

Original research article

# Preliminary evaluation of deslorelin, a GnRH agonist for contraception of the captive variable flying fox *Pteropus hypomelanus*<sup>☆</sup>

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## Abstract

**Background:** This study was conducted to study the effects of a gonadotropin-releasing hormone (GnRH) agonist, deslorelin, on luteinizing hormone (LH), testosterone (males), semen characteristics and pregnancy in the variable flying fox *Pteropus hypomelanus*.

**Study Design:** Male ( $n=3$ ) and female ( $n=5$ ) bats received a 4.7-mg implant and were housed with untreated bats (eight females and three males, respectively). Plasma was collected twice monthly and analyzed for hormone concentrations, and semen was collected from untreated and treated males 1 month preimplantation, 3 months postimplantation and 4 months postimplantation.

**Results:** Administration of a GnRH challenge 1 month postimplantation showed an attenuated response in treated ( $n=4$ ), but not in untreated ( $n=4$ ), male and female bats. Plasma LH was lower in treated versus untreated males ( $p=.04$ ), but not in females. Testosterone was lower in treated versus untreated males ( $p<.001$ ). Spermic ejaculates were obtained from treated males, although no untreated females became pregnant during the 8-month study. One treated female became pregnant 6 months after implantation.

**Conclusion:** Deslorelin is a useful and reversible contraceptive for *P. hypomelanus*.

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**Keywords:** Contraception; Deslorelin; Semen; Luteinizing hormone; Testosterone; Bats

## 1. Introduction

For group-housed and easily bred captive bat species, reversible contraception is essential to limit population growth, prevent genetic overrepresentation and improve genetic management. Only one previous study, using melengestrol acetate, has examined contraception in any bat species (*Pteropus rodricensis*) [1]. Although no treated bats conceived during the study, the behavioral effects of the drug were the main focus, and little physiological data were

provided. Furthermore, weight gain was a side effect of the progestin contraceptive [1]. One alternative to progestin contraceptives are gonadotropin-releasing hormone (GnRH) agonist implants, which have been used with considerable success in several carnivore species, lasting between 6 months and 2 years [2,3].

As in other mammals, bat reproduction is controlled by the hypothalamic–pituitary–gonadal axis [4]. GnRH from the hypothalamus directs secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary [5]. LH particularly causes ovulation in females and testosterone production in males, which is essential for sperm development [5].

In male horseshoe bats (*Rhinolophus ferrumequinum*), the effect of GnRH on the secretion of gonadotropins is enhanced in the spermatogenic period — a time of sperm production during the summer — prior to mating in early autumn [5,6]. Seasonal studies of pituitary function in

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several species of male bats demonstrated a rise in LH during the prebreeding (December–March for *Pteropus poliocephalus*) [7], spermatogenic (*R. ferrumequinum* [5,6]; March–May for *Miniopterus schreibersii* [8]) and mating (November–December for *Pteropus scapulatus* and April–May for *Pteropus alecto*) [7] periods. This is coincident with elevated circulating testosterone concentrations that also rose and peaked at different times during the reproductive cycle, depending on the species [5–8]. Plasma testosterone concentrations are correlated with testicular testosterone concentrations in wild *P. poliocephalus* [9] and with testis size in some captive male *Pteropus* (*P. poliocephalus* [10]; *Pteropus vampyrus* and *Pteropus pumilus* [11]). In female *P. poliocephalus*, *P. scapulatus* and *P. alecto*, the pituitary content of LH is significantly elevated during their respective mating seasons (April–May in *P. poliocephalus* and *P. alecto*; November–December in *P. scapulatus*) [7]; in *M. schreibersii*, plasma LH concentrations are highest during follicular development, peaking prior to ovulation (April and May) [12]. A study on negative feedback in gonadectomized males and females showed that LH is under negative feedback regulation from gonadal steroid hormones in *Pteropus* species (*P. poliocephalus*, *P. scapulatus* and *P. alecto*) [7]. These data support the link between GnRH, LH, reproductive hormones and reproductive activity in bats, including *Pteropus* species. Incidentally, there is no evidence of sperm storage, delayed implantation or delayed development in *Pteropus* species [7].

Chronic administration of GnRH agonists causes the pituitary GnRH receptors to down-regulate after an initial stimulation, resulting in desensitization of the pituitary gonadotrophs [13]. Therefore, it is predicted that treatment with GnRH agonists will result in an initial stimulation of LH and FSH, followed by a receptor down-regulation in pituitary gonadotrophs, a concomitant inhibition of the synthesis and release of LH, and subsequent decreased gonadal steroid hormone production. In turn, spermatogenesis and follicular development will cease. The goal of this study was to determine the effects of a GnRH agonist, deslorelin contained in Suprelorin® implants, on plasma LH and testosterone concentrations, testicular size, semen and sperm parameters, and pregnancy in the variable flying fox *Pteropus hypomelanus*. Based on Lube Bat Conservancy's (Gainesville, FL) production of pups at every month of the year, captive *P. hypomelanus* does not appear to have a defined breeding season. Aggression among the males, however, usually increases approximately in October (D. LeBlanc, personal communication), so deslorelin treatment was initiated in September.

