Development and proliferation of feline endometrial glands from fetal life to ovarian cyclicity

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A B S T R A C T

In this study it was determined the progression of uterine gland development from late gestation to puberty in domestic felids. Cell proliferation patterns for luminal (LE), glandular epithelium (GE) as well as stroma (S) were also described. Twenty-four uteri from female kittens: 45 and 65 days of gestation and 1 to 5, 8, 12, 16, 20 and 24 weeks postnatally were obtained. Uterine cross-sections were submitted for routine histological and immunohistochemical quantification of proliferating cell nuclear antigen (PCNA) techniques. Although prenatal uteri presented no indication of adenogenesis, 1 week old uteri revealed an incipient budding of the LE. During the second week budding increased and a mild degree of tubu-logenesis of the GE into the stroma was detected. From the third to fifth weeks coiling, branching and cross-sections of glands appeared. These latter findings were more evident in week 8 when GE began to penetrate through much of the S to week 24. PCNA immunostaining revealed that DNA synthesis decreased throughout the study in the 3 cell compartments; ($P < 0.01$). Luminal proliferation began prenatally, it maintained up to postnatal week 8 to markedly decrease to puberty ($P < 0.01$). From postnatal week 3 up to week 8, GE mitotic activity was elevated becoming low thereafter ($P < 0.01$). Stroma actively proliferated prenatally ($P < 0.01$), diminishing up to week 8 ($P < 0.01$) and again during the last weeks ($P < 0.01$) of the study. It was concluded that, in domestic felids, proliferation of LE begins prenatally, histological uterine adenogenesis commenced during the first postnatal week and both events concluded by postnatal weeks 5–8.

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1. Introduction

In eutherian mammals, the uterus develops as a specialization of the paramesonephric or mullerian ducts, which gives rise to the oviducts, uterus, cervix, and anterior vagina [1]. The mature uterine wall is comprised of two functional compartments, the endometrium and the myometrium. The endometrium is the inner mucosal lining of the uterus and is derived from the inner layer of ductal mesenchyme. Histologically, the endometrium consists of two epithelial cell types, luminal epithelium (LE) and glandular epithelium (GE).

The development and differentiation of most reproductive tract organs is completed during the fetal period; however, the uterus is not fully developed or differentiated at birth. Uterine glands begin to develop as invaginations of LE that progressively invade the mesenchyme, ultimately resulting in an extensive network of epithelial glands throughout the stroma (S). The timing of these developmental events differs among species and reflects differences in uterine maturity at birth [2] which could be a function of gestation length.

Although, in most mammals, uterine gland morphogenesis is primarily a post-natal event there are species differences in the time window at which adenogenesis begins [2]. In general, uterine gland development involves extensive coiling and branching morphogenesis [2], it is not before puberty that endometrium achieves complete histological and functional maturity [3]. In this respect, it was hypothesized that domestic felids are not an exception among mammals. Direct evidence of the essential nature

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of endometrium and their uterine secretions came with the demonstration that endometrial glands are required for establishment and maintenance of pregnancy [4].

Very scarce information is available regarding postnatal uterine development in felids [5]. Indeed, the time course of endometrial gland development and patterns of differentiation and proliferation of uterine glandular and LE during late gestation, neonatal, infantile and pubertal life in the cat have not been reported.

The ability of neonatal progestin treatment to inhibit uterine gland formation and fertility in ruminants and mice [6–9] suggests that this type of treatment could have promise as a contraceptive methodology in companion animal species. Importantly, it is known that progestin effects are critically dependent on age at initiation of treatment, requiring initiation before the beginning of uterine adenogenesis [6,8,9]. Thus, a complete understanding of the time course of feline adenogenesis will facilitate the development of a rational strategy of neonatal steroid administration to permanently inhibit uterine adenogenesis and produce sterility in abandoned domestic cats. In the present study it was determined the progression of uterine gland development from late gestation to just after puberty in domestic felids. Secondly, cell proliferation patterns for luminal and GE as well as S were also described.

2. Materials and methods

2.1. Animals

Twenty-four uteri from mixed-breed female kittens: 45 days of gestation (n = 2), just before parturition (approximately 65 days of pregnancy; n = 2) and 1 (n = 2), 2 (n = 2), 3 (n = 2), 4 (n = 2), 5 (n = 2), 8 (n = 2), 12 (n = 2), 16 (n = 2), 20 (n = 2) and 24 (n = 2) weeks postnatally were obtained from the animals born in the Institutional Feline Colony of the Veterinary School of the National University of La Plata (NULP) which had either died due to reasons that would not affect their uterine development or been submitted for elective ovariohysterectomy. Twenty − four weeks old females had just attained puberty. All experiments were approved by the Institutional Animal Care and Use Committee of this University (42.3.14P, 2014).

