Outcomes from an anti-gonadotropin-releasing hormone immunotherapeutic trial in large flying foxes (Pteropus vampyrus)

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OBJECTIVE
The anti-GnRH immunotherapeutic product Improvest was administered to intact male large flying foxes (Pteropus vampyrus) under managed care for androgen mitigation, leading to a decrease in agonistic behaviors, falls, and injuries from conspecific attention.

ANIMALS
12 males were included in this study.

PROCEDURES
Eleven bats received subcutaneous (SC) Improvest interscapular, and 1 animal received Improvest SC in its leg. Assessments included clinical presentation, treatment, behavior, and urine and fecal glucocorticoid metabolites and testosterone (T5) concentrations.

RESULTS
Eleven of the 12 bats developed reactions, which included facial edema, localized irritation, swelling of the head and neck, and pruritus with varying degrees of skin ulceration and subsequent necrosis. Three of the animals required extensive treatments, and the 1 animal who received the injection in its leg was unaffected. Posttreatment, fecal glucocorticoid metabolite and/or T5 values were at or below the nonbreeding season baseline for 3 successive breeding seasons, and there was a reduction in agonistic interactions, falls, and injuries.

CLINICAL RELEVANCE
A behavioral characteristic of this species is to focus on areas of irritation that exacerbated the extent of the skin wounds. Some cases required medical, surgical, and behavioral intervention. Large flying foxes may be particularly sensitive to this immunotherapeutic when given subcutaneously in the interscapular region. Despite this reaction, the positive long-term effects on behavior and multiyear reduction of hormones suggest that the use of this immunotherapeutic warrants further investigation, although the results should be taken into consideration with other factors such as handling, treatments, chronicity of lesions.

Large flying foxes (Pteropus vampyrus) have complex social structures and colonies are normally harems of 1 male and several females. All-male colonies are common in the managed care population with aggressive interactions occurring during the breeding season due to significant sexual conflict, often resulting in trauma. These behaviors correlate with seasonal variations in urine glucocorticoid metabolites (UGM) and fecal testosterone (T5) and glucocorticoid metabolites (FGM).

The breeding season in the population of large flying foxes at Disney’s Animal Kingdom lasts approximately 10 to 12 weeks between August and October. Males defend their territories and display behaviors such as chasing, biting, hooking, and wing flexing at incoming males. These territorial behaviors usually begin 3 weeks before and after peak sexual activity. More intense social aggression behaviors include jabbing, biting, aggressive wrestling, and mounting that lead to injuries that increase through September and then decrease at the end of October. In that time, the number and severity of documented injuries and falls increase (Freeman, PhD, Disney’s Animals, Science and Environment, Bay Lake, FL, unpublished data, 2015). Fecal and urine T5 and FGM and UGM concentrations peaked in September.
and decreased in early to mid-October. Seasonal changes in T5, FGM, and UGM concentrations and social aggression led to injuries. Consequently, hormone suppression was expected to decrease agonistic behaviors and improve welfare.

Gonadotropin-releasing hormone (GnRH) immunotherapeutics have been in use for over 10 years in at least 20 species across a wide range of taxa. They produce antibodies against GnRH and block activity of the hormone. There are several types of available GnRH immunotherapeutics that have been successful in reducing aggression in males of multiple species, including swine, horses, elephants, and reindeer. These immunotherapeutics interrupt the action of GnRH by generating antibodies against GnRH, thus neutralizing GnRH molecules in the vasculature between the hypothalamus and anterior pituitary, eliminating the stimulation for gonadotropin release.

The anti-GnRH immunotherapeutic Improvest (Zoetis, Inc) is approved in the United States (NADA 141-322) in male pigs and gilts and is an alternative to surgical castration with reversibility. Reversibility has been shown to vary based on species, with a recent study in giraffe that found the effects reversible after 1.5 years of treatment. Improvest is a GnRH immunological product that elicits antibodies against GnRH and abrogates the effects of endogenous GnRH. Although Improvest is approved specifically for administration to male pigs and gilts, the Association of Zoos and Aquariums Reproductive Management Center suggests that it may be useful in a wide range of other taxa, especially Artiodactyla. It has been used in a number of species including the domestic goat, domestic horse, domestic deer, Asian elephant, African elephant, greater one-horned rhinoceros, southern white rhinoceros, and giraffe.