## 2. Materials and methods

### 2.1. Animals

Twenty-six adult variable flying foxes housed at Lube Bat Conservancy were used in this study. Initially, 6 males

and 13 females were housed in single-sex groups. Three months after implantation with deslorelin (Suprelorin® implants; Peptech Animal Health, Australia), three treated males were housed with eight untreated females, and three untreated males were housed with five treated females. An untreated bachelor group ( $n=7$ ) was used as control for semen collection and sperm analysis. Bats were housed in naturally lighted outdoor enclosures (99.2 m<sup>2</sup>) that included a central octagonal nighthouse (17.9 m<sup>2</sup>) into which bats were locked when overnight temperatures dropped below 5°C. Supplemental heat was provided when overnight temperatures dropped below 18°C. Bats received a daily ~300-g portion of diet that was prepared using 25.8 kg of apple, 4.9 kg of pear, 9.8 kg of banana, 7.5 kg of grapes, 8.6 kg of cantaloupe, 6.6 kg of carrot or sweet potato, 4.7 kg of kale and 4.1 kg of Lube Fruit Bat Supplement (HMS Zoo Diets, Inc., Bluffton, IN). Water and salt licks were available ad libitum. This study was preapproved by the Lube Bat Conservancy Institutional Animal Care and Use Committee (CP06-4).

### 2.2. Blood sampling and Suprelorin® implantation

Bats were hand-captured in random order and anesthetized with isoflurane (5% for induction, then 1.5–2.5% for maintenance) in oxygen using a face mask. At 2-week intervals, starting in September and ending in February, blood (2 mL) was collected from the brachial vein of male and female bats using heparinized 3-mL syringes and 25-ga needles. The blood was immediately centrifuged at 3000 rpm for 5 min, and the plasma was placed in 1.8-mL sterile cryogenic vials (Nalge Nunc International, Rochester, NY) and frozen (–20°C) until hormone analysis.

Immediately after the collection of the first blood sample in September, the upper back of the treated bats was disinfected with chlorhexidine scrub, and one Suprelorin® implant containing 4.7 mg of deslorelin was placed subcutaneously in three males and five females using a 10-ga needle. After 3 months, the bats were housed as described above.

### 2.3. Morphometric measurements, semen collection and evaluation

With the use of calipers, testis length and width were determined on anesthetized males prior to each electroejaculation. Testis volume was calculated ( $V=L \times W^2 \times 0.524$ ) [14], and the two testis volumes (right and left) were added to provide a combined testicular volume per bat. Semen was collected from anesthetized treated ( $n=3$ ) and untreated ( $n=10$ ) males prior to and 3 months after deslorelin implantation (August and December, respectively) using a slightly modified protocol established for flying foxes [15], and semen was collected again from treated bats 4 months postimplantation (January). Following the induction of anesthesia, the bladder was palpated and urine was gently expressed. Any urine released during electroejaculation was separated from the semen temporally as it was released prior to ejaculation, and it was subsequently dried with a

paper towel. Some cross-contamination of the semen by the urine in the urethra was still possible. Semen was collected by electroejaculation (Canine EJ; Minitube of America, Verona, WI) using a rectal probe (6 mm in diameter, with three 15-mm strip electrodes dorsally located 5 mm from the tip of the probe) designed for use in *P. hypomelanus*. The stimulation protocol consisted of 10 stimulations at 3, 4 and 5 V, followed by 10 stimulations at 4, 5 and 6 V, and 10 stimulations at 5 and 6 V. Each stimulation was separated from the following by a 1-s interval, and each series was separated from the following by a 1-min rest. Semen was collected directly into microcentrifuge tubes (Perfector Scientific, Inc., Atascadero, CA).

The pH values of urine and semen were determined using pH strips (EM Science, Gibbstown, NJ), and discrimination between the two was based on these readings. The volume was measured using an adjustable calibrated Eppendorf pipetter (Brinkman Instruments, Inc., Westbury, NY). The presence of coagulum was noted. Sperm percent motility and concentration were assessed by examining 10  $\mu$ L of ejaculate at 37°C using the Computer-Assisted Semen Analyzer Sperm Vision® (Minitube of America), which was calibrated for bat semen (J.P. Verstegen, personal communication). Progressive motility status (0=no forward progression to 5=rapid and straight forward progression) was assessed subjectively. Ten microliters of semen was fixed in 1% glutaraldehyde, and 5  $\mu$ L of fixed semen was used to prepare a wet-mount slide. Sperm morphology was assessed by phase-contrast microscopy (original magnification  $\times$ 1000) as either normal or structurally abnormal. Acrosome integrity was assessed by preparing slides with 10  $\mu$ L of air-dried methanol-fixed semen and by staining with a fluorescein-labeled pea lectin stain *Pisum sativum* [16]. One hundred cells were counted to calculate the percentage of acrosome-intact or damaged sperm.

#### 2.4. GnRH challenge

GnRH challenge was performed with two male and two female treated bats and two male and two female untreated bats 1 month after implantation (October). Bats were hand-captured and anesthetized as described previously. One milliliter of blood was collected prior to administration of 1 mcg/kg gonadorelin diacetate tetrahydrate (Cystorelin®, 50 mcg/mL; Rhone Merieux, Inc., Athens, GA) intravenously into the right cephalic vein (25-ga needle, 0.5-mL syringe). Bats were returned to their nighthouse, recaptured 30 min postinjection and reanesthetized, and 1 mL of blood was collected. Bats were then maintained under anesthesia, during which testes were measured and combined testicular volume was calculated. One milliliter of blood was collected 45 min postinjection.

