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Influence of exogenous gonadotropin-releasing hormone on seasonal reproductive behavior of the coyote (*Canis latrans*)

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Abstract

Wild *Canis* species such as the coyote (*C. latrans*) express a suite of reproductive traits unusual among mammals, including perennial pair-bonds and paternal care of the young. Coyotes also are monestrous, and both sexes are fertile only in winter; thus, they depend upon social and physiologic synchrony for successful reproduction. To investigate the mutability of seasonal reproduction in coyotes, we attempted to evoke an out-of-season estrus in October using one of two short-acting gonadotropin-releasing hormone (GnRH) agents: (1) a GnRH analogue, deslorelin (6-D-tryptophan-9-(*N*-ethyl-L-prolinamide)-10-deglycinamide), 2.1 mg pellet sc; or (2) gonadorelin, a GnRH (5-oxoPro-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2) porcine hypothalamic extract, 2.0 μg/kg im once daily for 3 consecutive days. A transient increase in serum concentrations of estradiol and progesterone (1 and 2 wk, respectively) was detected after treatment with deslorelin but not gonadorelin. Also, socio-sexual behaviors reminiscent of winter mating (including courtship, mate-guarding, precoital mounts, and copulatory ties) were observed among the deslorelin group. During the subsequent breeding season (January and February), however, preovulatory courtship behavior and olfactory sampling appeared suppressed; emergence of mounts and copulations were delayed in both deslorelin and gonadorelin treatment groups. Furthermore, whereas 8 of 12 females treated in October ovulated and produced healthy litters in the spring, 4 naïve coyotes failed to copulate or become pregnant. Thus, perturbation of hormones prior to ovulation in species with complex mating behaviors may disrupt critical intrapair relationships, even if fertility is not impaired physiologically.
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Keywords: *Canis latrans*; Coyote; GnRH; Mating behavior; Reproductive seasonality

1. Introduction

Coyotes (*Canis latrans*) are indigenous wild canids widely distributed throughout North America. They are socially monogamous, territorial [1–3], and seasonally monestrous [4–7]. Both sexes are obligated to a single breeding season, extending January to March (depending on latitude), and become sexually inactive in the summer [4–6]. Spermatogenesis in coyotes begins late October or November, and mature spermatozoa may be found from December to May [4,5,8–10]. Meanwhile, ovaries from coyotes examined in January contain tertiary follicles, but cortices were otherwise unremarkable (without stigmata or ruptures). Ovulation is spontaneous, synchronous, and bilateral; thereafter, ovaries are dominated by corpora lutea [4,6] and nonpregnant females enter a compulsory pseudopregnancy [11]. Coyote pups are

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typically born March to May, after a gestation of 60 to 63 d [4,5], and both parents care for the young [1,2,5]. By July or August, ovaries are regressive but not yet fully regenerated, testes have atrophied, and epididymides are void of mature sperm [4,5]. Accordingly, endocrine profiles supported the histologic evidence that coyote reproduction was confined to a singular seasonal event [7,10,11].

Other *Canis* species express reproductive traits similar to the coyote [12–15], but the domestic dog (*C. familiaris*) is a notable exception. Male domestic dogs are fertile year-round, and bitches are typically aseasonal, with an interestrus interval 5 to 12 mo in duration [16]. A seasonal shift in reproductive recrudescence also was reported among first generation (F1) coyote-dog hybrids. Specifically, F1 hybrid offspring became sexually active in the fall (October to December) [5,8,17–19], and one female bred again in May [5]. Variability in reproductive strategies of wild canids has been ascribed to changes in social or environmental conditions, particularly within genera other than *Canis* [20–22]. Thus, canid reproductive tactics appeared to be adaptive, capable of responding to alterations in selective pressures or environmental conditions.

The mechanism controlling reproductive seasonality in wild *Canis* has not been elucidated, although presumably it is similar to that described in other species. Seasonal changes in environmental factors act as cues allowing an animal to coordinate arrival of offspring with availability of critical resources. Photoperiod activates neuroendocrine messengers, which in turn stimulate a cascade of physiologic and behavioral events; however, receptors vary in sensitivity depending on photoperiod and sequence of exposure to hormones [23–26]. In female coyotes, for example, Hodges [10] reported that in vitro cultures of pituitary cells showed a dose-dependent seasonal difference in sensitivity to gonadotropin-releasing hormone (GnRH). Pituitary cells collected from coyotes in winter produced significantly more luteinizing hormone than cells harvested in April to October, but only when challenged with higher doses of GnRH.

Environmental cues (physical or social) also synchronize endogenous circadian and circannual rhythms; yet conversely, the same factors may be ineffectual at suppressing or provoking a biological process from its entrained pattern [24,25]. Consequently, experimental manipulations elicit disparate effects when applied at different moments in the reproductive cycle [25,26]. In the domestic bitch, administration of exogenous GnRH has advanced estrus and ovulation [27,28] but, paradoxically, has acted also as a contraceptive [29,30]. Furthermore, before achieving a desired suppressive effect, signs of proestrus and estrus have been observed in dogs [29–31] and wolves [32]; pregnancies after treatment have been reported [31,32].

