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Effect of GnRH analogs in postnatal domestic cats

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ABSTRACT

The aim of this study was to reproductively assess the clinical and hormonal effects of a GnRH agonist (AG) and an antagonist (AN) administered during the postnatal period in domestic cats. Forty-eight male and female postnatal kittens were randomly assigned to deslorelin acetate 1.6 mg subcutaneous (AG; $n = 16$), acyline 33 μg/100 g subcutaneous weekly for 3 months (AN; $n = 16$), or control (CO; $n = 16$) which remained untreated. The cats were followed up (behavioral observation, physical examination, fecal sexual steroid determinations, mating test, and pregnancy diagnosis) up to puberty. Puberty was delayed (weeks) in the AG animals (62.9 ± 3.5; $P < 0.01$) but not in the AN (15.5 ± 1.7; $P > 0.05$) when they were compared with CO kittens (13.4 ± 0.4). Fifteen (15/16) of the AN and CO animals, and only 11 of 16 cats of the AG group were fertile ($P > 0.1$). No differences were found in body weight ($P > 0.1$) and measurements ($P > 0.1$), libido ($P > 0.1$) and in the appearance of side effects ($P > 0.1$; except a pyometra in an AG female) among groups. In both AG- and AN-treated males (testosterone; $P < 0.01$) and females (estradiol-17β; $P < 0.01$) fecal hormone concentrations were lower than in CO group during the first five postnatal weeks but not later. It is concluded that the neonatal administration of these AG and AN decreased fecal sexual steroids during the first postnatal weeks causing, the agonists but not the antagonist, a significant, reversible delay in puberty appearance.
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1. Introduction

Although, several approaches (i.e., surgical, hormonal, immunologic, chemical, and so forth) have been tried to control the undesirable domestic cat *(Felis catus domesticus)* reproduction [1], the ideal contraceptive has not been found for this species yet.

GnRH analogs, which include agonists and antagonists, have been produced by substitutions of amino acids in the native GnRH molecule [2]. From the beginning of this century, prolonged reversible chemical sterilization in domestic carnivores has been achieved by desensitizing the pituitary by the administration of long-term release formulations of GnRH super agonists [2,3]. However, in mature animals, this procedure results in an initial increased release of LH and FSH (flare-up effect), which is generally regarded as an inherent disadvantage because it delays gonadal suppression by 7 to 14 days [2,3]. Deslorelin (6-D-tryptophan-9-[N-Ethyl-LProlinamide]-10-desglycinamide, (Suprelorin; Virbac, Carros, France)) acetate is a potent and safe, long-term release GnRH agonist that is commercially available in the veterinary market of Australia and many European countries.

Conversely, GnRH antagonists bind to gonadotrope GnRH receptors and compete successfully with endogenous GnRH molecules for specific membrane receptor occupancy. This leads to an immediate arrest of gonadotrophin secretion without the undesirable initial "flare effect" that agonists produce [4]. Acyline (Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(Ac)-D4Aph(Ac)-Leu-ILys-Pro-DAla-NH2) is a safe, potent and long acting, third generation GnRH antagonist [5]. Although, both GnRH agonists and antagonists have shown to efficiently and safely control estrous cycle, male

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fertility, and libido of mature and immature domestic carnivores [2,5], they have never been tested during feline postnatal period.

In mammals, neonate gonads are very active after delivery [6–8]. In this respect, domestic cats are not an exception as birth is followed by the presence of elevated fecal sexual steroid concentrations during the first five postnatal weeks [9].

In both altricial and precocial mammalian species, endogenous sexual steroids have organizational actions during early postnatal life as shown in mouse, rat [10], and sheep [11] and, therefore, the postnatal stage can be considered a critical window of reproductive vulnerability [12]. Interference with the normal pituitary–gonadal function during this period impacts adversely on genital tract development and subsequent adult reproductive function [13]. GnRH analogs have been used as endocrine disruptors in rodents and monkeys during the postnatal time window (TW) with sterilizing effects [13,14].

