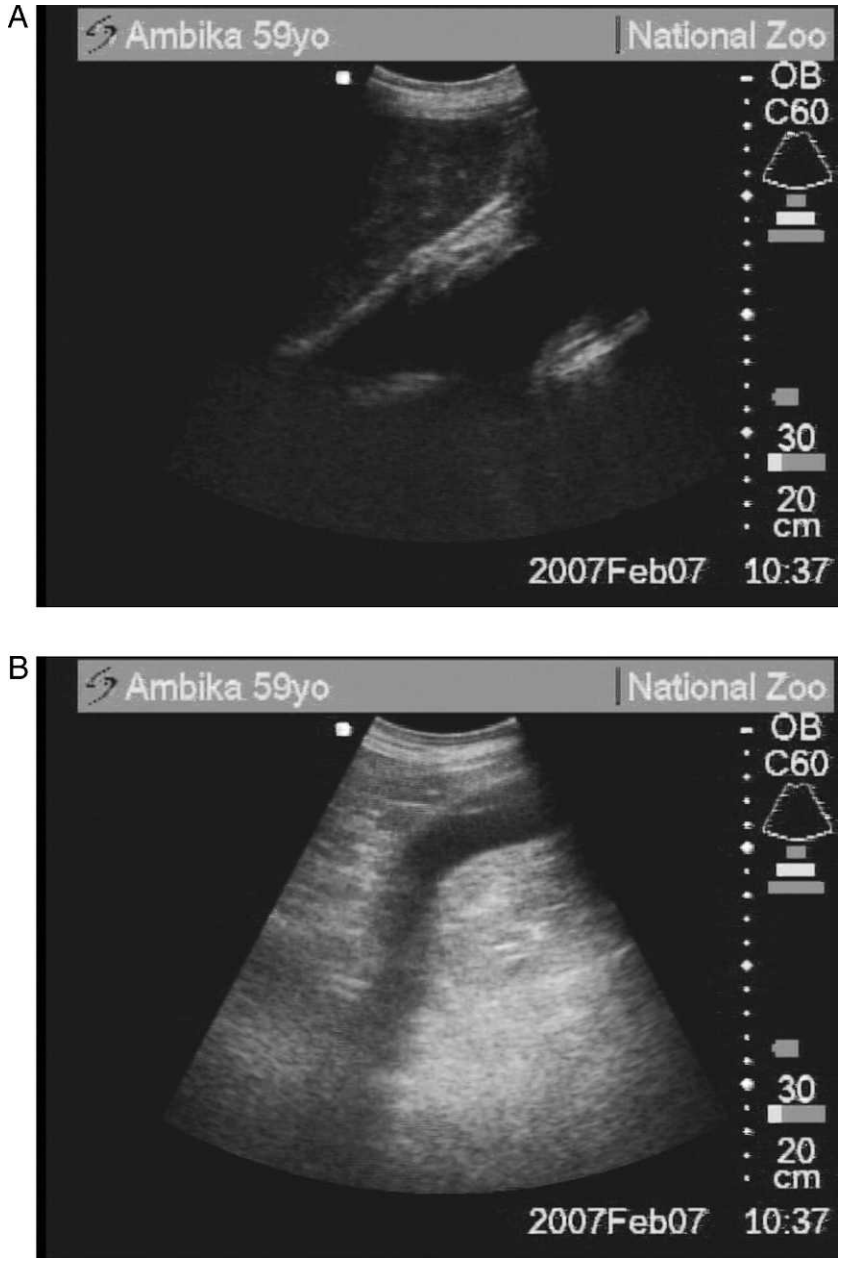

Figure 1. Ultrasound images from the 7 February 2007 examination, showing (**A**) the distended vaginal canal at the top of the image and a suspected blood clot enlarging to the left from the cervix with the urinary bladder below and (**B**) an enlarged blood vessel adjacent to the distended vagina. Images provided by Dr. Dennis Schmitt.

from samples collected approximately weekly from 4 January 2006 to 6 January 2010.