The objective of this study was to disrupt the estrus cycle of coyotes and describe the physiologic and behavioral consequences that could occur if pair-mates were desynchronized. We hypothesized that coyotes were physiologically prepared to initiate a new ovarian cycle in the fall. If true, GnRH given in late anestrus would evoke a premature estrus. Two short-acting agents were employed, because the sensitivity of a purebred coyote to treatment protocols used in domestic dogs could not be predicted. Herein we report our results and discuss how hormone manipulation might impact normal intrapair relationships, emphasizing the importance of healthy mating behavior as well as physiology for successful reproduction in wild canids.

2. Materials and methods

2.1. Animals

Coyotes were captive born or wild caught as pups and reared at the National Wildlife Research Center (NWRC) facility in Millville, Utah, USA (41°68′ N, 111°82′ W). All animals were housed in outdoor enclosures with natural lighting. Male-female pairs resided in 0.1-hectare pens with access to sheltered den boxes. Three pens formed a clover-shaped cluster separated by double fencing and concrete barriers; all pairs were within visual and audible range of other coyotes.

The animals were fed a commercially prepared carnivore diet (Fur Breeders Agricultural Cooperative, Sandy, UT, USA) once daily, and fasted 1 day per week. Water was provided ad libitum. Vaccinations were given annually against canine distemper, hepatitis, leptospirosis, parvovirus, parainfluenza, type 2 coronavirus, adenovirus, and rabies. Routine parasite control was administered as indicated. Animal care and research protocols were approved by the Institutional Animal Care and Use Committees at Utah State University (IACUC No. 1114) and the NWRC (QA987).

Eighteen mated coyote pairs recruited into this study were either established (n = 10), residing with each other during a previous breeding season, or recently introduced (n = 8) the month prior to initiation of treatment and observations. Sexually experienced females (n = 12) ranged in ages from 3 to 6 yr, whereas maiden coyotes (n = 6) were 18 mo to 3 yr of age at the

time of treatment (October 10 to 12, 2002). Average weight of female coyotes was 11.1 kg (range, 7.6 to 13.8 kg).

A previous longitudinal study [11] noted the ovarian cycles within this colony to be synchronous. During the 2000–2003 breeding seasons, the coyotes commonly entered estrus mid-January to mid-February. Behavioral estrus (the period of sexual receptivity when a female permits her mate to copulate) ranged from 8 d before ovulation to 10 d after ovulation, but at the individual-animal level, females remained receptive an average of 7.6 ± 1.4 d.

2.2. Treatment groups and controls

2.2.1. Deslorelin

Deslorelin (6-D-tryptophan-9-(*N*-ethyl-L-prolinamide)-10-deglycinamide), a synthetic analogue of GnRH, has been incorporated into a biocompatible inert matrix and formed into an implantable pellet for sustained-release (developed by Peptech Animal Health, North Ryde, NSW, Australia). In this study, the commercially available product, Ovuplant (distributed by Fort Dodge Animal Health, Fort Dodge, IA, USA), provided 2.1 mg deslorelin acetate in a short-acting subcutaneous (2.3×3.6 mm) pellet.

In the first treatment group, six female coyotes each received a single interscapular Ovuplant pellet (mean dose, 0.2 mg/kg deslorelin per animal), October 10 to 12, 2002. To prepare the insertion site, a small patch of fur was clipped and the skin cleansed with alcohol and povidone-iodine then allowed to dry. A small incision ($\leq$0.5 cm) made with a sterile surgical blade eased initial penetration of the implanter syringe needle through the epidermis; the pellet was then placed in the subcutaneous space, according to the manufacturer's instructions. The implant site was subsequently inspected each time the coyote was handled, and no gross adverse reactions were noted.

Within this cohort, three of six females were sexually experienced and residing with their established mates. Each female had whelped a healthy litter the previous spring (March to April, 2002). Among the maiden females, one female was 2 yr of age, and two were 18 mo old; the males selected to be their mates were also sexually naïve.

2.2.2. Gonadorelin

In the second treatment group, six female coyotes were given daily intramuscular injections, 2.0 μg/kg gonadorelin diacetate tetrahydrate (Cystorelin; distributed by Merial Ltd., Iselin, NJ, USA) for 3 consecutive days, October 10 to 12, 2002. Gonadorelin is a GnRH (5-oxoPro-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2) porcine hypothalamic extract with a short half-life in vivo. Thus, daily administration of gonadorelin was intended to mimic the endogenous GnRH pulses that naturally evoke reproductive recrudescence.

Within this cohort, three of six females were sexually experienced and in an established pair-bond; furthermore, all three had been pregnant the previous spring (March to April, 2002). The maiden females were 3 yr or 18 mo old (one and two females, respectively), and were introduced to sexually mature but equally naïve males in September 2002.