The use of Improvest has not been reported in bats, but its promise of reversibility and as an alternative to surgical castration made it an attractive option. The objective of the present study was to assess the effectiveness of this immunotherapeutic at suppressing T5 and leading to a decrease in agonistic behaviors, falls, and injuries in a colony of male large flying foxes under managed care.

Materials and Methods

Animals

The colony was made up of 12 intact male large flying foxes from 9 to 18 years old (average = 13.2 years). Individual males were easily identifiable by shape and fur coloration although each had individual microchips placed interscapular in the subcutaneous (SC) space. The bats were housed in a habitat with indoor and outdoor areas at Disney’s Animal Kingdom in Lake Buena Vista, Florida. Social interactions were recorded daily and included any aggression, falls, and injuries during daily keeper observations (2014 to 2018). The bats were fed daily a mixture of fresh fruits and vegetables, and they had ad libitum access to water. Disney’s Animal Care and Welfare Committee approved the study protocol (No. IR1412). The animals were transferred to a different facility in early 2019, thus ending the period of hormone and behavioral evaluation.

Immunotherapeutic administration

A single, 1.0-ml Improvest GnRH immunotherapeutic injection (gonadotropin-releasing factor analog-diphtheria toxoid conjugate; distributed by Zoetis, Inc) was administered SC under manual restraint during the first week of June 2016, approximately 2 months prior to the expected onset of the breeding season. Following immunotherapeutic injection treatment, animals were closely monitored by animal care staff for any behavioral changes or the development of clinical signs. The first bat, identified as “B,” was given the injection over the thigh and vocalized on administration, prompting an anatomical change in injection location to reduce perceived discomfort. Subsequent injections for all 11 remaining animals were given in the interscapular region. The original protocol involved a booster at 3 to 4 weeks postinitial injection. This was not performed in any of the animals due to the adverse reactions associated with the first treatment.

Sample collection

Morning fecal and urine samples were obtained 5 times per week between 7 and 9 AM (for additional detail on sample collection and processing, see Freeman et al). Data from 12 males (1 animal was removed from the [n = 13] group referred to in Freeman et al) were used to determine normal nonbreeding and breeding season patterns of UGM, FGM, and T5, and their correlations with social and reproductive behaviors, falls, and injuries throughout the year. These data from the previous untreated year served as a baseline for comparison for the present study with the groups’ hormone values acting as their own untreated control. There was no formal, separate, or simultaneous untreated or vehicle-control group in the present study.

Beginning in June of the Improvest treatment year (2016), morning urine and fecal samples were collected 1 to 3 times weekly during behavior observations to monitor hormone changes. Following immunotherapeutic treatment administration, sample collections for hormone monitoring were limited to AM urine collections (7 to 9 AM) for UGM and T5 in June and July to better assess posttreatment glucocorticoid changes. This was followed by fecal collections only (7 to 9 AM collected off the indoor holding floor) during the breeding seasons (August through the end of October from 2016 to 2018) for evaluation of efficacy to alter breeding season patterns of FGM, T5, and a reduction of agonistic behaviors, falls, and injuries (Figure 1).

To assess behavioral changes post-Improvest treatment, husbandry and medical records were reviewed from 2014 to 2018. Agonistic behaviors
use in male large flying foxes (*P. vampyrus*), and all measurements closely followed previously published protocols, inter- and intra-assay controls, and sample dilutions. A lower limit of 1.00 ng/g value was applied to fecal T5 measurements that fell below the readable range of the standard curve (between 80 and 100% B/Bo at the minimal 1:3 sample dilution).

