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Histologic effect of a postnatal slow-release GnRH agonist on feline gonads

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ABSTRACT

In postnatal domestic cats, GnRH agonists suppressed fecal concentrations of sexual steroids and delayed puberty. The aim of this study was to describe the gross and microscopic morphometric effects of a single administration of the GnRH agonist, deslorelin acetate, on the gonads of postnatally treated cats. Twenty-seven postnatal male (n = 14) and female (n = 13) kittens were randomly assigned to one of the following treatment groups within the first 24 hours of birth: deslorelin acetate (1.6 mg, subcutaneous; DA, n = 16) or control that remained untreated (CO, n = 11) and spayed or castrated immediately after the onset of puberty. After surgical removal, the gonads were gross and histologically assessed. Sertoli cells also were examined immunohistochemically. Comparisons between the treatments were carried out by the Student *t* test. Gross gonadal wet weight and volume as well as gonadosomatic index were significantly lower in the DA than those in the CO males; these same parameters were not different in females. Primordial (461.4 + 3.0 vs. 1074.3 + 117.5; $P < 0.01$), primary (59.1 + 13.5 vs. 165.4 + 24.6; $P < 0.01$), and secondary (17.5 + 2.6 vs. 31.17 + 8.1; $P < 0.05$) follicles per mm^2 were decreased in DA than in CO gonads. Epididymal sperm motility and morphology were normal in all but two DA cats that had too few sperm to be evaluated. Germinal epithelial height (μm; 39.68 + 0.92 vs. 72.7 + 1.2; $P < 0.01$) and most of their cellular components as well as the Sertoli (cm^3; 0.1 + 0.02 vs. 0.24 + 0.05; $P < 0.01$) cells were diminished in the DA cats. Gonadotropin-releasing hormone agonist endocrine disruption during the neonatal critical reproductive time window may have a potential as a contraceptive agent in domestic felids.

1. Introduction

Domestic cat (*Felis catus domesticus*) overpopulation is a relevant social and sanitary global problem. Although several approaches (i.e., surgical, hormonal, immunologic, chemical, and soforth) to this problem have been tried [1], the optimal option for the control of the undesired reproduction has not yet been found for this species.

Among the nonsteroid hormonal possibilities, long-term-release GnRH agonists are the most widely used in practice. Depot or prolonged administration of GnRH agonists acts through desensitization of the GnRH receptors, which eventually results in downregulation and a decrease of receptors after an initial short stimulation period [2].

In both altricial and precocial mammalian species, endogenous sexual hormones have organizational actions during early postnatal life as shown in mice, rats [3], and sheep [4] and, therefore, the postnatal stage can be considered a critical window of reproductive vulnerability [5]. Interference with the normal pituitary-gonadal function during this period impacts adversely on genital tract development and subsequent adult reproductive function [6].

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Gonadotropin-releasing hormone analogs have been used as endocrine disruptors in rodents and monkeys during the critical postnatal time window causing sterilizing effects along with gonadal alterations [6,7]. Diminished gonadal weight [8], reduction in seminiferous tubule diameter [9], epithelium [10], and number of Sertoli cells [11], decreased number of growing and large antral follicles [12,13] have been reported after the neonatal administration of GnRH analogs.

Deslorelin, 6-D-tryptophan-9-(N-ethyl-L-prolinamide)-10-desglycinamide acetate, a potent GnRH agonist that shown to be effective and safe for feline reproduction control [2], has recently been tested in postnatal cats at a single dose of 1.6 mg [14]. Deslorelin acetate suppressed the high concentrations of fecal estradiol-17β and testosterone present in immature cats during the first five postnatal weeks [15]. Furthermore, in that study, puberty was delayed for more than 1 year and infertility occurred in 30% of the treated felids [14]. Although further knowledge is still necessary, the early administration of GnRH agonists may have a potential as a contraceptive or a sterilizing agent in this species. To the authors' knowledge, there are no studies assessing the gonadal consequences of a GnRH agonist administered during the critical neonatal time window in felids. Thus, the aim of this article was to describe the gross and microscopic morphomertrical effects of a single administration of deslorelin acetate on the ovaries and testes of postnatally treated domestic cats.

