Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Histopathological Classification of Canine Endometritis

Jing-Xuan Wang
Northeast Agricultural University
Yao Xu
Northeast Agricultural University
Hao-Ran Wang
Northeast Agricultural University
Zhi-Yuan Liu
Northeast Agricultural University
Qi-Da Zhao
Northeast Agricultural University
Rui-Fang Liu
Northeast Agricultural University
Xin-Yu Wang
Northeast Agricultural University
Xu-Dong Song
Dr. Song Pet Hospital
Jian-Hua Xiao (Xiaojianhua@neau.edu.cn)
Northeast Agricultural University

Research Article

Keywords: Dog, Endometritis, Pathology, Classification

Posted Date: May 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-521483/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: Endometritis is a common disease of the reproductive system in canine dams. The purpose of this study was to develop a standardized histopathological diagnostic classification criteria for canine endometritis. Forty bitches were ovariectomized, and their endometrial tissues were pathologically classified by imaging, anatomical and hematological diagnostic evaluation. Expression of IL-1β, IL-6, and IL-8 mRNAs in uterine tissues were also detected by RT-PCR.

Results: Endometritis was grouped into one of four classifications: Cystic endometrial hyperplasia (CEH) (no inflammation), mild inflammatory infiltrate, severe inflammatory infiltrate (hyperplastic) and severe inflammatory infiltrate (atrophic). Direct comparison of the experimental results was complicated by the varying degree of disease severity in individual animals and the diagnostic criteria currently in use by researchers.

Conclusions: It is concluded from this study that most of the dogs with clinical manifestations were characterized by endometrial hyperplasia with severe inflammatory infiltration, while the endometrial hyperplasia with mild inflammatory infiltration was not significant. In addition, endometrial cystic hyperplasia-like lesions were also found in asymptomatic bitches who underwent physiological sterilization.

Background

Endometritis is an inflammatory condition of the endometrium of the female reproductive system. It is the most common and complex pathological disease in female dogs, which has a profound impact on the reproductive performance of female dogs [1].It is generally believed that the primary factors leading to development of the disease include disorders in reproductive hormone endocrine and receptor regulation, infections with pathogenic bacteria, and genetic predisposition [2, 3]. The disease usually occurs in female dogs after estrus, mating, miscarriage and childbirth with a higher incidence in older dogs [4]. Disease incidence has been reported at anywhere from 15% – 50% in dams older than 8 years old [5, 6], and frequently occurs in dogs with repeated infertility and no reproductive history [3, 5]. Early diagnosis of uterine disease is very important, as it can reduce the development of fatal sepsis [7].

The classification of endometritis is based on vaginal mucus viz., mild, mucopurulent, purulent and endometritis with mucopurulant or purulent fluid in uterus and severity of infection viz., acute, sub clinical and chronic endometritis [7].In general, acute endometritis occurs frequently in the postpartum period, and the diagnosis can be made based on clinical presentation and testing of vulvar secretions. Chronic endometritis can be diagnosed based on vaginal and rectal examination. The vaginal discharge is presented during estrus and laboratory vaginal testing [8]. Gross pathological examination of the uterine tissue of dogs with endometritis showed slight to severe dilatation of the uterine horns and an indeterminate amount of inflammatory exudate in the uterine cavity [9]. Different degrees of swelling and congestion were observed in the uterine wall, and significant necrotic lesions, sometimes accompanied

by hemorrhage, were observed in severe cases. Histopathologic examination of tissue sections showed varying degrees of degeneration of the intimal epithelium, sometimes with focal necrosis and shedding [10]. The lamina propria had varying degrees of inflammatory cell infiltration, stromal cells, edema, and necrosis. When the uterus has a severe infection, inflammation can spread to the uterus, which can result in dilatation of the uterine glands [11].

Early pathological studies have found that in the chronic endometritis, the probability of hyperplastic lesions in the uterine glands is greater, which leading to suppurative degeneration occurs in the deterioration of uterine inflammation, with a large amount of pus accumulated in the uterine cavity [12]. Therefore, chronic suppurative endometritis is usually defined as CEH/pyometra complex [13, 14].CEH has an increased number of endometrial glands, increased size, and thickened endometrial hyperplasia. Hyperplastic and hypertrophic endometrial glands have enhanced secretory function. Depending on the viscosity of the uterine contents, it is determined whether sterile fluid accumulates in the glands and uterine cavity, thus forming hydrops [1, 15, 16]. Only when the uterine contents are infected, CEH shows more obvious clinical symptoms. For example, the mucosa sometimes causes abdominal distension, which is called pyometra [17]. In a study published by De Bosschere et al., it was demonstrated that endometrial hyperplasia and pyometra were indeed two independent disease entities. The authors also developed specific histological criteria according to the pathological features, such as Endo/Myo ratio, the degree of inflammatory response, and the degree of changes in the uterine glands. In their study, the endometrium of 4 bitches showed mild inflammatory cell infiltration, and the percentage of glandular cavity to endometrial area was 15.60% ± 4.09. The observed Endo/Myo ratio was 1.26 ± 0.29, which classified this group of cases as the endometritis group. The endometrium of 23 bitches showed severe infiltration with inflammatory cells. These inflammatory cells were widely present in the uterine cavity, endometrial glands, endometrial stroma, and uterus. Cyst formation accounted for 39.2% ± 15.60% of the endometrium, and the Endo/Myo ratio was 1.20 ± 0.51. These cases were classified as having pyometra. The inflammatory reaction within the endometrium and uterus was severe in 3 bitches. The percentage of glandular cavity to endometrial area was 3.60% ± 3.34. Histologically, there was severe endometrial atrophy. The Endo/Myo ratio was 0.12 ± 0. This group of cases was classified as (atrophic) pyometra. On the basis of the observed histological alterations the different cases could be allocated to 7 different classes. The groups mild CEH, severe CEH, endometritis, (hyperplastic) pyometra, (atrophic) pyometra and mucometra contain clinically healthy bitches and ill bitches with no uterine inflammation [18]. According to the histological classification criteria used by De Bosschere et al., the presence or absence of clinical signs in the affected dogs, and B ultrasound imaging and hematology. The cases were divided into two groups: dogs free of any clinical signs (including healthy, endometrial hyperplasia) and those exhibiting clinical signs (including no inflammation, mild inflammatory infiltration, proliferative severe inflammatory infiltration, and atrophic severe inflammatory infiltration).