### Statistical analyses

Urine and fecal T5 and UGM and FGM measures were not normally distributed (even following log-transformation), and datasets to be compared consisted of unequal N and unequal variance (heteroscedasticity) and dispersion, resulting in the use of nonparametric statistical analyses using ranks that are more robust to these effects to measure the stochastic equality of the datasets being compared.

### Urine hormone assessment of the immediate post-Improvest reaction

Evaluation of the initial hormone response during the posttreatment period (urine collection only; June to August 2016) was conducted by testing each posttreatment month separately using pairwise Mann-Whitney rank sum tests at the *P* < .05 level of significance with their respective month in the year prior (pretreatment control; June to August 2015). Monthly median (x; “x-tilde”) UGM and urinary T5 with quartiles (25%, 75%) were provided as the approximate confidence interval (CI) lower and upper bound.

### Fecal hormone assessment of Improvest contraception efficacy

FGM and fecal T5 values were assessed to determine if breeding season (August to October) values over the next 3 years (2016 to 2018) were reduced relative to values from the colonies’ pretreatment (2015) breeding season values or the nonbreeding season (off-season) baseline comparison as the relative “off-season control” dataset for statistical analysis of relative efficacy to reduce hormone values to at or below the nonbreeding season values (Figure 1). This was done using a Kruskall-Wallis one-way analysis (ANOVA) of variance on ranks (*H* statistic) followed by post hoc, all pairwise multiple comparisons (Dunn’s method; using a significance level of *P* < .05). For comparison against that off-season control (non-breeding season months November to July), an estimation of effects analysis relative to the off-season (common control group) was used that included the heteroskedastic-robust, nonparametric Brunner-Munzel test for stochastic equality between 2 groups (also known as a generalized Wilcoxon test) to assess if the compared groups have the same frequency of elevated values. When comparing hormone results from the n = 1 male “B” (who received the GnRH immunotherapeutic SC in the leg) against the whole group (n = 12), breeding season fecal T5 and FGM data for “B” are reported as mean (x) ± SD.

### Estimation of Improvest treatment effects on FGM and fecal T5 suppression utilized Data Analysis with Bootstrap-coupled ESTimation (DABEST), which is an estimation of effect analysis performed through R code (dabestr) or using the web application DABEST.
estimationstats.com. This produced Gardner-Altman estimation plots with multigroup comparisons (Cumming plot) to enable a direct visualization and comparison of mean differences between groups with a shared control using the 2015 pretreatment year’s nonbreeding season (“off-season”) as the common control group for all comparisons (Figure 1). Data were plotted using a raw data swarmplot to represent the underlying sample density distribution and jitter and mean ± SD (vertical-gapped lines). Effect sizes of the mean differences between groups were plotted as bootstrap sampling distributions (n = 5,000 bootstrap sampling) using Cliff’s delta (d), which provides a measure of ordinal dominance, or how often the values from the test group are larger (or smaller) than the values from the control group. The mean difference between groups is expressed as a unitless measure of the probability that the means of 2 distributions overlap. Cliff’s d was chosen as it is more robust than Cohen’s delta (d) for datasets with unequal N and dispersion, and does not require strict assumptions of the underlying distributions.

A Cliff’s d of 0 indicates 100% overlap, whereas +1 or −1 indicates no overlap of the distributions. Because the magnitude of effect size using Cliff’s d is more like a proportion (relative from 0 to 1, or unitless) and different from the better-known Cohen’s d effect size (which presents interpretation of effect size in terms of the nonoverlap between 2 normal distributions), the Cohen’s d equivalent of small, medium, and large effects (d = 0.2, 0.5, and 0.8, respectively) is provided (defined here as Cliff’s delta equivalents of d < |0.15| = small, d up to |0.33| = medium, and d > |0.5| = large). Results are reported with the Cliff’s delta effect size d, difference, and 95% CI (lower bound; upper bound). The permutation and Brunner-Munzel P value(s) reported are the likelihood(s) of observing the effect size(s), if the null hypothesis of zero difference between the test and control group is true.

