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Induction of ovarian activity and ovulation in an induced ovulator, the maned wolf (*Chrysocyon brachyurus*), using GnRH agonist and recombinant LH

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ABSTRACT

Assisted reproductive techniques, such as ovarian manipulation and artificial insemination, are useful for enhancing genetic management of threatened wildlife maintained *ex situ*. In this study, we used noninvasive fecal hormone monitoring to investigate (1) the influence of pairing with a male on endocrine responses of female maned wolves (*Chrysocyon brachyurus*) to a GnRH agonist (deslorelin) and (2) the efficiency of recombinant LH (reLH) on ovulation induction in females housed alone. Deslorelin (2.1 mg Ovuplant) was given to females that were either paired with a male (n = 4) or housed alone (n = 7); the implant was removed 7 to 11 days postimplantation. Three of seven singleton females were injected with reLH (0.0375 mg) on the day of implant removal, whereas the remaining females (n = 4) did not receive the additional treatment. Fecal samples were collected 5 to 7 days/wk from all females starting 11 days prior to hormone insertion until at least 70 days post implant removal for a total of 11 hormone treatment cycles. Fecal estrogen and progestagen metabolites were extracted and analyzed by enzyme immunoassay. Evidence of ovulation, demonstrated by a surge of estrogen followed by a significant rise in progestagen, occurred in all paired females. Three of the four singleton females that did not receive reLH treatment exhibited no rise in progestagen after an estrogen surge. All singleton females treated with reLH exhibited a rise in fecal progestagen after injection, indicating ovulation. In conclusion, deslorelin is effective at inducing ovarian activity and ovulation in paired female maned wolves; however, exogenous reLH is needed to induce ovulation in females housed alone. The findings obtained from this study serve as a foundation for future application of artificial insemination to enhance genetic management of this threatened species *ex situ*.

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1. Introduction

The maned wolf (*Chrysocyon brachyurus*), a neotropical canid, is listed as 'Near Threatened' by the International Union for Conservation of Nature [1]. The main threats to this species are road mortality, persecution, disease risks from domestic dogs, and most significantly, agricultural

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conversion of habitat throughout its home range in the grasslands of South America [1]. It is expected that the wild population (~20,000) will continue to decline because of ongoing habitat loss and degradation [1]. Therefore, it is imperative that the *ex-situ* population be maintained as a hedge against extinction.

Assisted reproductive technologies, especially artificial insemination (AI), serve as valuable tools for genetic management of wildlife maintained *ex situ* [2]. For example, AI is routinely incorporated into the captive breeding program of the black-footed ferret (*Mustela nigripes*) resulting in the production of approximately 100 ferret kits from genetically valuable males [3]. This success has significantly increased the representation of founder lineages and thus, enhancing genetic diversity of *ex-situ* and *in-situ* populations [3]. Artificial insemination also is successfully used in many wild felid species e.g., the cheetah (*Acinonyx jubatus*), ocelot (*Leopardus pardalis*), and leopard cat (*Prionailurus bengalensis*) [4]. However, the application of this technology in wild canids has been very limited because of challenges in predicting the onset of ovulation and effectively manipulating the female reproductive cycle [5].

Stimulation of ovarian activity and ovulation significantly improves timed-AI success in rare species such as the cheetah [6], Eld's deer (*Panolia eldii*) [7], and black-footed ferret [4]. The success of AI can be partially attributed to knowledge gained through research on the domestic counterparts of these species [4]. A potential method for inducing estrus in the domestic dog (*Canis lupus familiaris*) [8,9] and gray wolf (*Canis lupus*) [10] is the use of deslorelin (2.1 mg Ovuplant; Peptech Animal Health, NSW, Australia), a short-acting GnRH agonist that is manufactured to induce ovulation in mares. In domestic dogs, deslorelin implants achieve 100% proestrus within 6 to 10 days of implant placement with 100% ovulation [11] and can produce 70% pregnancy rates after AI [9]. Similarly, the use of deslorelin in gray wolves successfully induces fertile estrus, resulting in 100% pregnancy from natural breeding and 33% pregnancy after AI [10].

To date, there have been no studies on induction of ovarian activities in the maned wolf. Yet, previous research indicates that the reproductive biology of this species is unique compared with other canids [12]. Data obtained from noninvasive monitoring of fecal progestagen metabolite profiles of female maned wolves reveal that individuals housed with a male exhibit a classical rise (for canids) in progestagen concentrations following an estrogen peak during estrus. However, the progestagen levels in wolves housed alone remain at baseline concentrations throughout the breeding season, indicating that ovulation has not occurred [12]. Thus, singleton females may require an ovulatory agent to induce ovulation as they respond differently to ovarian stimulation regimens than wolves housed with a male.