In addition, Bukowska et al. found that IL-8, IL-1α, IL-1β, IL18RAP, IL1RN and IL-6 were up-regulated anywhere from 11 - 77 fold, depending on the gene and animal, in the uterus of dogs with suppurative endometritis by microarray analysis [19-21]. Therefore, the content of cytokines in tissue and serum directly or indirectly reflects the degree of uterine inflammatory response. Referring to the research

method, RT-PCR was used to detect the mRNA expression levels of IL-1 β, IL-6 and IL-8 in full thickness tissue samples that were either healthy, had proliferative severe inflammatory infiltrates, and those with atrophic severe inflammatory infiltrates [22]. Variable inflammatory infiltration was observed in different types of endometritis.

At present, the research on canine endometritis in China mostly focuses on cases, epidemiological and bacteriological surveys, clinical diagnosis, and treatment. The associated uterine gross- and histo-pathological studies, and cervical opening and closure data are mostly descriptive. Therefore, in addition to the routine investigation of cases and clinical diagnosis of the affected dogs, this study focused on the gross and histological classification of the uteri of 30 confirmed and pathological hysterectomies alongside 10 asymptomatic and spayed healthy dams. Endometritis was characterized here based on the uterine inflammatory response and other lesion characteristics to provide a molecular reference to enhance disease prevention and control programs.

Results

Clinical symptoms

According to the clinical investigation, 30 dogs presented with varying degrees of clinical manifestations at the time of treatment, including depression, loss of appetite, fever, thirst and greed, polyuria, abnormal vulvar secretions, increased abdominal circumference, gastrointestinal disorders and other signs. Among them, 70% had an open cervix and 30% had a closed cervix. The dogs with a closed cervix had a poor presentation condition.

Ultrasonography

Figures 1A and 1B are typical B-ultrasound-confirmed image maps in this case collection[23]. The uterine horns were significantly dilated, and dark regions (shown by white arrows) were observed in the uterine cavity, suggesting uterine fluid. In panel 1A, the uterine wall was significantly hyperplastic and thickened (shown by red arrows), and the free side of the endometrium was wrinkled in appearance. In Figure 1B, the dark areas in the uterine cavity account for a relatively large area, and the uterine wall is significantly thinned (shown by the red arrow) .

Endo/Myo Ratio

Based on the clinical presentation, each of the 40 cases was divided into one of two groups, those with clinical endometritis and those that were healthy. Through the measurement of the Endo/Myo ratio, it was observed that the ratio of all cases in this test was within the parameters described by DeBosschere et al. Therefore, the two groups of patients were further classified anatomical, pathological and hematological diagnosis. For the no clinical signs group (healthy class, Endo/Myo ratio was 0.753 ± 0.044, n = 7; endometrial hyperplasia class, Endo/Myo ratio was 1.057 ± 0.048, n = 3) and clinical signs group (no inflammation, Endo/Myo ratio was 1.057 ± 0.048, n = 1; mild inflammatory infiltration,

Endo/Myo ratio was 0.940 ± 0.172, n = 9; proliferative severe inflammatory infiltration, Endo/Myo ratio was 1.255 ± 0.234, n = 12; atrophic severe inflammatory infiltration, Endo/Myo ratio was 0.474 ± 0.146, n = 8).

Gross pathological examination

In Figure 2A, an example of a healthy uterus from this study is presented, the appearance of uterine tissue is small, the uterine horns have a uniform diameter, no bulging, a smooth endometrial surface, and a healthy pink color. Figure 2B shows an uterus with endometrial hyperplasia and a small uniform diameter with no apparent bulging appearance of the uterine horn. The uterine wall was smooth with normal coloration, and there was no endometrial proliferative lesions were observed in the uterine wall. These observations were not significantly different from that of the healthy uterus at the gross observation level.

Figure 2C shows an inflammation-free uterus, and such cases originate from affected dogs with abnormal secretions from the vulva. The uterine horn was bulging slightly, and the uterine cavity contained turbid fluid. No obvious lesions were present on the surface of the uterine wall. Figure 2D shows mild inflammatory cells infiltrating the uterus, insignificant uterine horn bulge, less uterine cavity content, darker uterine wall color, and grain obviously. Figure 2E shows a proliferative severe inflammatory infiltrate of the uterus with exposed endometrium, which is covered with transparent or translucent vesicles of different sizes on the endometrial surface, accompanied by many papillary vegetations. Several blood clots were observed attached to the endometrial surface. Figure 2F shows atrophic severe inflammatory infiltration of the uterus, and dissection shows endometrial atrophy in the uterine horn with dark red honeycomb depressions on the surface.

Histopathological examination

Figure 3A shows a representative healthy uterus with intact endometrial epithelium and a typical monolayer columnar epithelial structure on microscopic examination. The number of endometrial lamina propria glands is small with typically sized glands. The glandular epithelial cells are cuboidal, the structure is intact without destruction, and the glands contain an appropriate amount of secretions. The stromal cells in the lamina propria are evenly distributed, devoid of inflammatory cells, and evidence of intrastromal vessels congestion is absent.

Figure 3B represents the characteristic proliferative lesions in the endometrium. The structure of the intimal epithelium was intact, and some glands of lamina propria showed different degrees of dilation. The lamina propria is mostly occupied by both superficial and deep uterine glands, with the area varying greatly between individual glands. Although the glandular dilation was large, the structure was intact, with glandular epithelial cells transitioning slightly from cuboidal to flat, and reduced stromal cell composition.

Figure 3C shows an inflammation-free uterus with mild endometrial atrophy, intact epithelial structure. The lamina propria uterine glands were uniformly distributed, and no significant dilatation was observed.