Results

Postimmunotherapeutic response

At 24 hours postinjection, all animals, except bat “B” were anorexic and depressed (not interacting or training as expected). At 48 hours, some animals were slightly more active and eating/drinking, but several were unchanged. Initially, testicular swelling was considered a clinical sign, but with further assessment, it was identified that testicular size, appearance on ultrasound were normal (compared to previous images), and the appearance was dependent on temperature and hanging position. All bats but “B” developed signs of pruritus in the area of the injection site; the entire colony was treated with an antihistamine (2 to 3 mg/kg, PO, twice a day; diphenhydramine distributed by Johnson & Johnson Inc).

At 96 hours, 1 animal still had not improved behaviorally and was treated with fluids, dexamethasone (0.5 mg/kg, IM, once; distributed by Bimed) and maropitant citrate (1 mg/kg, SC, twice a day; Cerenia; distributed by Zoetis, Inc). Another individual began exuberantly scratching himself on the head and was given a behavior-modifying drug (haloperidol (0.6 mg/kg, PO, 3 times a day; distributed by Janssen Pharmaceuticals, Inc) to reduce the behavior. Ten bats developed varying degrees of facial or cranial edema, and 1 animal was treated with dexamethasone and maropitant citrate (Figure 2). In subsequent days, the animals’ behavior continued to improve, with many maintaining a general pruritus and some showing an area of self-barbering at the injection site (Figure 2A). Two weeks after treatment, bat “B” had short-term pruritus suspected secondary to a wound sustained from a conspecific and was placed on oral meloxicam (0.2 mg/kg, PO, once a day; distributed by Boehringer Ingelheim) for a 7-day course. One month postimmunotherapeutic, with continued self-trauma, the entire colony was placed on prednisone (0.5 mg/kg, PO, twice a day; compounded by Taylor’s Pharmacy) and ranitidine (1.75 mg/kg, PO, twice a day; compounded by Taylor’s Pharmacy), including bat “B” for 7 days, although this was not extended for him.

Excisional and/or punch biopsies from affected regions of 3 affected individuals were characterized by the presence of extensive deep dermal and hypodermal coagulative necrosis and variably extensive epidermal ulceration and necrosis. Inflammation was variable and consisted predominantly of macrophages, lymphocytes, and plasma cells with fewer numbers of neutrophils, which were primarily located near areas of
epidermal ulceration. There was no evidence of vasculitis or fibrinoid vascular necrosis affecting the regional dermal or hypodermal vascular structures. The presence of the adjuvant was not documented within macrophages. A biopsy taken from 1 bat, a month postpresentation of clinical signs, was identified with a nearly complete loss of the panniculus with replacement by an extensive band of fibrosis (Figure 2B).

**Treatments**

Treatment plans were tailored to individual animals and focused on managing clinical signs and wound management with surgical, topical, and systemic treatments to support healing. Six individuals were relocated to the veterinary hospital for medical care for 3 to 8 weeks. To mitigate self-mutilation, treatments included midazolam (0.25 mg/kg, twice a day; Richmond Vet Pharma), butorphanol (0.05 to 0.2 mg/kg, PO, 2 to 3 times a day), and/or haloperidol (0.6 mg/kg, PO, 3 times a day; Janssen Pharmaceuticals) in conjunction with the placement of Elizabethan and foot collars. Tramadol (5 mg/kg, PO, twice a day; Sun Pharmaceutical Industries, Inc) provided additional analgesia. Antibiotic treatment included enrofloxacin (15 mg/kg, PO, once a day; Elanco Global), and azithromycin (10 mg/kg, PO, once a day; Pfizer). For inflammation, injectable prednisolone sodium succinate (1 mg/kg, IM, once; Solu Delta Cortef; Zoetis) was initiated followed by a prolonged (but individually varied) oral prednisolone (0.25 to 0.5 mg/kg, PO, 1 to 2 times a day; Lloyd Laboratories) course. Surgical resections of large necrotic areas were necessary to expedite healing in several cases. Topically, ointment with nystatin-neomycin sulfate-thiostrepton-triamcinolone ace-tonide (Animax) and honey (Medihoney; Integra LifeSciences) were used. Other treatments included diphenhydramine (2 to 3 mg/kg, PO, twice a day; Johnson & Johnson Inc), ranitidine (1.75 mg/kg, PO, once a day; Pharmaceutical Associates). Use of diphenhydramine did not appear to improve clinical signs. Longer courses of corticosteroids subjectively reduced overall swelling but time was likely a factor as well. Additionally, on multiple occasions, swelling recurred when the steroids were tapered too early. Three animals with preexisting conditions were euthanized during the treatment period. Euthanasia was performed by sedation with isoflurane gas anesthesia followed by injection with pentobarbital (1 mL per 4.5 kg; Virbac Animal Health). These patients were undergoing frequent quality-of-life assessments by a formal, in-house, welfare toolkit before the anti-GnRH immunotherapeutic treatment, which was pursued with the goal of mitigating some of their prior social stressors.