2.2.3. Normal saline

Six female coyotes received single interscapular subcutaneous injections of 0.5 mL sterile 0.9% physiologic normal saline (NS), October 10 to 12, 2002. Within this control cohort, four pairs were established, and the females had been pregnant the previous spring. Meanwhile, the other two females, although experienced and pregnant in 2001, were paired with new mates in September 2002 (one male was sexually experienced, the other naïve).

2.2.4. Colony reference group

Socio-sexual behavior and reproductive hormone profiles during breeding within this captive colony (including sexually experienced coyotes recruited for this experiment) were studied and reported elsewhere [11]. Briefly, during 2000–2003 breeding seasons (January to February), behavioral observations of 32 pairs of coyotes were recorded and categorized as described in Section 2.3. In addition, peripheral blood samples were collected during late proestrus, estrus, and early diestrus from a subset of 18 females; 10 mated female coyotes and 8 sequestered females (housed near their mates but separated to prevent copulation). Quantitative serum estradiol, progesterone, and prolactin concentrations were assayed, and intercohort (pregnant versus pseudopregnant) comparisons analyzed [11]. Socio-sexual behaviors and reproductive hormones were then aligned by each individual coyote's estimated day of ovulation (back calculated from day of parturition, assuming a 62-d gestation) and combined by cohort, thus characterizing a behavioral and physiologic reference profile for this population.

2.3. Mating behaviors

The coyotes were habituated to low-level human activity prior to the beginning of the study, and

behavioral observations began 1 wk prior to treating the females in early October. All enclosures could be viewed through binoculars or spotting scope from sites 100 to 500 m away and were continuously scanned: from 0800 to 1000 and from 1500 to 1700, October 3 to December 28, 2002; and throughout available daylight, 0700 to 1800, January 5 to March 28, 2003.

Observers would continuously scan the enclosures, viewing one pen, documenting any interactive behavior occurring between the mated coyotes, then scan the next pen. Because this process rarely took more than 30 sec per pen, all pens were viewed at least once every 5 to 10 min. Also, an observer would only record a behavior once even if a coyote pair continued the behavior for an extended period of time (e.g., copulatory ties might last 5 to 45 min). However, if the behavior was terminated then reinitiated, the observer would record it as distinct events (e.g., multiple mounts often precede a copulatory tie).

Characterization of social and sexual behavior [11,14,33] was standardized between observers and recorded. Documented appetitive and sexually explicit coyote mating behaviors included (a) olfactory sampling (sniff/lick of the female's anogenital region by the male, female solicitation with diverted tail, and sniff/lick of the male's inguinal area by the female); (b) precoital mounts or mounting attempts; and (c) copulation lock-tie. Observed affinitive social behaviors included (a) courtship (non-antagonistic play-wrestling and play-chases, allo-grooming such as licking the face, ears, or back, also body-rubs, hip-pushes, or sleeping curled against each other); and (b) mate-guarding (the male shadowing the female around the pen walking or trotting with his head and shoulders adjacent to her flank, or when in view of a neighbor the male would stand on the female with stiff forelegs on her back, or stand over her as she lay on the ground).

2.4. Specimen collection and handling

To evaluate ovarian response to the GnRH treatments, blood samples for quantitative estradiol and progesterone assays were routinely collected. An initial baseline sample was obtained while the animal was being handled for, but prior to, first treatment, and weekly thereafter for 9 wk (October 10 to December 19). Further sampling, however, was temporarily suspended until the coyotes' native breeding season and estrus began (January to February 2003). In winter, a serum sample was collected 1 to 3 d after a mated pair's first observed copulatory tie; and another 2 wk later. In the event a pair(s) was not observed in a copulatory tie, a sample was collected on a random day in mid-February (approximately 64% of pairs were observed in a tie, January 24 to February 13), followed by a second sample 2 wk later.

Peripheral blood samples were collected from the cephalic or saphenous veins by venipuncture. Samples were collected during 0800 to 0930 before the animals were fed and without sedation or anesthesia. For quantitative estradiol and progesterone analysis, whole blood was collected in an evacuated tube and allowed to clot at room temperature (20 to 24 °C) for 30 to 120 min. Serum was separated from the blood cells by centrifugation (3000 × g, 10 min, 20 to 24 °C), divided into aliquots then stored at ≤−20 °C until testing.

Pregnancy was determined by presence or absence of relaxin in plasma; therefore, anticoagulated (sodium heparin or lithium heparin) whole blood samples were also collected. In a previous study, relaxin was detectable after Day 28 of gestation in the plasma of all coyotes later seen with pups, whereas pseudopregnant coyotes were consistently negative [34]. Thus in the current study, heparinized samples were collected 4 to 5 wk after the first observed copulatory tie, and females initially testing negative were resampled 2 wk later. Samples were promptly centrifuged (3000 × g, 5 to 10 min, 20 to 24 °C), and the separated plasma was stored at ≤−20 °C until testing.