Statistical methods

To determine this elephant's estrous cycle patterns, baseline progestagen concentrations were calculated by using an iterative process previously developed for Asian elephants.[8] All data points with values above the mean plus two times the standard deviation (SD) were removed, and the process was repeated until no values that exceeded the mean plus two times the SD remained. The remaining data points defined the

baseline, and all points above that were considered part of a luteal phase. The highest point in each luteal phase was considered the peak. Estrous cycle duration was calculated as the number of weeks from the first increase in serum progestagens until the next increase. Peaks of LH and FSH levels also were determined by using the same iterative process by excluding values that exceeded the mean plus two times the SD.[8]

Titer and hormone data were subjected to statistical time series analysis and also were modeled with the ASTRA program with SPSS Statistics (ver. 20, 2011, IBM Corp. Armonk, New York 10504, USA) used for simple descriptive statistics, tests of assumptions, and general linear model hypothesis testing and modeling.[50] Autoregressive integrated moving average models were fit to the data for each of the variables. By using these models, the differences in cyclicity over time were examined by trend analysis methods that are related to ordinary least-squares regression.[50] In addition, the relationship over time intervals before and after vaccine introduction was examined. The variables were divided into six intervals based on the timing of vaccine administrations.

RESULTS

Health and ultrasound examinations

The elephant's appetite, attitude, activity, and weight had remained at normal levels with no recurrence of colic throughout the study period. The anemia fully resolved by 14 February 2007, and CBC parameters remained within normal ranges. Chemistry panel values remained unremarkable other than a period of mild intermittent hyperglycemia of undetermined etiology, with a low-level glucosuria in May 2009. Rare trace positive blood results on urine dipstick have been identified without elevations in red blood cells on microscopic urine sediment examination.

Episodes of passage of small volumes of mucohemorrhagic discharge after urination were noted occasionally in 2006 (prevaccination) as well as in 2007 and 2008. Cytologies of this suspected vaginal discharge revealed mild hemorrhage, mild granulocytic, and histiocytic inflammation, and small numbers of gram-positive cocci widely scattered in mucous suggestive of mild vaginitis. Pigment identified within the macrophages was consistent with hemosiderin and suggested breakdown of the ultrasonographically identified blood clot or potentially breakdown of clots from more recent undetected bleeding. This discharge continued intermittently until mid October 2008, approximately 1 mo before administration of the final vaccine booster, and has not been noted in the following 3 yr since this report.

A transient mild localized reaction occurred at both injection sites within 24 hr after the first GnRH vaccination; firm, nonpainful, subcutaneous swellings persisted for approximately 2 mo. A warm, firm, subcutaneous swelling developed as well at both injection sites after the final vaccine administration, which, in contrast to the first vaccination reaction, was sensitive to touch and caused guarded neck movement. The elephant's apparent discomfort resolved with treatment with ibuprofen (6,000 mg p.o. s.i.d.) for 3 days, but the firm subcutaneous swellings have persisted 2 yr later. Other than these local reactions, no negative adverse effects associated with the vaccine had been noted.

A transrectal ultrasound evaluation on 2 April 2007, approximately 11 wk after initial presentation and 6 days after administration of the first GnRH vaccination, revealed an approximately 10-cm-diameter, well-encapsulated, relatively homogenous, soft tissue mass in the cranial vagina. Blood vessels were identified in the capsule but not within the mass itself. Fluid and pseudomembranous tissue surrounded the mass, but there was no evidence of ongoing hemorrhage. Due to the location of the vaginal mass, there was concern that enlargement could compress the urethra and cause obstruction to urine flow and potential renal compromise. The uterus was again difficult to visualize in its entirety but appeared small, irregular, and misshapen, with prominent, visibly enlarged blood vessels, interpreted as being more suggestive of an infectious or neoplastic process than of menopausal changes. Fibrosis and cysts were identified in both kidneys.