#### 2.5. Plasma hormone analysis

Plasma LH concentrations were determined using an enzyme immunoassay with monoclonal anti-bovine anti-

serum (LH 518-B7; J. Roser, University of California, Davis, CA), biotinylated LH (NIADDK-OLH-26, AFP-5551B; National Hormone and Peptide Program) and streptavidin–peroxidase conjugate (Roche Molecular Biochemicals, Indianapolis, IN) in microtiter plates (MaxiSorp®; Nalge Nunc International) coated with anti-mouse gamma globulin (Sigma Chemicals, St. Louis, MO). The LH antibody has been shown to be highly specific for LH while having low species specificity, reacting with LH from a variety of mammals [17]. Bovine standards (NIH-bLH-B10, AFP-11743B; National Hormone and Peptide Program) were prepared in buffer (for 1000 mL: 2.42 g of Trizma base, 17.9 g of NaCl, 1.0 g of bovine serum albumin and 1.0 mL of Tween 80 at pH 7.5) in accordance with previously published techniques [18]. The LH assay was validated using a serial dilution (range, 1:1 to 1:512) of pooled male and female *P. hypomelanus* plasma to construct a displacement curve parallel to the standard curve. To ensure that the bat plasma itself did not interfere with specific antigen–antibody binding, an accuracy check (also known as ‘recovery’), whereby aliquots of pooled diluted (1:2) bat plasma were added to known hormone concentrations and assayed, was performed. Measured hormone (after subtracting the amount of measured hormone in plasma and buffer only) was plotted against known hormone to yield a regression line of  $r^2=0.9863$  ( $y=0.8024x+3.4819$ ), indicating a high agreement between measured hormone and true hormone. The recovery of known amounts of LH (0.038–5.0 ng/mL) added to pools of diluted plasma (1:2) was 96%. Bat samples were diluted 1:2 in buffer and assayed in duplicate. Intra-assay coefficients of variation were <10%; inter-assay variations were 19% for high controls and 18% for low controls.

Plasma testosterone concentrations were determined with IMMULITE® Total Testosterone (Diagnostic Products Corporation, Los Angeles, CA) solid-phase competitive chemiluminescent enzyme immunoassay, using the reagents and supplies provided with the system [19]. Analytical recovery, specificity and other assay parameters are as published for humans [19]. Assay sensitivity was 20 ng/dL. To confirm that the bat plasma did not interfere with the assay, parallel dilution curves were generated with samples in phosphate-buffered saline and with samples in charcoal-stripped bat plasma.

#### 2.6. Pregnancy diagnosis

Pregnancy in anesthetized bats was determined by palpation [20] and ultrasonography of the reproductive tract [21] 8 months following deslorelin implantation and 5 months after treated bats were initially housed with untreated bats using a portable unit (SonoSite, Bothell, WA) and a 7.5-MHz probe. After this exam, males and females were housed separately. Pregnancy status was reevaluated in August.

Table 1

Average values ( $\pm$ SD) for *P. hypomelanus* semen and sperm characteristics in untreated ( $n=4$  bats; four ejaculates) and deslorelin-treated bats 3 months (December) and 4 months (January) postimplantation ( $n=3$  bats; four ejaculates)

	Untreated	Treated
Ejaculate volume ( $\mu$ L)	98.3 $\pm$ 101.2	66.3 $\pm$ 78.0
Sperm concentration ( $\times 10^6$ /mL)	117.5 $\pm$ 69.4	297.0 $\pm$ 246.5
Sperm motility (%)	79.1 $\pm$ 22.9	33.3 $\pm$ 25.0
Progressive motility index	4.0 $\pm$ 0	2.3 $\pm$ 1.3
Structurally normal (%)	24.0 $\pm$ 16.5	5.4 $\pm$ 4.7
Acrosome integrity (%)	40.0 $\pm$ 17.0	43.3 $\pm$ 36.3 <sup>a</sup>

<sup>a</sup> These values are based on only two ejaculates from two bats. For both untreated and treated bats, it was not always possible to count 100 cells when determining percentages for morphology and acrosome integrity.

## 2.7. Statistical analysis

Plasma hormone data and semen and testes data are presented as mean $\pm$ SD. Sample sizes were insufficient to allow for the use of statistical procedures to determine differences between preimplantation and postimplantation for treated bats. Differences in hormone concentrations between untreated bats and treated bats postimplantation were determined using the Kruskal–Wallis one-way analysis of variance on ranks, performed by SigmaStat Version 3.0 software [22]. Failure to find statistically significant differences, however, may be due to variability among the small number of animals, as opposed to the absence of a treatment effect.

## 3. Results

### 3.1. Morphometric measurements, semen collection and evaluation

Semen was not able to be collected from every animal each time. Semen was collected from untreated bats in five of six attempts and from treated bats in seven of nine attempts; statistical analysis was not possible because of low numbers. Urine was easily distinguished from semen, and their mean pH $\pm$ SD were 7.0 $\pm$ 0.7 and 8.2 $\pm$ 0.7, respectively. Total ejaculate volumes were  $\leq$ 250  $\mu$ L. Ejaculates were initially clear and thin, but transitioned rapidly into a thick white fraction that coagulated and contained particles that were likely prostatic bodies.

Interestingly, ejaculate consistencies in the treated males postimplantation were thinner and more liquid than those of the untreated and bachelor males, likely as a result of less vesicular secretions. Similarly, fewer prostatic bodies were noted in treated and older bachelor male ejaculates, with the exception of the last ejaculate from one treated male.

All treated males produced spermic ejaculates after deslorelin implantation; although sperm concentration was higher in treated bats than in untreated bats (Table 1), sperm motility and normal morphology appeared lower in treated versus untreated bats (Table 1). One treated bat showed low sperm percent motility and progressive motility status on the first postimplantation collection (December; Table 2), but improved motility and status on the second postimplantation collection (January; Table 2). Sperm concentrations were much higher in this male than in any other (treated or untreated) and contributed to the higher average concentration in treated males.