2.5. Laboratory assays

Quantitative progesterone blood concentrations were assayed by competitive binding enzyme immunoassay (EIA; Progesterone EIA, DSL-10-3900, Diagnostic Systems Laboratories, Inc., Webster, TX, USA) using the procedure previously described and validated for coyotes [11,35]. All specimens from an individual coyote, collected in the fall and winter, were tested together in a single run. Samples were tested in duplicate with an intra-assay coefficient of variation (CV) threshold ≤10%. Kits from a single reagent lot were used, and the interassay mean CV was 7.8%.

Serum estradiol was quantitatively measured by radioimmunoassay (RIA) at the Colorado State University Endocrine Laboratory (ARBL/Foothills Campus, Fort Collins, CO, USA). In this double-antibody assay, ether-extracted estradiol from coyote sera competed with ^{125}I-labeled estradiol-17β for a fixed amount of rabbit anti-estradiol antibodies. Anti-rabbit IgG was added, and the amount of radioactivity in the captured antigen-antibody precipitate was measured. Samples were compared with a standard curve; the amount of radioactivity being inversely proportional to

the quantity of estradiol present in the unknown coyote sera. The stated ''lowest detectable limit of estradiol'' by this assay was 2.62 pg/mL.

Canine relaxin was qualitatively assayed by solid-phase enzyme-linked immunosorbent assay (ELISA; ReproCHEK, Synbiotics Corporation, San Diego, CA, USA) using the procedure previously validated and described for the coyote [34,35]. Relaxin present in the plasma of pregnant coyotes produced a blue color within microtiter wells; meanwhile, plasma from nonpregnant coyotes produced distinctively weaker (or no) color development by comparison. All initial-negative or indeterminate results were confirmed by retesting with a new sample.

2.6. Data analysis

Coyote mating behaviors were categorized, aligned by the day of treatment (in fall) or the estimated day of ovulation (in winter) for each individual female, then compiled by study cohort. In addition to intergroup comparisons, patterns of behavior recorded in this study were also compared to data similarly collected and documented for the captive colony at large during 2000–2003 breeding seasons [11,35]. Because the social and sexual behavior of the treatment-control (NS) animals in winter did not appear affected (multivariate analysis of variance, $P > 0.05$) by participation in the fall portion of the study, their data were included in the reference data set representing expected coyote breeding behavior, hereafter (unless otherwise noted) referred to as Colony. Accordingly, the patterns of mating behavior in winter among deslorelin-treated and gonadorelin-treated animals showed similar deviations from the expected estrus profile, therefore the two treatment groups were combined herein for contrast with other coyotes within this colony (exception: two deslorelin-treated and two gonadorelin-treated animals were excluded from winter behavioral analysis because unlike the rest of the cohort, these females did not copulate or become pregnant).

The approximate day of ovulation for an individual may be estimated by back-calculating from the day of parturition or by monitoring changes in serum progesterone concentrations [11,35]. In the current study, all colony and NS coyotes produced healthy full-term litters, as did four of six deslorelin-treated and four of six gonadorelin-treated females. Therefore, the estimated day of ovulation for these individuals was based on an assumed gestation of 62 d. However, to evaluate the possibility of a residual treatment effect on ovulation in the four females that did not copulate or produce pups, we compared the progesterone concentrations of these individuals to progesterone profiles previously described in an affiliated study of mated female coyotes in this colony [11].

Multivariate analysis of variance (MANOVA) with repeated-measures was used to analyze steroid hormone profiles and detect differences between study groups and between successive weeks (Statistical Analysis System [SAS], version 8.2; SAS Institute Inc., Cary, NC, USA). This statistical procedure provided a Wilks' lambda likelihood ratio assessment of the main effects, treatment (deslorelin, gonadorelin, normal saline), and time (Week 0 through Week 9) on estradiol and progesterone concentrations but also tested for a treatment-time interaction. Concurrently, differences in hormone concentrations between successive weeks, and weekly intergroup comparisons (least square means: Bonferroni correction) analyses were performed. A variance ratio test was used to compare behaviors (courtship, olfactory sampling, precoital mounts, copulatory ties, and mate-guarding) of treatment pair-mates to colony mating behaviors. Unless otherwise noted, we assumed a level of statistical significance of $P < 0.05$.

3. Results

3.1. Fall

Short-acting exogenous GnRH was given to 12 female coyotes (deslorelin, n = 6; gonadorelin, n = 6) in anestrus, approximately 34 to 38 wk after their last ovulation and 15 to 18 wk before their next estrus. Within 1 wk after treatment (Week 0 to Week 1), an ovarian hormone response was detected, but only in the deslorelin cohort ($F_{18,14} = 2.31$, $P = 0.059$). Deslorelin mean serum estradiol concentrations increased ($F_{2,15} = 11.76$, $P = 0.001$) from baseline preimplant concentrations (<2.6 pg/mL) to 22.4 ± 6.3 pg/mL (mean ± SEM). In contrast, females in the NS group remained relatively unchanged (intragroup mean estradiol, 5.7 ± 2.9 pg/mL to 3.4 ± 2.1 pg/mL) during this period, and estradiol concentrations within the gonadorelin treatment group were consistently < 2.6 pg/mL (Fig. 1).