There was a significantly reduced stromal cell composition, and no uterine lesions or inflammatory infiltrates were noted upon microscopic examination. Figure 3D shows mild inflammatory cell infiltration of the uterus, occasional partial detachment of the endometrial epithelium, and flat folds with dilation of the superficial glands of the lamina propria. Marked congestion of blood vessels was observed along with local inflammatory cell infiltration in the stroma. Figure 3E shows a proliferative uterus with severe inflammation. Complete detachment of the endometrial epithelium and extreme dilatation of the lamina propria uterine glands. This resulted in glandular rupture into adjacent glands and infolding into the glandular lumen to form a labyrinthine view. In addition, morphological hypertrophy with vacuolation of glandular epithelial cells was observed. Figure 3F shows atrophic severe inflammatory infiltration of the uterus. Most of the endometrial epithelium is necrotic and detached, and the lamina propria uterine glands are extremely atrophic with inapparent lumen. Stromal cells are reduced, and the entire thickness of the endometrium is covered with a large number of inflammatory cells.

Complete blood count (CBC) value

WBC test results. In the detection of 30 dogs, the proportion of the abnormal total white blood cell count and the abnormal neutrophils were high. In addition, among the indicators with abnormal values, except the number of lymphocytes and eosinophils, the proportion of abnormal increase was greater than the proportion of abnormal decrease. See Table 3 for the variation range and detection value distribution of each indicator.

RBC test results. In the detection of 30 dogs, the number of cases with normal index was more than the number of cases with abnormal index. The highest number of cases had abnormal red blood cell counts. See Table 4 for the variation range and detection value distribution of each indicator.

Expression of the pro-inflammatory cytokines IL-1β, IL-6 and IL-8

Figures 4A, 4B, and 4C show that the mRNA expression levels of IL-1 β, IL-6 and IL-8 in uterine samples with histological features of inflammation were significantly higher than those in healthy uterine tissue samples. Among them, the three genes in the uterine tissue of endometrial proliferative/atrophic severe inflammatory infiltration were significantly increased compared with the other two groups ($p < 0.001$). Expression of IL-1β and IL-8 genes in the uterine tissues with mild inflammatory infiltration were significantly and extremely significantly increased compared with the healthy group ($p < 0.05$ and $p < 0.01$), respectively. In uterine samples with severe inflammatory infiltration, IL-1 β and IL-6 mRNA in the endometrial atrophy type were significantly increased ($p < 0.05$ and $p < 0.001$), respectively. Expression levels of IL-8 were not significantly changed.

Pathological classification

After testing the uterine tissues of 10 clinically asymptomatic healthy dams who underwent sterilization, and 30 clinically symptomatic dogs with endometritis in terms of clinical symptoms, imaging

examination, hematological examination, and histopathological examination. The disease cases were divided into 2 groups and 4 sub-group classifications, which are outlined in Table 5.

Discussion

The most common clinical signs of endometritis in this survey were abnormal vaginal discharge, anorexia, depression, abnormal secretions in the vulva, and increased abdominal circumference [6]. In addition, combined with the onset time and clinical signs of the affected dogs, it can be inferred that the affected dogs have progresses to chronicity. Along with endometritis, there were 2 cases of abnormal estrous cycles, suggesting a possible that genetic, toxicological, and/ or environmental factors leading to hormonal dysregulation and the resulting diseases [24]. Most studies have found that abnormal regulation of reproductive hormones can promote the occurrence of mammary tumors, and the incidence of non-sterilized dams is 3 – 7 times higher than that of sterilized dams. Thus, there appears to be a certain correlation between dysregulation of reproductive hormones and the occurrence of endometritis [25]. In addition, the clinical features of elevated body temperature in affected dogs are atypical, albeit still within the normal range in most cases. Of the 9 affected dogs with a closed cervix, 6 had elevated body temperatures, with the highest body temperature of 40 ° C. Conversely, fever was not observed in dogs with an open cervix.

When diagnosed clinically by ultrasound, the healthy uterine wall is generally moderately or hypoechoic. Because the canine uterus is thin and small, it is typically only feasible to image during estrus, pregnancy, and when lesions occur [26]. The uterus is located on the dorsal side of the bladder, and in the early stage of endometritis, the uterine horn is not significantly dilated, making pathological observation of the uterine wall difficult. When there is fluid accumulation in the uterine cavity, images show a well-defined liquid dark area near the bladder. In this case, the diameter of uterine horn, liquid dark areas with different area ratios in the uterine cavity, and pathological changes of hyperplasia and thickening or atrophy and thinning of the uterine wall were also observed in the B ultrasound image. The uterine wall was significantly thinned in the free side of the endometrium. Therefore, conducting comprehensive laboratory panels on the affected dogs in clinical practice not only provides an auxiliary reference for the disease development and the degree of canine endometritis, but also provides a scientific and accurate basis for the establishment of the physical condition and treatment plan of the affected dogs. Ultimately, this may substantially improve the prognosis of the affected dogs.

The normal development of the endometrium during the estrous cycle in dams has been well and properly described by Barrau et al [27]. We can see that from proestrus to early estrus, the thickness of the uterine wall showed a trend of first increase and then decrease. At the same time, both the endometrium and the uterus follow this evolutionary process [28]. In proestrus, Endo/Myo ratio is high due to edema and endometrial hyperplasia. During diestrus, Endo/Myo ratio decreased significantly, and the ratio increased slightly during metestrus and anestrous. From estrus to the early stages of metestrus, the uterine glands divide and proliferate, become hypertrophic, and form highly branched and curled uterine

glands, along with hypertrophy of uterine glandular cells. The size of uterine glandular cells in metestrus decreases faster than that of the uterine glandular cavity, which leads to the development of glands [18].

