

# Vasopressin secretion in response to osmotic stimulation and effects of desmopressin on urinary concentrating capacity in dogs with pyometra

Reidun Heiene, DVM, PhD; Ilse K. van Vonderen, DVM; Lars Moe, DVM, PhD; Guro S. Mølmen, DVM; Nina H. Larsen, DVM; Hans S. Kooistra, DVM, PhD

**Objective**—To determine vasopressin (VP) secretory capacity during osmotic stimulation and the response to desmopressin treatment in dogs with pyometra and control dogs.

**Animals**—6 dogs with pyometra before and after ovariectomy and 6 control dogs.

**Procedure**—Urine osmolality (Uosm) was measured during 12 hours. Values measured on the first day defined the basal Uosm pattern. On the second day, dogs were given desmopressin to induce a desmopressin-stimulated Uosm pattern. On day 3, the VP response to osmotic stimulation was examined.

**Results**—Median Uosm on day 1 was 340 mOsm/kg (range, 104 to 1,273 mOsm/kg) and 807 mOsm/kg (range, 362 to 1,688 mOsm/kg) in dogs with pyometra before and after surgery, respectively, and 1,511 mOsm/kg (range, 830 to 1,674 mOsm/kg) in control dogs. Median Uosm during desmopressin treatment was 431 mOsm/kg (range, 168 to 1,491 mOsm/kg) and 1,051 mOsm/kg (range, 489 to 1,051 mOsm/kg) in dogs with pyometra before and after surgery, respectively, and 1,563 mOsm/kg (range, 1,390 to 2,351) in control dogs. In dogs with pyometra, threshold for VP secretion was lower before surgery (median, 340 mOsm/kg; range, 331 to 366 mOsm/kg) than after surgery (median, 358 mOsm/kg; range, 343 to 439 mOsm/kg) or in control dogs (median, 347 mOsm/kg; range, 334 to 360 mOsm/kg). Highest maximum plasma VP values were found in dogs with pyometra.

**Conclusions and Clinical Relevance**—Dogs with pyometra had increased urine concentration in response to desmopressin but not to the degree of control dogs, whereas VP secretory ability was not reduced. (*Am J Vet Res* 2004;65:404–408)

Urinary concentrating capacity depends on the ability of hypothalamic osmoreceptors to respond to changes

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From the Department of Companion Animal Clinical Sciences, Norwegian School of Veterinary Science, PO Box 8146 dep, 0033 Oslo, Norway (Heiene, Moe, Mølmen, Larsen); and the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.154, NL-3508 TD, Utrecht, The Netherlands (van Vonderen, Kooistra). Dr. Mølmen's present address is Veterinærvakta Tretten AS, 2635 Tretten, Norway. Dr. Larsen's present address is Dyrehospitalet Brynsveien 98, 1352 Kolsås, Norway. Drs. Mølmen and Larsen were fifth-year veterinary students at the time of this study.

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Address correspondence to Dr. Heiene.

in plasma osmolality (Posm), atrial and carotid bifurcation baroreceptors to respond to changes in blood pressure or blood volume, and release of the antidiuretic hormone vasopressin (VP) via the hypothalamic-neurohypophyseal axis. Pituitary release of VP is determined mainly by Posm.<sup>1</sup> Additionally, for an animal to concentrate urine, renal medullary hypertonicity must be generated and maintained and there must be an adequate number of functional nephrons with an appropriate response to VP.<sup>1</sup>

Most bitches with pyometra have polyuria-polydipsia (PU-PD) as a prominent clinical feature,<sup>2-6</sup> which typically resolves during the initial weeks after ovariectomy.<sup>7</sup> The mechanism behind PU-PD in bitches with pyometra is not clearly understood. Research has focused on the potential role of the endocrine status of bitches,<sup>8,9</sup> bacterial endotoxins, secondary changes in the kidneys, and changes in VP secretion.

Development of pyometra is specifically related to the typical endocrine pattern of the canine estrous cycle. The estrous cycle of bitches differs considerably from that of females of other species. In bitches, the estrous cycle is characterized by a follicular phase with ovulations, followed by an extended luteal phase that lasts approximately 75 days and a nonseasonal anestrus that lasts between 2 and 10 months.<sup>10</sup> In contrast to most other mammalian species, the duration of the luteal phase in nonpregnant bitches is comparable to that of pregnant bitches.<sup>11,12</sup> Pyometra typically develops during the luteal phase and is associated with cystic endometrial hyperplasia caused by repeated exposure of the endometrium to progesterone. However, plasma progesterone concentrations during the luteal phase are similar in healthy dogs and dogs with pyometra.<sup>13</sup> Exogenous progestagens administered to prevent estrus may also predispose bitches to pyometra.

