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Failure of a single dose of medroxyprogesterone acetate to induce uterine infertility in postnatally treated domestic cats

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ABSTRACT

In mice and sheep, neonatal administration of progesterone or progestins inhibited development of uterine glands. The aims of the present study were (1) to describe uterine gland development on postnatal Days 6 to 8 and (2) to evaluate the effects of a single postnatal administration of a progestin on reproduction and adult uterine glands morphology and function in domestic cats. Necropsy was performed on three 1-week-old female cats which had died unrelated to this study. Ten female kittens were randomly assigned within the first 24 hours of birth to: medroxyprogesterone acetate 10 mg/animal subcutaneously (MPA; n = 6) or placebo (PLC; n = 4) and followed up until puberty when they were mated. Twenty-four days after the end of estrus, ovulation and pregnancy were diagnosed by serum progesterone measurement and ultrasonography, respectively. Then, all the cats were ovariohysterectomized. After necropsy or surgery, the excised organs were histologically evaluated. Seven queens ovulated (4 of 6 MPA and 3 of 4 PLC; $P > 0.1$) and were pregnant ($P > 0.1$). Four MPA cats presented endometrial hyperplasia and one of them developed a pyometra. The 1-week-old females presented uterine glands in the stage of budding and incipient penetration of the glandular epithelium into the underlying stroma. The MPA-treated queens revealed that the area occupied by uterine glands per square-micrometer (0.55 ± 0.2 vs. 0.49 ± 0.2; $P > 0.1$) and the height of the glandular epithelium (μm; 24.5 ± 6.7 vs. 24.4 ± 7.2; $P > 0.1$) did not differ from those of the PLC group. Neither significant gross nor microscopical differences were also found for ovaries ($P > 0.1$). It is concluded that 1-week-old kittens had an incipient stage of uterine gland development and that a single postnatal supraphysiological dose of MPA did not alter uterine adenogenesis in this species. Furthermore, this treatment seemed to predispose to uterine disease without prevention of fertility.

1. Introduction

Domestic cat (*Felis catus*) overpopulation is arguably one of the largest global problems facing animal advocates. Although several approaches (i.e., surgical, hormonal, immunological, chemical, etc) have been tried [1], the optimal option to control undesired reproduction has not been found for this species yet. In this regard, the ideal contraceptive should be efficient, safe, nonsurgical, and suitable for administration as a single dose at a reasonable cost. A simple and permanent method for sterilizing cats would benefit both animals and society.

In mammals such as sheep, pigs, dogs, and rodents, the uterus is not fully developed or differentiated at birth, and uterine gland morphogenesis is primarily a postnatal event [2,3]. For example, canine uterine adenogenesis is initiated by the end of neonatal Week 1 and is completed by Weeks 6

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to 8 [4]. No information is available concerning domestic cat uterine adenogenesis.

Uterine glands originate as shallow gland buds from the luminal epithelium that invaginate to form tubules through the stroma toward the myometrium when they begin to coil and branch. Although the initial timing of these events may be influenced by gestation length [2,3], final endometrial maturation and growth may not occur until after puberty and, perhaps, even after pregnancy [5].

Uterine glands secretory products are required for establishment of uterine receptivity and conceptus preimplantation, elongation, and survival [6]. Thus, organizational alterations during endometrial gland formation may lead to infertility because of pregnancy loss as blastocysts hatch normally but fail to survive [6].

Exposure of the developing urogenital tract to steroids can affect structure and function of adult tissues compromising reproductive performance [7]. Particularly, progestin administration during the critical postnatal organizational period can alter the developmental trajectory of the uterus with lasting consequences. Exposure of neonatal ewe lambs from birth to 32 weeks of age to the synthetic progestin, norgestomet, ablated endometrial gland morphogenesis [8]. In mice, application of progesterone from postnatal Days 3 to 9 blocked adenogenesis and caused adult infertility [9].

The ability of neonatal progestin treatment in sheep and mice to produce uterine infertility suggests that an approach of this kind may provide a permanent contraceptive strategy with application in companion animal species for addressing overpopulation problems.

To test this hypothesis, the aims of the present study were (1) to describe uterine gland development on postnatal Day 7, (2) to evaluate the effects of a single postnatal administration of a progestin on reproduction and adult uterine glands morphology and function, and (3) to assess the clinical safety of this treatment in domestic cats. For this purpose, medroxyprogesterone acetate, a potent, long-term effect progestin which is inexpensive and worldwide available [10], was chosen as the endocrine disruptor.