**Hormone response**

The initial hormone response measured in urine following GnRH immunotherapeutic injection and subsequent move to the hospital for treatment of the adverse reactions resulted in an increase in the post-treatment group-median UGM and urine T5 measurements in the first posttreatment month (June 2016) when compared to their respective pretreatment baseline control month (Table 1). UGM measures did not differ from the prior year’s control values in

<table>
<thead>
<tr>
<th>Table 1—Group median urinary glucocorticoid metabolites (UGM; ng/ml/SG) and testosterone (T5; ng/ml/SG) measurements in the first 3 months immediately following a single anti-GnRH immunotherapeutic Improvest treatment in a group of n = 12 intact all-male large flying foxes (Pteropus vampyrus).</th>
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</thead>
<tbody>
<tr>
<td><strong>Confidence interval</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Urinary glucocorticoid metabolites (UGM)</strong></td>
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<tr>
<td>Pretreatment control</td>
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<tr>
<td>2015 June</td>
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<td>2015 July</td>
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<td>Posttreatment</td>
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<td>2016 June</td>
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<td>2016 July</td>
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<td>2016 Aug</td>
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<tr>
<td><strong>Urinary testosterone (T5)</strong></td>
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<td>Pretreatment control</td>
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<td>2015 June</td>
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<td>2015 July</td>
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<td>Posttreatment</td>
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<td>2016 June</td>
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<td>2016 July</td>
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<td>2016 Aug</td>
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</table>

<sup>a</sup>Urinary steroid measures were corrected for specific gravity (SG). <sup>b</sup>Lower and upper bound confidence intervals represent the 25 and 75% quartiles. <sup>c</sup>Initial posttreatment changes in UGM and urinary T5 were assessed by comparing matched-monthly hormone values in June to August 2016 with the groups’ prior, pretreatment year (2015) serving as their own control. <sup>*</sup>Significant difference (<i>P</i> < .05) between the pre- vs posttreatment test month from the previous year (2015).
the subsequent 2 months (July and August 2016). Posttreatment urine T5 was lower than the control in the second posttreatment month (July 2016) but was not different from the control by month 3 (August 2016).

Median FGM and fecal T5 values for the group of n = 12 males were reduced to values lower than the 2015 pretreatment breeding season for 3 subsequent breeding seasons after the single Improvest immunotherapeutic treatment [Kruskall–Wallis ANOVA on ranks FGM: H(4) = 172.86, P = < .001; fecal T5: H(4) = 176.274, P < .001]. In fact, values remained lower than or equal to the off-season pretreatment 2015 baseline control FGM values for years 1 to 3 posttreatment (Table 2).