In this study, the endometrial/uterine ratio of mild and severe inflammatory infiltration as well as endometrial hyperplasia was greater than was observed in healthy uteruses, and the endometrial/uterine ratio of severe inflammatory infiltration to endometrial atrophy was smaller than that of a healthy uterus. However, the results also contain large individual differences. Hyperplastic severe inflammatory uterine infiltrate is the result of glandular deformation and fibroblast stromal proliferation and inflammatory reactions. DeBosschere et al. performed histological examination of the uterus of 26 healthy bitches and 42 bitches with suspected pyometra using a computerized image analysis system, some of which had clinical signs of pyometra. However, inflammatory responses were not observed in the uterus, and the authorsconcluded that such bitches would be misdiagnosed as endometritis, which confirmed the difficulty of diagnosing endometritis by simple clinical examination [18]. In this study, among 30 dogs with relevant clinical signs and liquid contents in the uterine cavity as determined by B ultrasound examination, one dog had no inflammatory reaction in the uterus by histopathology, and no other characteristic lesions, but atrophy of the endometrial layer. This situation is consistent with what was reported by DeBosschere et al [18]. The remaining uterine tissue samples from affected dogs showed mild to severe inflammatory infiltrates. Among them, the uterine histopathology of mild inflammatory infiltration was similar to that described by DeBosschere et al., but the endometrial to thickness ratio was not significantly increased [18]. In uterine tissue samples with severe inflammatory infiltration, diffuse inflammatory cell infiltration was observed throughout the endometrial layer and, in some cases, the inflammatory response in the endometrial layer often spreads to the myometrium. According to the characteristics of the lesions, they are divided into two categories: proliferative and atrophic. Hyperplastic uterine lesions include severe dilation of the uterine glands, and the endometrial to uterine thickness ratio and radius are significantly increased. Compared with the proliferative type, atrophic uterine glands were severely atrophic, and the ratio of endometrial thickness to uterine thickness was significantly reduced. The above findings corroborate those reported by DeBosschere et al [18, 29].

In this study, gene expression of IL-1β, IL-6 and IL-8 in uterine samples with histological characterization of inflammation were significantly increased. The gene expression of IL-1 β and IL-6 in endometrial atrophic cases was significantly increased in uterine samples with severe inflammatory infiltration. The results of IL-6 gene expression were intermediate between endometrial proliferative and atrophic types of uterine tissues with severe inflammatory infiltration were similar to those of Hagman et al[29, 30].

The ideal clinical classification method should be based on the natural history of the disease, the degree of lesion infiltration, the severity of symptomsclinical signs, and the final outcome of the affected organ., and The diagnosis should guide clinical treatment by reflecting based on the severity, progression, and prognosis of the lesion. Although the classification of endometritis has undergone many years of in-depth evolution, and there is still no comprehensive and satisfactory classificationa clear classification of the spectrum of disease presentations is lacking. The new classification proposed here may be carried

out based on histopathology, imaging and molecular biology. It is believed that the study of endometritis classification will be further developed and refined in the future.

Material And Methods

Animals

Ovariohysterectomy was performed in 40 adult female dogs (ranging in age from 9 months to 10 years) of different breeds, either for the purpose of spaying (n = 10) or because of clinical signs indicative of endometritis (n = 30). Clinical symptoms considered indicative for endometritis were depression, fever, decreased appetite and even waste, increased desire to drink, frequent urination, abnormal secretions in the vulva, increased abdominal circumference, gastrointestinal disorders and other manifestations.

Samples

Samples of both uterus horns were collected at 3 randomly selected places and fixed in a 10% phosphate-buffered formaldehyde solution for 48 hours and thereafter were processed routinely. They were embedded in paraffin, sectioned at 10 um and stained with haematoxylin-eosin (HE). An endometrium/myometrium (Endo/Myo) ratio was observed and calculated under an optical microscope (OLUMPUS, BX53, Japan). The relative changes in thickness of endometrium and myometrium in pathological samples were compared with normal uterine samples [18]. On the basis of histologicai criteria, the bitches were divided into 5 groups, as described in the results section and as shown in Table 5.

Blood Samples

One milliliter of venous blood was collected from dogs and anticoagulated with dipotassium EDTA salt. The white blood cells and erythrocytes from 30 patients were immediately counted and analyzed using the BC-5000vet automatic veterinary five-part differential blood cell analyzer.

RT-qPCR Assay

Total RNA was isolated from canine uterine tissue using the TRIzol kit (Invitrogen, Carlsbad, CA, USA), and the concentration of RNA was determined by micro absorptiometry [22]. Reverse transcription was performed using 1 μg of total RNA and the master mix in Table 1 to amplify cDNA samples. The primer sequences used are presented in Table 2. The PCR cycling conditions were as follows: one cycle of 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, and 20 s at 60° C. All reactions were performed in triplicate. Amplification data were analyzed using the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH expression as an internal control [31].

Statistical analysis method of trial data

All RT-qPCR data were statistically analyzed using the SPSS 17.0 software. At the same time, GraphPadPrism7.0 software was used for statistical processing, and one-way analysis of variance was performed on the data. All data are expressed as mean ± standard deviation (SD). Analysis of significance were evaluated using t-test or ANOVA. Values with $p<0.05$ were considered statistically significant. All data are representative of 3 independent experiments.

Abbreviations

CEH: Cystic endometrial hyperplasia; Endo/Myo: Endometrium/myometrium;

CBC: Complete blood count ;HE: haematoxylin-eosin; WBC: Total leukocyte count; Neu: Neutrophil count; Lym: Lymphocyte count; Mon: Monocyte count; Eos: Eosinophil count; Bas: Basophil count; RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PCT: Plateletcrit.

Declarations

Acknowledgement

We thank Yao Xu for her contribution to this research.

We thank the Dr. Song Pet Hospital (Harbin, China) who contributed cases to this research.

Author' contributions

All authors have directly participated in the planning, execution & analysis of this study. Jing-Xuan Wang, Yao Xu, Hao-Ran Wang and Xu-Dong Song performed animal management and cell experiments. Zhi-Yuan Liu, Qi-Da Zhao, Rui-Fang Liu and Xin-Yu Wang collected and analyzed the data. Jing-Xuan Wang ,Yao Xu and Jian-Hua Xiao were responsible for experimental design and manuscript writing. All authors reviewed and approved the final manuscript.