In humans, pregnancy influences the set point of the osmoregulatory system, sometimes causing an abnormal increase in gestational plasma vasopressinase activity or a gestational form of nephrogenic diabetes insipidus.<sup>1</sup> In dogs, there is evidence for in vivo antagonism between VP and prostaglandins in the kidneys.<sup>14</sup> Prostaglandins are involved in the regulation of several renal functions at the cellular level. The prostaglandin F<sub>2α</sub> analogue sodium cloprostenol induces PU-PD in bitches during the luteal phase.<sup>15</sup> Endotoxin may also play a role in the development of PU-PD in bitches with pyometra. It has been reported<sup>16</sup> that PU-PD may develop in dogs after several weeks of daily injections of endotoxins. Endotoxins can influence the secretion of VP in pigs and goats,<sup>17,18</sup> but to our knowledge, a mechanism for the

effect of endotoxins on the kidneys or hypothalamus during pyometra in bitches has not been described.

Renal tubular abnormalities, including hyaline droplet degeneration, cellular swelling and vacuolization, basement membrane thickening, and accumulation of a lipofuscin-like pigment, have been reported in studies<sup>4,19</sup> of dogs with pyometra. They may all play a role in the development of PU-PD in dogs with pyometra, but studies with control dogs of comparable age are lacking.

Specific staining of neurosecretory cells in the hypothalamus from bitches with pyometra and PU-PD in 1 study<sup>20</sup> did reveal a reduction of staining material believed to represent VP granules, which contrasted with findings in another similar study.<sup>21</sup> Thus, indirect evidence supports the hypothesis that the defect in concentrating ability is not related to a defect in VP production, but plasma VP concentrations and sensitivity of the osmoregulatory system have not been reported in bitches with pyometra. The objectives of the study reported here were to determine the VP secretory capacity in response to osmotic stimulation and the effects of desmopressin treatment on urinary concentrating capacity in dogs with pyometra and control dogs.

## Materials and Methods

**Animals**—Six bitches with pyometra and 6 control bitches of comparable age were included in the study. After physical examination and laboratory tests were conducted, the diagnosis of pyometra was confirmed by use of ultrasonography. Additional inclusion criteria were polydipsia, a stable clinical condition that allowed for postponement of surgery, and willingness of owners to let their dogs participate in the study. Control dogs did not have a history of illness, and results of routine physical examination and laboratory tests did not reveal evidence of health problems. The signalment and clinical data of the dogs with pyometra in this study were similar in terms of clinicopathologic data and duration of disease to groups of dogs with pyometra described in other studies.<sup>4,6,22-23</sup> Two dogs were difficult to classify with respect to PU-PD, which is consistent with another description of dogs with pyometra.<sup>6</sup>

Dogs with pyometra ranged from 7 to 10 years of age, whereas the control dogs were 7 to 9 years old. The WBC concentrations in the dogs with pyometra ranged from  $4.3 \times 10^9$  cells/L to  $61.6 \times 10^9$  cells/L (median,  $18.0 \times 10^9$  cells/L), whereas WBC concentrations in the control dogs ranged from  $3.8 \times 10^9$  cells/L to  $13.0 \times 10^9$  cells/L (median,  $7.7 \times 10^9$  cells/L). Serum creatinine concentration in dogs with pyometra ranged from 62 to 145  $\mu\text{mol/L}$  (median, 72  $\mu\text{mol/L}$ ), whereas concentrations in control dogs ranged from 58 to 88  $\mu\text{mol/L}$  (median, 77  $\mu\text{mol/L}$ ). For the 6 dogs with pyometra, duration of the condition was 1 to 14 weeks (median, 4 weeks), body temperature at the time of initial examination ranged from 37.4° to 40.3°C (temperature was not recorded for 1 dog), and the protein-to-creatinine ratio ranged from 0 to 13 (median, 0). A clinical condition that was more severe than was apparent from the medical history and results of initial examination was discovered in 2 dogs with pyometra. One dog had extreme hypoalbuminemia that changed from 10 g/L on the day of admission to 6 g/L after surgery, although edema or ascites were not evident. That dog developed edema and azotemia during the first few months and uremia within 1.5 years after surgery. One dog was a clinical suspect for vasculitis at the time of testing but recovered uneventfully from surgery. That dog had PU-PD until it was lost from follow-up monitoring 1 year later.

