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Immunohistochemical study of IL-18 in canine cystic endometrial hyperplasia

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Introduction and aim. Interleukin 18 (IL-18) is a proinflammatory pleiotropic cytokine belonging to IL-1 superfamily (1). Its presence in the endometrium has been demonstrated in different species, where it varies with the cycle stage. It was also demonstrated to be over-expressed in pregnant uteri (2). In women, IL-18 expression changes in some pathological conditions of the uterus (3). IL-18 synergizes other local cytokines to modulate tissue angiogenesis, tumor progression and inflammation. The aim of this study was to evaluate IL-18 immunoexpression in canine endometrium during late stages of endometrial involution and in Cystic Endometrial Hyperplasia (CEH).

Materials and Methods. A total of 40 dog uterine samples were collected from ovariohysterectomy specimens. After routine processing and staining with haematoxylin and eosin, samples were grouped as postpartal (PP; n=8) or as having CEH (n=32), which were further classified according to Dow´s (4) morphological grades (I and II, sub-clinical; III, with endometritis; IV atrophic and hypertrophic, with pyometra; respectively n= 6, 7, 6, 7, 6). Immunohistochemistry was performed with a specific polyclonal primary antibody raised against canine IL-18 molecule (AF2924; R&D systems), at a 1:100 dilution for 2h. An intensity score from 0 to 3 (negative, mild, moderate, strong) was used in the stroma, inflammatory cells, surface epithelium and glandular and cystic epithelium. Intracellular location of the labeling was annotated apart.

Results. IL-18 immunostaining was detected in both the epithelial and stromal cells, in all stages of CEH *and* in postpartal samples. In postpartal samples, the endometrial stroma showed a strong to moderate intensity of immunoreaction although the epithelia remained negative. In CEH samples, the stroma showed a more intense staining pattern in the deep layers of the endometrium, despite that some variations were found between lesional grades: faint intensity in grade I, strong intensity in grade II and the prevalence of moderate immunoreactivity in grades III and IV. Further, it was also observed a tendency for the loss of expression around the glandular epithelium, particularly in grades I and II. In CEH samples, IL-18 expression was found in both the cytoplasm and/or nucleus of the epithelial cells, and the distribution of positive cells often showed an irregular mosaic pattern in grades III and IV. For most epithelial cells, the positive reactivity was restricted to the nucleus. However, deep endometrial glandular epithelium tends to be negative for the molecule. In the atrophic CEH of grade IV a marked reduction of the endometrial thickness associated to pyometra and compression, made the scoring of the glandular epithelium difficult. The presence of a positive inflammatory infiltrate occurred in all CEH grades but grade I; in CEH clinical stages, infiltrated immune cells were positive for IL18 and predominantly showed mild to moderate immunostaining.

Conclusions. Althought the distribution of positive labelling was quite variable in epithelial cells, the immunolabellingpattern suggest that IL-18 can play a role in the pathology of the CEH process.

References. 1) Lebel-Binay et al., Eur Cytokine Netw 2000;11:15-26. 2) Ashworth et al., Reproductive Biology and Endocrinology 2010;8:33. 3) Luo et al., J Reprod Immunol 2006;72:108. 4) Dow, C. The Veterinary Record 1957;69: 1409-1414.

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