Funding

This study was supported by National Natural Science Foundation of China (No. 31772807).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was granted by The Tab of Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University. Permission was given for the retrospective analysis of the histopathological classification of canine endometritis to VDS for clinical trial testing. This briefing note confirmed that testing of animals for the purpose of clinical research was permitted under appropriate ethical review. On submitting samples to Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University, veterinary practices agree that any sample may be used to investigate this disease. No animals died in this study.

The samples were collected in accordance with standard veterinary procedures and with the written informed consent of the owners and the approval of the Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University. All methods were carried out in accordance with relevant guidelines and regulations for the use of animal subjects. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing Interest

All the authors declare that they have no competing interests.

References

1. Allen WE: **Fertility and obstetrics in the dog**. 1991.
2. Hoffmann B, Riesenbeck A, Klein R: **Reproductive endocrinology of bitches**. *Animal Reproduction Science* 1996, **42**(1-4):275-288.
3. Jitpean S, Hagman R, Holst BS, Hglund OV, Egenvall A: **Breed Variations in the Incidence of Pyometra and Mammary Tumours in Swedish Dogs**. *Reproduction in Domestic Animals* 2012, **47**(6):347-350.
4. Waters DJ, Kengeri SS, Maras AH, Suckow CL, Chiang E: **Life course analysis of the impact of mammary cancer and pyometra on age-anchored life expectancy in female Rottweilers: Implications for envisioning ovary conservation as a strategy to promote healthy longevity in pet dogs**. *Veterinary Journal* 2017:25.
5. Asa CS, Bauman KL, Devery S, Zordan M, Camilo GR, Boutelle S, Moresco A: **Factors Associated With Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility**. *Zoo Biology* 2014, **33**(1):8-19.
6. Niskanen M, Thrusfield MV: **Associations between age, parity, hormonal therapy and breed, and pyometra in Finnish Dogs**. *The Veterinary record* 1998, **143**(18):493-498.
7. Purohit GN: **Postpartum endometritis in dairy cows: current status of diagnosis, therapy and prevention**. *Theriogenology Insight* 2015, **5**(1):1-23.

8. Hagman: **Molecular aspects of uterine diseases in dogs**. *Reproduction in domestic animals = Zuchthygiene* 2017, **52 Suppl 3**:37.
9. Post K: **Cystic Endometrial Hyperplasia/Pyometra Complex**. *Canine Internal Medicine Secrets* 2007:341-343.
10. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ: **Defining Postpartum Uterine Disease and the Mechanisms of Infection and Immunity in the Female Reproductive Tract in Cattle**. *Biology of Reproduction* 2009, **81**(6):1025-1032.
11. Schlafer DH, Gifford AT: **Cystic endometrial hyperplasia, pseudo-placentational endometrial hyperplasia, and other cystic conditions of the canine and feline uterus**. *Theriogenology* 2008, **70**(3):349-358.
12. Santana CH, Santos DO, Trindade LM, Moreira LG, Santos RL: **Association of Pseudoplacentational Endometrial Hyperplasia and Pyometra in Dogs**. *Journal of Comparative Pathology* 2020, **180**:79-85.
13. Dow, C.: **Experimental reproduction of the cystic hyperplasia-pyometra complex in the bitch**. *Journal of Comparative Pathology & Therapeutics* 1959, **69**:237,IN217-250,IN218.
14. Sandholm M, Vasenius H, Kivistö A: **Pathogenesis of canine pyometra**. *Journal of the American Veterinary Medical Association* 1975, **167**(11):1006-1010.
15. Haschek WM, Rousseaux CG, Wallig MA: **Female Reproductive System**. *Fundamentals of Toxicologic Pathology (Second edition)* 2010:599-631.
16. Eilts BE: **Cystic Endometrial Hyperplasia and Pyometra**.
17. Mcentee K: **The Uterus: Atrophic, Metaplastic, and Proliferative Lesions**. *Reproductive Pathology of Domestic Mammals* 1990:167-190.
18. Bosschere HD, Ducatelle R, Vermeirsch H, Broeck W, Coryn M: **Cystic endometrial hyperplasia-pyometra complex in the bitch: should the two entities be disconnected?** *Theriogenology* 2001, **55**(7):1509-1519.
19. Bukowska D, Kempisty B, Zawierucha P, Jopek K, Jaśkowski J: **Microarray analysis of inflammatory response-related gene expression in the uteri of dogs with pyometra**. *Journal of Biological Regulators & Homeostatic Agents* 2014, **28**(4):637-648.
20. Dekel N, Gnainsky Y, Granot I, Mor G: **Inflammation and implantation**. *American Journal of Reproductive Immunology* 2010, **63**(1):17-21.
21. Gargett CE, Ye L: **Endometrial reconstruction from stem cells**. *Fertility & Sterility* 2012, **98**(1):11-20.
22. Gutierrez, Mauriat, Guenin, Pelloux, Lefebvre, JF, Louvet, Rusterucci, Moritz, Guerineau: **The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants**. *PLANT BIOTECHNOL J* 2008, **2008,6(6)** (-):609-618.
23. Bhadwal MS, Mirakhur KK: **Ultrasonographic assessment of clinical conditions of the canine uterus**. *Indian Journal of Veterinary Surgery* 2001.