Thus, the aim of this study was to reproductively assess the clinical and hormonal effects of a GnRH agonist, deslorelin acetate and a GnRH antagonist, acyline, administered during the postnatal period in domestic cats.

2. Materials and methods

2.1. Animals

Forty-eight kittens (24 males and 24 females; 11 litters) which were born in our Institutional cat colony were included in this study. The animals were sexed at birth, identified, reared free in indoor catteries (three rooms 4×3 m, with 14 hours of light per day, and appropriate enrichment), weaned at the age of 30 days and fed with dry commercial premium kitten-food and water *ad libitum*. The kittens were socialized by a group of trained students. This study was reviewed and approved by the Animal Care and Use Committee of the Veterinary School of the National University of La Plata, Argentine and all experiments were conducted under the guidelines established in The Guide for The Care and Use of laboratory Animals, USA.

2.2. Pharmacologic protocols

The kittens of the same gender and litter were randomly assigned to one of the following treatment groups within the first 24 hours of birth: Deslorelin acetate 1.6 mg (Suprelorin; Virbac, Carros, France) subcutaneous (GnRH agonist [AG]; $n = 16$), acyline (Contraception & Reproductive Health Branch Center for Population Research, NIH, Bethesda, MD, USA) 33 μg/100 g subcutaneous which was repeated weekly until the 3 months of age (GnRH antagonist [AN]; $n = 16$), or control (CO; $n = 16$) which remained untreated. The agonist and antagonist pharmacologic protocols were selected according to previous studies in prepubertal [15] and adult [16] domestic carnivores.

2.3. Follow up

All the kittens were followed up until the first pubertal signs appeared. Follow up included behavioral observation, physical examination, clinical side effects, vaginal cytology, and fecal sample collection for sexual steroid determinations.

The felids were observed more than 1.5 hours twice a day looking for typical sexual behavior and libido, which was classified as absent, diminished, or normal. The cats were also physically examined (testicular volume [cc; [17]], balano–preputial separation [18], penile spines, body weight [kg], height at withers [cm], and crown-rump length [cm]) once a week. In females, vaginal cytology [19] was carried out three times per week from the third month of age.

In males, early puberty was defined as complete balano–preputial separation [18] and the appearance of penile spines, whereas in females, by the finding of more than 80% superficial keratinized cells and a clean background in vaginal smears [19].

2.4. Fecal collection, extraction, and hormone determinations

Fecal samples were weekly collected and frozen for testosterone (T) or estradiol-17β (E2) determinations in the males and females, respectively. For this purpose, each cat was confined in an individual cage with a clean sanitary litter one night per week. During the first 4 weeks of age, the neonates had to be rectally stimulated by a thin plastic suppository attached to a string to obtain the sample.

Fecal steroids were extracted as based on general methods described by Brown, et al. [20], and T (ng/mL) and E2 (pg/mL) were determined by electrochemiluminescence immunoassays (Elecsys Testo II and Estradiol II; Roche Diagnostics, Mannheim, Germany). All fecal data were expressed on a wet-weight basis [9].

2.5. In-vivo fertility tests

As the felids attained puberty, they were exposed to a fertile, nonrelated cat of the opposite gender during the whole female's estrus (approximately 1 week) up to three consecutive opportunities (i.e., heats). Libido (classified as mentioned previously) and matings were observed and/or diagnosed by the presence of spermatozoa in the vaginal smears. A total of 21 days after the end of estrus, gestation was diagnosed by ultrasound examination in all the cases [21]. At the end of the study, all the animals underwent gonadectomy and were placed for adoption.

2.6. Statistical analysis

Fertility after mating, libido, and safety (i.e., no clinical side effects) were compared among treatments groups by Fisher's exact test, whereas age at puberty was analyzed by one-way ANOVA followed by Tukey comparison test.