Bat testicular volumes varied between and within groups throughout the study, and no differences were observed between untreated and treated males (Fig. 1). Of the untreated and treated bats, four (two treated and two untreated) of six had decreased testicular volume in October (Fig. 1), 1 month postimplantation, but the decrease appeared more marked in the two treated bats whose testicular volumes decreased by 81% and 55% (compared to 20% and 18% for the two untreated bats, respectively). The testicular volume of the third treated bat could not be determined in October because one of the testes could not be palpated and measured. His testicular volume decreased by 21% from the pretreatment volume in December. After the initial decrease, the treated bats' testicular volume began to increase over time, however with only 46% and 32% decreases from the pretreatment volume for the first and second treated bats, respectively, and an increase of 21% for the third treated bat in January (Fig. 1).

Based on dominance rankings given by animal care staff, there appeared to be no correlation between dominance and testicular volume in August (pretreatment), with the exception of the least dominant bat that also had the smallest testicular volume (Table 3). After averaging testicular volumes for each bat in October, December and January, and after considering treated and untreated males separately, again it appeared that there was no correlation with

Table 2

Spermatozoa motility, progressive motility status and concentration for deslorelin-treated male *P. hypomelanus* preimplantation (August), 3 months postimplantation (December) and 4 months postimplantation (January)

Bat	August			December			January		
	Spermatozoa motility (%)	Progressive motility status	Concentration ( $\times 10^6$ /mL)	Spermatozoa motility (%)	Progressive motility status	Concentration ( $\times 10^6$ /mL)	Spermatozoa motility (%)	Progressive motility status	Concentration ( $\times 10^6$ /mL)
1	51	4	N/A	16.5	2	192	0	0	N/A
2	N/A	N/A	N/A	24	2	549	70	4	443.6
3	N/A	N/A	N/A	22	1	3.2	0	0	<1

N/A, not available.

Table 3

Comparison of dominance, testicular volume and sperm quality (based on sperm concentration and motility) among *P. hypomelanus* males ( $n=6$ )

August		October–December		
Dominance rank	Average testicular volume (cm <sup>3</sup> )	Dominance rank	Average testicular volume (cm <sup>3</sup> )	Sperm quality rank
1	8.4	T-1	3.8	2
1	5.5	T-2	4.1	1
3	6.5	T-3	1.4	3
4	7.4	U-1	5.0	3
4	4.4	U-2	7.7	1
6	1.4	U-3	3.4	2

Sperm quality for August ejaculates was not ranked due to insufficient sample size. After deslorelin treatment (September), treated (T) and untreated (U) males were ranked separately.

dominance, with the exception of the least dominant bat in each group, which had the smallest testicular volumes (Table 3). Similarly, there appeared to be no correlation between dominance and sperm quality (based on concentration and motility) in untreated or treated males (Table 3).

### 3.2. GnRH challenge results

In response to the GnRH challenge, plasma LH concentrations in male and female untreated bats increased by an average of  $69\pm 6.8\%$  (males: 64% and 77%; females: 63% and 72.5%) 30 min postinjection. In contrast, plasma LH concentrations in treated bats increased by  $6.8\pm 15.8\%$  (males:  $-13\%$  and  $8.5\%$ ; females: 6% and 25.5%).

### 3.3. Plasma hormone concentrations

Concentrations of plasma LH (mean $\pm$ SD) in treated males after implantation ( $1.2\pm 0.68$  ng/mL; range, 0.38–2.6 ng/mL) were significantly lower ( $H=4.32$ ,  $df=1$ ,  $p=.04$ ) than the concentrations in untreated males ( $1.7\pm 0.90$  ng/mL; range, 0.40–3.3 ng/mL). In contrast, the concentrations of LH (mean $\pm$ SD) in treated females postimplantation ( $1.6\pm 0.75$  ng/mL; range, 0.43–3.3 ng/mL) were not significantly different ( $H=0.20$ ,  $df=1$ ,  $p=.66$ ) from the concentrations in untreated females ( $2.3\pm 2.1$  ng/mL; range, 0.48–8.9 ng/mL). Postimplantation concentrations of LH (mean $\pm$ SD) were not lower than preimplantation concentrations (preimplantation: males,  $1.4\pm 0.86$  ng/mL; range, 0.46–2.1 ng/mL; females,  $2.1\pm 1.1$  ng/mL; range, 0.71–3.8 ng/mL). Notably, however, whereas average LH concentrations appeared to increase from October to mid-January in untreated males, average concentrations in treated males did not appear to increase over time (Fig. 2). Average LH concentrations did tend to increase from September to mid-January in treated females, but they did not exceed the preimplantation average (Fig. 3). In addition, both male (Fig. 2) and female (Fig. 3) treated bats' average LH concentrations showed slightly less variability over time compared to untreated bats. Two untreated females had much higher LH concentrations (mean $\pm$ SD) than any of the other female bats:  $4.6\pm 1.0$  ng/mL (range, 2.9–5.4 ng/mL) and  $6.7\pm 1.9$  ng/mL (range, 3.5–8.9 ng/mL) for Females A and B, respectively, versus  $1.3\pm 0.5$  ng/mL (range, 0.48–2.7 ng/mL) for the remaining untreated females.