2. Materials and methods

2.1. Animals

Three short-hair female kittens born in the Institutional Colony of the Veterinary School of the National University of La Plata which had died because of traumatic reasons between days 6 and 8 after birth were used to study early postnatal uterine gland development.

2.1.1. Experiment 2

Ten female kittens (6 litters) born in the same colony were included in the trial to evaluate the effects of a single postnatal administration of a progestin on reproduction and adult uterine glands morphology and function. These latter animals were identified, reared free in indoor catteries (3 rooms 4 × 3 meters, with 14 hours of light per day and appropriate enrichment), weaned at the age of 40 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, 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 females of the same litters were randomly assigned to one of the following treatment groups within the first 24 hours of birth: medroxyprogesterone acetate 10 mg/animal (Singestar MP, Konig, Argentina) subcutaneously (sc; MPA; n = 6) or placebo: 0.2-mL corn oil sc (PLC; n = 4).

2.3. Follow-up

All the animals were followed up until the first pubertal signs appeared. During the follow-up period, the cats were observed looking for sexual behavior 1.5 hours twice a day and physically examined including weighing once a week. Clinical side effects were also recoded. Vaginal cytology [11] was carried out 3 times/week from the third month of age onward. Puberty was defined by the finding of >80% superficial keratinized cells and a clean background in the vaginal smears accompanied by the typical estrous behavior [12].

2.4. In vivo fertility tests

As the female cats attained puberty, they were exposed to a fertile tomcat during the whole estrous period. Matings were observed and/or diagnosed by the presence of spermatozoa in the vaginal smears. Twenty–four days after the end of estrus, peripheral blood samples were taken for ovulation assessment by electrochemiluminescence immunoassay determination of serum progesterone (P4; Elecsys Progesterone II; Roche Diagnostics, Mannheim, Germany; P4 > 5 ng/mL; [12]). The lower detection limit and the sensitivity of this assay are 0.03 and 0.15 ng/mL, respectively. Within-run precision was less than 5%. Pregnancy diagnosis was carried out by ultrasonography in all cats [13].

2.5. Ovariohysterectomies

After assessment of pregnancy or nonpregnant state, all queens were ovariohysterectomized, and their ovaries and uteri subjected to histology. For the surgery, the animals were premedicated with atropine sulfate, (Atropine Sulfate, John Martin; 0.04 mg/kg, sc), acepromazine maleate (Acedan, Holliday; 0.03 mg/kg sc), and butorphanol (Torbutol Plus, Fort Dodge; 0.2 mg/kg, intramuscularly); anesthesia was induced with sodium thiopental (Pentovet TM, Richmond; 8 mg/kg, intravenously) in all the females. Once the females were endotracheally intubated, anesthesia was maintained with isoflurane and oxygen in a closed system. A midline laparotomy was performed to excise the ovaries and uteri. After surgery, ketoprofen (Ketofen, Fort Dodge; 1 mg/kg) was injected sc (once) and then orally every

24 hours during 4 additional days. All the queens were placed for adoption.

2.6. Gross and histomorphometric examination

Immediately after necropsy (experiment 1) or surgery (experiment 2), genital tracts were excised. The ovaries and uteri were macroscopically examined and weighed.

The ovaries were sectioned longitudinally, placed in Bouin's fixative for 12 hours, and then changed to alcohol 70 and processed routinely with paraffin embedding. After processing, 5-μm serial sections were cut, mounted on slides, stained, deparaffinized in xylene, rehydrated in graded 70% ethanol solutions, and stained with hematoxylin and eosin.

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) [14]. 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 (×20) per animal.

Uteri were sectioned longitudinally for internal inspection. Cross sections (approximately 0.5–1 cm) were taken from a point midway between the external bifurcation and the tip of each uterine horn and processed as described for the ovaries. In the case of pregnant cats (see in the following sections), placentation sites were avoided from the cross-sections.

The uteri were examined for the presence or absence of endometrial glands. The area occupied by uterine glands per square-microns of endometrium over the total area of each microscope field was measured by planimetry. The height of the glandular uterine epithelium was assessed counting 100 cells in a total of 10 images per uterus taken with a ×10 objective.