A follow-up transrectal ultrasound was performed on 19 April 2009, approximately 5 mo after the last booster was administered and when serum monitoring had indicated cessation of ovarian cyclicity in conjunction with a rise in GnRH antibody titers. A 10-cm diameter vaginal cyst just beyond the opening to the vestibule was observed. Both ovaries appeared small and inactive (Fig. 2A). The uterus was small and indistinct, with a visible decrease in size of the uterine vessels compared with the previous studies (Fig. 2B). The vaginal soft tissue mass identified in April 2007 appeared to have resolved, and no clot or active hemorrhage associated with the reproductive organs was identified. The ultrasound findings supported the hormonal data that indicated cessation of ovarian cyclicity and reproduc-

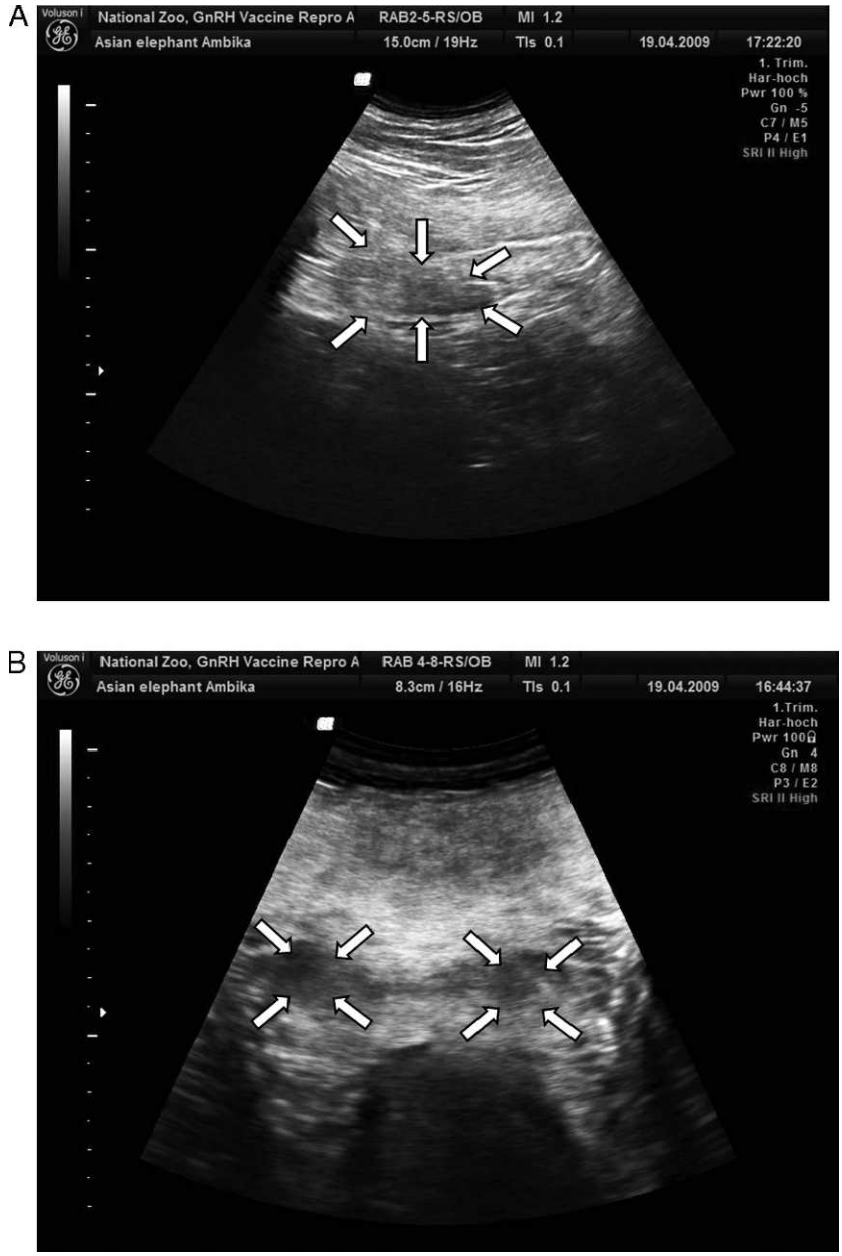

Figure 2. Ultrasound images from the 19 April 2009 examination, showing (**A**) an inactive ovary and (**B**) the inactive uterus in cross section. Images provided by Dr. Thomas Hildebrandt.

tive quiescence. No progression of the renal changes was identified.