24. Kutzler, Anne M: **Estrous Cycle Manipulation in Dogs**. *Vet Clin North Am Small Anim Pract* 2018:S0195561618300172.
25. Blendinger K, Bostedt H, Hoffmann B: **Hormonal state and effects of the use of an antiprogestin in bitches with pyometra**. *Journal of Reproduction & Fertility Supplement* 1997, **51**(1):317-325.
26. Tsumagari S, Ishinazaka T, Kamata H, Ohba S, Tanaka S, Ishii M, Memon MA: **Induction of canine pyometra by inoculation of Escherichia coli into the uterus and its relationship to reproductive features**. *Animal Reproduction Science* 2005, **87**(3-4):301-308.
27. Barrau M, Abel JH, Verhage HG, Tietz WJ: **Development of the endometrium during the estrous cycle in the bitch**. *American Journal of Anatomy* 1975, **142**(1):47-65.
28. Pineda MH, Pineda MH, Dooley MP: **Reproductive patterns of dogs**. 2003.
29. Tong PY, Cong A, Pather S: **Hysteroscopy for pyometra – treading on treacherous grounds**. *Gynecology and Minimally Invasive Therapy* 2018, **7**(4):185-.
30. Hagman R, Rönnberg E, Pejler G: **Canine Uterine Bacterial Infection Induces Upregulation of Proteolysis-Related Genes and Downregulation of Homeobox and Zinc Finger Factors**. *Plos One* 2009, **4**.
31. Rekawiecki R, Kowalik MK, Kotwica J: **The expression of progesterone receptor coregulators mRNA and protein in corpus luteum and endometrium of cows during the estrous cycle**. *Animal Reproduction Science* 2017:102-109.

Tables

Table 1: Reverse transcription reaction system

Reaction system	Volume（μL）
5× Drime Script RT Master Mix	4
RNA（1-10ug）	RNA Mass（1-10ug）/ Measured concentration
RNase Free dH2O	16-V_{RNA}

Table 2: Primer Information

Primer Name	Primer Sequence（5’-3’）	GenBank Number
GAPDH	F：GATGGGCGTGAACCATGAG	NM-001003142.2
	R：TCATGAGGCCCTCCACGAT	
IL-1β	F：TGTGAAGTGCTGCTGCCAAGAC	NM-001037971.1
	R：ACAGAGCTGGTGGGAGACTTGC	
IL-6	F：TGCTCCTGGTGATGGCTAC	NM-001003301.1
	R：ATTATCCGAACAGCCCTC	
IL-8	F：AACACACTCCACACCTTTCCATCC	NM-001003200.1
	R：TCCAGGCACACCTCATTTCCATTG	

Table 3: Leukocyte number test results

White blood cell count	Units	Reference range	Variation range	Normal value	Abnormal value	
				Percentage of case（%）	Percentage of cases（%）	
					Higher than normal	Below normal value
WBC	10^{-9}/L	6.00-17.00	2.17-48.84	46.67	40.00	13.33
Neu	10^{-9}/L	3.62-12.30	1.67-41.45	40.00	43.33	16.67
Lym	10^{-9}/L	0.83-4.91	0.08-24.35	66.66	16.67	16.67
Mon	10^{-9}/L	0.14-1.97	0.11-7.36	86.67	10.00	3.33
Eos	10^{-9}/L	0.04-1.62	0.00-2.79	76.66	6.67	16.67
Bas	10^{-9}/L	0.00-0.12	0.00-0.19	93.33	6.67	0.00

WBC:Total leukocyte count; Neu:Neutrophil count; Lym:Lymphocyte count; Mon:Monocyte count; Eos:Eosinophil count; Bas:Basophil count.

Table 4: RBC test results

RBC detection indexes	Units	Reference range	Variation range	Normal value	Abnormal value	
				Percentage of cases□%□	Percentage of cases□%□	
					Higher than normal	Below normal value
RBC	10-12/L	5.10-8.50	3.94-7.81	80	0.00	20.00
HGB	g/L	110-190	90-197	86.67	3.33	10.00
HCT	%	33.0-56.0	28.1-53.9	83.33	0.00	16.67
MCV	fL	60.0-76.0	64.9-78.5	90	10.00	0.00
MCH	pg	20.0-27.0	19.9-26.8	96.67	0.00	3.33
MCHC	g/L	300-380	283-382	80	3.33	16.67
PCT	%	0.090-0.580	0.022-0.355	83.33	0.00	16.67

RBC:Red blood cell count; HGB: Hemoglobin; HCT:Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC:Mean corpuscular hemoglobin concentration; PCT: Plateletcrit.

Table 5: Pathological classification criteria of uterine lesions

Group	No clinical symptom group		Clinical Symptom Group		
Classification	Healthy	Neointimal hyperplasia	Mild inflammatory infiltration	Severe inflammatory infiltration □hyperplastic□	Severe inflammatory infiltration □atrophic□
Inflammation	no	no	Mild	Severe	Severe
Histology of cysts	no	Partial presence of lamina propria	Individual presence in lamina propria	Very much	no
Endo/myo Ratio	0.753±0.044	1.057±0.048	0.940±0.172	1.255±0.234	0.474±0.146
Stromal cell component	Uniformly distributed	Decrease	Local inflammatory infiltrate	Essentially disappeared	Atrophy, decreased

