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Temporal changes in neutral endopeptidase/CD10 immunoexpression in the cyclic and early pregnant canine endometrium

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ABSTRACT

CD10 is a multifunctional transmembrane neutral endopeptidase (NEP) that is considered to be a reliable marker of ectopic human endometrial stroma. Available information on NEP/CD10 protein expression in animal endometria is scarce. This study focused on the immunolocalization of NEP/CD10 in the canine uterus and on its temporal changes during the estrous cycle and early pregnancy (Days 11 to 23 post-LH surge) in healthy females. NEP/CD10 expression was found in the canine endometrial stroma in all stages of the estrous cycle, showing cyclic differences both in intensity and in distribution pattern. A small population of negative stromal cells in subsurface position was also observed. This population shared some morphological characteristics with the human predecidual cells, which became positive in progesterone-associated stages of the cycle. In addition, positive immunolabeling was also observed in canine myometrial stroma. In early pregnancy, the basal glandular epithelia and the syncytium cords remained negative to this marker contrasting with the trophoblast and the lacunar epithelium. A weak to moderate intensity of immunolabeling was observed in the decidual cells, whereas stromal immunolabeling was more intense at the delimitation of the syncytium cords. In conclusion, CD10 is consistently expressed in the canine endometrial stroma and myometrium but not in the endometrial epithelia. The characteristic pattern seen in early pregnancy also suggests a role for this molecule in the process of embryo invasion at implantation.

1. Introduction

The mammalian endometrium is a highly complex tissue that undergoes accurately defined, cyclic morphological changes in response to sex steroids stimulation. The ultimate goal is to guarantee embryo survival, implantation, and the success of pregnancy [1,2].

Although under the control of sex steroids, endometrial cyclic changes are ultimately controlled by several autocrine and paracrine factors that include a multitude of local molecules that determine the proliferation of the epithelial endometrial elements, epithelial–stromal cells interaction, and invasiveness, angiogenesis, apoptosis, differentiation, and immune cells' infiltration, among

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others [3–5]. A correct equilibrium of these molecules, both in sequential changes and in quantity, is essential for fertility [6].

Endometrial stroma cell functions are not limited to maintaining the endometrial structure; the stroma is involved in epithelial development and proliferation [7], cell adhesion, tissue remodeling, and organ immune competence [8]. These are notorious during the cyclic changes and at implantation, particularly in species with deciduae placenta.

CD10 protein is a membrane-associated neutral peptidase, also known as neprylisin, enkephalinase, common acute lymphoblastic leukemia antigen, or neutral endopeptidase (NEP) [9,10]. CD10 is a 90- to 110-kDa cell-surface zinc-dependent metalloprotease shown to be expressed by a wide variety of cell types and tissues, including the uterus [11]. CD10 functions as a cell-surface enzyme acting to reduce the cell response to some peptide factors, including oxytocin, endothelins, and interleukin 1 [12]; through cleavage and inactivation of these peptides, NEP/CD10 reduces its local concentrations and decreases their effects [13-15].

NEP/CD10 has been implicated in the regulation of growth and differentiation in many cellular systems, in which it plays an important role in the maintenance of homeostasis [16–18] and in carcinogenesis and tumor progression [19–23], possibly mediated through its role on angiogenesis [24], in cell cycle activity [25], and apoptosis [26].

In human, NEP/CD10 is frequently used as a reliable immunohistochemical marker of normal endometrial stroma [27,28] and is used for diagnosis of several neoplasic [28–30] and non-neoplasic [31,32] gynecological conditions. Yet, NEP/CD10 functions in the endometrium remain poorly understood.

Although clinical conditions such as endometriosis are not proven to exist in dogs, additional knowledge on the location and cyclic variation of this endopeptidase in the canine endometrium may be valuable, especially in pathological alterations such as cystic endometrial hyperplasia or when fertility may be compromised. In domestic animals, although previous work by Riley et al. [33] reported the presence of this enzyme in the sheep uterus, limited information is available on uterine pattern of the CD10/NEP protein expression.

The purpose for this study was to determine the pattern of NEP/CD10 protein expression in the normal canine endometrium by using an immunohistochemical technique and to investigate whether this pattern changes during the estrous cycle and in early pregnancy (Days 11 to 23 post-LH surge). By establishing the normal pattern of CD10 expression in canine endometrium, this study will further provide reference data that might be essential in the study of endometrial diseases, especially in angiogenesis and stromal-epithelial cross-talk.

2. Materials and methods

2.1. Tissue collection and preparation

Forty-eight post-pubertal, healthy nonpregnant bitches and 16 pregnant females of different breeds and ages ranging from 10 months to 6 year old were used in this study. Endometrial tissue collected at ovariohysterectomy (OVH) was used with the owners' informed consent in accordance to the International Ethical standards.

For immunohistochemistry, samples from the uterus were fixed in 10% formalin immediately after the surgery. Transversal fragments were collected from each uterine horn, embedded in paraffin wax, sectioned at 3 μm, and stained with hematoxylin and eosin for histological staging of the estrous cycle and for excluding uterine disease. Samples showing histological signs of delayed uterine involution (glandular dysplasia and increased number of macrophages in the presence of large vessels within the *stratum vasculare*) or endometrial disease (such as cystic endometrial hyperplasia or pyometra) were excluded from the study. For the pregnant group, transversal samples were collected from zonary invasion areas and interplacental areas (or paraplacenta). When the collected samples were not distinguishable, longitudinal sections were obtained. For Western blotting (WB), adjacent 1-cm-thick uterine sections were collected from nonpregnant samples and immediately snap-frozen in liquid nitrogen before being stored at −70 °C, until analysis.

Before surgery, a vaginal cytological specimen was obtained, and a blood sample was collected from the jugular vein into a controlled vacuum tube (*Serum-gel*, S-Monovette, Sarstedt, Nümbrecht, Germany), centrifuged, and stored at −20 °C until analysis. Serum progesterone levels were determined by chemiluminescence immunoassay system (Immulite; DPC-Diagnostic Products Corp., Los Angeles, CA, USA).

2.2. Estrous cycle and pregnancy staging

Nonpregnant animals were initially selected on the basis of vaginal cytology. At OVH, the stage of the estrous cycle for each bitch was determined by ovaries inspection and later confirmed based on the histological examination of the ovaries and by progesterone levels that were used in fine-tuning the histological staging [8]. Uterine samples for the proestrus (n = 9), estrus (n = 8), diestrus (n = 20), and anestrus (n = 10) were used in this study. Considering that in carnivores implantation is not an early event [34], with dog embryos interacting with the endometrium around postovulatory Day 16 [35], the diestrus was further divided into two stages: an early diestrus period (n = 10) with rising progesterone levels and with young, cavitary CLs in the ovaries, and a full diestrus period (n = 10) with high progesterone levels and mature, compact, and active CLs in the ovaries.

Pregnancy samples were obtained from females with unwanted pregnancies up to 3 weeks post-coitus submitted to OVH. Pregnancy samples were then staged on the basis of cumulative information gathered from diestrus-compatible cytology, known unwanted breeding plus high progesterone levels and the coexistence of small-sized (<3 cm) uterine swellings. When uterine swellings were not noticed, but knowledge of coitus existed, pregnancy estimated of less than 17 days was determined based on embryo collection by flushing each uterine tube and uterine horns separately as described by Tsutsui et al. [36]. Pregnancies were further divided into