Serum GnRH antibody titer, progestagen, and LH and FSH profiles

GnRH percent binding data in sera diluted at 1:100 is presented in Figure 3A. Time series regression indicated a significant change in binding over time, and a quadratic trend fit to these data showed a monotonically increasing trend ($P = 0.001$). Dividing these time-dependent observations for antibody titers into six time periods starting before the first dosage resulted in significantly different and increasing means ($P < 0.001$), with SDs that increased until time period 4, then decreased (Table 1). The change in GnRH titer remained nonsignificant until after the sec-

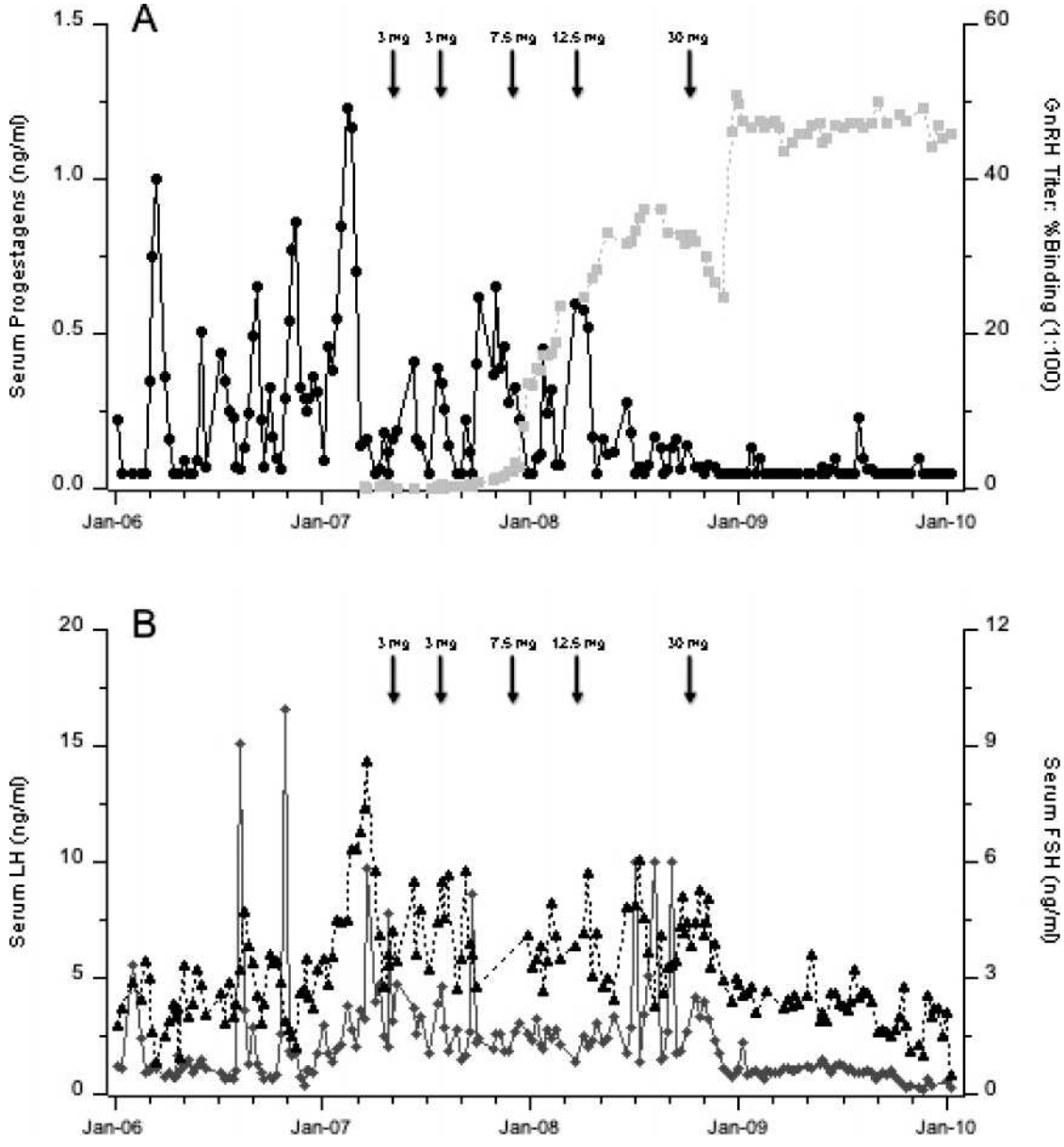

Figure 3. Profiles of (**A**) serum progesterone (●) and gonadotropin-releasing hormone (GnRH) titers (■) indicated by the percentage binding of sera diluted at 1:100; and (**B**) serum luteinizing hormone (LH) (♦) and follicle-stimulating hormone (FSH) (▲) concentrations in an Asian elephant administered a GnRH vaccine. Vaccination dosages were administered at each of five time points indicated by the arrows.

ond 3-mg dose was given on 3 July 2007 in time period 3, after which titers exhibited a continuous and significant increase. The slope of this linear fit was significant as were the two sets of combined observations ($P = 0.001$). The variances, like the means, also were significantly different across these periods ($P = 0.0001$). Although a quadratic trend could be fit, it was apparent that there was a significant change induced after time period 3. In considering all data, there were 2 distinct groups of time periods, which consisted of time periods 1 through 3, and time periods 4 through 6 (Table 1).