Ovulatory agents, including hCG and LH, have been used to induce ovulation in the domestic dog with varying success. For example, hCG fails to support luteal function [13] and has no positive effects on ovulation rates in domestic dogs [14]. However, Write [15] reports that ovulation in domestic dogs is induced by hCG within 26 to 30 hours of administration. Although these ovulatory agents have not been tested in wild canids, the combination of eCG and hCG has been commonly used to stimulate ovarian activity and induce ovulation in wild felids [6]. However, evidence suggests that these protocols can cause hyperstimulation of follicles [16] and estrogen production [17], which can ultimately be detrimental to fertilization, embryogenesis, and implantation [6]. Continued use of eCG with hCG can also result in refractoriness [18,19] and can trigger an immune response [20]. Recently, a recombinant form of equine LH (reLH) that is purified and free of other pituitary hormones has been shown to effectively induce ovulation within 48 hours of administration in domestic mares (*Equus ferus caballus*) [21]; however, reLH has not been tested in domestic or wild carnivores.

In the present study, we used noninvasive hormone monitoring to investigate the influence of pairing with a male (housing condition) on endocrine responses of female maned wolves to a GnRH agonist (deslorelin). We also assessed the effectiveness of reLH on ovulation induction in females housed alone when it was administered following deslorelin (2.1 mg Ovuplant).

2. Materials and methods

2.1. Animals

Eight adult female maned wolves housed in various zoological institutions across the United States were included in the study (Table 1). Animals were housed according to guidelines from the Association of Zoos and Aquariums's Maned Wolf Species Survival Plan. All individuals, except #3015, were provided dry, commercial dog food (ProPlan Lamb & Rice) with supplemental fruits (apple, tomatoes, papaya, and banana), vegetables (lettuce), protein sources (mice, rats, fish, and chicks), and water *ad libitum*. Wolf #3015 was fed a blended mixture of Bil-Jac Senior Kibbles, Natural Balance (beef), and fruits. All animal procedures were approved by the Smithsonian Conservation Biology Institute's Institutional Animal Care and Use Committee (IACUC), the White Oak Conservation Center's IACUC, and Houston Zoological Garden IACUC.

2.2. Ovarian stimulation

Ovarian stimulation was performed in eight females (four paired with a male and four unpaired wolves) during the 2007 to 2011 North American breeding seasons (October to February) for a total of 11 stimulation cycles (paired females, $n = 4$; unpaired females, $n = 7$). All females were implanted (Day 0) with a GnRH agonist, deslorelin (2.1 mg, Ovuplant) in the vestibular mucosa of the vulva ($n = 2$) as originally described in the dog [9] or in the subcutaneous layer of the ear ($n = 9$). Implantation in the ear was tested for the ease of hormone placement and removal, as the maned wolf has a rather small vulva vestibule. Furthermore, the maned wolf has a propensity to excessively lick wounds, and implantation in the vestibular region would be more accessible than the ear. Due to logistical reasons (e.g., staffing at participating institutions), implants were removed on Day 7 ($n = 3$), 9

Table 1
Demographic information about female maned wolves (n = 8) participating in an ovulation induction study from 2007 to 2011.

Studbook no.	Institution	Age at the time of implant (y)	Management	Treated with reLH	Date implanted	Implant site	Duration of implant (days)
1891	HZP	11.0	Paired	no	10.07.07	Vulva	11
3015	DKZ	4.0	Paired	no	10.21.10	Ear	11
2539	SCBI	7.0	Paired	no	10.08.10	Ear	9
2539	SCBI	6.0	Paired	no	11.24.09	Ear	7
2257	HZ	9.0	Unpaired	no	10.07.07	Vulva	11
2926	SCBI	5.0	Unpaired	no	11.02.10	Ear	9
2612	SCBI	4.0	Unpaired	no	11.24.09	Ear	7
2613	SCBI	4.0	Unpaired	no	11.24.09	Ear	7
2612	SCBI	5.0	Unpaired	yes	10.13.10	Ear	9
2613	FRWC	5.0	Unpaired	yes	10.13.10	Ear	9
2945	WOCC	6.0	Unpaired	yes	11.01.11	Ear	9

Females are listed in chronological order of treatment; three wolves were treated more than once.
Abbreviations: DKZ, Dickerson Park Zoo; FRWC, Fossil Rim Wildlife Center; HZP, Houston Zoological Park; reLH, recombinant LH; SCBI, Smithsonian Conservation Biology Institute; WOCC, White Oak Conservation Center.

