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The GnRH antagonist acyline prevented ovulation, but did not affect ovarian follicular development or gestational corpora lutea in the domestic cat

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Abstract

Two experiments were conducted to investigate the effects of the GnRH antagonist acyline (330 μg/kg, given sc) on ovarian follicular development and ovulation, as well as on pregnancy maintenance in domestic cats. In the first experiment, seven queens in proestrus (total of 24 proestrus periods), were randomly assigned to treatment with either acyline (ACY; $n = 17$) or a placebo (PLC; $n = 7$). All queens were mated with a fertile tomcat. In the ACY and PLC groups, cessation of estrus occurred (mean ± SEM) 7.0 ± 1.3 and 7.0 ± 1.7 d after treatment ($P > 0.1$), ovulation occurred in 2 of 17 and all seven estrus periods ($P < 0.05$), and pregnancy rates were 1 of 16 and 7 of 7 ($P < 0.05$), respectively. In the ACY and PLC groups, intervals from treatment to the onset of the ensuing proestrus were 18.4 ± 1.7 and 120 ± 17.2 d. In the second experiment, 14 pregnant queens were randomly allocated, according to their mating date, to treatment with acyline in early pregnancy (from 20 to 25 d, $n = 3$), mid pregnancy (from 26 to 45 d; $n = 4$), late pregnancy (> 45 d; $n = 3$), or injection of a placebo in early ($n = 1$), mid ($n = 2$), or late pregnancy ($n = 1$). Ultrasonographic assessments of the uterus were done every second day for 2 wk post treatment, and serum progesterone (P_4) concentrations were determined before treatment, and at 7 and 14 d after treatment. No pregnancies were prematurely terminated and post-treatment P_4 concentrations did not differ among treatment groups ($P > 0.1$). In conclusion, in the domestic cat, GnRH withdrawal by acyline prevented ovulation when given in early follicular phase (proestrus), but did not significantly affect luteal function during pregnancy.

Keywords: Acyline; GnRH analogue; Pregnancy; Estrus Cycle; Feline

1. Introduction

Controlling domestic cat populations is a complex problem, with feral and abandoned cats present in most cities and towns. Veterinarians are often consulted regarding suppression of estrus or termination of pregnancy in cats. Furthermore, pharmacological control of the feline estrus cycle may be needed for assisted reproductive technologies. In that regard, suppression of cyclic activity prior to ovarian stimulation by exogenous hormones may improve the stimulatory response [1].

Most pharmacological options for estrus cycle interruption and pregnancy termination in the queen include protocols that require numerous parenteral

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treatments or compounds with substantial side effects [2–4]. Therefore, there is a need to develop a safe, single-dose pharmacological compound, highly efficacious for suppressing ovarian function, which would be useful for contraception and pregnancy termination.

Gonadotrophin releasing hormone (GnRH) antagonists competitively block GnRH receptor sites in the adenohypophysis, exerting an immediate inhibitory action on the gonadal axis, making them particularly useful when a rapid effect is required (e.g., estrus interruption or pregnancy termination [5,6]). Furthermore, GnRH antagonists lack the undesirable initial stimulation (flare effect) of the gonadal axis, inherent in the use of GnRH agonists [7].

The effects of the third-generation GnRH antagonist, acyline [acetyl-D2Nal-D4ClPhe-D3Pal-Ser-Aph(ac)-DAph(Ac)-Leu-Lys(lpr)-Pro-D-Ala-Nh2] have been recently described in female dogs; a single high dose effectively terminated estrus and pregnancy (in the absence of side effects) [8,9]. However, data regarding the use of GnRH antagonists in cats are scarce and controversial. In a first study, the GnRH antagonist antide (two injections of 6 mg/kg 15 d apart) induced short-term suppression of ovarian follicular recruitment and ovulation, thus inhibiting estrus cyclicity [1]. More recently, prevention of ovulation and synchronization of the follicular phase, but only incomplete down-regulation of ovarian activity, was achieved with the same drug and dose [10]. To our knowledge, there are no reports regarding the effects of GnRH antagonists on feline pregnancy. The objective of the present study was to investigate the effects and clinical safety of the GnRH antagonist acyline in cats, when administered during the early follicular phase (proestrus) and at various stages of pregnancy.