Dogs with pyometra were rehydrated during a period of 2 to 4 hours prior to ovariectomy. During surgery, cystocentesis of the bladder was performed, and a renal biopsy specimen and uterine swab specimen were obtained for bacteriolog-

ic culture. The renal biopsy specimen was stained by use of H&E and periodic acid-Schiff stains and examined by use of light microscopy. Only 2 of the dogs had bacteruria, but bacteria were cultured from the uterus of each of the 6 dogs with pyometra (4 with *Escherichia coli*, 1 with *Streptococcus canis*, and 1 with *Pasteurella multocida*). Results of histologic examination of renal tissues were considered normal in 1 dog with pyometra, whereas the other 5 had evidence of pathologic changes (2 with low-grade membranous glomerulonephritis, 1 with severe interstitial pyelonephritis, 1 with subacute glomerulitis and tubulointerstitial nephritis, and 1 with interstitial nephritis).

**Testing procedures**—Clinicopathologic data were obtained on the day of arrival at the clinic. Urine osmolality (Uosm) was measured every 2 hours for a 12-hour period on day 1 of the study. Dogs had ad libitum access to water during the measurement period. Values measured on day 1 were used to define the basal Uosm pattern.<sup>26</sup>

On day 2, serial measurements of Uosm were performed at the same time points; however, dogs were treated with desmopressin<sup>3</sup> (1 drop in the conjunctival sac at the beginning of the measurement period and again 6 hours later). These values were used to define the desmopressin-stimulated Uosm pattern.

On day 3 of the testing procedure, the VP response to osmotic stimulation was investigated by IV infusion of hypertonic saline (20% NaCl) solution throughout a 2-hour period at a rate of 0.03 mL/kg/min.<sup>27</sup> One dog with pyometra was inadvertently given hypertonic saline solution as a bolus injection, causing maximal VP secretion in the middle of the test procedure.

Blood samples for measurement of plasma VP concentration and Posm were collected at 20-minute intervals from a jugular vein of each dog into chilled EDTA-coated tubes and immediately placed on ice until centrifuged. Plasma was obtained by centrifugation at 4°C and stored at -20°C until assayed. The 3-day testing procedure was performed twice in the dogs with pyometra (before and 1 month after ovariohysterectomy). The testing procedure was performed only once in the control dogs.

**Measurement of Posm and Uosm**—Plasma osmolality and Uosm were measured in duplicate by use of the freezing-point depression technique.<sup>b</sup> Vasopressin was extracted by adding 5.2 mL of chilled (4°C) 96% ethanol to 0.8 mL of plasma; samples were then incubated during end-over-end rotation for 30 minutes at 4°C. After incubation, samples were centrifuged at 5,000  $\times$  g for 30 minutes at 4°C. Supernatant was collected and dried overnight by use of a rapid vacuum concentrator. Extracts were dissolved in 0.8 mL of assay buffer. Mean  $\pm$  SD recovery of VP was 75  $\pm$  1%. Vasopressin concentrations were measured by use of a radioimmunoassay<sup>c</sup> validated for samples obtained from dogs. Serial dilutions of an extract of canine plasma from a dog with a high VP concentration were plotted, which resulted in a curve parallel to the standard curve. Limit of detection of the radioimmunoassay was 1 pmol/L. The intra-assay coefficient of variation was 12% at 8 pmol/L, and the interassay coefficients of variation were 20% at 1.5 and 4 pmol/L and 10% at 8.5 pmol/L.

**Data analysis**—The effect of desmopressin treatment on urinary concentrating capacity was evaluated by calculating the mean of Uosm values for each of the dogs for days 1 and 2. Median values of individual means within each group of dogs were compared for days 1 and 2.

The relationship between Posm and plasma VP concentration was determined by use of regression analysis. The slope of the regression line was used to describe the sensitivity of the osmoregulatory system, and the intercept with a value of 5 pg/mL provided a measure of the threshold value.<sup>28</sup>

Because of the limited number of dogs and wide variability in results among bitches, we did not perform statistical testing between groups with regard to changes in Uosm values, plasma VP concentrations, osmotic threshold, or sensitivity of the VP response.