Fecal T and E2 concentrations, body weight, and measurements were analyzed by repeated measures ANOVA followed by Tukey comparison test. All the comparisons among the three groups were carried out until the first pubertal signs appeared in CO-treated animals, from that time point on, the analogs groups were statistically described. To further characterize fecal hormones and scrotal volume, two consecutive TWs were defined and

compared (postnatal weeks 1–5 [TW1, feline critical TW; nine] vs. week 6 to the end of the comparison period [TW2]). Data were expressed as mean ± standard error of the mean, and the level of significance was set at $P < 0.05$ (SPSS 17.0; SPSS, Chicago, IL, USA).

3. Results

The appearance of the first signs of puberty was delayed in the AG-treated animals (62.9 ± 3.5 weeks; range, 42–91 weeks; $P < 0.01$) but not in the AN (15.5 ± 1.7 weeks; range 16–20 weeks; $P > 0.05$) when they were compared with CO kittens (13.4 ± 0.4 weeks). Mean age at puberty of AG cats was 58.0 ± 4.7 and 67.8 ± 4.8 weeks for the male and female animals, respectively. The same values for AN animals were 16.8 ± 2.9 weeks for males and 14.2 ± 0.5 for females.

Scrotal volume differed throughout the TWs among treatments groups ($P < 0.01$), being CO values higher than AG ($P < 0.01$) and also AN ($P < 0.05$) during TW2 but not during TW1 ($P > 0.1$ for both analogs; Fig. 1). In both AG- and AN-treated animals, there was also an increment of this volume 2 or 3 weeks before puberty (data not shown).

Libido at puberty onset was slightly decreased to normal in all the cats without differences among groups ($P > 0.1$) and all the analogs and CO pubertal animals were mated. Fifteen (15/16) of the AN and CO animals and only 11 of 16 cats of the AG group demonstrated to be fertile after puberty achievement and mating ($P > 0.1$). The three AG females that were not fertile had short (<20 days) interestrous intervals after matings.

No differences were found in body weight ($P > 0.1$; Fig. 2A, B), withers height ($P > 0.1$), or body length ($P > 0.1$) among groups up to the first pubertal signs in male (14.3 ± 0.3 weeks) and female (13.3 ± 0.4 weeks) CO animals. From that time point on, nine of 16 AG-treated cats developed the typical gonadectomized phenotype including overweight (body score 7/9–8/9). Within 15 to 25 days before achieving the first pubertal signs, these animals gradually began to lose body weight to normality (body score 5/9–6/9).

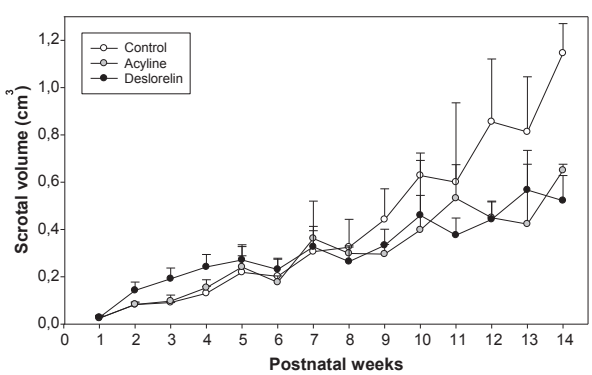

Fig. 1. Scrotal volume (mean ± SEM; $P < 0.01$) of 24 male kittens treated postnatally (week 0) with deslorelin acetate (AG), acyline (AN) or nontreated (CO) and followed up until the first pubertal signs appeared in CO-treated animals. There is an increase in CO values over AG ($P < 0.01$) and AN ($P < 0.05$) from week 6 to the end of the study. SEM, standard error of the mean.