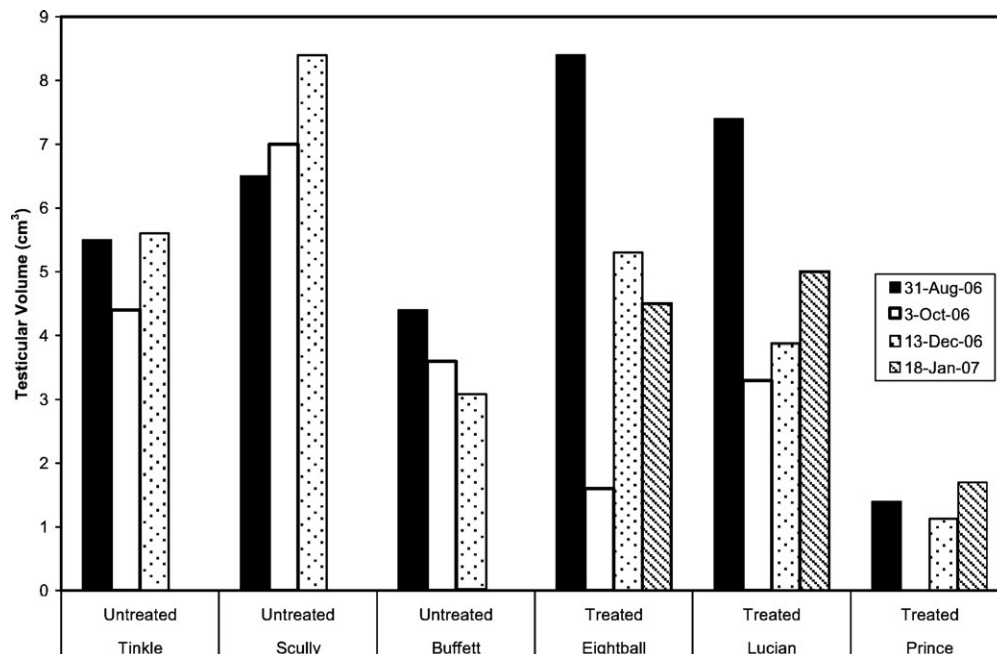


Fig. 1. Combined testicular volumes for untreated and deslorelin-treated male *P. hypomelanus* relative to implantation in September. In October, decreases in testicular volume appeared more marked in two treated bats whose testicular volumes decreased by 81% and 55% compared to 20% and 18% for two untreated bats.