2.2. Histological examination

Immediately after necropsy or during surgery genital tracts were excised. Uterine cross-sections were taken between the external bifurcation to the tip of each uterine horn and placed in Bouin's fixative for 12 h 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. The uterine tissues were examined for the presence or absence of endometrial glands as well for the description of their stage of development according to Gray et al. (2001) [2]. All histological images were obtained from a microscope Olympus BX50, Tokyo, Japan; 10X through an attached digital RGB video camera (Evolution VF Color, Q Imaging, USA) and digitalized in a 24 bit true color TIFF format.

2.3. Immunohistochemical quantification of PCNA

Uteri cellular proliferation was assessed at various ages by immunohistochemical quantification of proliferating cell nuclear antigen (PCNA). Sections (3 μm) were mounted on slides coated with [3-(Methacryloyloxy)propyl]trimethoxysilane (M6514, Sigma, St. Louis, MO, USA), passed through a decreasing graded alcohol scale and incubated with 0.03% H_2O_2 in methanol (purum P99.0%) for 30 min at room temperature. Sections were then rinsed twice in PBS and exposed to microwave with a power of 750 W 10 min, to antigen retrieval was used a buffer citrate solution (pH 6.0). For PCNA localization, sections were then incubated with mouse monoclonal anti- PCNA antibody (clone PC 10, ascites fluid, Sigma Chemical, St. Louis, MO; 1:3000) at room temperature for 1 h. The En VisionVR 151 detection system1HRP system labeled anti-mouse polymer (Dako Cytomation) was applied for 30 min. Liquid DAB (DakoVR Cytomation) was used as chromogen and hematoxylin for counterstaining. Negative control sections were prepared by omitting primary antibody.

The percentage of cells with PCNA - positive (dun) nuclei, was determined for luminal and GE as well as S by counting 500 cells within 4 quadrant tissue sections from each uterine sample.

2.4. Statistical analyses

The percentage PCNA nuclear labeling (% PCNA - positive cells) for each cell type (luminal glandular or stroma) were grouped as prenatally (PRE; 45 and 65 days of gestation), neonatally (POS1; 1 to 8 postnatal weeks) and remaining weeks (POS2; 20 to 24 postnatal weeks) and compared by analyses of variance followed by Tukey comparison test. To further characterize results of each cell compartment, a Spearman correlation test was carried out between PCNA percentages and cat age (parturition was considered as age 0; SPSS 17.0, SPSS, Chicago, IL, USA). Results were presented as mean ± SEM and the level of significance was set on $P < 0.05$.

3. Results

Histogenesis of the feline endometrium from gestational life to just after puberty is shown in Fig. 1. Both at gestation day 45 and immediately before birth (approximately 65 days of pregnancy; Fig. 1A) the uteri presented no indication of adenogenesis. One week old uteri revealed nascent endometrial glands represented by an incipient budding of the simple columnar LE (Fig. 1B'). During the second week of age budding of LE increased and a mild degree of tubulogenesis of the GE into the underlying stroma was first detected. A similar scenario appeared throughout the third (Fig. 1B''), fourth and fifth postnatal weeks in which the tubulogenesis was more marked along the LE border and coiling and branching began to be visible appearing the first cross-sections of rudimentary glands beneath the LE. An increase in the number and depth of gland cross-sections was evident at postnatal week 8 (Fig. 1B'''). From that week up to the end of the study, coiling and branching of the GE gradually penetrated through much of the endometrial S (Fig. 1C).

