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Inhibition of the Reproductive System by Deslorelin in Male and Female Pigeons (*Columba livia*)

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Abstract: Veterinary practitioners frequently encounter disorders of the reproductive system in avian patients. Management of these disorders relies on manipulating reproduction by modifying the environment, diet, and social interactions, and by the use of pharmacologic agents and surgery, with varying levels of success and side effects. An alternative is to use the gonadotropin-releasing hormone (GnRH) agonist deslorelin to suppress the pituitary–gonadal axis. To determine the efficacy of deslorelin in domestic pigeons (*Columba livia*), male (n = 10) and female (n = 10) birds each were implanted intramuscularly with a single long-acting implant containing 4.7 mg deslorelin. Untreated males (n = 11) and females (n = 10) were used as controls. The baseline serum concentration of luteinizing hormone (LH) was assayed at 7, 28, 56, and 84 days after treatment, and egg production was recorded weekly. In females, deslorelin administration significantly reduced serum LH concentrations compared to pretreatment levels at 7, 28, 56, and 84 days ($P < .05$). In males, deslorelin significantly reduced LH concentrations at 7, 28, and 56 days ($P < .05$). Female birds treated with deslorelin laid significantly fewer eggs over the course of the study (mean = 1.46, SEM = 0.84) compared with controls (mean = 5.54, SEM = 0.88). Deslorelin treatment had no discernible effect on body weight. Deslorelin is effective for controlling egg laying in female pigeons for at least 49 days, but further research is required to determine the effects on male fertility and the duration of action in both sexes.

Key words: deslorelin, GnRH agonist, luteinizing hormone, LH, reproduction, avian, pigeon, *Columba livia*

Introduction

While commercial facilities and conservation programs involving avian species focus on procreation, veterinary practitioners dealing with pet birds often need to suppress reproductive function and related behavior. In addition to primary diseases of the gonads in both sexes, chronic egg laying, egg yolk coelomitis, dystocia, and cloacal or uterine prolapses are common disorders related to reproduction in female birds.[1–3] Management of these disorders relies on the ability to control reproduction effectively. Reproduction has been managed by modifying the environment, diet, and social interactions, by pharmacologic agents, and by surgical intervention, with varying levels of success and side effects.[1–3] A safe and effective method of reproductive control, thus, still is needed in pet birds.

One alternative approach is to use a gonadotropin-releasing hormone (GnRH) "super-agonist" to suppress the hypothalamic-pituitary-gonadal (HPG) axis. Deslorelin (4.7 mg, Suprelorin, Virbac

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Animal Health Australia, Regents Park, NSW, Australia), a synthetic long-acting GnRH agonist, has been assessed in a variety of mammalian species,[4–20] although there are very limited reports of its use in birds.[21–23] Endogenous GnRH is released from the median eminence of the hypothalamus, binding to receptors on the gonadotroph, and facilitating the production and secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the systemic circulation.[24,25] Gonadotropins exert their action on the gonads, ultimately inducing ovulation in the female, and spermatogenesis and testosterone secretion in the male.[24,25] The gonadotropins also stimulate the production of gonadal hormones (androgens, estrogens, progestagens, and inhibin) that then exert negative feedback on the hypothalamus and consequently inhibit GnRH secretion.[24,25] Clearly, blocking the action of GnRH can effectively shut down the whole HPG axis. This can be done with antagonists, but, remarkably, also with super-agonists, such as deslorelin, that initially hyperstimulate the pituitary GnRH receptors, and greatly increase the production of LH and FSH.[24] However, prolonged stimulation of GnRH receptors leads to reduced numbers of receptors, blocking the action of GnRH pulses on the gonadotrophs, preventing the secretion of LH pulses and decreasing FSH secretion, leading to down-regulating the HPG axis.[24]

In the present study, we tested whether deslorelin could be used temporarily to control reproduction in male and female domestic pigeons (*Columba livia*). To monitor the HPG response, we measured the circulating concentrations of LH, which previously have been assessed in the domestic pigeon.[26] Additionally, this species is considered to be a seasonal breeder,[27] although birds are capable of reproducing throughout the year given appropriate resources.[27,28]

Materials and Methods

Animals and husbandry

Ethical approval for the study was granted by the Animal Ethics Committee of the Department of Employment, Economic Development and Innovation (DEEDI) of the Queensland government. A local breeder provided 41 adult racing pigeons that then were housed in outdoor aviaries in Brisbane (27.4667° S, 153.0333° E), Queensland, Australia. All birds were examined under general anesthesia achieved by mask induction with isoflurane and oxygen. Each bird was weighed, given a numbered leg ring, and sexed by direct visualization of the gonads by laparoscopy. All birds were given prophylactic treatments with oxfendazole (10 mg/kg PO once) and praziquantel (10 mg/kg PO once), toltrazuril (25 mg/kg PO once; Baycox, Bayer Australia Ltd, Pymble, NSW, Australia), topical pyrethrin and methoprene (Avian Insect Liquidator, Vetafarm Pty Ltd, Wagga Wagga, NSW, Australia), and doxycycline (50 mg/kg IM once). Once in the aviary, the flock was treated with ronidazole (600 mg/L water q24h for 7 days). All treatments were concluded 6 months before the start of the study.