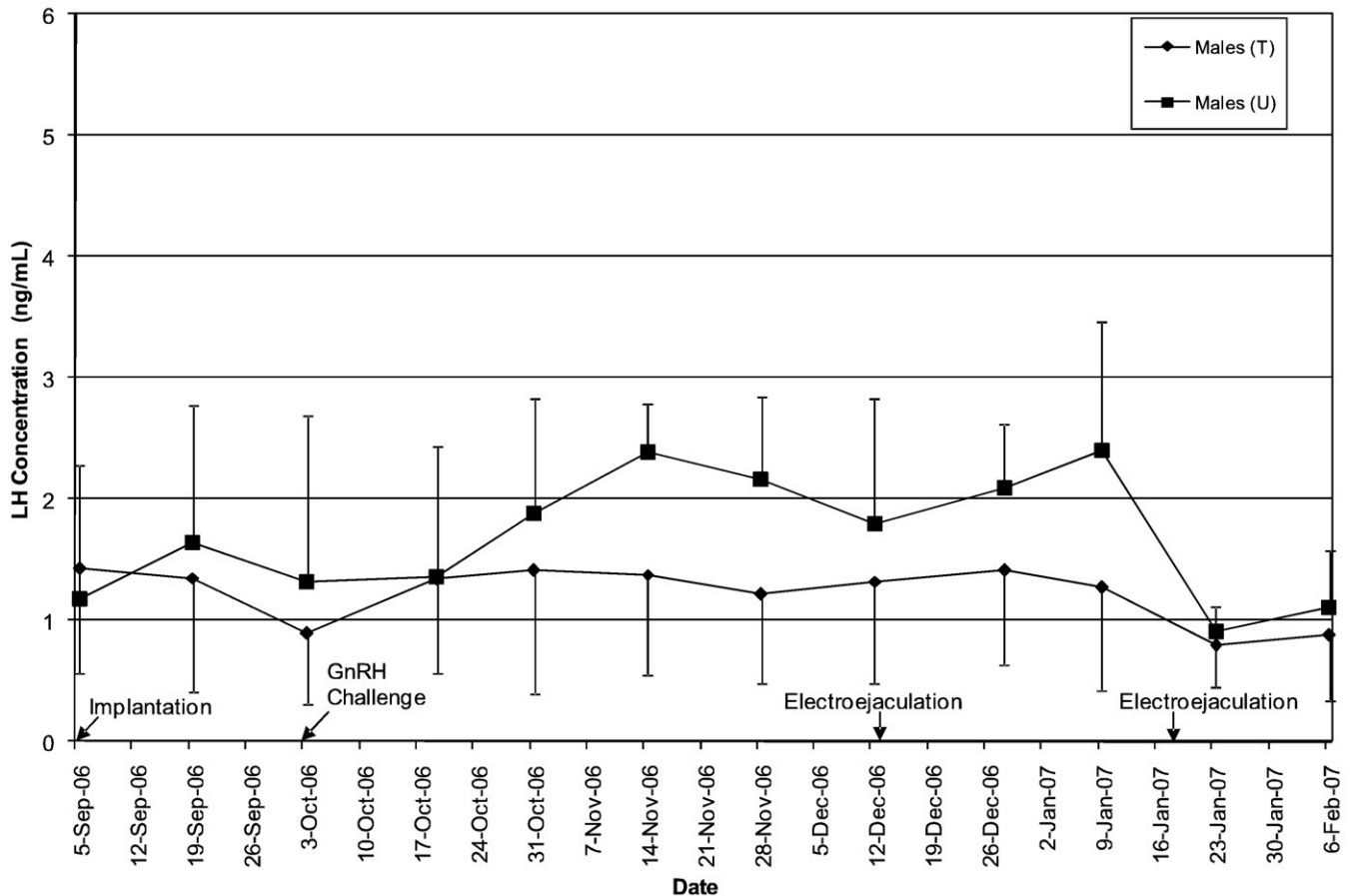


Fig. 2. Average plasma LH concentrations over time for untreated (U;  $n=3$ ) and deslorelin-treated (T;  $n=3$ ) male *P. hypomelanus*. LH concentrations in treated males after implantation (September) were significantly lower ( $p=.04$ ) than concentrations in untreated males.

Following implantation, plasma testosterone concentrations dropped dramatically in all of the treated males (Fig. 4). Plasma testosterone concentrations (mean $\pm$ SD) in treated males postimplantation ( $38.0\pm 41.3$  ng/dL; range, 5.8–239.0 ng/dL) remained significantly lower ( $H=44.93$ ,  $df=1$ ,  $p<.001$ ) than testosterone concentrations in untreated males ( $280.0\pm 260.2$  ng/dL; range, 53.9–1061.0 ng/dL) for the 5 months during which plasma was collected. Untreated males exhibited testosterone concentrations that fluctuated widely compared to treated males' testosterone concentrations that remained low and fluctuated minimally after implantation.

### 3.4. Pregnancy

Copulations with all five treated females were observed by animal care staff. Ultrasound and palpation 8 months after deslorelin implantation revealed a pregnancy in one of the five treated females, but in none of the untreated females housed with deslorelin-treated males. The pup was born in mid-August after a predicted gestation of  $\sim 5$  months (D. LeBlanc, personal communication). This indicates that conception occurred in March, 6 months after deslorelin implantation and 3 months after treated females were housed

with untreated males. When pregnancy status was reevaluated in August, no further pregnancies had occurred.

## 4. Discussion

The results of this study indicate that the GnRH agonist deslorelin is likely to be an effective contraceptive for the flying fox species *P. hypomelanus*. One female, which had demonstrated down-regulation of pulsatile LH 1 month after deslorelin implantation, became pregnant 6 months after treatment, consistent with the expected duration of efficacy for a 4.7-mg implant [3]. The four other treated females, however, had not become pregnant by the end of the study at 8 months postimplantation. In males, sperm motility and normal morphology were negatively affected in only two of three treated males, and semen continued to be present throughout the study, although ejaculate secretions were modified in all treated males. However, unexpectedly, no pregnancies occurred in the eight females housed with the three treated males.

The observation of fractionation of the ejaculate into a liquid component and a viscous component, together with the small volumes and sperm percent motility, is consistent