An ovarian response to treatment was also inferred from a change in mean serum progesterone concentrations, but as with estradiol, only in the deslorelin group ($F_{18,14} = 2.62$, $P = 0.037$). Two weeks after the deslorelin implant, progesterone increased from 22.3 ± 6.5 ng/mL to 46.5 ± 17.5 ng/mL. Whereas this episodic surge (from Week 1 to Week 2) was statistically

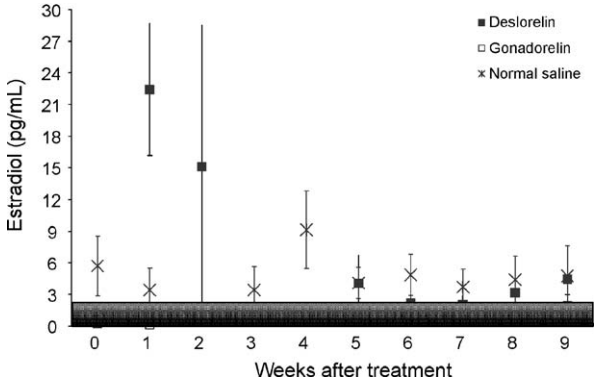

Fig. 1. Weekly (mean ± SEM) serum estradiol concentrations (pg/mL) in female coyotes sampled after treatment with deslorelin, gonadorelin, or normal saline (October to December, 2002). Week 0 represents pretreatment baseline concentrations. Semitransparent bar represents area below the detectable limit of the RIA (<2.62 pg/mL). All gonadorelin intragroup mean estradiol concentrations were <2.6 pg/mL.

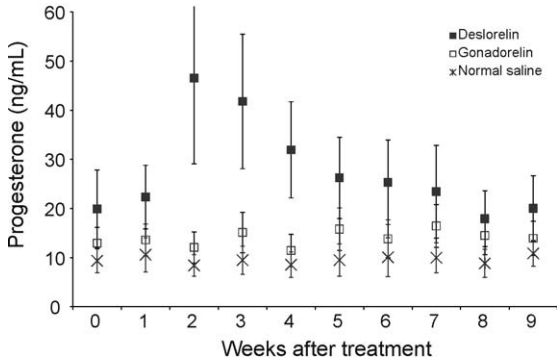

Fig. 2. Weekly (mean ± SEM) serum progesterone concentrations (ng/mL) in female coyotes sampled after treatment with deslorelin, gonadorelin, or normal saline (October to December, 2002). Standard error bars are not displayed for the gonadorelin group. Week 0 represents pretreatment baseline concentrations.

borderline ($F_{2,15} = 3.23$, $P = 0.068$), it was notably absent in the NS or gonadorelin profiles (Fig. 2).

Concurrent with endocrine expression, coyotes treated with deslorelin solicited and displayed (albeit sporadically) socio-sexual behaviors commonly restricted to the breeding season (Fig. 3). Specifically, physical interactions reminiscent of courtship (such as body-rubs, hip-pushes, and face-licking) were seen during the first week after implant. Later in the second and third weeks, females tolerated their mates' olfactory investigations; subsequently soliciting attention from their mates with diverted tails, and permitting the males' anogenital sniff/lick and precoital mount attempts. Also during this period, two males became defensive, shadowing their mates or standing over them. In one particular case, the male became aggressive, threatening the neighboring male by charging the fence with hackles, ears, and tail raised. Periodically, his