The length of the female's estrous cycle before vaccination (time period 1) averaged 14.1 ± 0.5 wk, with a mean peak progestagen concentration of 0.78 ± 0.4 ng/ml (range, 0.45–1.23 ng/ml).

Table 1. GnRH antibody titer, progestagen, LH, and FSH levels, means (SDs) and (ranges), during six time periods, pre- and postvaccination.[a]

Time period	Date range	Vaccine dose (mg)[b]	GnRH antibody Titer (% binding)			Progestagen (ng/ml)			LH (ng/ml)			FSH (ng/ml)		
			n	Mean (SD)	Range	n	Mean (SD)	Range	n	Mean (SD)	Range	n	Mean (SD)	Range
1	1/4/06–3/26/07	0	1	–0.09 ± 0	0.09	53	0.34 ± 0.30	0.05–1.23	53	2.32 ± 3.09	0.34–16.54	53	3.06 ± 1.62	0.83–8.59
2	3/27/07–7/2/07	3	15	–0.01 ± 0.23	–0.32–0.52	10	0.15 ± 0.11	0.05–0.41	10	3.85 ± 1.65	2.05–7.78	12	4.18 ± 0.91	2.77–5.77
3	7/3/07–11/28/07	3	21	0.83 ± 0.91	–0.35–3.28	17	0.28 ± 0.19	0.05–0.65	17	2.82 ± 1.71	1.51–8.63	12	4.25 ± 1.12	2.71–5.77
4	11/29/07–3/26/08	7.5	13	16.55 ± 6.57	2.68–27.13	15	0.20 ± 0.17	0.05–0.60	15	2.81 ± 1.82	1.44–9.14	14	4.27 ± 1.20	2.66–6.85
5	3/27/08–11/19/08	12.5	16	31.31 ± 3.02	24.67–36.11	27	0.14 ± 0.13	0.05–0.58	27	3.45 ± 2.52	1.35–10.00	30	4.03 ± 0.96	2.27–6.07
6	11/20/08–1/6/10	30	29	46.93 ± 1.68	43.66–50.83	56	0.06 ± 0.03	0.05–0.23	53	0.92 ± 0.36	0.16–2.25	48	2.23 ± 0.59	0.49–3.61

[a] GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SD, standard deviation.