2. Materials and methods

2.1. Effects of acyline on ovarian follicular development and ovulation

2.1.1. Animals and treatment groups

Seven postpubertal queens were housed in individual cages in the faculty cattery, exposed to a 10 h dark, 14 h light photoperiod, fed commercial cat food, and given water *ad libitum* for at least 3 mo before the beginning of the experiment. Estrus cycles and ovarian follicular development were monitored daily, based on behavior and vaginal cytology [11]. In a total of 24 proestrus (< 3 d) periods, seven queens were randomly assigned to receive either acyline (330 μg/kg, given sc; Contraception & Reproductive Health Branch Center for Population Research, NIH, Bethesda, MD, USA; ACY; $n = 17$), or given a placebo (corresponding volume of diluent sc; PLC; $n = 7$).

Acyline was provided in a lyophilized powder, which was suspended in sterile distilled water (concentration, 2 mg/mL). The dose was based on clinical studies in dogs [8]. All seven cats were used in both treatment groups. At least two estrus cycles were allowed to occur between treatments, to minimize confounding due to previous treatments. This study was approved by the Faculty Institutional Care and Animal Use Committee (IACUC).

2.1.2. Follow up

One day after treatment, female cats were housed with a fertile tom cat for 2 d, and assessed daily (observation of behavioral patterns for >1 h twice daily and once daily examination of vaginal cytology [11]) until cessation of estrus. The latter was defined as absence of behavioral and cytological signs of follicular activity. The occurrence of local or systemic side effects, matings, and the onset of the ensuing proestrus were also documented.

2.1.3. Blood samples and hormone determinations

In each cat, a blood sample was collected by peripheral venipuncture 14 d after estrus cessation, for determination of serum progesterone (P_4) concentrations to verify whether ovulation had occurred ($P_4 > 2$ ng/mL [11]). Serum P_4 was determined in duplicates by a solid-phase radioimmunoassay (Coat-A-Count, DPC®, Los Angeles, CA, USA). For this kit, the sensitivity at 95% binding was 0.1 ng/mL, and the intra-assay CV was 5.6%.

2.1.4. Ultrasonography

Twenty days after removal of the tomcat, females were examined by real-time ultrasonograhy, using an 8-MHz linear-array transducer (Toshiba Core Vision Pro, Tokyo, Japan) for pregnancy diagnosis [12].

2.2. Effects of acyline on luteal function during pregnancy

2.2.1. Animals and treatment groups

Fourteen pregnant queens were housed under the previously described conditions. Pregnancy was confirmed by ultrasonography [12]. According to the date of mating, females were randomly assigned to the following groups: early pregnancy group—acyline from pregnancy diagnosis to 25 d post mating (ACY-E; $n = 3$), mid pregnancy group—acyline 26–45 d post

mating (ACY-M; $n = 4$), late pregnancy group—acyline >45 d post mating (ACY-L; $n = 3$); and PLC (placebo, $n = 4$). Placebo was given (as described above) in early ($n = 1$), mid ($n = 2$), or late pregnancy ($n = 1$). The acyline dose (330 μg/kg sc) was based on clinical studies in dogs [9].

2.2.2. Ultrasonography, clinical, and endocrine monitoring

After treatment, ultrasonography was done every 2 d for 14 d, to detect signs of embryonic resorption or abortion [12]. Side effects were also recorded. To assess luteal function, blood samples for serum P_4 concentrations were collected by peripheral venipuncture on Days -1, 7, and 14 (Day 0 = treatment).

2.2.3. Statistical analysis

In the first experiment, a Student's t-test was used to compare, between the two groups, the interval from treatment to pro/estrus termination, and the interval from treatment to the onset of the next proestrus. Chi square analyses were used to compare, between groups, ovulation and pregnancy rates. In Experiment 2, effects of treatments on pregnancies were compared between groups (ACY vs. PLC) by Chi square, and serum P_4 concentrations were compared by ANOVA for repeated measurements. All statistical analyses were done with Sigma Stat (SPSS, Inc., Chicago, IL, USA) and the level of significance was set at 0.05.