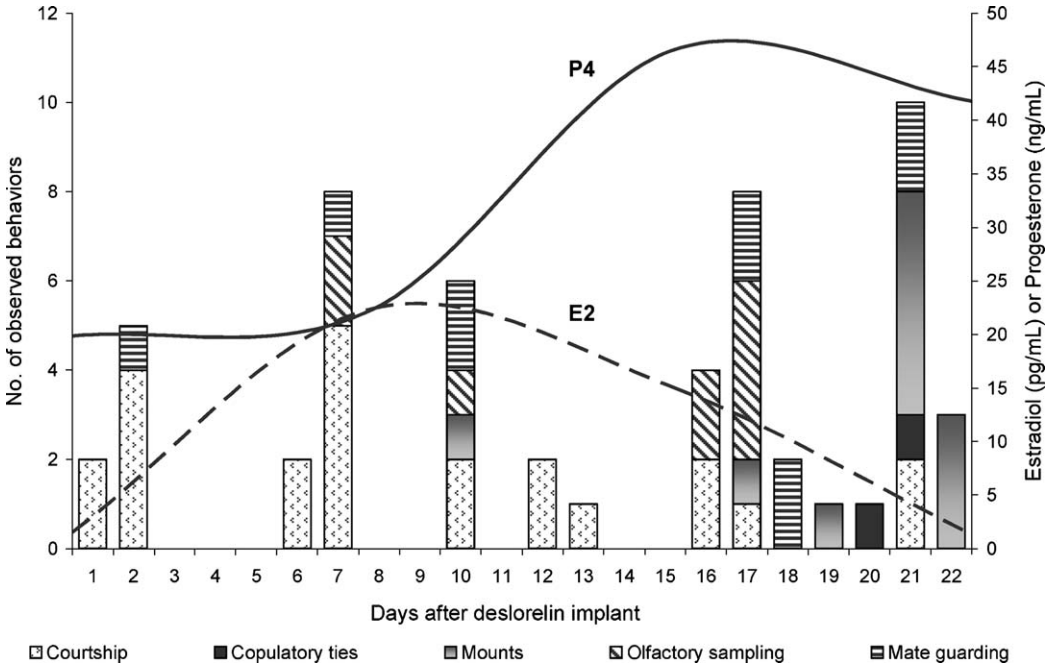

Fig. 3. Social and sexual behaviors observed among six mated coyote pairs after treatment of the females with deslorelin in October 2002. Mean steroid hormone concentrations are overlaid, demonstrating temporal relationships between behaviors and hormones.

threat displays also included reprimanding his mate when she went near the fence, snapping at her and driving her back.

In contrast, neither the NS nor the gonadorelin group engaged in any unseasonal behavior; the majority of their activities were independent from their mates. Routine activity usually consisted of patrol and investigation of their enclosures or surveillance of regular maintenance activities by humans. Occasionally, agonistic interactions over food or play objects were seen, but time spent in close proximity to a mate was short and inevitably focused on a task without specific affinitive or sexual intent.

The deslorelin cohort was most remarkable because of a brief period of out-of-season mounting and copulation, atypical among coyotes in the fall (Fig. 3). During Week 3 postimplant, four deslorelin pairs were observed in precoital mounts, and two pairs ultimately tied on Days 20 and 21 (one copulatory tie each pair); remarkably one of these latter pairs was previously inexperienced. In addition, one coyote pair engaged in several bouts of precoital mounting (most intensively on Days 21 and 22) including sustained pelvic thrusting and remounts. But in this case, the male became increasingly exhausted before ejaculation. A copulatory-lock was never confirmed for this pair, due to loss of visibility at nightfall, and the following day they did not engage in any further sexual activity.

Ironically, two cases within the deslorelin group failed to be stimulated; their lack of activity after treatment resembled NS and gonadorelin pairs more than those of their cohort. The two naïve 18-mo-old coyotes in this group experienced two- to threefold increases in progesterone concentrations similar to other females treated with deslorelin. However, compared with the increasingly intimate behavior demonstrated by the other females, the younger coyotes rarely engaged their mates. These females were observed in typical agonistic displays of passive and active submission (rolling-over or rapid chin-licking with head and tail held low below the top line), but we never witnessed elements of courtship such as allogrooming, non-agonistic body contact, or role reversal in play.

To determine if any observed (or unseen) copulations could have led to fertile matings, all females were tested and found negative for relaxin in December.

3.2. Winter

Deslorelin-induced behavioral and physiologic effects in the fall were transient and short-term, returning to normal (i.e., consistent with those of NS and gonadorelin pairs) before December. Yet as the coyotes entered their native breeding season (January to February), another affect of GnRH treatment was observed. Emergence of affinitive and appetitive behaviors within the deslorelin and gonadorelin groups appeared suppressed relative to the colony at large, particularly during the week prior to ovulation. Whereas activity within other colony pairs intensified, preovulatory courtship ($F_{19,17} = 5.56$, $P = 0.001$), olfactory sampling ($F_{20,11} = 41.48$, $P < 0.001$), mate-guarding ($F_{15,13} = 19.01$, $P < 0.001$), and mounting attempts ($F_{16,7} = 19.83$, $P = 0.001$) among GnRH pairs appeared relatively steady (Fig. 4).

In addition, the near absence of preovulatory copulations among GnRH-treated coyotes was unexpected and varied from the colony ($F_{20,10} = 3.49$, $P = 0.047$), resulting in an atypical pattern of sexual activity (Fig. 5). Among other colony pairs, 23.6% (43 of 182) of all observed copulatory ties occurred before ovulation; however within the GnRH groups, only 1 of 58 (1.7%) preovulatory tie was witnessed. Nonetheless, the length of behavioral estrus at the individual level was not statistically different ($P|t|_{0.05(2),23} \geq 1.34 = 0.194$, $F_{17,7} = 8.97$) between GnRH-treated coyotes (5.5 ± 0.7 d) and other colony females (7.6 ± 1.4 d), and ultimately fecundity was not impaired.

Among GnRH pairs observed in copulatory ties (8 of 12) in winter, all produced healthy full-term litters in spring; and litter size for GnRH females (mean ± SEM, 5.5 ± 0.7 pups) did not vary ($P|t|_{0.05(2),42} \geq 0.46 = 0.644$, $F_{7,35} = 2.14$) from the colony at large (5.4 ± 0.3 pups). There were, however, four notable exceptions, suggesting that GnRH may have had a more profound influence on sexually naïve females. Two deslorelin-treated and two gonadorelin-treated females were never observed in a copulatory tie, nor did they become pregnant. In addition to a lack of experience, these four females were coincidentally 22-mo-old litter mates.