(n = 5), or 11 (n = 3). For three of the unpaired females, 0.0375 mg (93.75 IU in 0.1 mL intramuscularly) reLH (AspenBio Pharma, Castle Rock, CO, USA) was administered at the time of implant removal on Day 9. Because most canids are spontaneous ovulators, the dosage of reLH for maned wolves was calculated based on dosages previously reported for inducing ovulation in clouded leopards (*Neofelis nebulosa*) and cheetahs (*Acinonyx jubatus*) [22,23].

2.3. Fecal collection and hormone extraction

Fresh fecal samples (within 12 hours of defecation) were collected from all females 5 to 7 days/wk starting at least 11 days before hormone insertion (to establish baseline values), and continued until 2 months after the removal of the implant (to account for gestation or pseudopregnancy) [12]. Food coloring (Wilton, Woodridge, IL, USA) was added to the diet of paired females to distinguish experimental feces from those of their mates. Fecal samples were stored at −20 °C until being transported to Smithsonian Conservation Biology Institute for hormonal analyses.

Fecal hormone extraction was performed as previously described [2]. After extraction, each sample was resuspended in 1 mL dilution buffer (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, 0.2 M NaCl, pH 7.0) to a final dilution of 1:10 for storage at −20 °C. The mean extraction efficiency for this study was 75.6 ± 0.3%.

2.4. Endocrine analyses

Fecal estrogen and progestagen metabolites were quantified using single antibody enzyme immunoassays, as previously described [24]. Briefly, fecal extracts were diluted in dilution buffer (estrogens, 1:50–1:400; progestagens, 1:500–1:40,000). Estrone conjugate (polyclonal antibody R 522-2; 1:40,000 working dilution) and pregnane (monoclonal antibody CL425; 1:10,000 working dilution) antibodies were obtained from the University of California, Davis, CA, USA. Serial dilutions of pooled fecal extracts produced displacement curves parallel to those of the appropriate standard. Interassay coefficients of variation for two internal controls were 14.2% (mean binding, 32.9%) and 8.7% (mean binding, 65.3%) for estrogen (n = 80 assays) and 10.9% (mean binding, 29.1%) and 6.2% (mean binding, 69.2%) for progestagen (n = 58 assays). Intra-assay coefficients of variations for both assays were less than 10%.

2.5. Data analysis

Longitudinal profiles of steroid metabolites were aligned to the day of deslorelin treatment (Day 0). Baseline concentrations of fecal progestagens were determined by an iterative process whereby progestagen values in excess of 1.5 standard deviations (SDs) of the baseline were removed from the dataset until no values exceeded 1.5 SD of the baseline mean [25]. Baseline progestagens were reported as the mean of the remaining values, and this process was repeated each year for each female. Ovulation was defined by a distinct estrogen peak followed by a rise in progestagen level 1.5 SD above baseline for at least 3 consecutive days. Comparisons of estrogen and progestagen concentrations among pre-, peri-, and postdeslorelin implant periods were performed using ANOVA followed by Tukey's multiple comparison test. Significant differences were set at $P < 0.05$. Data were reported as mean ± standard error of mean.

3. Results

In three of the four paired females, copulatory ties were not observed, although sexual behaviors, including anogenital investigation and mounting were seen. Concentration of gonadal hormone metabolites varied among females regardless of management conditions and hormone treatments (Figs. 1–3). Based on Figures 1 and 2, it appeared that the site and duration of the GnRH-agonist treatment had no effect on overall endocrine responses to hormone stimulation in both paired and unpaired females.

Among the paired females (n = 4), one wolf (#1891) exhibited a rise in estrogen metabolites on the day of deslorelin implant (Fig. 1A) that remained high during the hormone treatment. The elevated estrogen was followed by a rise in progestagen metabolite concentrations that remained elevated for the 60 days. In the remaining three paired females, estrogen metabolites peaked shortly after the deslorelin implantation and were followed by a rise