2. Materials and methods

2.1. Animals, pharmacologic protocols, and procedures

Twenty-seven, male (n = 14) and female (n = 13) kittens, which were born in our institutional cat colony and used for a previous clinical study [14], which included a larger number of 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 35 days, and fed with dry commercial premium kitten food and water *ad libitum*. The kittens were socialized by a group of trained students. The animals were randomly assigned to one of the following treatment groups within the first 24 hours of birth: deslorelin acetate 1.6 mg subcutaneous (Suprelorin; Virbac, France; DA, n = 16) or control that remained untreated (CO, n = 11) and spayed or castrated immediately after the onset of puberty. In males, early puberty was defined as complete balanopreputial separation 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 [14]. 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 of the experiments were conducted under the guidelines established in the Guide for the Care and Use of Laboratory Animals, USA.

2.2. Gross and microscopic morphometrical examination

Immediately after surgical removal, the gonads were measured (length, width, and depth; cm) and weighed (g). Gonadal volume (cm^3; [16,17]) and gonadosomatic index (%; [18]) were calculated.

Sperm were recovered from the epididymal tail sectioning using a scalpel blade. Forward progressive motility was subjectively assessed at × 400 magnification on a warm plate. Sperm morphology was evaluated with Giemsa staining, under × 1000 magnification using bright-field microscopy.

The ovaries and testes were sectioned longitudinally, placed in Bouin's fixative for 12 hours, then transferred to 70% alcohol, and processed routinely with paraffin embedding. After processing, 5-μm serial sections were cut, mounted on slides, dyed, deparaffinized in xylene, rehydrated in graded ethanol solutions, and stained with hematoxylin and eosin. Histologic images were obtained from a microscope (Olympus BX50; magnification: × 10 and × 40) through an attached video camera (Sony DXC 151A) and digitalized in a 24-bit true-color TIFF format. The images were analyzed using the Image-Pro Plus software.

Follicles were classified as primordial (small ovocyte surrounded by a single layer of squamous or cubic epithelial cells), primary (bigger ovocyte surrounded by a single layer of higher epithelial cells), secondary (two or more layers of granulosa cells and a theca cell layer), antral (fluid-filled antrum, mural and cumulus granulosa cells, and two or more layers of thecal cells), or atretic (degenerated granulosa cells and follicular fluid containing cellular debris [19]). The number of primordial, primary, secondary and antral follicles, CL, and atretic follicles per square millimeter was determined on a computer screen using 20 captured images (× 10) per animal.

Fifteen to 20 tubular round profiles were measured for each testis. The maximum, minimum, and medium tubular diameters (μm), major and minor axes (μm), area (μm^2), and perimeter (μm) of seminiferous tubules were measured at × 10 by planimetry (Image-Pro Plus). The germinal epithelium height (μm) also was recorded.

The volume (cm^3) of the different testicular tissue component was determined by stereology using a 441-intersection grid placed on × 40 magnification photographs. For this procedure, 15 fields were chosen randomly (6615 points) for each testis, and the proportion of each structure was calculated on the total testicular volume. Points were classified as one of the following: spermatogonia, primary and secondary spermatocytes, round spermatids, elongated spermatids, spermatozoa, Sertoli and Leydig cells, intertubular compartment, basement membrane, lumen, and cellular debris. The tubular–intertubular compartment proportion and the total length of seminiferous tubules (m) also were calculated [18]. Sertoli cells were stained immunohistochemically using an anti-vimentin antibody (monoclonal mouse antivimentin clone 9; Dako). The EnVision System Kit (Dako) was used as an immunohistochemical detection system. Positively stained cells were a golden dark brown 3,3-diaminobenzidine tetrahydrochloride–H_2O_2 reaction product. After counterstaining with alcoholic hematoxylin, the slides were dehydrated and mounted for examination. The cells of the tubules were counted in 20 microscopic fields for each testis.

Table 1
Testicular gross morphologic parameters (mean ± standard error of the mean) of male cats treated postnatally with 1.6-mg deslorelin acetate (n = 8) and nontreated controls (n = 6).

Parameter	Deslorelin	Control
Testis length (cm)	1.59 ± 0.05**	1.85 ± 0.05**
Testis width (cm)	1.31 ± 0.05*	1.47 ± 0.05*
Testis weight (g)	1.55 ± 0.07**	1.95 ± 0.05**
Testicular volume (cm^3)	1.29 ± 0.13**	1.85 ± 0.17**
Gonadosomatic index (%)	0.04 ± 0.00**	0.05 ± 0.00**

*P < 0.05 or **P < 0.01 in the same line indicates differences between the groups.