Totals of 20 females and 21 males were assigned randomly to experimental and control groups. The 10 males from the experimental group were housed with the 10 experimental females in an aviary. The male and female control groups were housed in a second, identical aviary. There was one additional male in the control group. Both aviaries were furnished with nesting sites and nesting materials. The aviaries were positioned outdoors and were naturally exposed to normal seasonal reproductive stimuli for this species, particularly increasing day length. All birds were offered a commercial formulated diet and, in the 4 weeks before the study started, they additionally were given a daily ration of dry, mixed seeds. At weekly intervals during the study, egg production was recorded. The eggs were removed each week to minimize the effect of incubation on LH secretion, as prolactin concentrations typically rise during incubation leading to a decline in serum LH concentrations.[29,30]

Deslorelin implantation and blood sample collection

The pigeons were acclimatized to the aviaries during the winter months and, in spring, birds from both groups were caught and anesthetized as described previously. To determine baseline values of LH, approximately 3 mL of blood were sampled from the basilic vein and placed in serum tubes. A deslorelin implant was inserted in the right pectoral muscle of each bird in the male and female experimental groups, using the trocar needle supplied with the implant. The small skin wound then was closed with skin adhesive. Birds from the control groups received 0.5 mL of sterile saline by injection into the right pectoral muscle after blood was sampled for baseline LH levels. All birds were weighed and recovered uneventfully from anesthesia. Blood samples and weight recordings of all birds subsequently were collected under isoflurane anesthesia at the same time on days 7, 28, 56, and 84 post treatment. Blood samples were centrifuged

at 1210*g* for 5 minutes and refrigerated at 4°C for 24 hours before the serum was frozen at –20°C until hormonal analysis.

LH assay

The serum concentration of LH was measured by using radioimmunoassay (RIA) techniques adapted from a published protocol that was based on chicken LH (PRC-AEI-s-1) and rabbit anti-chicken antibody (anti-ch-LH 3/3) preparations.[31] Validation of the assay involved serial dilutions of pigeon serum that were compared to standard quantities of LH. The resulting curves paralleled chicken LH standards, demonstrating appropriate cross-reaction of pigeon serum LH with the antibody preparation. Duplicate 50 μL aliquots of all samples were run in a single assay. The limit of detection was 0.04 ng/mL. Six replicates of 2 quality controls containing 0.15 and 2.9 ng/mL were included in the assay and were used to estimate intra-assay coefficient of variation of 5.8% and 2.8%, respectively.

Statistical analysis

For all statistical analysis, we used SPSS 19 for Windows (SPSS Inc, Chicago, IL, USA). The level of significance was set at $P < .05$. Data for males and females were analyzed separately. Values for LH concentration were compared between the control and experimental groups using independent samples *t* tests. A repeated measures ANOVA was used to assess changes in the bodyweight. The results were expressed as mean ± SEM. A 1-way ANOVA was used to assess differences in egg production between the female experimental and control groups.

Results

Serum LH concentrations

Mean LH concentrations in the female and male pigeons from experimental and control groups at each sampling time are presented in Figures 1a (females) and 1b (males). Before implantation, there were no significant difference in LH concentrations between the experimental and control groups, for either the male or female birds. Male and female pigeons implanted with deslorelin had significantly lower mean LH concentrations at 7, 28, and 56 ($P < .05$) days after implantation compared with the respective control groups. At 84 days after implantation, mean LH concentrations were not significantly different between treatment groups, for either sex. Over the length of the study,