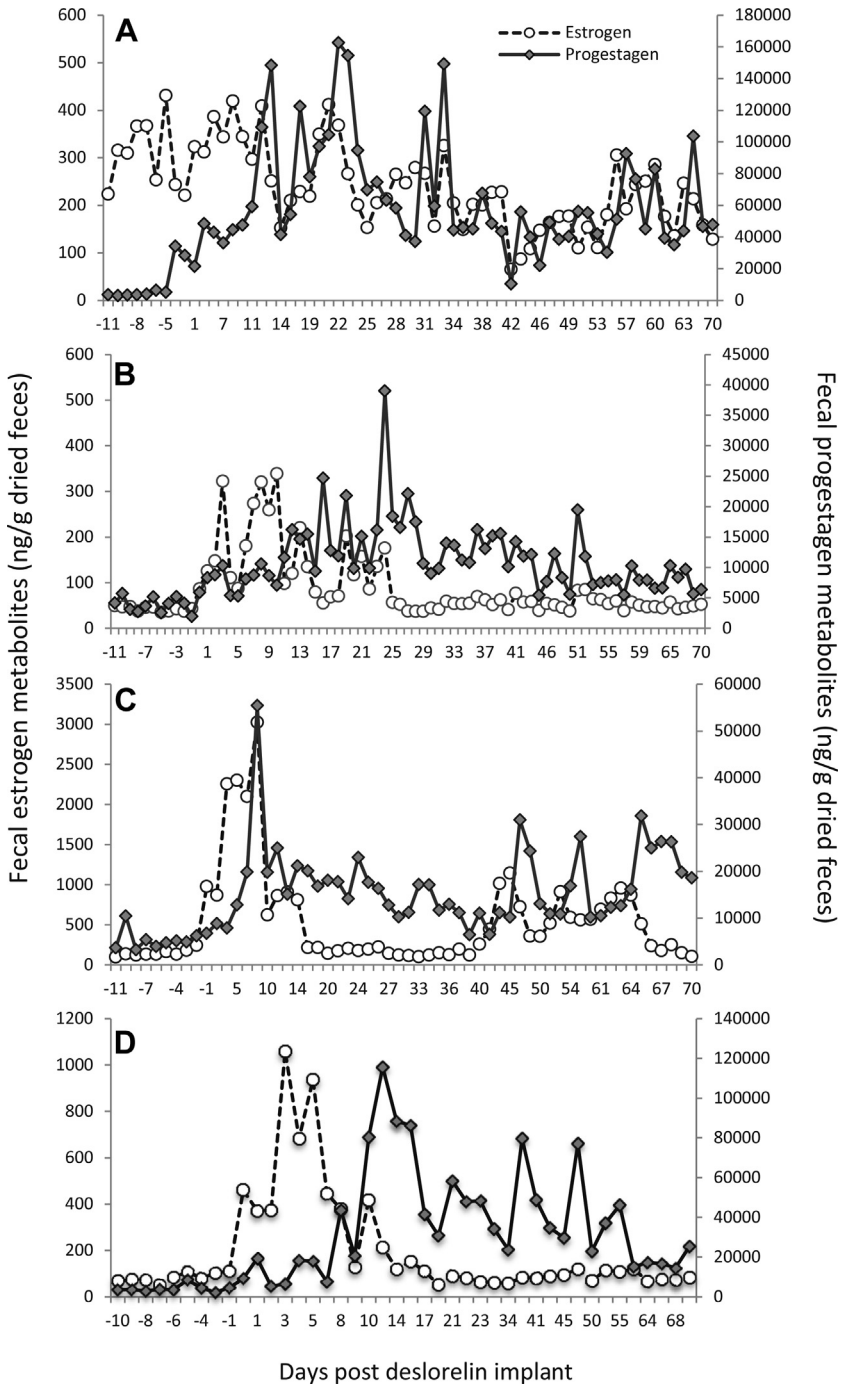

Fig. 1. Fecal estrogen and progestagen metabolites in paired maned wolves implanted with 2.1 mg deslorelin in the vulva mucosa for (A) 11 days or on the ear for (B) 11, (C) 9, and (D) 7 days.

in progestagen concentrations that remained elevated throughout the study period (Fig. 1B–D). When combining data from the four paired females, mean estrogen metabolite concentrations reached the highest level ($P < 0.01$) during deslorelin treatment (482.8 ± 109.3 ng/mL) compared with pre- (136.9 ± 17.0 ng/mL) and postdeslorelin administration (174.5 ± 24.3 ng/mL). Mean progestagen level was the highest during post- (45.3 ± 2.2 μg/g feces) and the lowest during predeslorelin treatment (18.1 ± 3.1 μg/g feces) with the concentrations during the peri-interval occurring at an intermediate level (34.2 ± 3.0 μg/g feces; $P < 0.01$). Peak estrogen and progestagen concentrations in paired females were observed between Day 3 and 9 (7.5 ± 1.5 days) and Day 9 and 16 (12.5 ± 1.4 days) postdeslorelin implant, respectively. None of the paired females became pregnant during the study.