All histologic images were obtained from a microscope (Olympus BX50, Tokyo, Japan; 10×) through an attached digital RGB video camera (Evolution VF Color; Q Imaging, USA) and digitalized in a 24-bit true-color tagged image file format. These images were analyzed using the (Image Pro Plus version 6.0; Media Cybernetics, Silver Spring, MA, USA).

2.7. Statistical analysis

Quantitative and qualitative differences between MPA and PLC groups were analyzed by the Fisher exact and the Student *t* tests, respectively. All data were expressed as mean ± standard deviation, and P values < 0.05 were considered significant.

3. Results

3.1. Experiment 1

All the 6- to 8-day-old kittens presented uterine glands in the stage of budding of luminal epithelium and incipient penetration of the glandular epithelium into the underlying stroma (Fig. 1).

3.2. Experiment 2

Neither age (43.7 ± 5.9 vs. 35.3 ± 5.8 weeks; $P > 0.05$) nor body weight at puberty (2760.1 ± 331.5 vs. 2720.0 ± 153.9 g; $P > 0.1$) differed between MPA and PLC groups. Five of the six MPA kittens presented a sharp "prepuce like" vulva and mild clitoris enlargement during postnatal weeks 5 to 20 when they gradually normalized.

All the pubertal females showed normal sexual behavior ($P > 0.1$), and when exposed to males during estrus, accepted repeated matings. Ovulation occurred in 7 (4 of 6 MPA and 3 of 4 PLC; $P > 0.1$) of the 10 queens after estrus. The 7 queens that ovulated demonstrated to be pregnant without differences between treatment groups ($P > 0.1$). Nonpregnant females were in interestrus at the time of ovariohysterectomy.

No permanent side effects were observed ($P > 0.1$) except one pregnant MPA queen that presented an open cervix pyometra after pregnancy diagnosis just before ovariohysterectomy was carried out.

No significant gross nor microscopical differences were found between treatments for any studied ovarian parameter (Table 1; $P > 0.1$). Uterine wet weight did not differ between groups (MPA 3.86 + 1.1 vs. PLC 4.10 + 1.9 g; $P > 0.1$). The gross internal evaluation of the excised uteri revealed a thick and irregular endometrium in 4 of 6 MPA cases (Fig. 2); one of them presenting accumulation of abundant pus (pyometra case mentioned previously). The presence of embryonic vesicles was also found in 4 MPA queens (including the previous one with pyometra in which the vesicles were empty). Conversely, none of the PLC queens, either pregnant or not, presented gross uterine abnormalities.

Microscopic assessment of the uteri of the MPA-treated queens revealed that the area occupied by uterine glands per square-micrometer of endometrium (0.55 ± 0.2 vs.

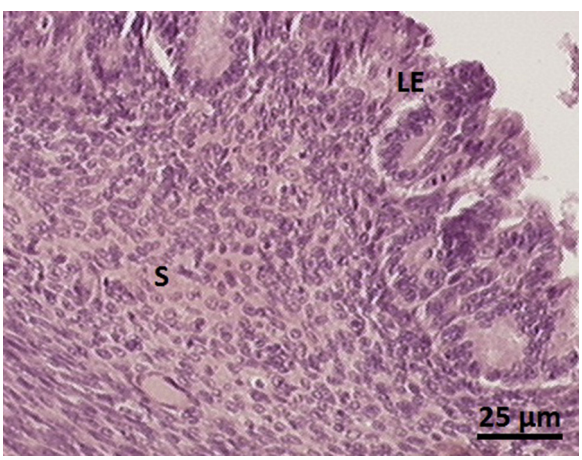

Fig. 1. Uterus (hematoxylin and eosin, 10X) of a 7-day-old female cat. Notice budding and incipient penetration of the glandular epithelium into the stroma. GE, glandular epithelium; S, stroma.

Table 1
Ovarian gross and histologic parameters (mean ± standard deviation) of female cats treated postnatally with medroxyprogesterone acetate 10 mg/animal (MPA; n = 6) or placebo sc (n = 6) and ovariohysterectomized after pubertal estrous cycle.