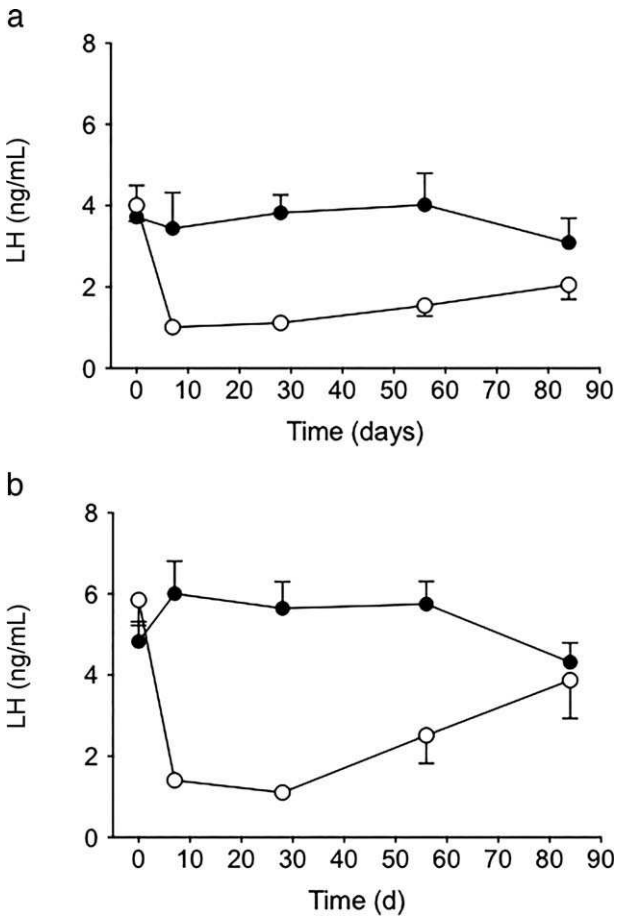

Figure 1. (a) Comparison in mean plasma LH concentrations between female domestic pigeons treated with 4.7 mg deslorelin (open circles, n = 10) and control pigeons (closed circles, n = 10). (b) Comparison in mean plasma LH between male domestic pigeons treated with 4.7 mg deslorelin (open circles, n = 10) and control pigeons (closed circles, n = 11).

serum LH concentrations did not change significantly in the control groups of either sex. In contrast, deslorelin administration was followed by a significant decrease in LH concentrations in female pigeons, which remained below pretreatment values at 7, 28, 56, and 84 days ($P < .05$). In males treated with deslorelin, LH concentrations declined significantly at days 7, 28, and 56 ($P < .05$) after treatment compared with pretreatment values, but the mean values for 84 days did not differ significantly between the groups ($P < .06$).

Egg production

The numbers of eggs produced in each aviary are detailed in Table 1. The experimental group produced only 19 eggs over the 84 days of the

Table 1. Number of pigeon eggs counted in the control (nontreatment) and experimental (4.7-mg deslorelin treated) aviaries pre- (day 0) and posttreatment.

Day	Control	Experimental
0	12	11
7	5	1
14	6	0
21	4	0
28	6	0
35	1	0
42	9	0
49	5	0
56	7	1
63	3	0
70	7	3
77	0	2
84	7	1
Total	72	19

study, which was significantly fewer than the 72 eggs from the control aviary ($P < .001$). The average weekly egg production for both groups also was examined, which demonstrated that birds treated with deslorelin (mean = 1.46, SEM = 0.84) laid significantly fewer eggs than females that did not receive implants (mean = 5.54, SEM = 0.88). In the experimental aviary, no eggs were produced from 14 to 49 days after implantation.

Body weight

The mean weights of the experimental and control groups are detailed for female pigeons in Figure 2a and for males in Figure 2b. For all time periods, mean weights did not differ significantly between the experimental and control groups for either males or females. Moreover, the mean body weights of male and female pigeons, in both experimental and control groups, did not change significantly from the baseline value or over time.

Discussion

In male and female pigeons, a single 4.7-mg deslorelin implant reduced serum LH concentration within 7 days. Low values were maintained in females for 84 days, clearly demonstrating the efficacy of this treatment in suppressing the HPG axis, and the suppression of LH secretion led to a reduced number of eggs laid. Indeed, for approximately 5 weeks, no eggs were produced by any deslorelin-treated birds. Importantly, the blood sampling protocol did not permit detection of an initial "flare" in LH secretion immediately after GnRH agonist administration. However, we concluded that deslorelin offers an excellent opportunity for blocking reproduction in female pigeons and, probably, also in males.