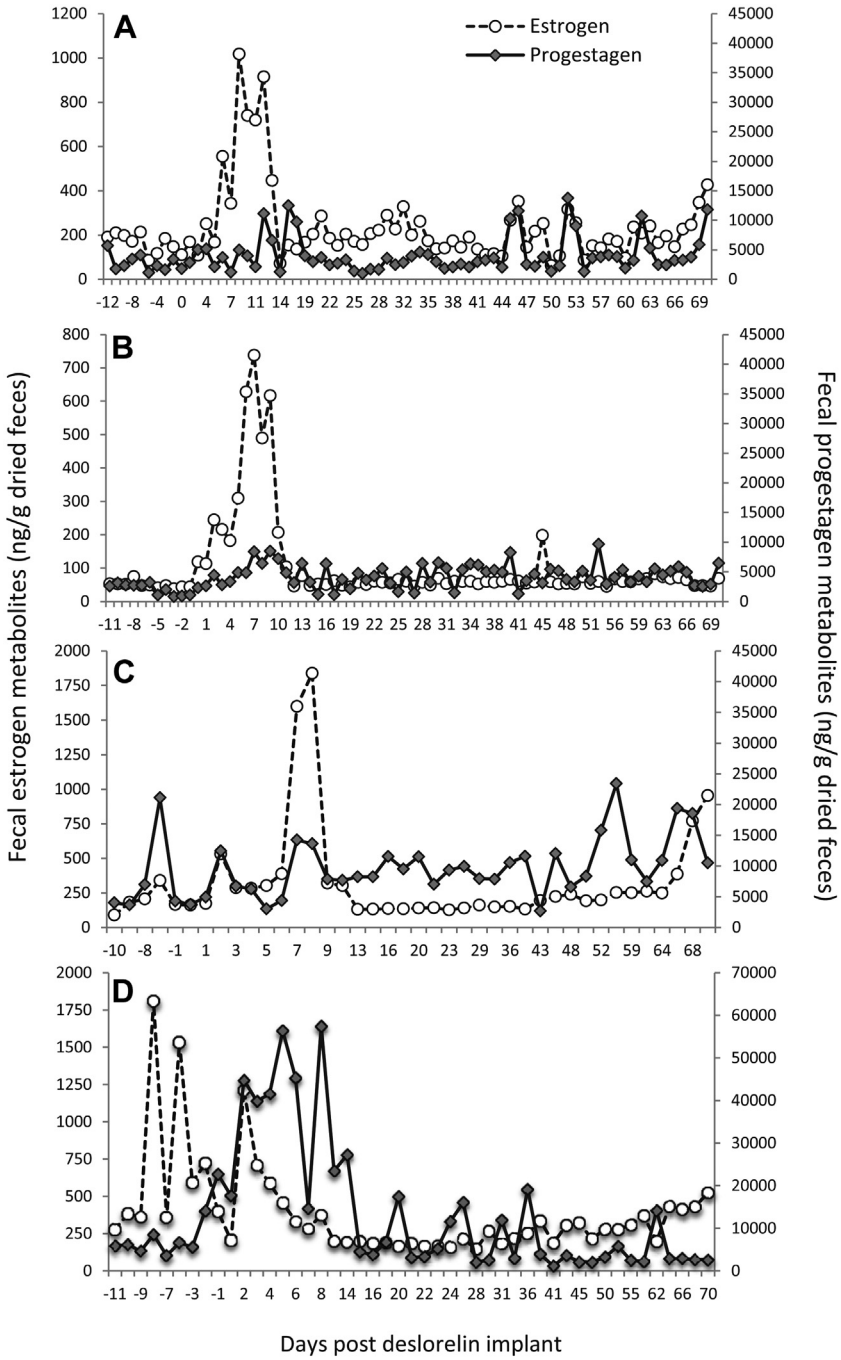

Fig. 2. Fecal estrogen and progestagen metabolites of singleton maned wolves implanted with 2.1 mg deslorelin in the vulva mucosa for (A) 11 days or on the ear for (B) 9, and (C and D) 7 days.

In singleton females, deslorelin treatment alone resulted in increased estrogen metabolites that reached the peak level (750–1750 ng/g feces) on Day 7 or 8 post implant in three of the four females (Fig. 2). In these three females, fecal progestagen metabolite concentrations did not increase and remained at the baseline level throughout the study period (Fig. 2A–C). In the remaining individual (Fig. 2D), fecal estrogen metabolite concentrations were elevated even before deslorelin treatment. Shortly after deslorelin placement, estrogen metabolite concentrations increased followed by a brief rise in progestagens (Days 2–14, totally 12 days), which then declined to the baseline level (Fig. 2D). When combining data from the four treated cycles and comparing steroid concentrations before, during, and after deslorelin implant, estrogen metabolite concentrations were highest during deslorelin (i.e., peri)

