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Effect of a GnRH antagonist on GnRH agonist–implanted anestrous bitches

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Abstract

Various combinations of gonadotropin-releasing hormone (GnRH) antagonists and long-acting GnRH agonists have been assessed in several species to prevent the ''flare-up'' effect that agonists cause on the pituitary-gonadal axis. To determine the effect of a single administration of the GnRH antagonist acyline in anestrous GnRH agonist–implanted domestic bitches, 19 dogs (canis familiaris) were randomly assigned to receive either 10 mg sc deslorelin acetate (DA; n = 6) or DA combined with 330 μg/kg sc acyline within the first 48 h (DA&ACY; n = 13). These bitches were examined daily for detection of posttreatment flare-up, manifested as an estrous response during the month after treatment. In the DA and DA&ACY groups, an estrous response was detected in 6 of 6 and 9 of 12 ($P < 0.5$) of the bitches, starting 5.3 ± 1.3 and 10.1 ± 1.8 d (mean ± SEM, P = 0.5), respectively, after treatment. Based on serum progesterone concentrations, ovulation occurred in 6 of 6 and 5 of 9 of these bitches (P = 0.1). None of the dogs had any local or systemic side effects related to the treatments. In five DA and six DA&ACY bitches that could be followed up after the trial, interestrus intervals were 385 ± 22.5 and 330 ± 69.1 d, respectively ($P > 0.1$). It was concluded that the current antagonist protocol prevented initial ovarian stimulation in one quarter of the treated dogs, whereas the stimulation period was postponed and ovulation was inhibited in approximately half of the remainder.

Keywords: Acyline; Anestrus; Canine; Estrus prevention; GnRH analogue

1. Introduction

An important aspect of canine reproduction control is prevention of estrous cycles. Temporal cyclic activity suppression has not only contraceptive indications but also medical and biotechnological applications. In that regard, management of hormone-dependent diseases and prevention of gonadal damage during cytotoxic therapies are examples of clinical indications. Furthermore, ovarian quiescence prior to stimulation of ovarian follicular development improved the success of assisted reproductive technologies in many species.

Unfortunately, canine estrous cycle prevention has not been safely and efficiently achieved. Traditional steroid contraception is accompanied by many side effects in this species [1]. Conversely, the slow-release gonadotropin-releasing hormone (GnRH) superagonists have been shown to safely and reversibly prevent estrous cycles for >1 yr by desensitization of pituitary receptors (downregulation) [2]. However, when these products are given to anestrous bitches, there is an initial stimulation of the pituitary-gonadal axis (so-called flare-up period), manifested as an estrous response before the axis is suppressed [3]. This immediate

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response is undesirable, and efforts have been made to prevent it by combining the agonists with serial progestin administration [3,4]. However, there is a need for safer, single-dose pharmacologic options.

Gonadotropin-releasing hormone antagonists competitively block GnRH receptor sites in the pituitary gland [5]; they have an immediate suppressive effect on the gonadal axis, which makes them particularly useful when rapid inhibition is required [6]. A single treatment (330 μg/kg) of the third-generation GnRH antagonist acyline [acetyl-D2Nal-D4ClPhe-D3Pal-Ser-Aph(ac)-DAph(Ac)-Leu-Lys(lpr)-Pro-D-Ala-Nh2] suppressed the progression of the estrous cycle to ovulation in proestrous bitches [7]. Combinations of GnRH antagonists and long-acting agonists to prevent the flare-up period have been assessed in other species with inconsistent results [8–11]. It was therefore of interest to determine the effect of a single administration of the GnRH antagonist acyline in anestrous bitches implanted with a GnRH agonist.

2. Materials and methods

2.1. Dogs

Nineteen 2 to 7 yr old, 18 to 31 kg, cross and purebred, client-owned, anestrous bitches were included in this study. Anestrus was defined as being >90 d after the last estrous cycle with typical anestrous vaginal cytology and vulvar findings [12]. Consent forms were signed by all owners.

2.2. Treatments

The bitches were randomly assigned to one of the following two treatment groups: deslorelin acetate (Suprelorin; Peptech Animal Health, Sydney, Australia) 10 mg sc (DA; n = 6), or the same dose and pharmaceutical form of deslorelin combined with acyline (Contraception & Reproductive Health Branch Center for Population Research, NIH, Bethesda, MD, USA) 330 μg/kg sc administered within the first 48 h (DA&ACY; n = 13). Deslorelin (6-D-tryptophan-9-[*N*-ethyl-L-prolinamide]-10-desglycinamide) acetate was supplied in the form of biocompatible implants (0.23 × 15.2 mm) in preloaded disposable syringes for sc administration. Acyline was provided in a lyophilized powder that was suspended in sterile distilled water (concentration, 2 mg/mL). The dosage used was chosen according to previous studies [7].

2.3. Follow-up

During the month after treatment, all bitches were examined daily for detection of a posttreatment estrous response, as well as any systemic or local side effects. Post–GnRH agonist estrous response related to the treatment was defined as vulvar swelling and bloody vulvar and typical vaginal cytology [12]. Additionally, the appearance of the first spontaneous estrous cycle after treatment was recorded in 11 dogs that could be monitored after the trial, and interestrus intervals were calculated.

2.4. Serum progesterone concentrations

In bitches with an estrous response after treatment, blood samples for determination of serum progesterone (P_4) were collected by peripheral venipuncture 3 wk after the onset of an estrous response to determine if ovulation had occurred ($P_4 > 5$ ng/mL [13]). The samples were centrifuged at 4000 × *g* for 15 min, and serum obtained and stored at –20 °C until assayed. Serum P_4 was measured by a solid-phase radioimmunoassay (Coat-A-Count, DPC; Los Angeles, CA, USA). Sensitivity at 95% binding was 0.1 ng/dL and the intra-assay and interassay CVs were 8.8% and 9.7%, respectively.

2.5. Statistical analyses

The proportion of bitches with an estrous response, ovulation, or side effects after treatment was compared between groups (DA vs. DA&ACY) by Fisher's exact test. For the interval from treatment to an estrous response and the duration of posttreatment interestrus intervals, a nonpaired Student's *t*-test was used to detect differences between the two treatment groups. Data were expressed as mean ± SEM. All statistical analyses were conducted with Sigma Stat (SPSS, Inc., Chicago, IL, USA) and the level of significance was set at $P < 0.05$.

3. Results

An estrous response appeared in 6 of 6 and 9 of 12 (P = 0.5) of the animals of the DA and DA&ACY groups 5.3 ± 1.3 and 10.1 ± 1.8 d ($P < 0.08$) after treatment, respectively. Ovulation occurred in 6 of 6 and 5 of 9 (overall, P_4 25 ± 7.1 ng/mL) of the bitches of the same groups (P = 0.1). None of the dogs had local or systemic side effects related to the treatments. Additionally, in the five DA and six DA&ACY animals