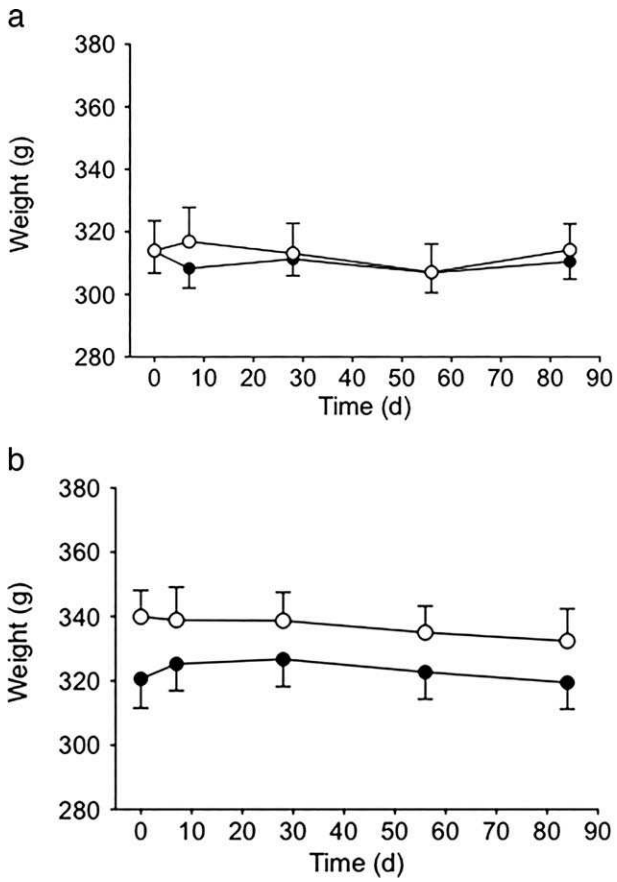

Figure 2. (a) Bodyweight of female pigeons treated with 4.7 mg deslorelin (open circles, n = 10) and control birds (closed circles, n = 10). Values are mean ± SEM. (b) Bodyweight of male pigeons treated with 4.7 mg deslorelin (open circles, n = 10) and control pigeons (closed circles, n = 11). Values are mean ± SEM.

While the mean serum concentrations of LH at 28 and 56 days in the female birds treated with deslorelin were below baseline levels, 1 egg was produced within this time frame. The egg production of individual birds was not monitored, which meant that direct correlations between LH concentrations and egg production could not be determined. As seen in other species, individuals might vary in their response to deslorelin and the outcomes are likely to be dose-dependant.[32] The duration of deslorelin action on LH levels cannot be demonstrated convincingly from the present study, because LH levels in neither sex returned to baseline values. In the males, the mean LH concentration at 84 days was not significantly different from that at baseline, suggesting that circulating LH had begun to return to normal

levels between 56 and 84 days. In the females, the restoration of egg production in one bird by 56 days post implantation suggested that suppression of female reproduction in pigeons is relatively short or depends on the individual physiology of the bird.

The use of another GnRH super-agonist, leuprolide acetate, has been reported previously to inhibit egg laying in cockatiels (*Nymphicus hollandicus*) for 12 to 19 days.[33] In Hispaniolan Amazon parrots (*Amazona ventralis*), fecal and plasma hormone levels declined after leuprolide acetate treatment, but did not differ significantly from baseline values 14 days after administration.[34] These studies suggest that the duration of action of GnRH super-agonists is shorter in avian than in mammalian species,[34] perhaps due to species variation in the pituitary response to exogenous GnRH, pituitary resistance to desensitization, or the high metabolic rate of birds. Critical differences exist between birds and mammals in the way their GnRH receptors respond to mammalian GnRH analogues, with some mammalian antagonists behaving as agonists.[35] Further complicating the current understanding of the pharmacology of GnRH analogues in avian species is the existence of 2 forms of GnRH in the chicken.[24,25,31] Possibly, persistent exposure to environmental reproductive stimuli might override exogenous pharmacologic action. Research involving ring doves (*Streptopelia risoria*), for example, has shown that courtship increases LH concentrations, leading to ovulation.[30] In the present study, LH values generally are similar to those reported elsewhere for ring doves and pigeons,[26,30] but the effect of exposure to untreated birds of the opposite sex could not be assessed because deslorelin-treated females were housed with deslorelin-treated males.

Parallel measurements of other HPG variables, such as testosterone and estrogen, or endoscopic evaluation of the reproductive tract are needed to provide further insight into the contraceptive effects of deslorelin. Concurrent observations of reproductive behaviors throughout the study may have provided additional information for clinicians. We used egg production to assess the effects of deslorelin on female fertility, but we did not include analogous measures of male reproductive capability. For mammals, testicular mass and semen quality have been used as additional parameters for monitoring the effects of deslorelin on fertility.[4,15,36] This approach clearly is needed for male pigeons, because in a variety of mammalian species, it appears that only pulsatile LH release is affected and that low rates of tonic LH secretion persist, which are sufficient to maintain testosterone production and reproductive capabilities.[13,15,18,36,37] Similar outcomes were obtained in studies involving red-winged blackbirds (*Agelaius phoeniceus*).[38]

As a broad indicator of adverse effects, we tested whether deslorelin treatment affected body weight. There was no clear effect of deslorelin on the weight of treated pigeons. It was initially thought that treatment with deslorelin would result in weight loss because sex steroid production would be inhibited, reducing anabolism. From clinical observations, no other indicators were observed that would prevent recommendation of deslorelin treatment.

Our observations showed that deslorelin offers excellent possibilities for the clinical management of avian reproduction, but further evaluation will be important for confirming the applicability of the implant in avian species and for verifying its reversibility. Measurement of blood LH concentrations beyond the time frame of this study, and greater sample sizes, could help establish the duration of action in male and female pigeons. The use of deslorelin should be considered by the veterinary practitioner when control of reproduction in avian patients is considered important.

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