Post hoc analysis using Dunn’s method for all-pairwise comparisons (P < .05), followed by Cliff’s delta (d) effect size analyses indicated a large effect size in reduction in FGM and fecal T5 concentrations posttreatment relative to their pretreatment (2015) breeding season, and for the next 3 breeding seasons (Figure 3; 2016 to 2018). Specifically, effects analysis of Cliff’s d values indicated that the treatment effect was greatest for FGM in year 2 where it was lower than the off-season baseline comparison. While values were lower than the breeding season pretreatment control (2015), years 1 and 3 (2016 and 2018) were not different from the off-season baseline comparison (Table 2). Fecal T5 was lower in posttreatment breeding seasons in 2016 and 2017 than the nonbreeding season control (2015). In those years, the suppression of fecal T5 in the second-year posttreatment (2017) was equivalent to that of the first (2016). Cliff’s d values showed that the treatment effect size on fecal T5 was largest in 2016, while still lower than breeding season values in 2018, fecal hormone values had recovered to the same values as the 2015 “off season” untreated control. A separate analysis of individual fecal T5 data from bat B that received the Improvest immunotherapeutic injection in the leg, showed that his average fecal T5 values posttreatment were also lower post-GnRH treatment (x = 11.65 ± 18.83 [September to October 2016; n = 23], x = 52.93 ± 107.18 ng/g [August to October 2017; n = 13], x = 60.51 ± 77.54 ng/g [August 2018; n = 4]) compared to his pretreatment breeding season values (October 2015; n = 4; x = 187.47 ± 203.58 ng/g fecal T5).

### Behavioral and medical intervention reports

Pretreatment, there were a total of 71 incidents of falls in the group recorded in 2014, and 110 in 2015. Looking at the breeding season only (August to October), 31 falls were recorded in 2014, and most occurred after social aggression (n = 18 aggression; 10 unknown preceding cause; 2 while flying; and 1 while locomoting along the aerial ropes). Similar proportions occurred during the 2015 breeding season. Thereafter, the incidence of falls reduced to 40% of the pretreatment 2014 through 2015 averages. Falls from aggression that resulted in trauma or injury decreased to rare events (rarely observed or 0 reports) posttreatment (2016 to 2018); documentation ended when the group was relocated to another facility in 2019.

There were 36 reports (over 4 years, 2016 to 2019) of bats requiring medical intervention due to negative interactions sustained before administration of the immunotherapeutic. Most of these injuries consisted of broken phalanges and trauma

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**Table 2**—Group median fecal glucocorticoid metabolites (FGM; ng/g) and testosterone (T5; ng/g) measures in the first 3 breeding season following a single anti-GnRH immunotherapeutic Improvest treatment in a group of n = 12 intact all-male Malayan large flying foxes (*Pteropus vampyrus*).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Year</th>
<th>N</th>
<th>Median (ng/g)</th>
<th>Cliff’s delta (d) effect size</th>
<th>Permutation P value</th>
<th>Brunner-Munzel Test statistic†</th>
<th>Brunner-Munzel p value</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Difference</td>
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<td>Upper bound</td>
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<tr>
<td>Fecal glucocorticoid metabolites (FGM)</td>
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<td>Pretreatment</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-season (control)</td>
<td>2015</td>
<td>166</td>
<td>56.80</td>
<td>0.68</td>
<td>0.51</td>
<td>0.79</td>
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<tr>
<td>Breeding season</td>
<td>2016</td>
<td>211</td>
<td>70.55</td>
<td>-0.02</td>
<td>-0.14</td>
<td>0.10</td>
<td>.762</td>
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<td>Posttreatment</td>
<td></td>
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<tr>
<td>Year 1</td>
<td>2017</td>
<td>122</td>
<td>2.61</td>
<td>-0.61</td>
<td>-0.72</td>
<td>-0.49</td>
<td>.000*</td>
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<tr>
<td>Year 2</td>
<td>2018</td>
<td>107</td>
<td>105.85</td>
<td>0.13</td>
<td>-0.02</td>
<td>0.27</td>
<td>.069</td>
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<td>Fecal testosterone (T5)</td>
<td></td>
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<tr>
<td>Pretreatment</td>
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<tr>
<td>Off-season (control)</td>
<td>2015</td>
<td>166</td>
<td>25.39</td>
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<td>Breeding season</td>
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<td>213</td>
<td>3.41</td>
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<tr>
<td>Year 1</td>
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<td>123</td>
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<tr>
<td>Year 2</td>
<td>2018</td>
<td>106</td>
<td>31.85</td>
<td>-0.08</td>
<td>-0.23</td>
<td>0.07</td>
<td>.288</td>
</tr>
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</table>

*Significant difference (P < .05) between the pre- vs posttreatment test month from the previous year (2015).