Figures

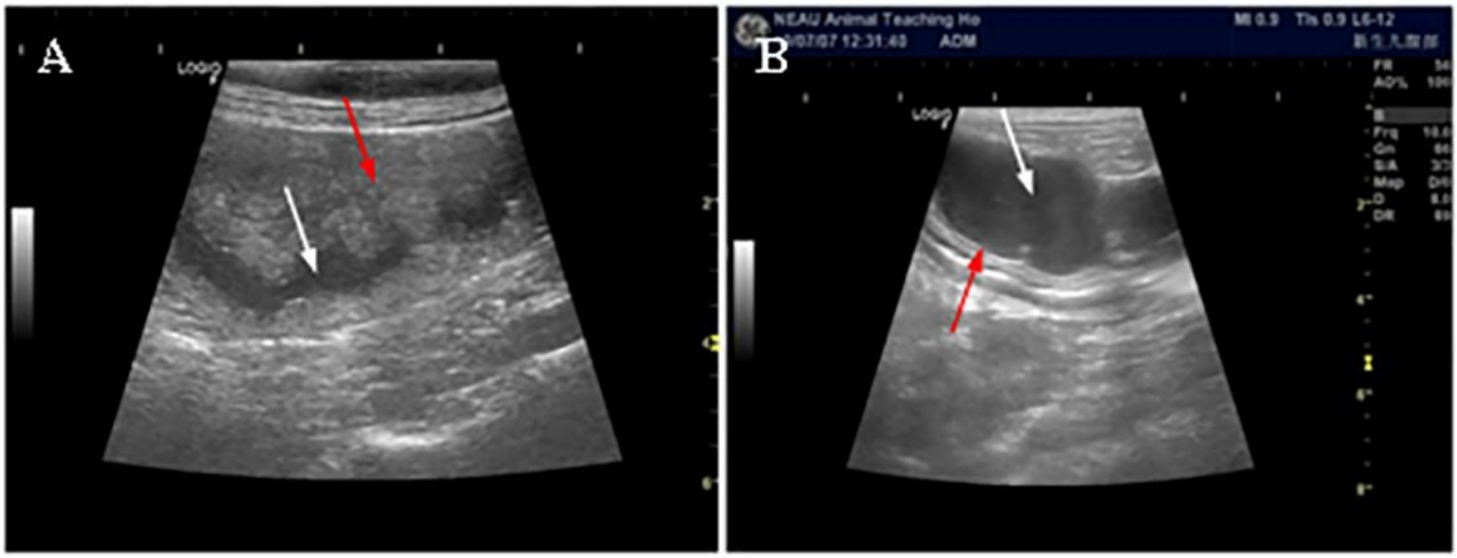

Figure 1

Ultrasonographic results.

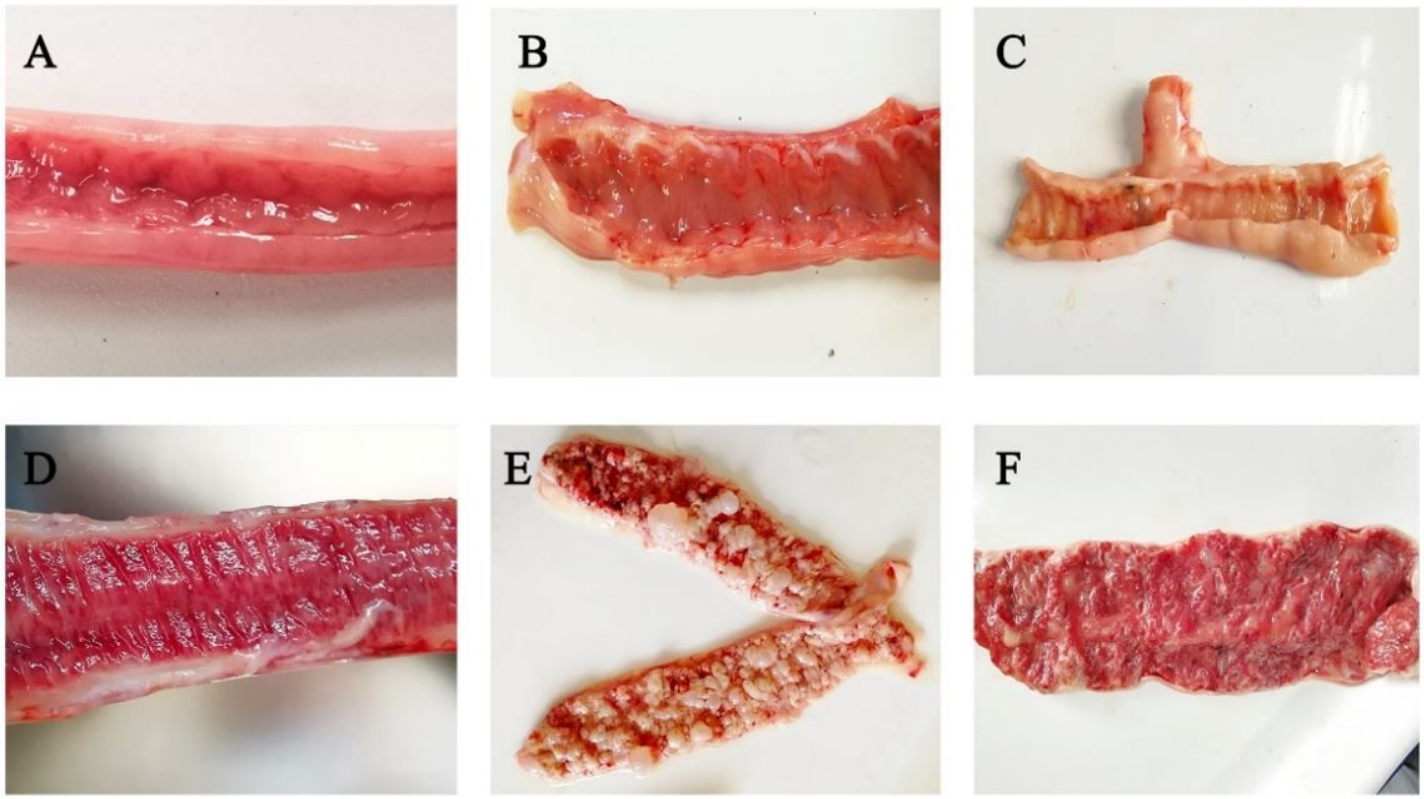

Figure 2

Uterine anatomy.