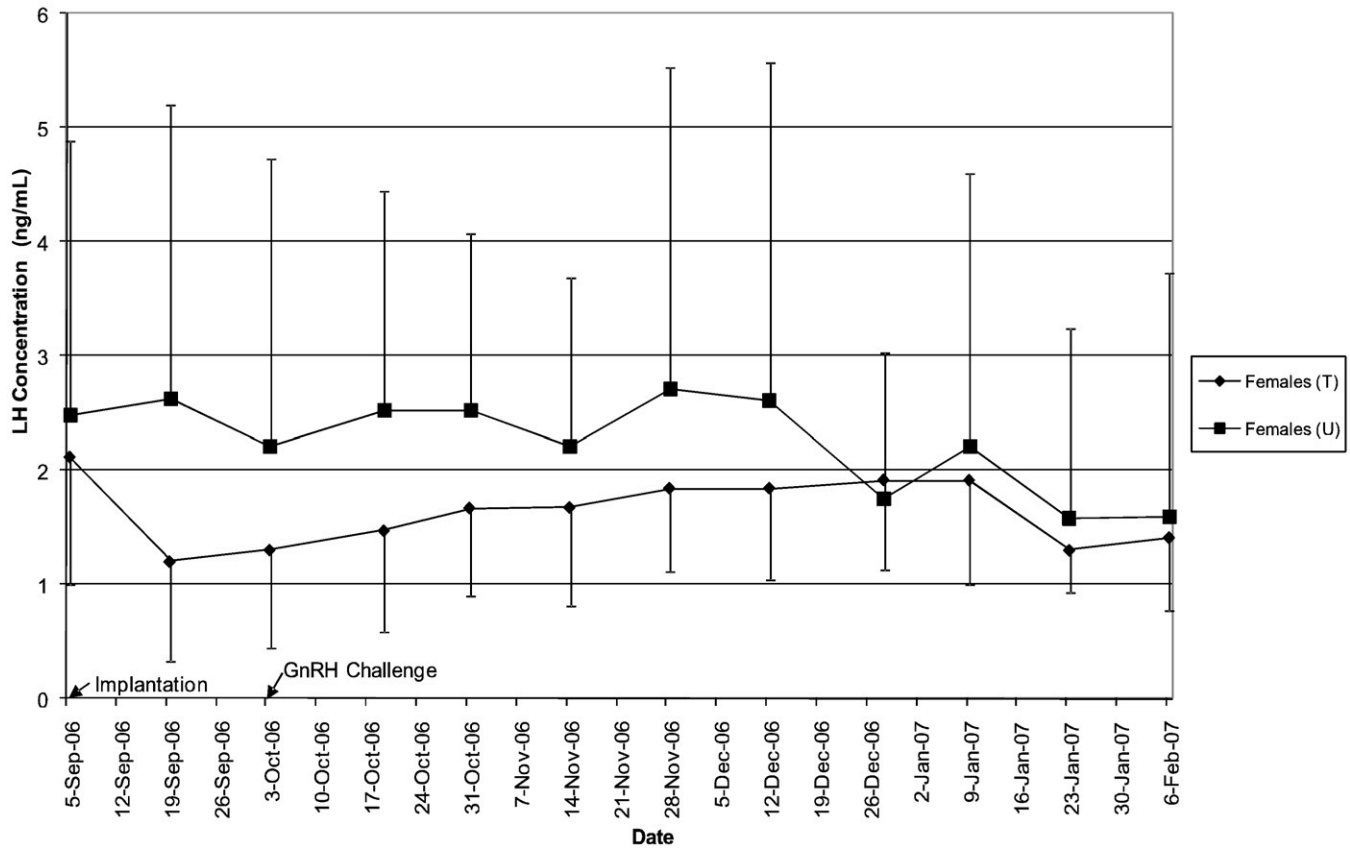


Fig. 3. Average plasma LH concentrations over time for untreated (U;  $n=8$ ) and deslorelin-treated (T;  $n=5$ ) female *P. hypomelanus*. LH concentrations in treated females after implantation (September) were not significantly different ( $p=.66$ ) from concentrations in untreated females; however, treated females' average LH concentrations showed less variability compared to those of untreated females.

with previous observations for this genus (*P. alecto*, *P. poliocephalus* and *P. scapulatus*) [15]. The observation of vesicular secretions in the white viscous ejaculate fraction is a potential indicator of a “normal” ejaculate. Observations of vesicular secretions are more common in wild *Pteropus* ejaculates collected during the breeding season than out of the breeding season [15]. In this study, vesicular secretions and the thickest ejaculates were only obtained, with one exception, from untreated nongeriatric males. Martin et al. [23] proposed that the secretions are essential for maintenance of sperm viability in the female reproductive tract and that males are only fertile when secretion is abundant. Since untreated females housed with treated males did not become pregnant, it is possible that deslorelin treatment sufficiently reduced the fertility of the males by affecting vesicular secretions. It is also possible that motility and fertility were reduced by the poor sperm morphology observed in the treated males. The percentage of normal spermatozoa in treated and untreated bats was lower than values reported for free-ranging bats of other species within the genus [15]. Improvement in sperm percent motility and progressive motility status between December and January for one treated male suggests that the effects of the implants may have begun to diminish after 3 or 4 months, although it

should be emphasized that no pregnancies occurred from treated males during the 8-month study.

The initial decrease in testicular volume, which was profound in two of the deslorelin-treated males relative to changes in untreated males, provides further evidence that the implants initially affected testicular function. While testicular volume was still decreased relative to pretreatment volumes for two of three of the treated males in December and January, the increase in testicular volume by December again suggests that the effects of the implant on the testes began to diminish after 3–4 months. While sperm was present, it is clear from the significantly decreased testosterone concentrations in the treated males that the implants did impact testosterone production for at least 5 months. Low testosterone production may have contributed to the diminished production of vesicular secretions and poor sperm morphology, but it did not appear to impact mating behavior. The records maintained by the animal care staff noted courtship behaviors and copulation from two of three treated males. The male that was not observed engaged in copulation had the second largest testicular volume among the treated males and had the highest sperm concentration.

Overall, this study's findings suggest that the implants were affecting plasma LH concentrations. Although tonic

