Theriogenology xxx (2016) 1–7

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Expression of 3β-hydroxysteroid dehydrogenase in ovarian and uterine tissue during diestrus and open cervix cystic endometrial hyperplasia-pyometra in the bitch

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ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form 1 February 2016
Accepted 9 February 2016

Keywords:
Canine
Cystic endometrial hyperplasia
Pyometra
3β-Hydroxysteroid dehydrogenase

ABSTRACT

The purpose of this study was to compare the expression of 3β-hydroxystreroid dehydrogenase (3β-HSD) in the uterus and ovary of healthy dogs and those with cystic endometrial hyperplasia and/or pyometra complex (CEH-pyometra). Eighteen female dogs were included in the study. Eleven bitches with open cervix CEH-pyometra were included in the CEH-pyometra group and seven diestrus bitches in the control group. For immunostaining a rabbit polyclonal, one raised against recombinant human type 2 (adrenal/gonadal) 3β-HSD was used. Progesterone (P4) concentrations were not statistically different between the groups. Strongly stained large interstitial cell groups in the ovarian medulla were observed particularly in CEH-pyometra group although these cells in the control group were weakly or moderately stained and existed singly or paired. The expressions of 3β-HSD in luminal epithelium (42.40 ± 22.40% vs. 18.42 ± 13.15%, $P < 0.05$) and glandular epithelium (32.80 ± 27.05% vs. 2.94 ± 7.79%, $P < 0.01$) of endometrium were significantly higher in CEH-pyometra group than those in the control group. The expression of 3β-HSD in CL was higher (29.38 ± 9.58% vs. 22.94 ± 4.97%) in CEH-pyometra group than that of control group although the differences were not significant ($P > 0.05$). Similarly, the significant increase in the expression of 3β-HSD in ovarian interstitial cells (33.86 ± 29.44 vs. 1.13 ± 2.97, $P < 0.05$) was found in CEH-pyometra group compared to the control group. The study revealed that 3β-HSD expression in the endometrium of canine CEH-pyometra was significantly high.

1. Introduction

Canine pyometra is an important disease of intact mature bitches and occurs after estrus [1]. It is thought that there is an association between pyometra and cystic endometrial hyperplasia (CEH) [2]. Cystic endometrial hyperplasia allows bacterial proliferation in the uterus at the end of estrus, and the degenerative process of development of endometrial hyperplasia is linked with formation of pyometra. Because the whole process is mediated by P4, it is considered a disease of diestrus [3,4]. Estrogen and progesterone administration were also linked with development of pyometra [5], whereas pregnancy has a protective effect especially in Rottweiler, Collie, and Labrador retriever breeds [6]. There are two forms of pyometra with either an open or a closed cervix. Bitches with open cervix pyometra present with a vaginal discharge, whereas the

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.02.006

ones with closed cervix pyometra present without a vaginal discharge [7]. There is also information about ovarian steroid hormonal effects in that estrogen opens the cervical canal and P4 closes [8]. In addition, it has been shown that the presence of P4 receptors in the uterine cervix is related to the cervical patency [9]. Progesterone increases secretoric activity of endometrial glands and decreases myometrial contractility, therefore causes closure of cervix [10].

Early diagnosis and appropriate treatment of pyometra are required to avoid disastrous consequences such as endotoxemia and specific renal abnormalities as a result of the effects of endotoxins [11]. In addition, the presence of systemic inflammatory response syndrome could be detected in canine pyometra that is associated with poorer prognosis [12,13].

Steroid hormones such as P4, mineralocorticoids, androgens, and estrogens have a crucial role in the development and growth of most tissues. The biosynthesis of these hormones requires the transformation of delta-5-3β-hydroxysteroids, namely, pregnenolone, 17-hydroxy pregnenolone, dehydroepiandrosterone, and androst-5-ene-3β-,17β-diol into 4-ene-3-ketosteroids, P4, 17-hydroxy progesterone, androstenedione, and testosterone, respectively. The membrane-bound 3β-hydroxysteroid dehydrogenase/5-ene-4-ene-isomerase (3β-HSD) catalyzes that conversion [14,15].