2.3. Statistical analysis

Descriptive data were expressed as mean ± standard error of the mean, and comparisons between the treatments (DA vs. CO) were made using the Student *t* test. P values less than 0.05 were considered significant (SPSS Inc.).

3. Results

The appearance of the first signs of puberty was delayed in the DA-treated animals when they were compared to CO kittens (54.4 ± 4.6 vs. 16.6 ± 0.9 weeks; P < 0.01). Gross testicular wet weight, measures, volume, and gonadosomatic index were significantly lower in the DA than those in the CO males (Table 1). These parameters were not significantly different in gonads from the DA and CO females (Table 2).

Primordial, primary, and secondary follicles per cm^2 were significantly decreased in DA compared to CO gonads. No significant differences were found for neither the number of CL nor atretic follicles between the treatments (Table 3; Fig. 1).

Epididymal sperm motility and morphology were normal in all but two DA cats that had too few sperm to be evaluated. Although microscopic tubular measures did not differ between the treatments, germinal epithelial height and most of their cellular components as well as the Sertoli cells per cm^3 and per seminiferous tubule were significantly diminished in the DA cats. These differences in the germinal epithelium were due to abundant cellular degeneration which was accompanied by mild luminal cellular detritus in the DA but not in the CO animals. Conversely, Leydig cells did not vary between the groups (Table 4; Fig. 2).

Table 2
Ovarian gross morphologic parameters (mean ± standard error of the mean) of female cats treated postnatally with 1.6-mg deslorelin acetate (n = 8) and nontreated controls (n = 5).

Parameter	Deslorelin		Control	
	Right	Left	Right	Left
Length (cm)	0.95 ± 0.05	0.9 ± 0.05	1.0 ± 0.03	0.9 ± 0.02
Height (cm)	0.58 ± 0.06	0.63 ± 0.05	0.72 ± 0.03	0.7 ± 0.03
Weight (g)	0.18 ± 0.01	0.18 ± 0.02	0.21 ± 0.02	0.2 ± 0.01
Volume (cm^3)	0.19 ± 0.03	0.21 ± 0.02	0.25 ± 0.02	0.23 ± 0.03
Gonadosomatic index (%)	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01

Table 3
Ovarian histologic structures (mean ± standard error of the mean) of the female cats of Table 2.

Structure (cm^2)	Deslorelin	Control
Primordial follicles	461.40 ± 34.01**	1074.26 ± 117.46**
Primary follicles	59.08 ± 13.48**	165.38 ± 24.60**
Secondary follicles	17.50 ± 2.58*	31.17 ± 8.10*
Antral follicles	9.64 ± 3.48	14.43 ± 5.31
CL	4.77 ± 1.41	4.0 ± 3.0
Atretic follicle	59.01 ± 13.94	51.48 ± 12.03

*P < 0.05 or **P < 0.01 in the same line indicates differences between the groups.

4. Discussion

This study reports the gonadal effects of a single dose of the long-term-release of the GnRH agonist, deslorelin acetate, administered in the critical postnatal time window in domestic cats. In agreement with the larger clinical study [14], in these animals, the prolongation of puberty attainment in the DA group was conserved.

In the male but not in the female cats, gross gonadal wet weight, volume, and gonadosomatic index were lower in