Parameter	MPA	Placebo
Length (cm)	0.95 ± 0.1	0.95 ± 0.1
Height (cm)	0.75 ± 0.2	0.63 ± 0.1
Weight (g)	0.19 ± 0.0	0.17 ± 0.0
Volume (cm^3)	0.3 ± 0.2	0.2 ± 0.0
Primordial follicles/mm^2	216.83 ± 31.1	216.5 ± 40.8
Primary follicles/mm^2	6.99 ± 2.8	7.64 ± 2.6
Secondary follicles/mm^2	0.8 ± 0.3	0.53 ± 0.3
Antral follicles/mm^2	0.1 ± 0.2	0,11 ± 0.2
CL/mm^2	0.31 ± 0.1	0.34 ± 0.3
Atretic follicles/mm^2	17.33 ± 4.8	14.5 ± 8.7

No significant differences were found between treatments ($P > 0.1$).

0.49 ± 0.2; $P > 0.1$) and the height of the glandular uterine epithelium (μm; 24.5 ± 6.7 vs. 24.4 ± 7.2; $P > 0.1$) did not differ from those of the PLC group (Fig. 3). Cystic endometrial hyperplasia was confirmed in 4 of the 6 MPA-treated cats.

4. Discussion

Previous reports [9,15] support the investigation of the potential use of neonatal progestins in small animal permanent contraception. This is the first study that describes feline endometrial development at the end of the first postnatal week and the effect of a single postnatal supraphysiological dose of a potent and time-released progestin, that is, medroxyprogesterone acetate on uterine glands.

In this respect, similarly to dogs [4], another altricial carnivore with similar gestation length and birth to puberty interval (for cat-sized dogs; [16]), cats presented an incipient stage of uterine gland development by the end of the first postnatal week. Furthermore, both uterine glands per square-micrometer and glandular epithelium height clearly increase to maturity with advancing age as shown in nontreated animals. The finding of an immature endometrium highlights the importance of testing the effect of postnatal progestins for contraceptive purposes in feline species. Thus, in this study, a single-dose, low-cost, practical protocol was assessed to induce uterine permanent infertility. For this purpose, medroxyprogesterone acetate was administered within the first day of life. As this progestin is known to suppress the gonadal axis for a minimum of 3 weeks [10] and assuming that cats are not an exception among mammals, the whole period of glandular development would be coved with this single treatment.

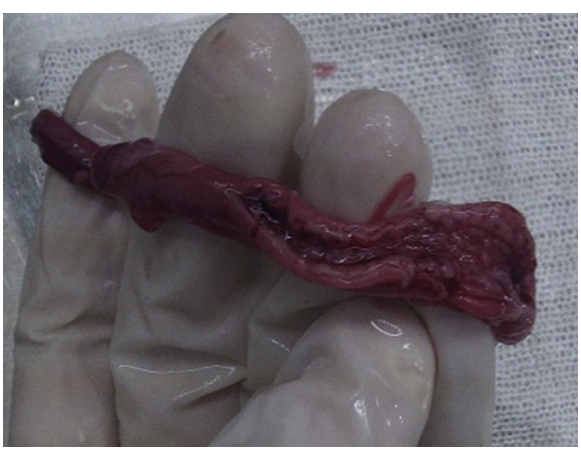

Fig. 2. Gross uterine internal view of a selected female cat treated postnatally with medroxyprogesterone acetate 10 mg/animal. Notice endometrial thickening and irregularity.

This early progestin treatment did not seem to affect age at puberty and somatic development, crudely assessed through body weight at puberty. The transient vulvar and clitoris abnormalities observed in most of the MPA-treated cats could be attributed to the androgenic effect of progestins [10].

In contrast to what has been reported for the neonatal administration of other steroids in rodents and dogs [17,18], in this study, progestins did not modify sexual behavior and libido during heat. The hypothalamic-pituitary-ovarian axis functionality was, apparently, not affected as ovulation occurred in almost all the animals, and ovarian morphology was normal. The anovulation, diagnosed in 2 MPA-treated queens, could be due to individual thresholds to vaginal stimulation in these pubertal animals. Furthermore, 1 PLC queen did not ovulate.

Histologic examination revealed that the supraphysiologic dose of the long-term release progestin did not alter uterine adenogenesis in these neonatal domestic felids as neither the area occupied by uterine glands nor the height of the glandular epithelium was reduced by the progestin. Similarly, it has very recently been shown that postnatal medroxyprogesterone acetate does not prevent uterine adenogenesis in domestic dogs [19].

The fact that all the MPA queens which had ovulated became pregnant further confirms the failure to induce a “functional” uterine gland knockout phenotype in felids. Conversely, adult uterine gland knockout ewes were unable to establish pregnancy [5]. Information of fertility after neonatal progestins is not available for canids.

