



## Diagnosis of endometritis in the bitch

*Diagnóstico da endometrite em cadelas*

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### Abstract

Endometritis refers to an inflammation of the uterine mucosa that does not extend beyond the stratum spongiosum. Recent studies indicate it is a common finding in 30-50% of bitches suffering from infertility/subfertility. This disorder is subclinical, and its diagnosis involves sampling the uterus, whether it is by performing a cytology from flushing the organ or histology on collected uterine biopsies. Its pathophysiology remains unclear in 2021. Some cases are associated with cystic endometrial hyperplasia, which leads to disruption in the uterine clearance mechanisms after breeding ; while other appear as a pure inflammatory process. While we don't have all the answers yet, there is no doubt today that these disorders must be included in the differential diagnosis of infertility in the bitch. The work that has already been done on this topic already offers some idea on how to approach these cases in our veterinary clinics.

**Keywords :** canine, infertility, endometritis, uterus

### Introduction

Endometritis refers to an inflammation of the uterine mucosa that does not extend beyond the *stratum spongiosum*. It is recognized as a condition that can lead to infertility in cows and mares, as these endometrial alterations might prevent fertilization and/or embryonic development by modifying the uterine environment. Same has been hypothesized in canines, and recent studies indicate it is a common finding in 30-50% of bitches suffering from infertility/subfertility. However, diagnosing this disorder in canines is currently seen as a challenge. On top of being a subclinical disorder [5], in cows and mares its diagnosis consists in collecting uterine samples for bacteriology and whether cytology or histology... which, in bitches, is definitely more complicated. In the last decade, different techniques have been described to do so in canines. We will review them here and discuss how they can be used to reach a diagnosis in bitches consulting for infertility/subfertility.

### Uterine cytology

These days, transcervical catheterization of the canine uterus under endoscopy is growing in popularity among veterinarians. While the technique was first developed for artificial insemination purposes, it also allows to sample the canine uterus, as shown in this study.

A rigid endoscope can be used to visualize the cervical opening of the bitches and cervical catheterization can be performed using a ureteral catheter (Ureteral CRU ch. 6 223602). The catheter is passed into the uterus and the lumen is flushed using a sterile saline solution (NaCl 0.9% 2mL/10kg). This solution is then re-aspirated and the liquid collected can be used to perform cytology and bacteriology.

In our study, samples were collected essentially during diestrus, as close as possible to when the infertility was diagnosed. The objective was to let the sample coincide with the leukocyte inhibition and suppressed activity of cellular immunity primed by progesterone in the uterus.

While catheterization of the cervix in itself was easy to perform, aspiration of the liquid infused inside the uterine lumen was not. In some cases, flushing had to be performed several times to obtain enough liquid for investigating the uterine status. The low volume recovered could lead to a misinterpretation of the results as the concentration of the different cell population varied dramatically according to the volume recollected.

In this study, endometritis was diagnosed in 10/26 infertile bitches. Among those 7 of them had a positive bacterial culture. That was in disagreement with studies done later on the same topic, where no

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bacteria were found in bitches suffering from endometritis. It is possible that bacterial contamination from the vagina might have occurred during the transcervical catheterization.

Also, four bitches that were sampled this way developed a pyometra later in diestrus, probably because of a higher sensitivity of the endometrium during diestrus. Therefore, anytime this type of sample is performed, it appears important to administer a medical treatment to prevent development of pyometra (aglepristone or PGF<sub>2</sub>-alpha) following the procedure.

### **Uterine biopsies**

#### *Full-thickness surgical uterine biopsies*

Mir et al performed surgical uterine biopsies by laparotomy in bitches suffering from infertility/subfertility. A midline laparotomy was performed under general anesthesia. A macroscopic examination of the uterus and ovaries was then performed by visual inspection and careful palpation. If a suspected lesion was found, a 1cm long wedge sample was collected at the lesion site with a No.10 scalpel blade and a forceps, ensuring that the endometrium has been sampled. When no macroscopic uterine lesion was found, the surgical biopsy was performed in the middle of each uterine horn using the same technique. A sterile swab was then introduced into each uterine horn for testing for infectious agents (bacteriology and PCRs).

In this study, 7/21 bitches were diagnosed with endometritis; no bacteria was isolated from the uterine lumen of those bitches. For the same reasons as previously mentioned, samples were preferentially done during diestrus and in order to prevent development of pyometra, bitches biopsied during diestrus were preventively treated with aglepristone (Alizine, Virbac), 10mg/kg after surgery (D0) and again on D1 and D7 or dinoprost (Lutalyse, Pfizer), 150 µg/kg/day IM for 5 consecutive days.

Today, some authors do recommend full-thickness uterine biopsies in order to properly diagnose endometritis as well as other subclinical uterine disorders in canines. The technique appears more accessible as any veterinary practice performing routine surgery could potentially sample the uterus this way. It is interesting to note that some complications can still occur: Mir et al reported that in one bitch, a uterine stenosis was found at the site of the biopsy. The main limitation lays in its invasive nature though, which might definitely refrain some owners.

#### *Transcervical endometrial biopsies*

Christensen et al sampled 20 intact adult bitches by performing transcervical endometrial biopsies. A rigid 43cm endoscope with a 5 Fr instrument port was used to visualize the cervix, and then a 5 Fr biopsy instrument was passed transcervically into the uterus.

Transcervical endometrial biopsies were obtained and compared to full-thickness uterine biopsies they obtained from the same bitch. The authors found no difference in the consistency of detecting uterine lesions.