PCNA immunostaining revealed that DNA synthesis decreased throughout the period of the study in the 3 cell compartments (r = −0.75, −0.78 and −0.73 for the LE, GE and S, respectively; P < 0.01; Fig. 2A, B, C). Luminal epithelium (Figs. 2 and 3A) proliferation began prenatally (23.1 ± 2.1%), it maintained slightly, but not significantly, higher during the neonatal POS1 period (25.3 ± 1.9%) to markedly decrease to minimal values up to puberty (2,3 ± 0,9%; P < 0.01). As GE (Figs. 2B and 3B) was not histologically distinct at birth, its immunostaining could be recorded from postnatal week 3 onwards. From that week up to week 8 (POS1), mitotic activity was elevated (23.4 ± 3.1%) becoming low thereafter (POS2; 4.7.±1.0%; P < 0.0). Stromal (Figs. 2 and 3C) proliferation was very active prenatally (26.1 ± 3.1%; P < 0.01), diminishing in POS1 (13.3 ± 1.5%; P < 0.01) and again during POS2 periods (3.1 ± 1.3%; P < 0.01) of the study.
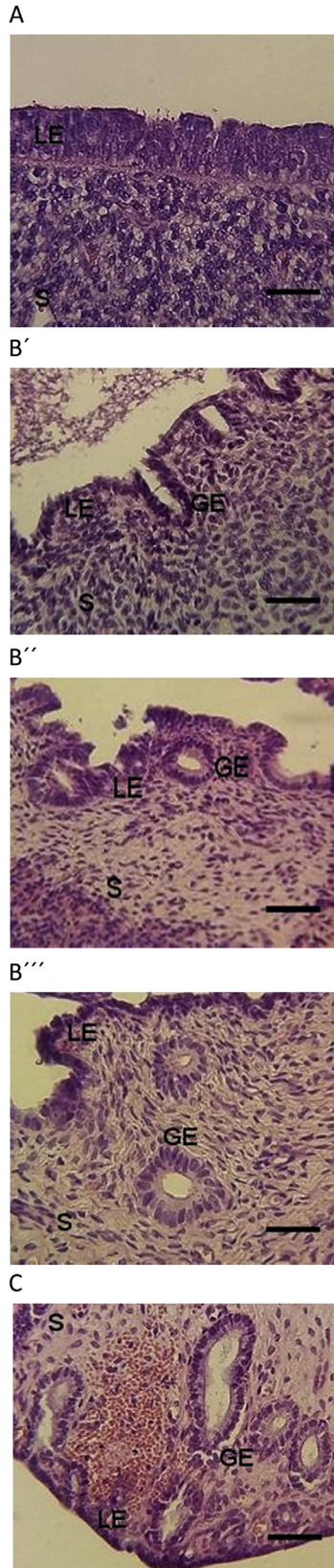
Fig. 1. Adenogenesis in feline uterus (hematoxylin and eosin, 40X). **A**: prenatal (PRE; before parturition), **B**: postnatal 1 (POS1; **B'**: 1 week, **B"**: 3 weeks, and **B'''**: 8 weeks) and **C**: postnatal 2 (POS2; 24 weeks old). GE: glandular epithelium, LE: luminal epithelium, S: stroma. The scale represents 20 μm.
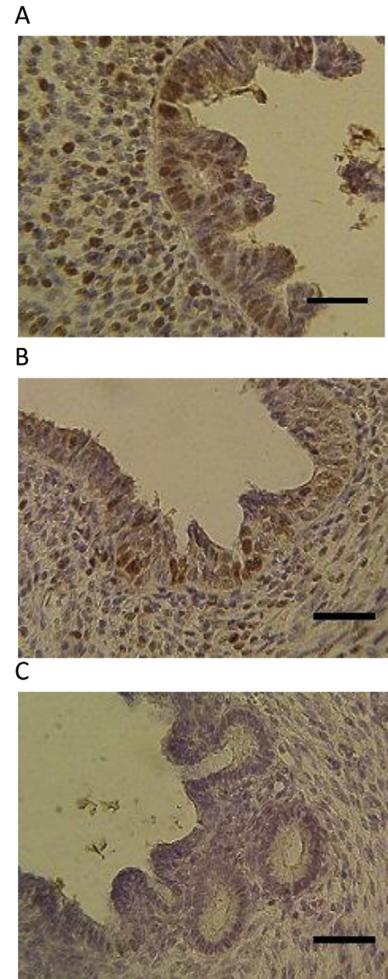

Fig. 2. Cell proliferation of feline uterus visualized by PCNA immunostaining (40X) as dun nuclei. **A**: prenatal (PRE), **B**: ≤ 8 weeks of age (POS1) and **C**: > 8 weeks (POS2). The scale represents 20 μm.

4. Discussion

In this study it is provided for the first time a complete overview of how uterine glands develop and proliferate from prenatal period to just after puberty in domestic felids. Although, a recent study provided a first step toward this goal by describing the incipient stage of uterine adenogenesis in one week old kittens [5] further knowledge was necessary for tuning up a permanent uterine contraceptive methodology in this species. Both murine and ovine models [6,8,9] showed that full inhibition of adenogenesis requires that treatments begin before the initiation of uterine gland development. Thus, a detailed prenatal uterine description was also included.