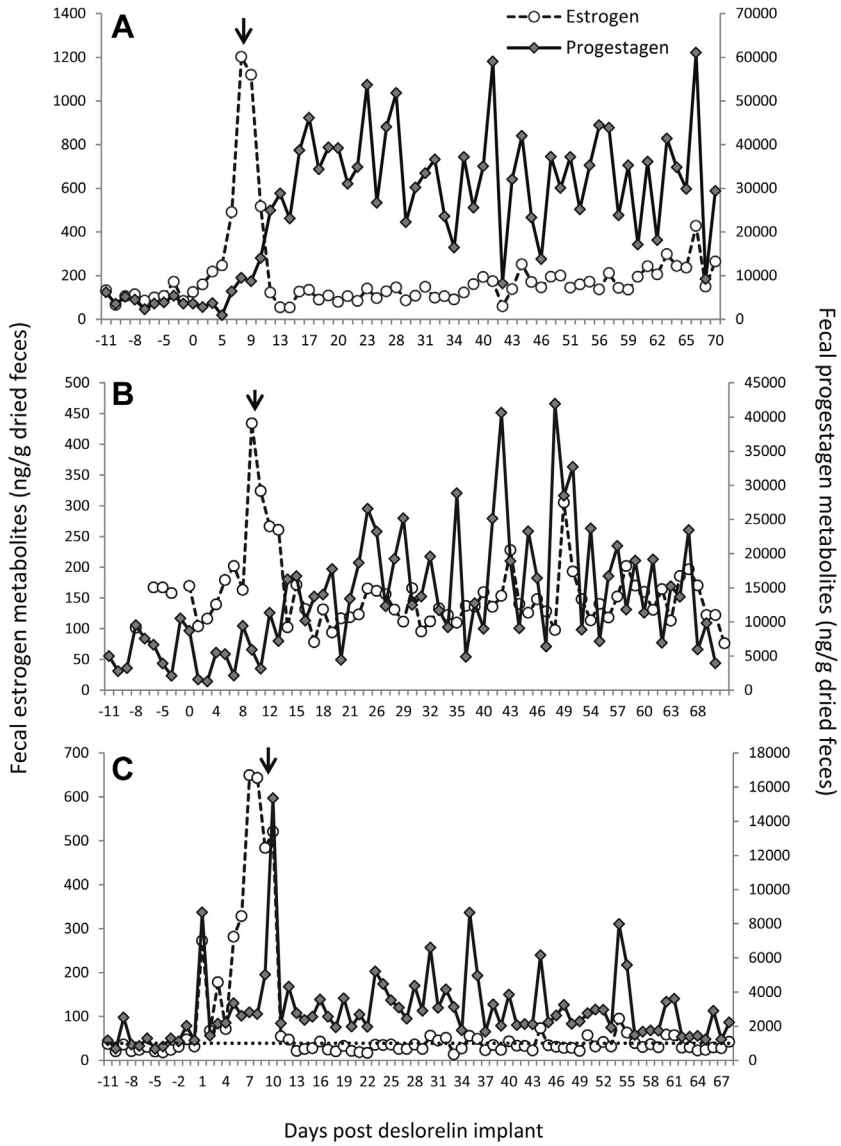

Fig. 3. Fecal estrogen and progestagen metabolites in three singleton maned wolves (A–C) implanted with 2.1 mg deslorelin in on the ear for 9 days followed by an injection of 0.0375 mg/mL reLH on the day of implant removal. Dotted line (C) represents baseline progestagen concentration. Arrows indicate the day of reLH injection. reLH, recombinant LH.

treatment (349 ± 50.8 ng/g feces) followed by prestimulation (278.6 ± 66.9 ng/g feces), with the posthormone implant concentrations being the lowest level (145.3 ± 9.5; $P < 0.01$ ng/g feces). Progestagen concentrations increased ($P < 0.01$) during deslorelin treatment (11.3 ± 2.5 μg/g feces) and then declined after the implant removal to the pretreatment levels ($P > 0.05$; pre, 4.9 ± 0.9 μg/g feces; post, 6.2 ± 0.5 μg/g feces).

Among the singleton females that received a deslorelin implant with a subsequent reLH injection ($n = 3$), estrogen peaked (450–1200 ng/g feces) on Day 8.6 ± 0.3, and this was followed by a rise in fecal progestagen metabolites starting on Day 10 ± 1.5 postdeslorelin treatment, and remained elevated above baseline for at least 65 days (Fig. 3A–C). It should be noted that one of the three females excreted much lower fecal progestagen metabolite concentrations than the other wolves (Fig. 3C). However, longitudinal monitoring demonstrated a distinct estrogen peak followed by the elevation of progestagens above the baseline concentrations for this female.

4. Discussion

Understanding ovarian function and responsiveness to exogenous hormones is essential for reproductive management of wildlife populations living *ex situ* [4,26], especially in a monestrous species, such as the maned wolf. In the present study, we demonstrated that management condition (housed with or without a male) significantly influenced the response of female maned wolves to a

GnRH-agonist, deslorelin, treatment. Although deslorelin stimulated ovarian activities (based on increased estrogen metabolite concentrations) in both paired and singleton females, this hormone treatment did not result in ovulation (i.e., progestagen remained at baseline level) in the latter. Administration of an ovulatory agent, such as reLH, was required for inducing ovulation in singleton females.