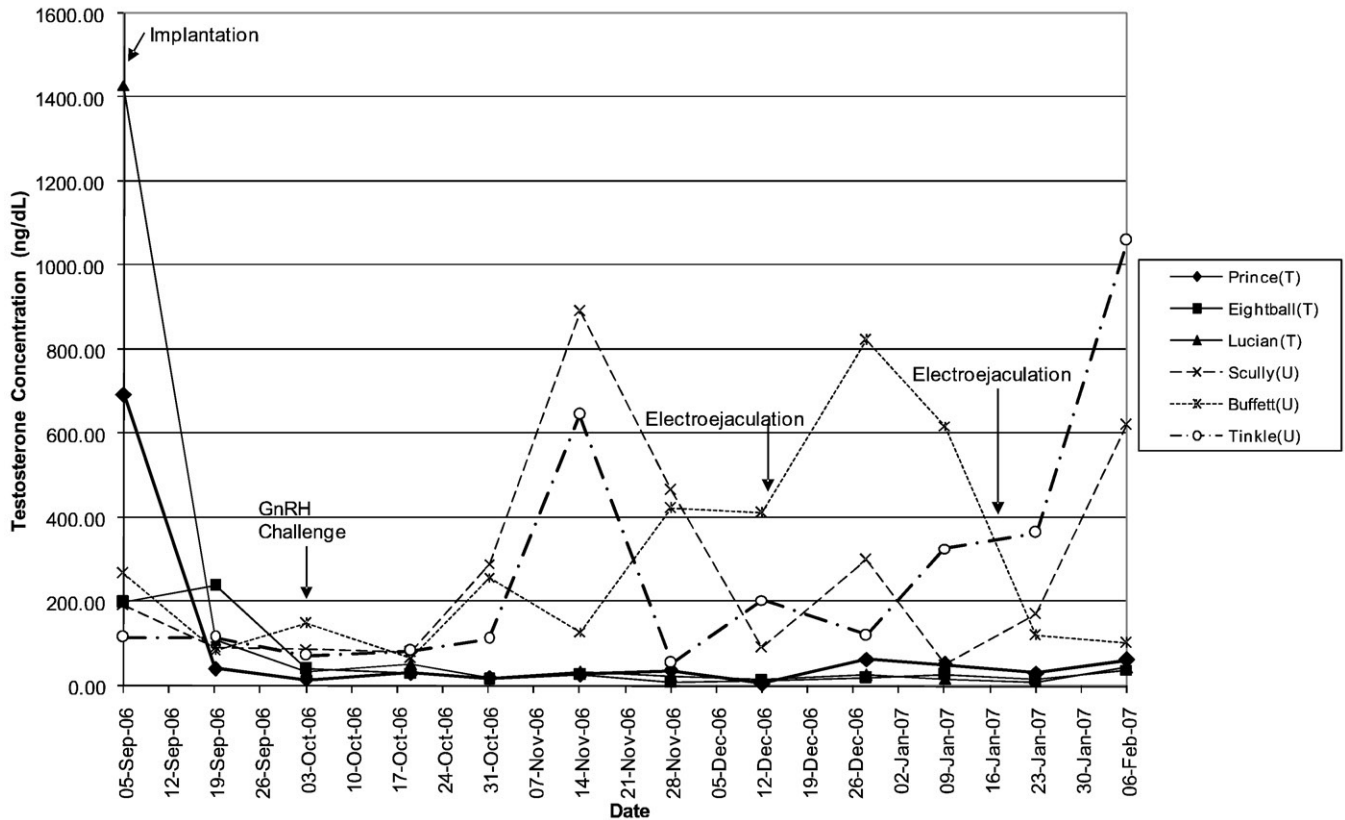


Fig. 4. Plasma testosterone concentrations over time for untreated (U;  $n=3$ ) and deslorelin-treated (T;  $n=3$ ) male *P. hypomelanus*. Testosterone concentrations in treated males after implantation (September) were significantly lower ( $p<.001$ ) than the concentrations in untreated males.

LH did not decrease in treated bats, average LH concentrations also did not increase in treated males as they did in untreated males, nor did average LH concentrations in treated females exceed their preimplantation average. There was also a statistically significant difference between LH concentrations in treated males postimplantation and untreated males. Although a similar statistically significant difference was not detected in the females, considerable individual variation in LH concentrations among the females, particularly among the untreated females, contributed to the lack of statistical significance. The large range of LH concentrations seen in untreated females is explained, in part, by two of these females that had much higher LH concentrations than any other female bat. There is no known reason why these two females' LH concentrations were elevated in comparison to those of other untreated bats. Although one tended to be the heaviest female, the other bat's weight was similar to the other females' weights (data not shown). Neither was noted by keepers to be particularly more dominant, and all the untreated females were wild-caught in 1990. In spite of the maintenance of tonic LH concentrations, a dramatic and sustained decrease in plasma testosterone concentrations occurred after deslorelin implantation in treated males.

The inability of the treated bats to produce an LH surge after the administration of exogenous GnRH is evidence

that decreased pituitary sensitivity was achieved. The response of the untreated bats to the GnRH challenge was not as great as has been observed in some species such as dogs (10 mcg/kg) [24] and hyena (*Crocuta crocuta*; 1 mcg/kg) [25], but was comparable to other species (dorcas gazelle, *Gazella dorcas*; 1 mcg/kg) [26]. Due to their small body size, only three blood samples could be drawn from these bats. It is possible that more frequent sampling may have measured a larger increase in untreated bats. Handling stress may have diminished the response [27], as might the isoflurane anesthesia. Other studies using GnRH agonists in domestic bulls [28], male antelope (dorcas gazelle; gerenuk, *Litocranius walleri*; scimitar-horned oryx, *Oryx dammah*) [26] and male tammar wallabies (*Macropus eugenii*) [29] have also found evidence of decreased pituitary sensitivity despite the maintenance of tonic LH concentrations. In these species, testosterone concentrations were increased [26,28,29], and testis size [26,29] and sperm production [26] were maintained even though pituitary desensitization was confirmed.

Domestic and nondomestic feline [2] and canine [3] species, however, have shown suppressed testosterone and semen production in response to implanted deslorelin as a result of the down-regulation of both pulsatile and tonic LH release. Male primates have shown variable responses. Rhesus monkeys (*Macaca mulatta*) responded to GnRH

agonist treatment with an initial rise in serum LH and testosterone, followed by a decline in both hormones to undetectable levels [30,31]. The response to exogenous GnRH was lost [30,31], testicular volumes decreased, spermatogenesis was inhibited and ejaculatory activities were absent [30]. In contrast, LH-releasing hormone agonists did not suppress plasma LH and testosterone concentrations or testicular function in marmosets (*Callithrix* spp.) [32,33], although pituitary desensitization did occur [33]. The results from this study, combined with those of previous studies, support the findings of Penfold et al. [26] and D’Occhio and Aspden [28], who suggested that the effects of GnRH agonists on the pituitary–testicular axis are species-specific in males. This is despite the down-regulation of pituitary receptors, which is common across species [26,28].

In conclusion, this study shows that the deslorelin contained in Suprelorin® implants has potential as a reversible contraceptive for both male and female variable flying foxes. It is likely to be an effective tool for the management of captive colonial bat populations.

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