Because of the known stimulating effect of progestins on normal endometrial glands [10], the appearance of endometrial hyperplasia and pyometra was quite expected in these pubertal cats. In this respect, it is worth to note that, although nonsignificant, the area occupied by uterine glands was higher in MPA group. Additionally, it should also be borne in mind that eventual long-term side effects of this treatment could not be discarded out of the time frame of this trial.

Although disproved hypotheses are seldom reported, it is believed that these results will contribute to the clarification of the limited role of postnatal progesterone on the development of a sterilizing strategy in this species. In this respect, the use of a larger progestin dose or serial administrations appears unfeasible as side effects could become more prevalent.

It is concluded that 1-week-old kittens had an incipient stage of uterine gland development and that a single postnatal supraphysiological dose of medroxyprogesterone acetate did not alter uterine adenogenesis in this species.

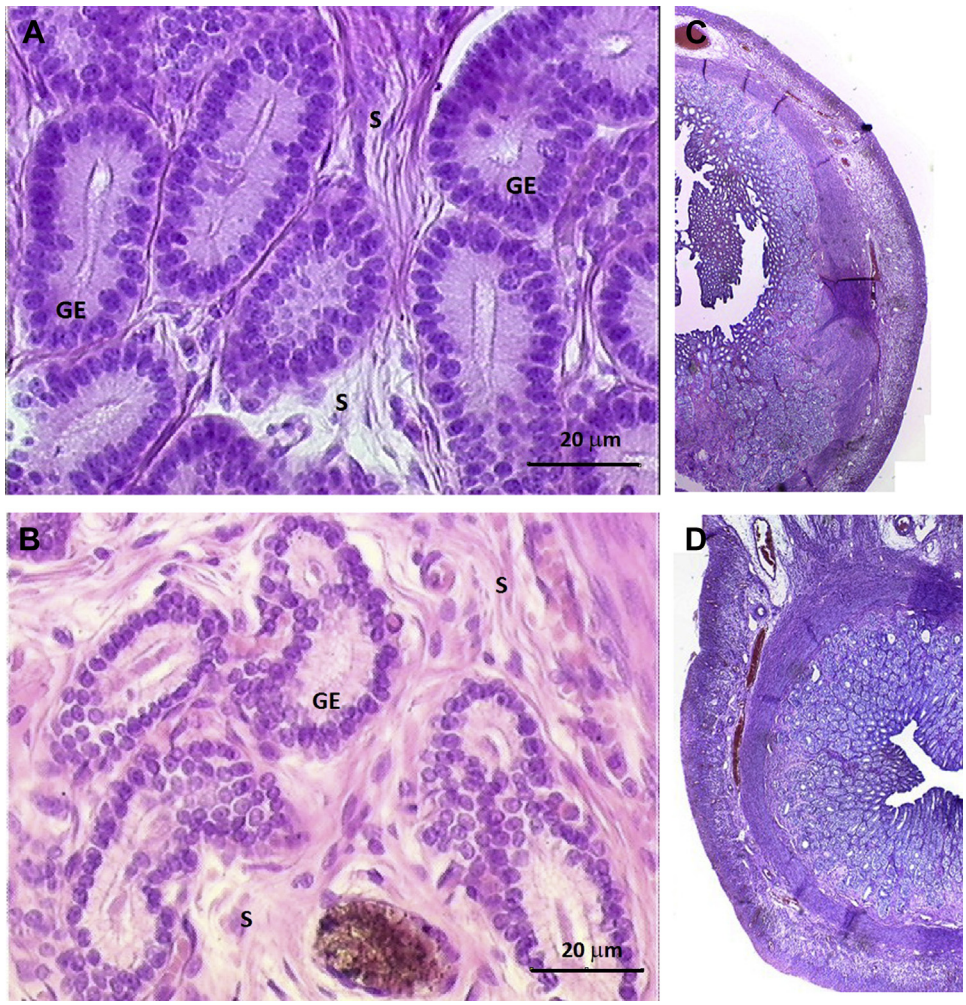

Fig. 3. Uterus (hematoxylin and eosin, 1.25X and 10X) of female cats treated postnatally with medroxyprogesterone acetate 10 mg/animal (A and C) or placebo (B and D) and ovariohysterectomized after pubertal estrous cycle. GE, glandular epithelium; S, stroma.

Furthermore, this treatment seemed to predispose to uterine disease without prevention of fertility.

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