ELSEVIER

Contraception 78 (2008) 336–345

Contraception

Original research article

Preliminary evaluation of deslorelin, a GnRH agonist for contraception of the captive variable flying fox *Pteropus hypomelanus*☆

Lara C. Metrione[a,*], John P. Verstegen[b], Darryl J. Heard[c], Dana LeBlanc[d], Allyson L. Walsh[d], Linda M. Penfold[a]

[a]*White Oak Conservation Center, Yulee, FL 32097, USA*
[b]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*
[c]*Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA*
[d]*Lubee Bat Conservancy, Gainesville, FL 32609, USA*

Received 27 October 2007; revised 7 April 2008; accepted 19 April 2008

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*.

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

☆ We did not receive a grant for this project. This was funded by the participating institutions White Oak Conservation Center, Lubee Bat Conservancy, and the University of Florida. Approval for this study was granted by the Lubee Bat Conservancy Institutional Animal Care and Use Committee (CP06-4).

* Corresponding author. The Ohio State University, Columbus, OH 43210, USA. Tel.: +1 240 401 7222.
E-mail address: metrione.2@osu.edu (L.C. Metrione).

0010-7824/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.contraception.2008.04.125

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 Lubee 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 Lubee 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^2) that included a central octagonal nighthouse (17.9 m^2) 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 Lubee Fruit Bat Supplement (HMS Zoo Diets, Inc., Bluffton, IN). Water and salt licks were available ad libitum. This study was preapproved by the Lubee 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