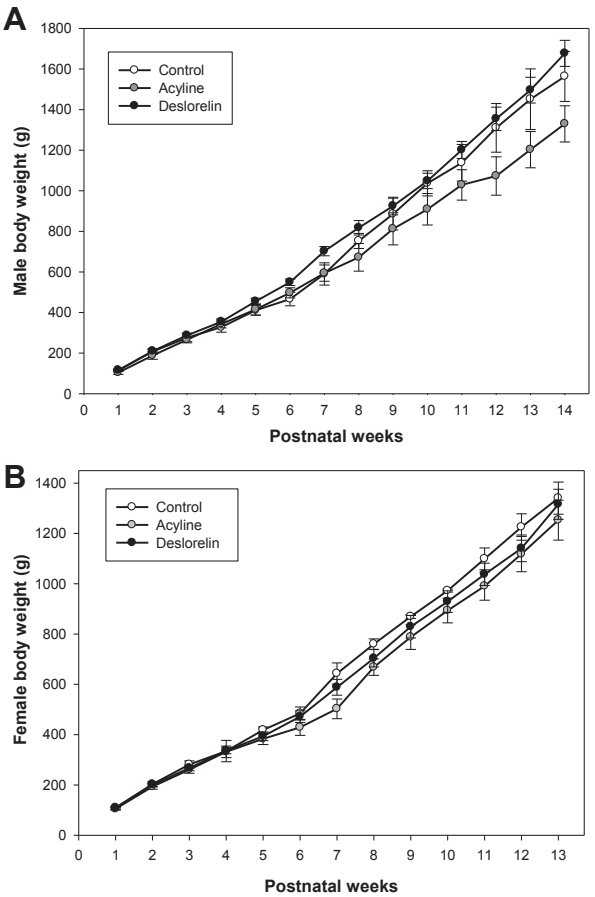

Fig. 2. Body weight (mean ± SEM) of 24 male (A; $P > 0.1$) and 24 female (B; $P > 0.1$) kittens treated postnatally (week 0) with deslorelin acetate, acyline, or nontreated (CO) and followed up until the first pubertal signs appeared in CO-treated animals. SEM, standard error of the mean.

No clinical (including post agonist "flare up") or behavioral adverse side effect was observed in any group during this trial ($P > 0.1$) except in one (1/8) AG-treated female. This latter cat achieved puberty at the age of 91 weeks, was mated, and 18 days later presented an open cervix pyometra. The gross internal evaluation of the excised uterus also showed four implantation sites.

Both fecal T ($P < 0.01$; Fig. 3) and E2 ($P < 0.01$; Fig. 4) concentrations differed among treatment groups throughout the follow-up period (until puberty of CO animals), being AG and AN different from CO ($P < 0.05$; for both hormones) but not between themselves ($P > 0.05$; both hormones). In AG- and AN-treated males ($P < 0.01$; Fig. 3, Inset) and females ($P < 0.01$; Fig. 4, Inset) fecal hormone concentrations were lower than in CO group during TW1 but not during TW2.

4. Discussion

In the present study, the clinical and hormonal reproductive effects of the postnatal administration of two GnRH