The naïve coyotes were very interactive with their mates, but nonsexually. They engaged in physical non-agonistic contact such as playing and chasing, but the females rarely solicited their mates (with diverted tail), and male olfactory investigation (vulval sniff/lick) was also uncommon. Furthermore, when a male attempted a precoital mount, the females immediately employed a variety of evasive tactics, such as passively sitting, lying down, or running off; aggressively growling and snapping; or spinning and diverting his attention with play (play-bow, feigned charges, or mock wrestling).

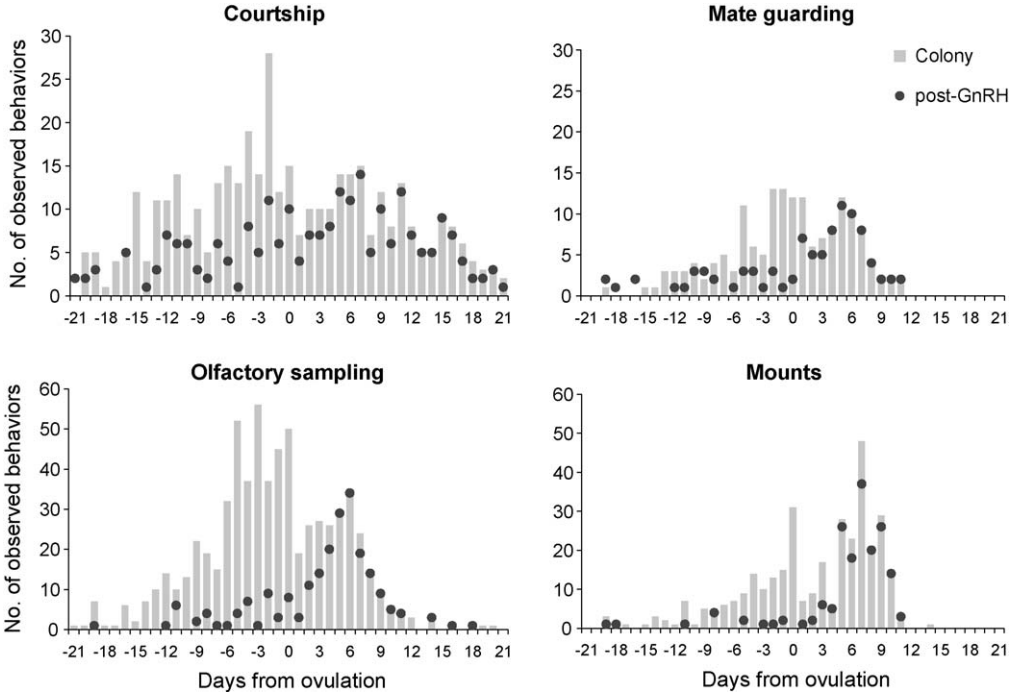

Fig. 4. Mating behaviors observed during the coyotes' physiologic breeding season (January to March), aligned to the estimated day of ovulation. Colony data represents 32 (untreated) coyote pairs observed during four seasons (2000–2003). Post-GnRH treatment data combines observations of four deslorelin postimplant pairs and four gonadorelin posttreatment pairs (January to February 2003). All colony and posttreatment females represented herein became pregnant.

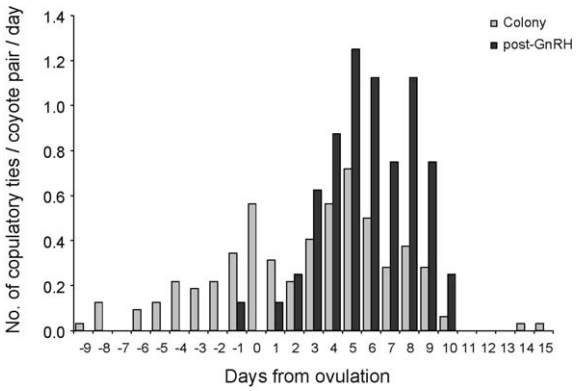

Fig. 5. Frequency of copulatory ties observed during the coyotes' native winter estrus, aligned to the estimated day of ovulation. Colony data represents 32 (untreated) coyote pairs observed during four breeding seasons (2000–2003). Post-GnRH treatment data combines frequency of ties observed in four deslorelin postimplant pairs and four gonadorelin posttreatment pairs (two pairs in each cohort failed to tie; January to February 2003).

4. Discussion

Anestrus is a relatively quiescent phase in the canine ovarian cycle, physiologically and behaviorally. It is also the phase that determines the overall interestrus length for an individual [16], and thus the most likely period regulating the timing of reproduction in coyotes. Although the mechanism controlling reproductive recrudescence in wild canids is poorly understood, our data provided evidence that the female coyote is physiologically and behaviorally prepared for mating in the fall, 4 mo prior to the native breeding season, and that sexually experienced males can recognize these changes in their mates and react accordingly.