The expression of 3β-HSD was confirmed in the human uterine endometrium by Rhee et al [16] especially in the glandular epithelium and decidua. It was detected in nonpregnant mouse endometrium at metestrus [17]. Moreover Ullmann et al [18] demonstrated the presence of 3β-HSD in the ovarian interstitial tissue, the CL, and the granulosa cells of antral and atretic follicles in the South American opossum. Its expression was even demonstrated in Purkinje cells of the cerebellum in canine distemper virus–infected dogs suggesting its association with demyelination in canine distemper virus infection [19]. Concerning the female dog, 3β-HSD expression in CL during early and late diestrus was presented by Kowalewski et al. [20].

Cystic endometrial hyperplasia-pyometra is a common disorder in dogs, and its pathogenesis is still worth to investigate in detail. Thus in the present study, we examined the expression of 3β-HSD in canine ovarian and uterine tissue during diestrus and open cervix CEH-pyometra complex as well as its relationship with the circulating concentration of P4 to light the possible role of enzymatic activity in the uterus. To our best knowledge, this is the first report concerning the expression of 3β-HSD in canine CEH-pyometra complex.

2. Materials and methods

2.1. Animals

The study was performed in accordance with the principles outlined in Decision no: 2009–12 of Ethical Committee of Animal Research of Turkey. Eighteen privately owned adult female dogs were assigned to the study. Groups consisted of bitches that had not been treated with endogenous progestins or estrogens in the past. All animals were subjected to ovariohysterectomy, either for treatment of CEH-pyometra complex or on request of their owners.

Cystic endometrial hyperplasia-pyometra group ($n = 11$) included bitches with open cervix CEH-pyometra aged 6.23 ± 0.67 years. The breeds were six mongrels, two Pekingeses, one Norfolk Terrier, one Doberman Pinscher, one Golden Retriever. The diagnosis of CEH-pyometra was on the basis of anamneses, physical, vaginoscopic, and ultrasonographic (Falco Vet, Pie Medical Imaging, Maastricht, The Netherlands) examination findings and blood test results.

The control group ($n = 7$) included diestrus bitches aged 2.14 ± 0.32 years and the breeds were five mongrels, one English Pointer, one Dogo Argentino. The ages of the groups differed significantly ($P < 0.01$). Because pyometra is considered a diestrus disease, the control group included healthy bitches confirmed to be in diestrus after vaginoscopic, cytologic, and ultrasonographic examinations [21]. Vaginal smears were obtained from the anterior vaginal wall with the use of a vaginoscope in order not to be contaminated with vestibulum vaginal material. Afterward, they were stained using the Papanicolaou technique [22] and evaluated with a light microscope (Leica Microsystems Inc., Illionis, USA).

The blood samples for P4 assesment were taken from the cephalic vein into heparinized tubes before the surgery for hematologic analyses. The leukocyte, lymphocyte, and monocyte counts were determined using a hemogram (Abacus Vet Junior, Diatron MI LtD, Budapest, Hungary).

2.2. P4 measurement

The plasma was separated after centrifugation at 1550 $\times g$ for 10 minutes, then transferred into labeled microcentrifuge tubes and stored at -20 °C until assayed. Progesterone concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) method using canine-specific commercial kits (MyBioSource, Inc., San Diego, CA, USA). All plasma samples were analyzed twice according to the manufacturer's recommendations. Both intra-assay and interassay variabilities for the assay were less than 15%. The ELISA plate was read at 450 nm on a microplate reader (Digital and Analog Systems, RS 232, Rome, Italy). The concentration of P4 was calculated with reference to a standard curve that was generated by plotting the average optical density (450 nm) obtained from each standard on the horizontal axis versus the corresponding each standard concentration on the vertical axis. Results were expressed as ng/mL of plasma.

2.3. Sample collection and histopathological examination

Both ovaries and cornu uteri of each dog were fixed in 10% neutral formalin immediately after the surgery, dehydrated through an alcohol series and embedded in paraffin. Tissue sections were cut at a thickness of 5 μm and processed for hematoxylin and eosin staining [23]. Sections were histologically examined to confirm healthy tissue and to verify the presence of CEH-pyometra. In addition, staging was performed according to the criteria of Dow