In spite of an active epithelium and stromal proliferation, feline fetus endometrium appeared very immature with no histological suggestion of early gland formation. These findings are in line with those described for mice, rats, sheep, pigs and cows [10—13] and different to from those of horses and human beings [3,14]. In these felids uterine adenogenesis initiated during the first postnatal week and uterine gland development appeared completed by week 4. From that week onwards, both glandular number and size increased up to postnatal weeks 5—8. In domestic dogs, uterine adenogenesis is initiated by the end of the first neonatal week and
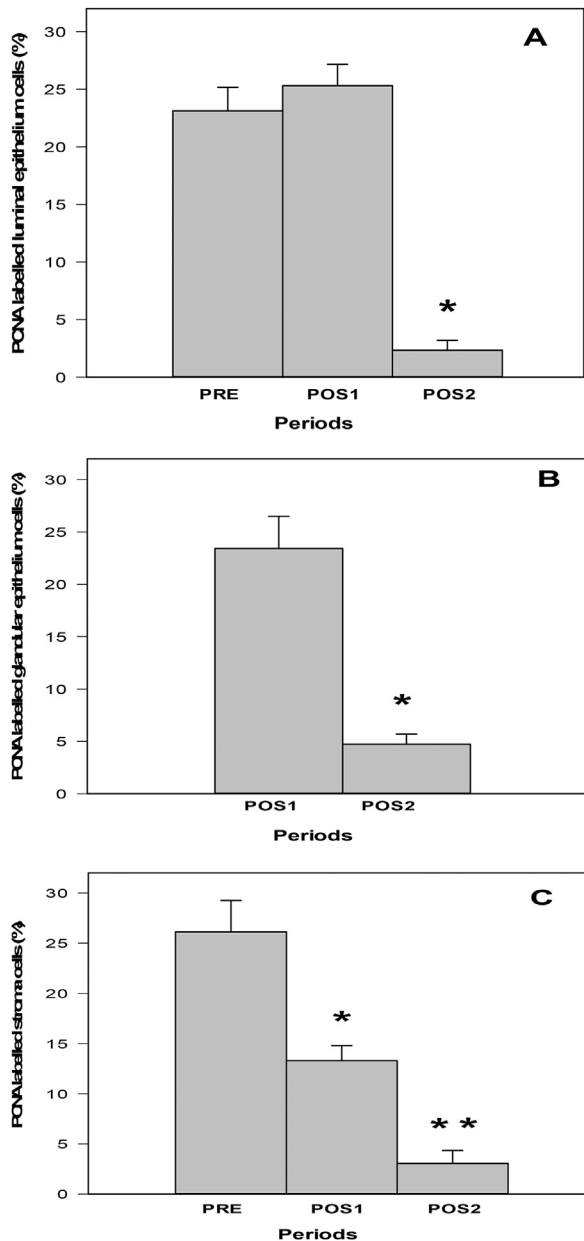
Fig. 3. Percentage PCNA labeled feline uterine cells (mean ± SEM) from prenatal life (PRE) up to puberty (POS1 and POS2 ≤ or > 8 weeks of age, respectively). Luminal epithelium (**A**; P < 0.01). Glandular epithelium (**B**; P < 0.01). Stroma (**C**; P < 0.01). Asterisks represent differences P < 0.01.

it is completed by postnatal weeks 6–8 [15]. This coincidence between carnivores' species was quite expected as dogs are also altricial carnivores with similar gestation length and life span to puberty assuming, for this latter parameter, a similar adult body size.

To assess the proliferative activity of the developing uterus, a well known cell cycle marker, namely proliferating cell nuclear antigen (PCNA) was used. Proliferating cell nuclear antigen main function is in DNA replication being elevated during the S and G2 phases of the cell cycle, but is very low in quiescent cells [16]. Based on data for PCNA labeling, in feline uterus luminal and stromal cell proliferation actively began prenatally. No information is available concerning canine prenatal uterine development and proliferation thus no comparisons can be made in these respects. After birth, and in agreement with the endometrial morphological development,

the highest mitotic activity was reached during the first 8 postnatal weeks by both luminal and GE to significantly decrease to minimal values thereafter in the 3 studied cell compartments. This proliferative pattern is also in line with what has been described for domestic dogs using MKI67 nuclear labeling [15].

Further work is required to unveil the reasons why the first attempt to cause feline uterine gland agenesis failed after both postnatal progestin [5] and testosterone administration, obtaining only a decrease in the area occupied by uterine glands and the height of the uterine epithelium using the latter treatment [17]. It may be the case that, in this species, prenatal steroid administration is necessary to fully inhibit the estrogen mitogenic effects [18] and, therefore, lead to an aglandular uterine phenotype that meets the sterilizing overpopulation demands.

It was concluded that although, in domestic felids, proliferation of luminal epithelium begins prenatally, histological uterine adenogenesis commenced during the first postnatal week and both physiological events concluded by postnatal weeks 5–8.

Conflict of interest

The authors do not have any financial nor personal relationships with other people or organizations that could inappropriately influence the study.

Acknowledgments

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