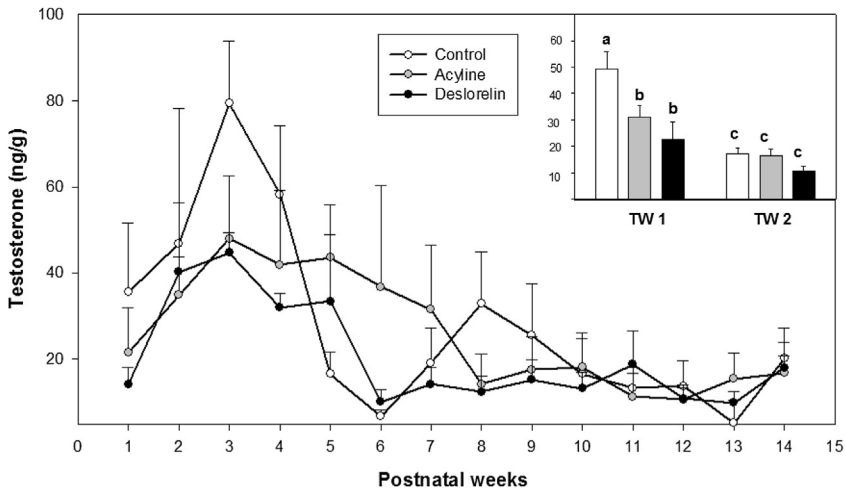

Fig. 3. Fecal testosterone (mean ± SEM; $P < 0.01$) concentrations of 24 male kittens treated postnatally with deslorelin acetate, acyline, or nontreated (CO) and followed up until the first pubertal signs appeared in CO-treated animals. Inset: Fecal testosterone concentrations (mean ± SEM) of postnatal weeks 1 to 5 (TW1) and 6 to 14 (TW2) of the same male kittens. Different letters over the columns represent differences $P < 0.01$. SEM, standard error of the mean; TW, time window.

analogs, an agonist and an antagonist, were assessed for the first time in newborn domestic cats.

Puberty was significantly delayed in all the AG but not in the AN-treated cats. It is difficult to explain why puberty was postponed after an analog administration and not the other. Considering that the period of administration (or drug release in the case of the agonist) was even longer for AN than that for AG i.e., 3 months vs. approximately 2 months, respectively—the differences could be attributed to an insufficient antagonist dose for this purpose. Furthermore, it is also known that the gonadal axis takes longer to recover after GnRH agonist than antagonist treatment [2].

In AG group, the puberty postponement obtained appears quite long (mean 16 months) considering the low deslorelin dose used i.e., one-third of the regular dose indicated for 6-month sterilization in mature animals. In another report, when 4-month-old prepubertal cats were administered the regular dose for 6-month effect, puberty was delayed to the mean age of 9 months (range, 281–428 days; [15]). These differences in the duration of the effect between the studies may reflect the developmental vulnerability of the TW here selected for the agonistic disruption. In this way, a potential new approach to feline contraception, i.e., the use of GnRH analogs as endocrine disruptors during the critical postnatal window of

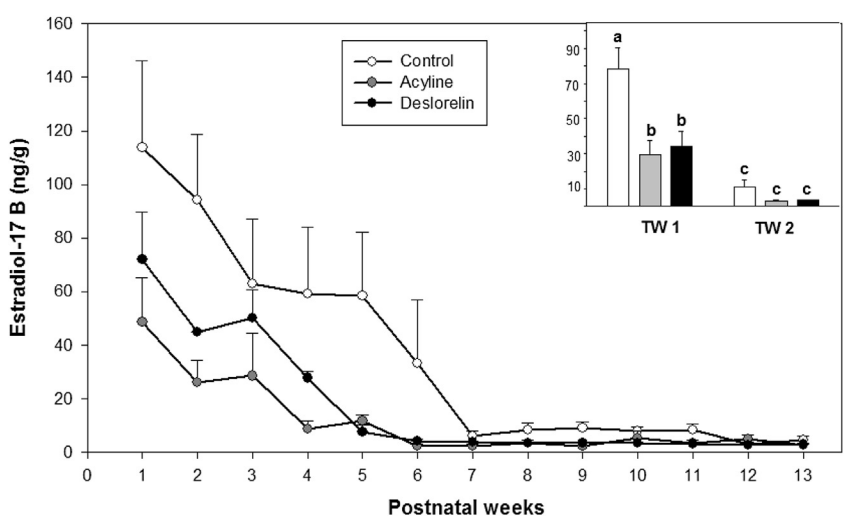

Fig. 4. Fecal estradiol-17 β (mean ± SEM; $P < 0.01$) concentrations of 24 female kittens treated postnatally with deslorelin acetate, acyline, or nontreated (CO) and followed up until the first pubertal signs appeared in CO-treated animals. Inset: Fecal estradiol-17 β concentrations (mean ± SEM) of postnatal weeks 1 to 5 (TW1) and 6 to 13 (TW2) of the same female kittens. Different letters over the columns represent differences $P < 0.01$. SEM, standard error of the mean; TW, time window.

reproductive development could be proposed. Similarly, neonatal immunization against GnRH provoked long-term impairment of gonadal function after antibody titers had fallen to undetectable levels in sheep and cows, further supporting the hypothesis that a critical developmental window also exists in precocious animals [22,23].