GnRH agonist, deslorelin, has been used to induce fertile estrus in the mare, domestic dog [8,9,27], and gray wolf [10]. This hormone treatment stimulates the release of FSH and LH from the pituitary gland that, in turn, promotes ovarian follicle growth and production of gonadal hormones. In the domestic mare, deslorelin is administered either subcutaneously [27] or in vulva mucosa [28] and ovulation normally occurs 48 to 72 hours posttreatment. In domestic dogs, subcutaneous administration leads to premature luteal failure in some females [29]. Specifically, although serum progesterone concentrations rise during estrus irrespective of implant sites, there are premature declines in steroid levels starting 1 to 4 weeks after hormone implant placement. In females exhibiting such declines, circulating deslorelin remains elevated (>100 pg/mL) 20 days after implant administration, whereas that of individuals administered via vulva mucosa decreased to undetectable levels after 12 days [30]. Thus, prolonged release of the deslorelin into circulation may cause the downregulation of pituitary gonadotropins and premature luteal failure in female domestic dogs administered deslorelin subcutaneously [27,30]. Furthermore, it has been shown that GnRH and its agonists bind to specific receptors on the luteal cells that, in turn, suppress the production of progesterone in the rat [31].

Although we did not directly compare administration sites (because of small sample size) in the present study, it appeared that implant site and duration did not impact maned wolves' response to deslorelin treatment. All females responded to deslorelin by exhibiting an estrogen surge posthormone implant insertion. Concentrations of estrogen metabolites excreted during deslorelin treatment in both paired and singleton individuals were comparable with levels observed during the peri-ovulatory period in naturally cycling females [24]. Thus, a 2.1-mg deslorelin implant was 100% effective at inducing ovarian activity in the maned wolf. However, ovulation depended on the presence of a male or reLH injection. In paired females, ovulation was estimated to occur between Days 9 and 16 (mean 12.5 $\pm$ 1.4 days) after implant insertion based on a rise in fecal progestagen concentrations after an estrogen surge. This interval is consistent with previous reports in the domestic dogs (10 $\pm$ 2.9 days [29]; 11–19 days [13]) and gray wolf (16–18 days [10]). Unlike the domestic dog, maned wolf progesterone metabolite concentrations remain elevated for 2 months after implant removal in all females regardless of implantation site. In the present study, deslorelin was inserted in the subcutaneous layer of the ear, whereas in the domestic dog, the hormone was implanted between the shoulder blades. Therefore, the variation in endocrine responses between the two studies may be because of (1) different implantation sites (shoulder blades vs. ears) and (2) species-specific reproductive biology (spontaneous vs. induced ovulator).

Unlike the paired females, progestagen metabolite concentrations remained at baseline after deslorelin treatment in three of four singleton maned wolves. In the remaining female, there was a brief rise in progestagen after deslorelin treatment. The difference in responses to deslorelin treatment among singleton females may be because of variation in ovarian status at the time of hormone implantation. Estrogen metabolite concentrations of the three females that failed to ovulate were at baseline levels at the onset of deslorelin treatment, whereas estrogens were elevated in the remaining wolf before treatment. Female maned wolves housed individually exhibit a cyclical rise in estrogen concentration throughout the breeding season [24]. Because estrogen is produced by the granulosa cells of growing follicles [32], it is likely that ovarian follicles still grow and produce steroids even in the absence of a male. Thus, if a deslorelin implant is placed in the presence of growing follicles, the release of FSH and LH in response to the GnRH agonist may be sufficient to support preovulatory follicle growth and ovulation. It is possible that this is what led to ovulation in the singleton female observed in the present study. Conversely, the estrogen surge followed by ovulation in this wolf may be because of social stimulation, as this individual was housed next to another female (#2612). In the island fox (*Urocyon littoralis*), which is also an induced ovulator, some females ovulate when housed with other females [33]. Nevertheless, the rise in progestagen concentration was short lived (12 days) in the singleton female wolf indicating that CL (if present) prematurely regressed. Thus, it is possible that the presence of a male is also important for maintaining luteal function. Alternatively, premature luteal regression observed in this female may be because of the site of hormone administration (subcutaneous). It has been previously shown in the domestic dog that a higher percentage (three of eight) of females receiving deslorelin subcutaneously exhibited premature declines in progesterone (luteal failure) compared with those administered via vestibular mucosa (one of eight) [29].