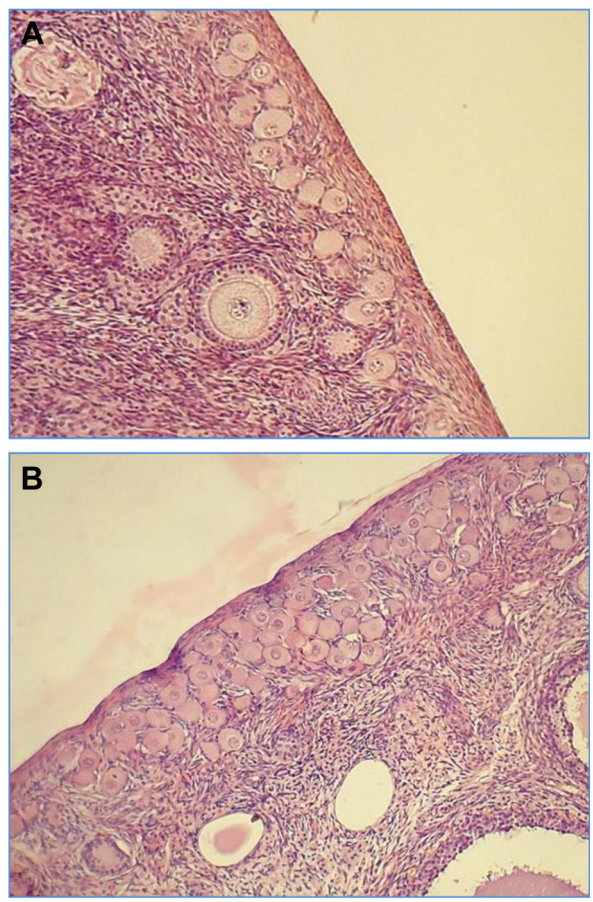

Fig. 1. Ovarian cortex for the queens and experiment described in Table 2. Notice the fewer number of primordial follicles is present in the deslorelin-treated (A) versus the control (B) cats. All sections were stained with hematoxylin and eosin (magnification: × 20).

Table 4
Testicular histologic structures (mean ± standard error of the mean) of male cats of Table 1.

Parameter	Deslorelin	Control
Major tubular axis (μm)	220.08 ± 6.42	216.72 ± 3.16
Minor tubular axis (μm)	176.87 ± 10.62	176.45 ± 2.65
Medium tubular diameter (μm)	189.28 ± 3.14	193.25 ± 2.46
Tubular area (μm^2)	39,666.55 ± 2480.61	44,394.93 ± 6297.69
Tubular perimeter (μm)	714.36 ± 70.82	627.09 ± 16.97
Germinal epithelium height (μm)	39.68 ± 0.92**	72.7 ± 1.2**
Spermatogonia (cm^3)	0.08 ± 0.02	0.15 ± 0.04
Primary spermatocytes (cm^3)	0.19 ± 0.042*	0.38 ± 0.06*
Secondary spermatocytes (cm^3)	0.01 ± 0.00**	0.01 ± 0.00**
Round spermatids (cm^3)	0.31 ± 0.08**	0.69 ± 0.09**
Elongated spermatids (cm^3)	0.06 ± 0.01**	0.11 ± 0.02**
Spermatozoa (cm^3)	0.01 ± 0.00**	0.04 ± 0.01**
Sertoli cells (cm^3)	0.1 ± 0.02**	0.24 ± 0.05**
Leydig cells (cm^3)	0.04 ± 0.01	0.10 ± 0.04
Intertubular compartment (cm^3)	0.06 ± 0.01	0.04 ± 0.01
Basement membrane (cm^3)	0.06 ± 0.01	0.07 ± 0.01
Cellular degeneration (cm^3)	0.18 ± 0.05	0.00 ± 0.0
Cellular debris (cm^3)	0.04 ± 0.01	0.00 ± 0.0
Lumen (cm^3)	0.06 ± 0.01	0.04 ± 0.01
Tubular–intertubular proportion	7.61 ± 4.94	8.97 ± 4.59
Total tubular length (m)	34.52 ± 6.64	51.97 ± 9.91
Sertoli cells per seminiferous tubule	17.45 ± 1.931**	26.65 ± 1.131**

*$P < 0.05$ or **$P < 0.01$ in the same line indicates differences between the groups.

the DA than those in the CO group. Decreased gonadal weight has been previously described for both male [8,11] and female [13] rats treated neonatally with GnRH analogs. The lack of differences found in these queens could be explained by the presence of big ovarian structures (i.e., CL, antral follicles) in both groups of females.

The dynamic regulation of mammalian folliculogenesis is a key component of the female reproductive process because the primordial to primary follicle transition is nonreversible. Then, the follicle continues growing until ovulation or destruction by atresia [20]. The rate of follicular assembly and the primordial to primary transition is of critical importance, and the abnormal control of primordial follicle assembly or development can lead to conditions such as premature ovarian failure [20]. In the domestic cat, primary oocyte formation takes place during a well-defined period of time with the onset occurring at approximately 40 to 50 days of fetal development and possibly being completed by 8 days after birth [21]. In other mammalian species, different endocrine disruptors have shown to affect the aforementioned mechanism, disrupting the normal hormone-dependent control within the ovary and neuroendocrine tissues [22].