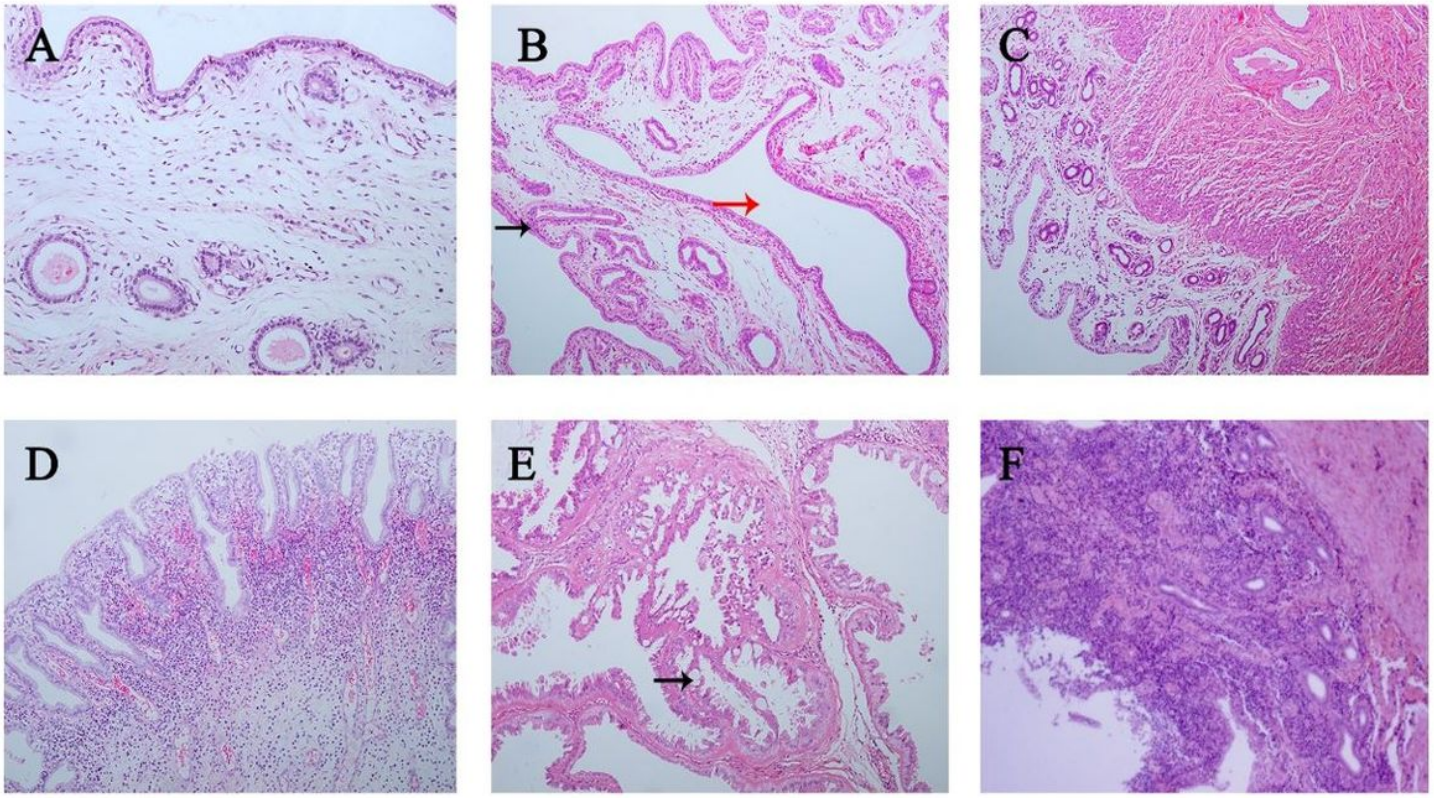

Figure 3

Histogram of uterus. A: Structure diagram of healthy endometrial epithelium and lamina propria (200 ×). B: Endometrial hyperplasia endometrial epithelium and superficial lamina propria (100 ×), black arrow refers to endometrial epithelial structure, red arrow refers to extremely dilated uterine glands. C: Non-inflammatory endometrium and structure diagram (100 ×), visible endometrial layer atrophy. D: Mild inflammatory cell infiltration local endometrial epithelial detachment, inflammatory infiltration in the superficial lamina propria (100 ×). E: Proliferative severe inflammatory infiltrate The superficial glands of endometrial lamina propria were severely dilated, the morphology of glandular epithelial cells was hypertrophic, and vacuolar degeneration (black arrow) (100 ×). F: Atrophic severe inflammatory infiltrative endometrial atrophy, diffuse inflammatory infiltrate throughout the thickness (100 ×).

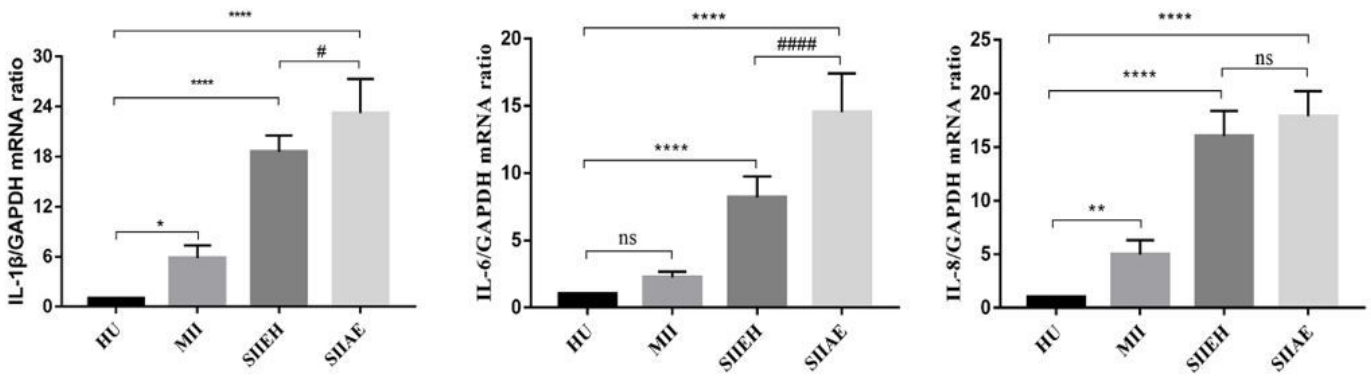

Figure 4

Inflammatory cytokines IL-1β, IL-6 and IL-8 mRNA expression results. * And # indicates that the difference between the two groups was significant, $p < 0.05$; ** indicates that the difference between the two groups was extremely significant, $p < 0.01$; **** and ####indicates that the difference between the two groups was extremely significant, $p < 0.001$; ns indicates that the difference between the two groups had no statistical significance. HU：Healthy Uterus；MII：Mild inflammatory infiltration；SIIEH: severe inflammatory infiltration of endometrial hyperplasia；SIIAE: severe inflammatory infiltration with atrophy of the endometrium.