[b] Vaccine doses were administered on the first day of each of the time periods 2 through 5.

Serum progestagen concentrations decreased after GnRH vaccination, with a definitive cessation in ovarian cyclicity and luteal function after the final 30-mg vaccine dose (Fig. 3A; Table 1). The variances, like the means, were significantly different across time ($P = 0.001$). A linear, not a quadratic trend could be fit to the mean values over the time periods ($P = 0.0001$). However, it was apparent that there was a significant change in progestagen concentrations after time period 3 (Table 1). When considering the prevaccine time period 1 as a baseline, Dunnett's test showed that time periods 3 and 4 were not different from baseline, whereas the subsequent time periods 5 and 6 were lower ($P = 0.013$). The average estrous cycle length through time period 4 was 13.9 ± 0.6 wk, with a mean peak progestagen concentration of 0.45 ± 0.06 ng/ml (range, 0.40–0.65 ng/ml). In time periods 5 and 6, after the 12.5-mg dose, only 4 clear cycles were observed; the average length had decreased to 10.1 ± 0.3 wk, and progestagen peak concentrations had decreased from a high in time period 4 of 0.60 ng/ml to successive declining peak concentrations of 0.28, 0.17, 0.16 and 0.14 ng/ml (Fig. 3A). Thereafter, serum progestagen concentrations remained at or near baseline (<0.07 ng/ml).

After an initial increase compared with the prevaccination baseline (time period 1), mean LH and FSH concentrations decreased, with the greatest effect observed after the final 30-mg booster, more than 1.5 yr after the initial vaccination (Fig. 3B; Table 1). When examined as serial data, only time periods 1 and 6 were statistically different from the remainder ($P < 0.001$). Mean LH, which was more variable, declined to 0.92 ng/ml at time period 6, from a baseline of 2.32 ng/ml, whereas mean FSH declined to 2.23 ng/ml at time period 6, from a baseline of 3.06 ng/ml. The decline in gonadotropin secretion occurred coincident with the suppression of serum progestagen concentrations. This decrease in mean concentrations was associated with a dampening of pulsatile LH and FSH secretion in time period 6, as evidenced by a decrease in the SDs (Table 1). Peak concentrations of LH exceeded 6 ng/ml nine times, and peak concentrations of FSH exceeded 4 ng/ml eight times through time period 5. No significant pulses of LH or FSH were observed in time period 6 after the 30-mg dose was given.

DISCUSSION

This is the first published report, to the authors' knowledge, on the effects of a GnRH

vaccine on health and reproductive function of a female Asian elephant. Altogether, GnRH antibody titer, serum hormone, ultrasonographic, and clinical results strongly suggest that the cessation of ovarian cyclicity and the associated decrease in uterine size and vascularity occurred as a result of GnRH vaccination. The significant increase in GnRH antibody binding was inversely related to a sustained suppression in LH and FSH secretion and in serum progestagens after the final booster was administered. Based on time-series analyses, effects of the vaccine on GnRH and progestagens were evident after the 12.5-mg booster (time period 5), approximately a year after the initial injection. Significant effects on LH and FSH secretion were observed after the final 30-mg booster. The vaccinations were well tolerated, with no adverse effects other than relatively minor localized reactions at the injection sites after two of the five vaccinations. Any appreciable discomfort associated with these subcutaneous swellings resolved quickly. Thus, the results of this current study suggest that GnRH vaccination is a safe and effective method for resolving significant health problems associated with uterine pathologies likely caused by continuous ovarian cyclicity.

Caution in not overinterpreting these results is advised, however, because only one female was studied. A definitive diagnosis of the underlying pathology that led to the strongly suspected hemorrhage into this elephant's reproductive tract and associated severe anemia could not be determined. In addition, it cannot be determined with certainty if this elephant would have hemorrhaged again without therapy. Although analysis of limited laboratory data suggests that female Asian elephants can continue cycling normally into their 60s (Brown, unpubl. data), it is possible that this geriatric female would have stopped cycling naturally without vaccine administration. However, the strong correlation in timing of the rise in GnRH antibody titer with the cessation of ovarian cyclicity, appearance of reproductive quiescence on ultrasound and resolution of intermittent mucohemorrhagic vaginal discharge makes this possibility seem unlikely.

