Available online at www.sciencedirect.com
ScienceDirect
Theriogenology
Theriogenology 66 (2006) 1325–1333
www.journals.elsevierhealth.com/periodicals/the

# Lactoferrin expression in the canine uterus during the estrous cycle and with pyometra

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Received 24 January 2006; accepted 26 April 2006

**Abstract**

The expression of lactoferrin, a non-specific antimicrobial defence, in the canine uterus during the normal estrous cycle and in bitches with pyometra was examined. Using polymerase chain reaction analysis, lactoferrin gene transcripts were detected in the endometrium at all stages of the estrous cycle, with the highest levels in estrus. In normal bitches, endometrial lactoferrin mRNA increased from proestrus to estrus ($P < 0.05$). Thereafter, it dramatically decreased from estrus to Day 10 of diestrus ($P < 0.05$), and stayed low at Day 35 of diestrus and anestrus; this was consistent with blood estrogen concentrations. Levels of lactoferrin mRNA were higher in bitches with pyometra than in normal diestrus ($P < 0.05$). With immunohistochemistry, distinct staining of lactoferrin was detected in the luminal and glandular epithelial cells of the endometrium at proestrus and estrus, but little staining was detected at Day 10 of diestrus. At Day 35 of diestrus and anestrus, a partial and weak reaction was present in the same region. In bitches with pyometra, the glandular epithelial cells and many cells in the uterine stroma were strongly stained. Staining cells in the stroma were morphologically similar to neutrophils. No lactoferrin staining was seen in the uterine stromal cells or myometrium in any section. These results suggest that, in the canine uterus, lactoferrin expression is related to the blood concentration of estrogen, and that the dramatic reduction in lactoferrin observed at the early stage of diestrus may impair antimicrobial defense. Also, enhanced expression of lactoferrin mRNA in the endometrium with pyometra may be associated with neutrophil invasion into the uterus to combat the infection.


*Keywords:* Lactoferrin; Uterus; Dog; Pyometra; Estrous cycle

## 1. Introduction

Pyometra is clinically the most important pathologic condition in the uterus of the bitch. It has been suggested that the basis of the disease is endometritis with cystic glandular hyperplasia, and that superimposition of pathogenic organisms often results in the development of pyometra [1,2]. There has been considerable debate over predisposing factors for this disease. The primary determinant is uterine dysfunction due to ovarian abnormality. Many workers have investigated changes in the concentration of ovarian steroid hormones in blood and changes in their receptors in uteri with pyometra [3–5]. However, they were not able to conclude that these are the only factors involved.

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0093-691X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.04.028

The bacterium most frequently isolated from the canine uterus is *Escherichia coli* [2,6,7]. Canine pyometra is most commonly observed in the first half of diestrus; the period during which severe pyometra can be induced by inoculation of *E. coli* into the uterus was limited to the early stage of diestrus [7–9]. Conversely, Watts et al. [10] showed that, in normal bitches, bacteria were always detected in the uterus during proestrus and estrus, but rarely at other stages of the estrous cycle. It has been believed that bacteria enter the uterus through the patent cervical canal during estrus [11]. Although bacteria are present, pyometra rarely occurs during proestrus and estrus. The canine uterus might therefore have some protective capability from bacterial infection during these intervals, which is subsequently lost in the first half of diestrus.

In our previous study, it was suggested that a reduction in Muc1 covering the apical surface of uterine luminal epithelial cells is associated with increased *E. coli* adherence in the canine uterus in the early stage of diestrus [12]. Also, cellular immunity, corresponding to the responses of peripheral blood mononuclear cells to *E. coli* isolated from the uterus of a dog with pyometra and the expression of gamma interferon in response to this bacterium, decreased at the same time in bitches [13]. However, non-specific humoral immunity, such as secretory leukoprotease inhibitor, lysozyme, defensins and lactoferrin which are expressed in the human endometrium and cervix [14,15], have not been investigated in the canine uterus.

Lactoferrin is an iron-binding glycoprotein of the transferrin family, first isolated from milk but found in most exocrine secretions [16,17]. This is an important component of the non-specific antimicrobial defenses of the mammary gland, milk, mucosal surface and neutrophils [18–22]. Lactoferricin, N-terminal residues of lactoferrin, has especially potent bactericidal activity for *E. coli* [20]. It has been reported that mice pretreated with bovine and human lactoferrin survived or tolerated challenge with a lethal or an infectious dose of *E. coli* [23,24], and that piglets prefed with lactoferrin tolerated challenge with *E. coli* lipopolysaccharide (LPS) [25]. The main antimicrobial effects of lactoferrin have been explained as inhibition of bacterial growth by limiting the availability of environmental iron [26], and disruption of the bacterial membrane by binding of lactoferrin to its components (e.g., LPS, porins) [27,28].

In the endometrium in the human [29] and the mouse [30], lactoferrin expression in the follicular phase was greater than in the luteal phase. The mouse and human lactoferrin promoter genes contain estrogen response elements (ERE) [20], and lactoferrin mRNA was rapidly induced by estrogen in these species [31]. However, this gene structure or estrogen response of the lactoferrin gene differs across animal species (e.g., ERE is absent in the bovine lactoferrin gene) [20,32].

A study of the expression of lactoferrin in the canine endometrium may provide a better understanding of the pathogenesis of pyometra in the bitch. Expression of lactoferrin in the canine uterus has not been studied, however. In the present study lactoferrin expression was examined in the uterus of normal bitches during the estrous cycle. The uterus of bitches with pyometra was also examined to evaluate the response of lactoferrin after infection, compared with normal cycling bitches.

## 2. Materials and methods

### 2.1. Animals and sample collection

In total, 30 female dogs were used in this study. Samples from the uteri of 25 purebred beagle bitches with normal estrous cycles and five mixed-breed bitches that had developed pyometra were used.

The normal bitches were housed and used in these experiments in accordance with the Guidelines for the Animal Welfare in Research and Education of Osaka Prefecture University. They were monitored daily to detect the onset of proestrus/estrus and breeding, and signs of estrus were recorded for each bitch for classification into five groups ($n = 5$ per group). These five groups were: proestrus, estrus, Day 10 of diestrus, Day 35 of diestrus, and anoestrus. The stage of the cycle was determined by reproductive records, vaginal cytology, inspection of the ovaries by laparotomy, and circulating progesterone concentrations. Blood samples were collected by jugular venipuncture just before anaesthesia, and the plasma was stored at −20 °C until analysed. Uteri were removed under general anesthesia, induced with ketamine hydrochloride (Ketalar™, Sankyo Co., Ltd., Tokyo, Japan) given i.m., following preanesthetic medication with atropine sulphate (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) and xylazine hydrochloride (Celactal™, Bayer, Tokyo, Japan), and was maintained by inhalation of halothane, nitrous oxide and oxygen.

Dogs with pyometra were all at a clinical stage of the disease and were presented to the Veterinary Teaching Hospital at Osaka Prefecture University. All of these dogs were in diestrus, determined based on the history given by the owner, and on histological examination of the uterus and ovaries. Their uteri were collected at surgery.

After removal, uteri were immediately placed on ice, and two complete rings of uterine horn, each about 1 cm