In this experiment, coyotes treated with a subcutaneous implant of deslorelin responded with increased secretion of ovarian steroid hormones. Elevated serum estradiol concentrations were detected approximately 9 to 12 d after treatment, followed by a rise in progesterone 18 to 26 d postimplant. This consecutive pattern of hormone synthesis (estradiol followed by progesterone) was consistent with follicular stimulation within the ovary and was reminiscent of the preovulation endocrine profile previously reported for the coyote [11]. During a normal ovarian cycle, however, progesterone remains elevated for approximately 9 wk; rising before ovulation and reaching peak concentrations 3 to 4 wk postovulation [11]. By contrast, deslorelin-induced progesterone surges appeared transient and damped, presumably

because either ovulation did not occur or the corpora lutea could not be sustained.

Although the elevation of estradiol and progesterone was brief, the steroids nonetheless appeared to exert a positive affect on the coyotes' socio-sexual behaviors. The subsequent expression of mating behaviors was also interesting because of the males' involvement. Affinitive behaviors (similar to courtship activity seen in winter) were first to emerge; specifically body-bumps, hip-pushes, allo-grooming, and play-solicitation. Such physical contact appears as ritualized social interactions but without obvious agonistic intent (i.e., without aggressive or passive gestures, and interactions do not end with either coyote being obviously dominant or submissive). Instead, the coyotes made contact and then separated equitably; or sometimes roles were reversed in exaggerated nonaggressive play.

Next, the appearance of appetitive and overtly sexual behaviors (such as male sniff-lick investigation of a female's anogenital region, mounting attempts, and copulations) was important because (1) emergence of proceptive and receptive behaviors in the females implied that upregulation of estradiol and progesterone had sufficiently stimulated neural receptors in the female; (2) the neural receptors responsible for such behavior were sensitive and available to steroid influence in the fall; and (3) physiologic and/or behavioral changes in the female were detectable by the male and evoked appropriate responses from him. Sexual interactions require interest, participation, and cooperation of both partners; and the behaviors we witnessed were not solely female initiated. To the contrary, males appeared stimulated by and responsive to their mates, most likely abetted by incipient seasonal synthesis of testosterone.

Overt physiologic and behavioral responses were undetectable after treatment within the gonadorelin cohort and ephemeral in the deslorelin group. However, a long-term consequence was realized in the behavioral suppression observed at the beginning of the winter breeding season. All preovulatory affinitive, appetitive, and sexual behaviors were depressed in comparison with the pattern predicted by our previous observations of other colony pairs [11]. Females treated with GnRH in the fall rarely solicited their mates' attention and often rebuffed their mates' sexual overtures in the winter. Interestingly, male-initiated anogenital olfactory investigations, mate-shadowing, and precopulatory mounting attempts were also reserved.

Ovulation in winter occurred nonetheless; and copulations were well-timed because 8 of 12 GnRH-treated coyotes became pregnant and delivered healthy pups. A review of historical records revealed no obvious discrepancy between the estimated day of ovulation in 2003 and previous seasons. Thus, treatment in the fall with deslorelin or gonadorelin (at the described dosages) did not ultimately delay or suppress ovulation in the subsequent breeding season.

Four coyote pairs, however, were notable exceptions and appeared to be more severely affected. In these cases, the females were all naïve and 2 yr old (as were their mates), and we never saw them copulate nor did any of them become pregnant. Unfortunately, the reason for reproductive failure in the younger coyotes cannot be adequately explained within the context of this study. Inexperience is a likely cause. Yet within the deslorelin cohort, there was a 3 yr old naïve pair that successfully bred; and another inexperienced pair (4-yr-old female with a 2-yr-old male) in the gonadorelin group reproduced. Alternatively, it is possible that none of these females actually ovulated, although random serum samples (collected from February 11 to March 7, 2003) were within or exceeded the expected seasonal range for progesterone. Interestingly, the females were sisters, and at least two of them have successfully bred since this study (D.A. Carlson, unpublished data). We therefore speculate that decreased sensitivity to steroid hormones contributed to the inhibited sexual receptivity of the females; and without prior experience, the females may have been confused by their mates' attempts to copulate, misunderstanding them to be nonsexual agonistic gestures or play solicitations.

The response of coyotes treated with exogenous GnRH advances our understanding of reproductive behavior in this species and raises concerns for future consideration. If females are capable of estrus October through February and males are fertile December through May, then we speculate changes in habitat or resource availability or cross-breeding with domestic dogs could elicit a strategic shift in coyote reproduction. Monogamy and paternal care of young are important tactics in the reproductive strategy of free-roaming coyotes. Dominant adult (alpha) coyotes also are vigilant defenders of their territories, regulating access to food and potential mates. Yet the role socio-sexual behaviors play in the establishment and reinforcement of coyote pair-bonds is not fully understood. If the length of the breeding season expands from weeks into months, and the advantage of monogamy over polygamy is lost, consequent destabilization of a pack's social hierarchy could facilitate breeding among individuals previously prohibited from mating thereby affecting local population densities. Further study of coyote reproductive behavior will help biologists

understand these mechanisms before alternative strategies emerge.

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