3. Results

3.1. Effects of acyline on ovarian follicular development and ovulation

Matings were confirmed in 23 of 24 estrus periods by either direct observation or detection of sperm in vaginal smears. One ACY-treated queen was not mated, because she could not be housed together with the tomcat. The mean (±SEM) interval from treatment to cessation of estrus was 7.0 ± 1.3 and 7.0 ± 1.7 d ($P > 0.1$) in the ACY ($n = 16$) and PLC ($n = 7$) groups, respectively. In one cycle, estrus was not terminated until 4 mo after treatment with ACY. This cycle was considered an outlier and excluded from the previous mean.

Ovulation occurred in 2/17 (11.8%) of the ACY-treated queens and in all seven queens (100%) of the PLC-group ($P < 0.05$). In the ACY and PLC groups, 1 of 16 that were mated (6.3%) and all seven cats, respectively, became pregnant ($P < 0.05$). Pregnancy, parturition, and neonates were normal in all cases. The interval between treatment and onset of next proestrus was 18.4 ± 1.7 d for the 14 nonovulating queens treated with ACY, and combined for all pregnant ACY- and PLC-treated cats was 120.0 ± 17.2 d.

The duration of interestrus intervals in non-pregnant ACY treated queens corresponded to historical data of controls. The treatment to next proestrus interval of the ovulating, nonpregnant queen from the ACY-group was 65 d. All five queens that could be monitored after the end of the study had normal estrus cycles.

3.2. Effects of acyline on luteal function during pregnancy

All 14 pregnant queens maintained pregnancy. Six of them that were monitored after the study gave birth to healthy kittens without complication, whereas the other eight were ovariohysterectomized after the trial. Uterine inspection revealed normal fetuses and placentas. Progesterone serum concentrations did not differ among treatment subgroups (early, mid, and late) throughout the trial ($P > 0.1$). In all cases, individual P_4 values were within the range for normal feline pregnancy [11].

4. Discussion

In the first experiment, all queens in the ACY group continued to have estrus behavior and cytology and accepted matings. There were no differences between the two treatment groups in duration of estrus, which was within the physiological range for cats [11]. Similarly, it was previously reported that antide, another GnRH antagonist, failed to inhibit estrogen surges in cats [1]. Responses to acyline were variable; ovulation and pregnancy were prevented in approximately 88% (15/17) and 94% (15/16) of mated cats, respectively. Prevention of ovulation by administration of GnRH antagonistics, as described in domestic dogs and cats [1,8,10], may be due to blockade of the preovulatory luteinizing hormone (LH) peak. Perhaps in the two estrus periods in which ovulation occurred, LH may already have reached peak concentrations before acyline treatment. Furthermore, it was noteworthy that administration of a GnRH antagonist did not cause luteal regression in a previous study [10].

Interestrus intervals after treatment with this short-term release formulation of a GnRH antagonist did not differ from those described for nonovulatory cycles in this species [11], confirming that GnRH antagonists do not affect follicular phase. It is also noteworthy that after GnRH antagonistic treatment, returns to proestrus were highly synchronous, as reported in both domestic cats and dogs [1,8,10]. Consequently, treatment with a

GnRH antagonist may be useful for synchronizing estrus in cats.

It is widely accepted that ovarian P_4 is the primary progestin required for maintenance of feline pregnancy, whereas placental P_4 may have a minor role during the last third of gestation [13–16]. It is also assumed that luteal function is autonomous during the initial stage of pregnancy [17], but clearly depends on luteotrophic hormones during the second third of gestation.

Prolactin is luteotrophic in mid and late pregnancy in cats [18,19], whereas the specific role of LH on gestational corpora lutea has not been described in this species. In the second experiment, serum P_4 concentrations were uniform and consistent; therefore, we inferred that acyline treatment did not affect luteal function at any stage of pregnancy. This finding indirectly contributed to elucidating the role of LH in maintaining feline pregnancy. In contrast to the domestic dog [9], LH does not seem to be necessary for CL support at any stage of gestation in the cat. A similar situation was described in the luteal phase of the nonpregnant cat [10]. Therefore, we inferred that prolactin was the primary luteotrophic hormone in this species.

In the present trials, both the local and systemic safety of the acyline treatment seemed to be assured; furthermore, normal cyclicity was apparently regained in some queens after both experiments. Therefore, we concluded that, in the domestic cat, the GnRH antagonist acyline prevented ovulation, although ongoing ovarian follicular development and gestational corpora lutea were not affected by the gonadotrophin withdrawal induced by this treatment.

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