†Posttreatment values are compared to the groups previous pretreatment year (2015) breeding season and off-season baseline values as their own control.
The anatomy of the interscapular region may have played a role in the irritation and subsequent reaction. Bats have typical mammalian integumentary glandular scent organs composed of sebaceous and sudoriferous glands. The specific location of these glands varies based on species but correlates with the animal’s habitat, behavior, and size.32 The shoulder glands of *Pteropus spp* are enlarged, androgen-sensitive sebaceous glands that have been found to secrete over 65 compounds.30 Due to the complexity of these glands, the interscapular space may not be the ideal location for subcutaneous injections with androgen-stimulating compounds despite its ease of access. The 1 animal, bat “B,” who received the immunotherapeutic SC treatment in the region of the thigh did not develop significant adverse effects, further suggesting the unique anatomy of the interscapular region may have played a role in the irritation and subsequent reaction seen in the other immunotherapeutic recipients.

An alternate theory is a focal interaction with innate bacteria in the skin initiated the behavior/reaction.34 However, vaccinations (rabies) and injectable therapeutics have been delivered subcutaneously in other bat species, including this group, with no side effects. No adverse reactions or clinical signs of disease were observed over a 6-month period in a colony of Brazilian free-tailed bats (*Tadarida brasiliensis*) after receiving an inactivated rabies vaccine subcutaneously.35 Another study looking at injectable anesthesia in Egyptian fruit bats (*Rousettus aegyptiacus*) found no injection site reactions when 5 medetomidine-midazolam combinations were administered subcutaneously in the interscapular region.34 No adverse effects were reported in black flying foxes (*Pteropus alecto*) injected subcutaneously with lipopolysaccharide (LPS).36 When given an intramuscular rabies vaccine, Egyptian fruit bats maintained adequate titers up to 1 year with no adverse side effects noted. The results of that study suggested that humoral immune response following subcutaneous administration may differ from responses induced by intramuscular vaccination.35,37 In the present case, all immunotherapeutics were given subcutaneously per the label instructions. The
1 bat, “B,” with no significant adverse reaction was administered the immunotherapeutic treatment in the leg; the other bats with interscapular administration developed a reaction. Thus, injection site location and components of Improvest may be a significant factor in the present case and the mutilative behavior of the large flying fox exacerbated the wounds.

Finally, a complex sensitivity/hyper-response to the adjuvant is possible. Addition of an adjuvant is necessary to promote an adequate immune response. Other GnRH immunotherapeutic products have used Freund’s complete adjuvant, which was a deciding factor to use Improvest (due to known reactions with Freund’s). The components of the immunotherapeutic include 0.2 mg GnRF Analogue-DT conjugate, 150 mg diethylaminoethyl-dextran hydrochloride (DEAE), and 1 mg chlorocresol sodium hydroxide. DEAE-dextran is a polycationic derivative of dextran. It is a white, hygroscopic powder, freely soluble in water and salt solutions, and used often as an adjuvant in immunotherapeutics. GnRH-immunotherapeutic treatments may have long-term effects since GnRH receptors are located in a variety of tissues. However, the effects in nonreproductive tissues have not been reported for either the anti-GnRH immunotherapeutics or GnRH agonists.