Our finding that the presence of a male was required for female maned wolves to ovulate after deslorelin treatment corroborated earlier reports that only paired females naturally ovulate; thus, this species is an induced ovulator [24,34]. Because most canids, with the exception of the island fox [33], ovulate spontaneously (in the absence of a male) [35], being an induced ovulator makes the maned wolf rather unique compared with other species within the family Canidae. Induced ovulation is most commonly reported in mammals among the order Carnivora, and predominantly occurs in solitary species that exhibit multimale mating systems such as felids [36]. Although the maned wolf is solitary in the wild, it is also monogamous [37] and thus, would not benefit from the postcopulatory mate choice that felids exhibit [36]. Because of this species' solitary nature and large home range, it is likely that the maned wolf benefits from induced ovulation by ensuring that ovulation only occurs when the male is in the vicinity.

Because the domestic dog ovulates spontaneously, administration of an ovulatory agent as part of an estrus induction protocol is considered unnecessary [11]. Nevertheless, an intravenous injection of LH between 7 and

12 days after the onset of FSH treatment does not always result in ovulation in domestic dogs, possibly due to the interfering effect of the exogenous hormone on the release of the endogenous counterpart [11]. In the present study, we demonstrated that a single reLH injection on the day of deslorelin removal was able to induce ovulation in unpaired females. These results are extremely encouraging for the usage of timed AI to preserve the genetics of valuable females that cannot be paired with a male due to reasons such as location, space limitations, or aggression. Furthermore, our findings may be applied to the reproductive management of another induced ovulator, the island fox [33].

With a small captive population and very limited access to female maned wolves for research, the numbers available for participation in this study were minimal. For this reason, further investigations to increase the sample size and identify a narrower window of ovulation for timed AI are required. In the present study, fecal progestagen metabolite concentrations of ovulating females (both paired and unpaired) surged, on average, on Day 12 with a range of 9 to 16 days. Thus, it is currently difficult to identify precisely when ovulation occurred using only fecal steroid metabolite data for the purpose of timed AI. However, determining the timing of an LH surge would narrow this window as ovulation in the dog occurs 48 to 60 hours after the LH surge [38]. A recent study in bottlenose dolphins (*Tursiops truncates*) has established methods to monitor urinary LH for timed AI using immunochromatographic assays originally designed to detect canine serum LH [39]. Thus, future research should explore the benefits of urinary LH in predicting the onset of ovulation for timed AI in maned wolves.

Although deslorelin has been used to induce fertile estrus in domestic dogs and gray wolves, previous studies on GnRH agonists have revealed some concern over uterine health [40,41] and premature luteal failure [29]. For example, long-acting deslorelin implants (4.7 mg, Suprelorin; Peptech Animal Health) used to suppress reproduction in sea otters (*Enhydra lutris*) lasted 2 to 3 years longer than expected [41]. In the present study, none of the four treated cycles in the three paired females resulted in pregnancy during the course of this study. However, it is unlikely that the lack of reproduction in these females was due to deslorelin treatment as one wolf (#1891) was past prime reproductive age (i.e., 3–8 years), one (#2539) was housed with a young male for 2 consecutive years (aged 1 year at initial pairing in 2009), and the other (#3015) was paired with an incompatible mate, and sexual behaviors were not observed. Thus, future studies are needed to investigate the influence of deslorelin on fertility in the maned wolf. It should also be noted that female #2539 continued to be housed with her mate (aged 3 years at the time of breeding), conceived, and gave birth to four healthy offspring in the following year. Thus, a 2.1-mg deslorelin implant did not adversely impact reproduction in subsequent breeding seasons, at least, for this maned wolf.

In the present study, there were variations in hormone metabolite concentrations among individuals, regardless of housing conditions (presence vs. absence of a male). The reasons for this variation are unknown, but it has been demonstrated that changes in dietary fiber consumption can significantly affect fecal hormone metabolite excretion [42]. When comparing steroid metabolite concentrations observed in the present study to those previously reported by our laboratory [22], the progestagen levels observed in paired females and unpaired individuals injected with reLH were lower than those excreted during the luteal phase in pregnant wolves that gave birth and raised young (mean ± standard error of mean, 68.9 ± 5.9 μg/g feces [24]). However, progestagen metabolite concentrations reported here were similar to those observed in females that lost pups soon after birth (39.0 ± 6.3 μg/g feces) and pseudopregnant females (11.3 ± 1.8 μg/g feces) [24].

4.1. Conclusions

The present study demonstrated that management conditions (being housed with or without a male) influenced the response of ovarian activity to induction in female maned wolves and that exogenous LH was required to induce ovulation in singleton females. The findings obtained from this research provide further evidence that the maned wolf is unique among canids in that the presence of a male is required for the female to ovulate. Finally, the findings also serve as a foundation for future studies focusing on developing improved strategies to induce ovarian activity and ovulation for timed AI to better manage this threatened species *ex situ*.

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