GnRH vaccination has potential for treating reproductive tract disorders, such as endometriosis and ovarian tumors, as well as prostate and breast cancer and other diseases driven by gonadal steroids.[21,34,46,53] In elephants, common pathologies of the reproductive tract, particularly in nulliparous females, include leiomyomata and cystic endometrial hyperplasia. Although these processes are thought to be generally benign, they are suspected to contribute to infertility and could lead to erosion of vessels and associated hemorrhage.[24,28] Cessation of cycling through GnRH vaccination would be expected to decrease the likelihood of negative outcomes with these pathologic conditions, and the likelihood of significant negative adverse effects is considered low.[11,16,18,20,26,39]

Many factors can influence the immune response to GnRH vaccination, including individual genetic and immune status variation, patient age, vaccine dose, species variation in regard to optimal adjuvants, and the conjugant and specific design used in the manufacture of the vaccine.[17,19,20,40,41,48,54] Preliminary evaluation of a GnRH vaccine for control of aggression and musth behavior in bull elephants has shown some degree of variation in the response of individuals, and this phenomenon has been noted in a variety of other species.[2,13,23,30,32,52] More work clearly is needed to optimize the dose and frequency of vaccination when using the Repro-BLOC formulation in elephants, because it took more than a year for the effects to be realized. It is not clear why this elephant required multiple boosters before the desired effect of vaccination occurred. The initial doses administered may have been too low, the dose intervals could have been suboptimal, the adjuvant used may not be as immunostimulatory in elephants as in beef cows, or this individual's immune response could have been compromised due to age or disease status. Other GnRH vaccines are available, some of which are being designed for multiyear contraceptive effects after a single injection and act more rapidly in tested species, within weeks (e.g., GonaCon™, National Wildlife Research Center, USDA-APHIS Wildlife Services, Fort Collins, Colorado 80521, USA), but they have not been evaluated in female elephants.[36,37,41,43] The initial doses of Repro-BLOC used in this study were based on data in cattle in which peak antibody titers were reached 2 wk after administration of a 1.5-mg booster (16 wk after initial injection of 1.5 mg).[11] Based on the progestagen and possibly the FSH results, suppression of ovarian activity in this elephant was not evident until after the 12.5-mg dose, whereas a significant effect on LH was evident only after the final 30-mg dose. However, it cannot be concluded that 12.5 mg is the optimal dosage or that the cumulative effects of multiple doses over time were not significant. But once the response occurred, it has been sustained, and the elephant continues to be reproductively suppressed. Ongoing routine progestagen monitoring that continues to date, con-

tinues to show that concentrations average less than 0.07 ng/ml, 3 yr after the final booster. The reversibility of this vaccine approach in elephants now remains to be determined.

Based on studies in other species, GnRH vaccination also offers exciting possibilities as a new option for potentially reversible contraception in female elephants.[3,18,30,36,41,43] In elephant bulls, GnRH vaccination has shown efficacy in suppressing testosterone production and musth behaviors, presumably by similarly inhibiting pituitary control of gonadal steroidogenic activity.[2,13,14] A carefully managed contraception strategy would be of particular value in areas of high elephant density in close proximity to humans to mitigate human-elephant conflicts and in game parks where protected space and resources are limited. It would be beneficial to compare the efficacy, duration of effect, reversibility, long-term health and genetic effects, and social and behavioral effects of GnRH vaccination in elephants with those of porcine zona pellucida vaccination, which prevents fertilization by blocking sperm binding to the egg and is currently used in female elephants for contraception.[2,12,14,15,38,47] Porcine zona pellucida vaccination would not be efficacious for resolving health problems associated with ovarian steroidogenic activity, however, because contracepted females continue to cycle.

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