Table 1—Effects of desmopressin treatment on urinary concentrating capacity and vasopressin (VP) secretory capacity (osmotic threshold and sensitivity of VP secretion) in response to osmotic stimulation in 6 dogs with pyometra before and 1 month after ovariohysterectomy and in 6 control dogs of comparable age

Dog	Mean urine osmality (Uosm) without (day 1) and with (day 2) desmopressin in treatment (mOsm/kg)						Osmotic threshold (mOsm/kg)			Sensitivity (pg/mL)/(mOsm/kg)			Maximum VP concentration in plasma (pg/mL)		
	Pyometra Before surgery		Pyometra After surgery		Control dogs		Pyometra Before surgery	Pyometra After surgery	Control dogs	Pyometra Before surgery	Pyometra After surgery	Control dogs	Pyometra Before surgery	Pyometra After surgery	Control dogs
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2									
1	783	1,491	1,430	1,546	1,393	1,396	336	NA	334	0.37	NA	0.27	11.6	NA	12.8
2	266	329	893	1,680	1,628	2,219	335	369	360	0.22	0.21	0.06	10.9	6.8	6.0
3	1,273	532	1,688	1,546	910	1,621	343	343	350	0.28	0.23	0.18	14.0	12.9	8.0
4	413	583	362	519	830	1,431	366	439	353	0.11*	0.04*	0.17	4.4	2.7	7.7
5	104	169	722	557	1,667	2,351	331	348	335	0.48	0.18	0.45	20.1	7.3	19.3
6	120	168	390	489	1,662	1,506	365	358	343	0.05*	0.12*	0.16	6.4	4.6	6.5
Median	340	431	807	1,051	1,511	1,563	340	358	347	0.25	0.18	0.18	11.3	6.8	7.9

For all dogs, median 12-hour mean Uosm was 901 mOsm/kg (range, 104 to 2,219 mOsm/kg), median osmotic threshold was 348 mOsm/kg (range, 331 to 439 mOsm/kg), median sensitivity was 0.18 (pg/mL)/(mOsm/kg) (range, 0.04 to 0.48 [pg/mL]/[mOsm/kg]), and median maximum VP concentration was 7.7 mg/mL (range, 2.7 to 20.1 mg/mL).

\*Values for the regression line did not differ significantly ( $P < 0.05$ ) from 0.

NA = Not available.

## Results

Median Uosm for the dogs with pyometra on day 1 was 340 mOsm/kg (range, 104 to 1,273 mOsm/kg), compared with a median value of 807 mOsm/kg (range, 362 to 1,688 mOsm/kg) for those same dogs 1 month after ovariohysterectomy and 1,511 mOsm/kg (range, 830 to 1,674 mOsm/kg) for the control dogs. Median Uosm during desmopressin treatment on day 2 was 431 mOsm/kg (range, 168 to 1,491 mOsm/kg) for the dogs with pyometra, compared with 1,051 mOsm/kg (range, 489 to 1,680 mOsm/kg) for those same dogs 1 month after ovariohysterectomy and 1,563 mOsm/kg (range, 1,390 to 2,351 mOsm/kg) for the control dogs (Table 1).

In most dogs, mean Uosm increased during desmopressin stimulation. However, substantial individual variation in response to desmopressin was evident in all groups. In 1 dog with pyometra before ovariohysterectomy, there was a minimal increase in Uosm during desmopressin treatment. After ovariohysterectomy, Uosm decreased during desmopressin treatment in 2 dogs, whereas in a third dog, Uosm was virtually identical before and during desmopressin treatment. In the group of control dogs, Uosm decreased during desmopressin treatment in 1 dog, whereas Uosm was virtually identical before and during desmopressin treatment in another dog (Table 1).

Median threshold value for VP secretion was 340 mOsm/kg (range, 331 to 366 mOsm/kg) and median sensitivity was 0.25 (pg/mL)/(mOsm/kg) (range, 0.05 to 0.48 [pg/mL]/[mOsm/kg]) for dogs with pyometra, compared with 358 mOsm/kg (range, 343 to 439 mOsm/kg) and 0.18 (pg/mL)/(mOsm/kg) (range, 0.04 to 0.23 [pg/mL]/[mOsm/kg]), respectively, for those same dogs 1 month after ovariohysterectomy. For the control dogs, median osmotic threshold for VP secretion was 347 mOsm/kg (range, 334 to 360 mOsm/kg) and median sensitivity was 0.18 (pg/mL)/(mOsm/kg) (range, 0.06 to 0.45 [pg/mL]/[mOsm/kg]). The VP response during osmotic stimulation varied among dogs (Fig 1; Table 1).