The reduction in the number of the primordial, primary, and secondary follicles found in this feline study could be due to a stimulation of the apoptotic effects by the agonist. It seems reasonable to assume that these queens will prematurely become infertile. These ovarian findings are in agreement with previous reports in neonatal rats and ewes, in which the exposure to different endocrine disruptors reduced the ovarian pool of primordial follicles [23,24]. Specifically and similarly to the present results, when neonatal rats received GnRH analogs, the number of growing and antral follicles was diminished without affecting the quantity of CL [12,13]. Albeit no statistical significance could be detected for antral follicles in this study of deslorelin-treated cats, their number was clearly lower than those in CO animals.

In male mammals, only immature Sertoli cells proliferate during fetal, neonatal life and in the peripubertal period. As the number of Sertoli cells determines the number of spermatozoa produced per day, it is important that the correct number of Sertoli cells is generated. Without the physical and metabolic support of the Sertoli cells, germ-cell differentiation, meiosis, and transformation into spermatozoa would not occur [25].

In this study, deslorelin caused the reduction of Sertoli cells and, therefore, of the germinal epithelium. Similarly,

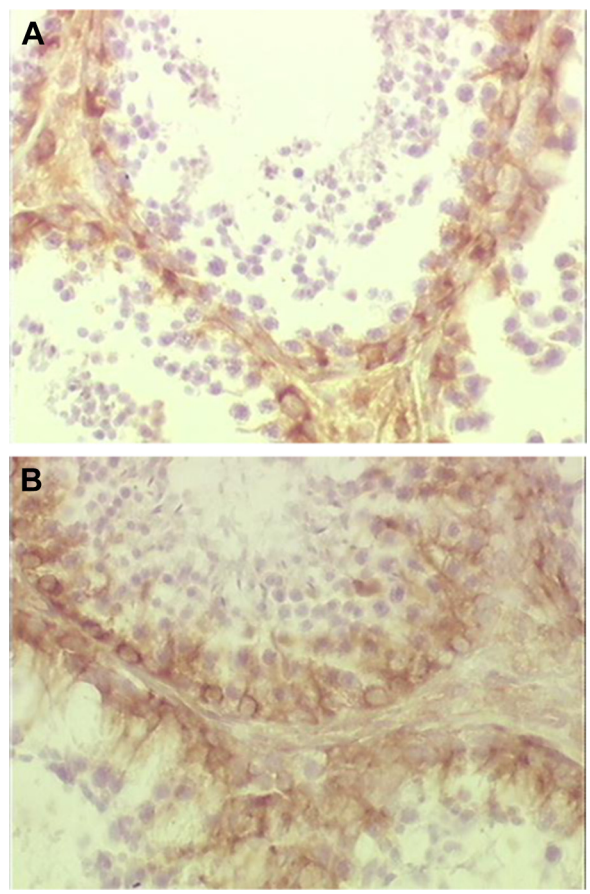

Fig. 2. Immunohistochemical stained Sertoli cells (tanned colored cells) positive to antivimentin antibody of the seminiferous tubules for the cats and experiment described in Table 1. Notice the fewer number of Sertoli cells and the diminution of the germinal epithelial height present in the deslorelin-treated (A) versus the control (B) cats (magnification: × 40). (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)

in marmoset and ewes, the neonatal GnRH analog treatment reduced total germ cell numbers because of suppression of Sertoli cell quantity and function [10,11]. Conversely, and in coincidence with what has been described for rats [25], Leydig cells seemed not to be determined by the number of Sertoli cells per testis. This implies that also in cats, final Leydig cell number may be determined before birth.

Finally, these findings in both genders further support the hypothesis that, as in other mammals, domestic cat gonads have "key elements" (e.g., primary oocytes and Sertoli cells) that complete their development or proliferation during early postnatal life, being susceptible to endocrine disruption and therefore, could be targets of permanent contraceptive strategies. In this respect, disruption of reproductive development during critical organizational periods, such as the postnatal, can alter the functional capacity of the adult animal [5].

It is concluded that the postnatal administration of deslorelin acetate decreased the number of primordial and growing follicles in female cats and Sertoli cells and germinal epithelium in male cats. Gonadotropin-releasing hormone agonist endocrine disruption during the early neonatal critical reproductive time window may have implications for the development of this methodology as a safe and permanent contraceptive strategy. Further studies are warranted to fine tune these protocols in postnatal domestic felids.

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Competing Interests

None of the authors of this paper 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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