Hormone monitoring immediately posttreatment showed that UGM and urinary T5 were elevated during the first post-GnRH immunotherapeutic treatment month (June 2016) compared to the pretreatment control comparison (June 2015). Both UGM and FGM and urine and fecal T5 begin to elevate during July in the normal breeding season in untreated males, and thus, both glucocorticoid metabolites and T5 were expected to rise as part of the normal breeding season hormone changes until the immunotherapeutic treatment takes effect. From studies in other species, it was expected that it would take at least 4 weeks (and possibly a booster) to see any effect of the treatment on the reduction of reproductive steroids, so it is likely that the higher UGM (and urinary T5) observed was a combination of effects as a result of the immunotherapeutic treatment itself, its subsequent side effects (stress associated from handling and administration), as well as the move to the clinic and the daily/weekly restraint and treatments required for wound care. Although urine T5 values were statistically higher in June 2016 versus the control (June 2015), urine T5 values are only a small proportion of that excreted in feces (which can be 100-fold higher), and the median increase observed relative to the control (median June 2016 urine T5 vs control, June 2015 was 1.95 and 0.53 ng/ml/SG, respectively) may not be biologically significant.

FGM and T5 measurements in the first breeding season immediately post-GnRH immunotherapeutic treatment (August to October 2016) were reduced to values lower than the previous (untreated; 2015) breeding season, for the 3 successive breeding seasons (2016 to 2018), and were in fact lower than the nonbreeding season (“off-season”) control in 2016 (T5) and 2017 (FGM and T5), and a similar pattern was observed in the first-treated male “B” that received the injection in the leg. Effects analyses showed that the amplitude of the effect of suppressed fecal T5 was decreasing with each successive year and was equivalent to the “off-season” baseline control by 2018. However, in early 2019 the bats were moved to a different facility and were not monitored further to determine if a full reversal of its effects on androgen suppression occurred.

Treatments for adverse reactions were tailored to each individual case and focused on behavioral, topical, and systemic management; some of these treatment protocols were extensive. Three individuals with chronic, preexisting quality of life impairments were euthanized during the study period in lieu of further treatment. Various therapies were used for each case, including steroids; thus, there is potential that may have had secondary effects. This necessitated consideration for steroid impact on T5 suppression over the immunotherapeutic treatment. To look at this, a literature review was performed and indicated that steroid use can reduce testicular functions causing decreased sperm output and T5 secretion in humans with 1 study finding that long-term systemic corticosteroid therapy reduced T5 concentrations by 18%. These studies do not support long-term or complete suppression of T5. Based on these results, the long-term T5 suppression seen in the bats in the current study is most likely a result of the GnRH immunotherapeutic treatment. Reinforcing this notion is another study that found suppressed testicular function due to androgen abuse was mostly reversible (apart from testicular volume reduction) and full recovery took 9 to 18 months after taking consistently high doses. In context, steroid treatments here were lower in dosage than those in the studies cited above. Impacts from medical treatments and varying degrees and duration of inflammatory response could have affected adrenal or gonadal production of hormones. However, this is not likely because the range of inflammatory responses did not parallel a similar range of hormonal change. In summary, the animals in the present study had reduced FGM and T5 concentrations, and social aggression for at least 3 successive breeding seasons following a single GnRH immunotherapeutic treatment.

Male large flying foxes treated SC interscapular with Improvest GnRH immunotherapeutic treatment developed variable adverse effects ranging from mild pruritus and swelling of facial features, to ulceration and necrosis at the injection site with some requiring surgical debridement. Severe self-induced trauma and associated inflammation at the injection sites were documented with histopathology. An infectious etiology was not identified and an immune response to the treatment or the adjuvant could not be substantiated. The 1 animal that was administered the injection in the leg did not develop similar adverse effects. These bats are predisposed to self-mutilation behaviors and the interscapular space should be avoided with this treatment. Although there was no formal control group, the group served as their own control by comparing values to the
previous breeding season with no treatment. Despite the complications, fecal T5 concentrations were suppressed along with associated low FGM values, indicating further study is warranted. With these lower hormone values, falls and injuries resulting from agnostic interactions were reduced for at least 3 breeding seasons following a single 1.0-ml Improvest hormone injection.

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