In 2 dogs with pyometra, there was not a substan-

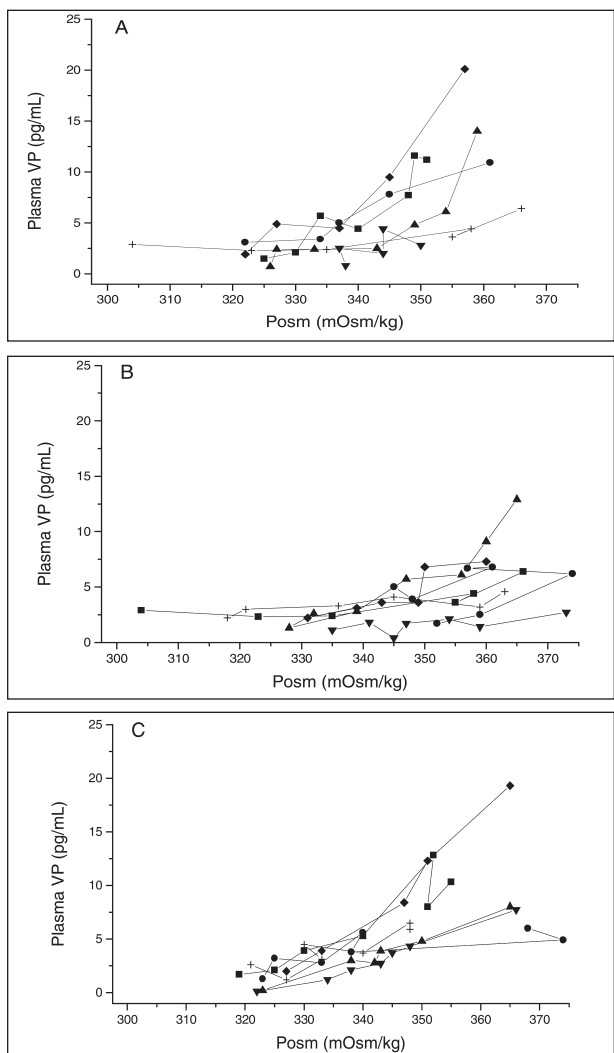


Figure 1—Vasopressin (VP) secretion in response to osmotic stimulation with hypertonic saline (20% NaCl) solution in 6 dogs with pyometra before (A) and 1 month after (B) ovariohysterectomy and in 6 control dogs of comparable age (C). Each symbol represents results for 1 dog. Posm = Plasma osmolality.

tial VP response before or 1 month after ovariohysterectomy. These were the 2 dogs with the most severe clinicopathologic abnormalities. The highest maximum plasma VP values were found in dogs with pyometra before ovariohysterectomy. In all 6 dogs with pyometra, maximum plasma VP values were higher before ovariohysterectomy than 1 month after surgery (Fig 1; Table 1).

## Discussion

Analysis of results of the study reported here revealed that dogs with polyuria attributable to pyometra were able to increase urine concentration in response to desmopressin treatment, but not to the same extent as healthy dogs. A substantial variation among dogs was seen for the Uosm response to desmopressin treatment and VP response to increased Posm. Maximum plasma VP concentrations in all dogs with pyometra were higher before ovariohysterectomy than 1 month after ovariohysterectomy.

Large variation among dogs in the small groups of the study made it necessary to report the results without extensive statistical analysis. Furthermore, this restricted interpretation of group data.

Vasopressin release during osmotic stimulation was comparable in dogs with pyometra and healthy dogs. In some dogs with pyometra, VP release seemed to be increased, but it returned to expected values after ovariohysterectomy. The threshold value for VP secretion was decreased and sensitivity of the VP response was increased in some dogs with pyometra, compared with values for the control dogs. Analysis of the data suggests that VP secretory ability is adequate in dogs with pyometra. Changes in threshold and sensitivity of the osmoregulatory system have been reported<sup>1,28</sup> for several endocrine disorders, including the syndrome of inappropriate VP release.<sup>29</sup> The results observed in our study could potentially result from interference with the endocrine secretory feedback system, a direct mechanical or biochemical interference with VP receptors, or interference with the formation of aquaporin water channels in renal tubular cells.<sup>30</sup> An increase in the VP response caused by osmotic stimulation may also have been attributable to hypovolemia associated with polyuria.<sup>31</sup>