While Christensen et al performed their uterine biopsies blindly, those samples can also be obtained while performing a hysteroscopy as described by Fontaine et al. In this study, the cervix of the bitch was visualized under endoscopy using a rigid uretero-renoscope (27002K; 9.5Fr, 43cm length, Storz Germany) and transcervical catheterization performed using a ureteral catheter (Ureteral CRU, 5Fr, Rusch, France). Filtered air was insufflated to allow distension of the cervix in order to pass the scope inside the uterus and visualize the endometrium. Bitches were systematically sedated before examination, using medetomidine (Domitor, 0.5 µg/kg IV).

If several authors reported complications after manipulations or collection of samples at the uterine level during diestrus, we did not report any particular side effects as long as the same precautions mentioned while performing full thickness surgical biopsies were taken.

#### *Interpretation of uterine biopsies*

The main limitation we see when it comes to uterine biopsies today is how those are interpreted. Many pathologists indeed might not be accustomed to this. It therefore seems important to work with pathology labs that have some experience in reading endometrial biopsies, in mares for instance.

Mir et al also discussed in their paper the importance of the type of fixative agent that is used for this type of sample. Some pathologists might ask for the samples to be fixed with Bouin's solution to



avoid formation of artifacts, while others might be totally fine with using 10% formalin. Before performing a uterine biopsy, it therefore appears important to identify the pathology lab that will be used for interpretation and to discuss with the pathologist what will be his requirements. Because of the invasive character of the procedure, this discussion must happen before the sampling is performed.

### **Ultrasounds?**

In Fontaine et al and Mir et al, the bitches that were diagnosed with endometritis had an ultrasounds examination prior to the sampling of the uterus and this scan performed during diestrus did not reveal anything in any of those bitches.

Freeman et al however used B-mode ultrasonography to investigate the prevalence of free fluid in the uterine lumen in bitches, as delayed clearance of the fluid has been documented in bitches suffering from cystic endometrial hyperplasia and is described as a risk factor for development of endometritis in mares.

They found that the presence of uterine fluid either on D5 or D14 after ovulation was significantly associated with a reduced likelihood of pregnancy. In pregnant bitches, uterine luminal fluid present on D0 was associated with a smaller litter size.

They proposed that ultrasonographic detection of uterine luminal free fluid after mating may be a useful prognostic indicator for pregnancy outcome and may allow detection of females prone to develop post mating-induced endometritis.

Recently, England et al reported that Doppler ultrasound could also help here. In normal bitches indeed, there is a significant increase in uterine artery blood velocity and a decrease in the resistance index after mating, indicating vasodilatation. In bitches with CEH, the baseline resistance index has been reported to be significantly higher than in normal bitches; bitches suffering from CEH also show a decrease in the resistance index of lower magnitude than what it is reported in normal ones. They hypothesize that this lower baseline uterine perfusion and this blunted vasodilatation response to mating in bitches with CEH can contribute to the development of mating-induced endometritis by disturbing the normal post-mating clearance mechanisms in place in the uterus. Doppler ultrasound could therefore help spot bitches that might be predisposed to develop this kind of disease.

### **And what about therapeutic diagnosis?**

#### *NSAIDs*

Based on the results of previous studies, Borges et al hypothesized that in contrast to what had been suggested in previous studies, infectious agents may rarely be present inside the uterus, even in cases of endometritis. Thus it may be useful trying to lower endometrial inflammation by using non-steroidal anti-inflammatory drugs (NSAIDs). Recent studies on the localization of COX-2 enzymes in bitches with endometritis actually showed that in those cases, an increased expression of this enzyme in the luminal epithelium, glandular epithelium and stromal cells of the uterus was noted. COX-2 being involved in the pathophysiological mechanisms, the use of anti-COX2 NSAIDs appears therefore as a potential solution when dealing with those cases.

In this study, 15 bitches with a history of infertility had an intra-uterine insemination twice between 1 and 3 days post-ovulation. All bitches were treated with anti-COX2 NSAID (carprofen or meloxicam) in the 5 days following the second insemination. Altogether, 12/15 bitches gave birth to a normal litter (mean litter size was 7.7 puppies).

Nizanski et al followed the same line of thoughts. They administered meloxicam from D2-D4 post ovulation and D15-17 post-ovulation after intra-uterine insemination was performed. 10/23 bitches that were suffering from infertility/subfertility did conceive following this treatment (mean litter size was 6.8±4.5 puppies).

#### *Antibiotics ?*

Some authors have mentioned that “mating-induced endometritis in bitches with endometrial hyperplasia appears to affect fertility by reducing the uterine vasodilatory response to mating and delaying clearance of uterine fluid as a result of decreased uterine contractions, but the effect can be ameliorated in part by the postmating administration of antibiotics.” They have recently reported that they



studied the outcome of 50 bitches with ultrasound-detected CEH. All those bitches were treated with oral amoxicillin/clavulanic acid twice daily for 4 days starting on the day of breeding. Following this, they reported a 94% pregnancy rate, with a mean litter size of  $8.1 \pm 0.3$  puppies, similar to expected breed averages. Their hypothesis is that the antibiotic treatment helped with the early resolution of post-breeding endometritis.

In 2021, subclinical endometritis has to be included in the differential diagnosis of infertility in the bitch. Today, there is no doubt about this. Many questions however remained unanswered, and its pathophysiology is still unclear. While most recent publications recommend to perform surgical biopsies as a diagnostic tool, the fact that it is invasive and complex does refrain the clients as well as some practitioners. Some have turned to the therapeutic diagnosis option, which obviously still leaves some unanswered questions. If on the bright side, there are definitely multiple options to consider to reach a diagnosis, further studies are definitely needed to clarify and codify how to approach this disorder in canines.

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