Additionally, bearing in mind the dose-related effect that both GnRH analogs have [5], it may be the case that higher neonatal doses could further postpone or even prevent feline puberty. Further trials are certainly guaranteed to test this hypothesis.

Anywise, in practice, the AG puberty delay obtained in this study may be useful for breeding cats, which could be phenotypically evaluated before their reproduction. Conversely, in crossbred cats, the administration of serial agonists' implantations e.g., every 9 to 10 months might offer a longer period of sexual immaturity.

Additionally, a wide range in puberty postponement was found in AG-treated (42–91 weeks) animals. This variability has been extensively reported for GnRH analogs treatments, final effects of which represent a complex interaction of several factors including their potency, dose and releasing rate, animal reproductive stage, and also idiosyncrasy [5].

Similar to what has been reported for sexual behavior in some postnatal GnRH analog–treated monkeys [24], in this trial, there were no differences in libido manifestations among the cats of the three groups. The slightly diminished sexual behavior found in some felids of the three groups is a usual finding in pubertal animals [25] and cannot be attributed to any specific treatment.

In line to what has been described for adult animals concerning the reversibility of analogs' effects [2], fertility seemed to be conserved after treatment wanes off in most of these pubertal AG- and AN-treated cats. Conversely, five AG-treated cats proved to be infertile after matings. Although, the three AG-treated infertile females apparently did not ovulate, the exact cause of infertility after mating in one, one, and five animals of the CO, AN, and AG groups, respectively, was not determined and cannot be directly ascribed to the pharmacologic treatments.

Acyline and, in general, deslorelin acetate appeared to be safe in feline neonates, which, in coincidence to what has been extensively reported for mature animals [2,3,5], they had no clinical side effects after their administration. Only one AG-treated female developed a pyometra after mating. We have previously reported a pyometra in a prepubertal deslorelin-treated female cat [15]. Although, highly suggestive, the direct relationship between the AG and the uterine disease was not investigated in this trial.

Importantly, no AG-treated animal presented any sign compatible with early stimulation (flare-up effect) of the gonadal axis evidencing the complete gonadal and/or the axis immaturity during the selected postnatal TW. Conversely, 7% of the prepubertal queens presented a post-AG estrous response when treated at the age of 4 months in a previous report [15].

Although, growth rate was not affected by the analogs up to the age of 3 to 3.5 months, more than half of the adult nonpubertal AG-treated cats presented typical external genitalia and body weight (including fat distribution) of neutered animals exteriorizing their hypogonadic state before puberty attainment. As expected, increasing scrotal volume was one of the first signs of puberty attainment in the three groups of animals.

Fecal steroid measurement has been widely employed in endocrinology of wild and domestic felids, being well suited for use in longitudinal protocols as they provide a noninvasive, time-integrated measure of blood hormones over several days [26]. In concordance with other mammals, elevated (i.e., adult concentrations) fecal sexual steroid concentrations have been described in domestic cats during the first five postnatal weeks [9].

Although statistically different from control animals, neither AG nor AN treatments caused basal (i.e., as in the second TW) fecal steroid values during the first postnatal weeks indicating the administration of insufficient doses for strong contraceptive purposes. A deeper suppression of the gonadal axis would be necessary during this TW to definitively unveil the exact organizational and/or activational roles of the postnatal sexual steroids in domestic felids.

4.1. Conclusions

It is concluded that the neonatal administration of the long-term release AG, deslorelin acetate, or the AN, acyline, partially decreased fecal sexual steroids during the first postnatal weeks safely causing, the agonists but not the antagonist, a significant, reversible delay in puberty achievement.

Acknowledgments

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Both A Carranza and M Faya worked equally in this study.

Competing interests

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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