We did not detect a substantial increase in plasma VP concentration during osmotic stimulation before or after ovariohysterectomy in 2 dogs with pyometra. In both dogs, only a small increase in Uosm was detected during desmopressin treatment, excluding deficiency of VP as the sole cause of PU-PD. Values for Uosm did not reach 400 mOsm/kg after ovariohysterectomy in these 2 dogs, whereas values of 700 to 1,700 mOsm/kg were reached after ovariohysterectomy in the other dogs with pyometra. Examination of renal biopsy specimens obtained during ovariohysterectomy revealed tubulointerstitial nephritis and pyelonephritis in these 2 dogs, whereas membranous glomerulonephritis or no abnormalities were found in the other dogs with pyometra. Thus, polyuria after ovariohysterectomy in these 2 dogs may have been attributable to permanent renal damage. Both dogs were in poorer clinical condition than was apparent from the medical history and initial clinical examination. The severity of their con-

dition may explain why these dogs did not have good urinary concentrating capacity during control testing after ovariohysterectomy.

Renal lesions in dogs with pyometra are believed to be temporary because PU-PD in most affected dogs improves within a few weeks after surgery.<sup>7,19</sup> However, polyuria may remain after surgery, as was seen in the 2 dogs of our study. Thus, the question remains as to whether polyuria is a direct result of the renal lesions.<sup>5,16,19</sup> Tubular abnormalities in the kidneys of dogs with pyometra may interfere with the cellular action of activated VP receptors. However, the glomerular and tubular lesions described in dogs with pyometra have typically been based on the results of studies<sup>4,19</sup> in which investigators did not include an age-matched control group. In 1 study<sup>5</sup> with age-matched control dogs, changes were similar in the kidneys of healthy dogs and dogs with pyometra, suggesting that renal lesions in dogs with pyometra are not directly associated with polyuria.

The low Uosm values during serial measurements on day 1 clearly documented polyuria in the dogs with pyometra. After ovariohysterectomy, Uosm improved in 4 of 6 dogs. In control dogs and dogs with pyometra, desmopressin treatment increased urine concentration. Therefore, it can be concluded that the healthy dogs did not concentrate urine to a maximal extent. Although endogenous VP production was sufficient, administration of desmopressin increased urine concentration. Administration of desmopressin did not return Uosm to normal values in dogs with pyometra, suggesting partial renal resistance to VP. These data are compatible with the downregulation of VP receptors, interference with VP receptor function, or interference with the formation of aquaporin water channels in renal tubular cells. Administration of desmopressin 1 month after ovariohysterectomy did not cause Uosm values to improve and be similar to values of the control dogs, suggesting that renal resistance to VP may not have been fully reversed at the time of testing.

Although we did not measure circulating endotoxin concentrations in this study, results of bacterial culture make it unlikely that all of the bitches with PU-PD were endotoxemic. Administration of *E coli* toxin to clinically normal dogs induces decreased renal concentrating ability and polyuria, which persists as long as exposure to the toxin is maintained.<sup>16</sup> Two studies<sup>32,33</sup> have revealed varying prevalence of endotoxemia in bitches with pyometra by use of differing methods for detection. In 1 of those studies,<sup>33</sup> the plasma endotoxin concentration was significantly higher in dogs with pyometra that had more severe clinical effects. Although *E coli* predominates, other bacteria (including gram-positive bacteria) are involved in pyometra, as observed here and in other studies.<sup>4,5,32,34</sup> In some dogs with pyometra, blood endotoxin concentrations are low, and in dogs in which *E Coli* are not isolated, polyuria is also evident.<sup>5,33</sup>

Urine concentration was increased in dogs with pyometra in response to desmopressin but did not reach the Uosm values of the control dogs. Pyometra was not associated with decreased ability to secrete VP. These results are consistent with interference with the

function of VP receptors or interference with the formation of aquaporin water channels in renal tubular cells of dogs with pyometra.

<sup>a</sup>Minirin, Ferring AB, Malmö, Sweden.

<sup>b</sup>Fiske One-Ten osmometer, Fiske Associates, Norwood, Mass.

<sup>c</sup>Arginine-vasopressin RIA, Nichols Institute, Wijnchen, The Netherlands.

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