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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2026.57.1.118-123

Ultrasonographic, Hormonal and Molecular Insights into Canine Cystic Endometrial Hyperplasia–Pyometra Complex

S. Dvija[1*], R. S. Abhilash[1], C. Jayakumar[1], Amritha Aravind[1], K. Raji[2], and Lali F. Anand[3]

[1]Department of Animal Reproduction, Gynaecology and Obstetrics, [2]Department of Veterinary Physiology, [3]Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India

Citation: Dvija, S., Abhilash, R. S., Jayakumar, C., Aravind, A., Raji, K. & Anand, L. F. (2025). Ultrasonographic, hormonal, and molecular insights into canine cystic endometrial hyperplasia–pyometra complex. *Journal of Veterinary and Animal Sciences* 57 (1), 118-123
https://doi.org/10.51966/jvas.2026.57.1.118-123

Received: 12.09.2025 Accepted: 18.11.2025 Published: 31.03.2026

Abstract

Pyometra is a common and potentially life-threatening uterine disorder in intact bitches, characterised by cystic endometrial hyperplasia, bacterial infection, and systemic inflammatory responses. The present study evaluated ultrasonographic, hormonal, and molecular changes in pyometra-affected bitches in comparison with clinically healthy dioestrus controls. Twelve intact bitches comprising six healthy dioestrus controls (Group I) and six confirmed CEH-pyometra cases (Group II). Serum progesterone concentration were significantly lower in pyometra cases (24.12 ± 5.27 ng/mL) compared to normal dioestrus bitches (2.65 ± 0.79 ng/mL). Ultrasonographic examination demonstrated a marked increase in uterine horn diameter (12.40 ± 1.70 vs. 20.65 ± 4.60 mm) and wall thickness (1.70 ± 0.42 vs. 3.31 ± 0.28 mm), while Doppler imaging revealed reduced resistive index (0.79 ± 0.02 vs. 0.57 ± 0.02) and pulsatility index (2.82 ± 0.26 vs 1.63 ± 0.17). Molecular analysis by qRT-PCR revealed a six-fold increase ($p<0.05$) in progesterone receptor (PR) expression in uterine tissue in group II animals. In conclusion, CEH-pyometra arises from a multifactorial interplay of uterine alterations, where low serum progesterone alongside high uterine receptor expression proposes compensatory upregulation that sustains disease progression, thereby underscoring its pathogenesis and highlighting potential avenues for future diagnostic and therapeutic strategies.

Keywords: *Canine pyometra, cystic endometrial hyperplasia, progesterone receptor*

Cystic endometrial hyperplasia (CEH) is widely recognised as the precursor lesion to pyometra, a potentially life-threatening reproductive disorder reported in intact bitches. Repeated exposure to progesterone during the dioestrus phase promotes endometrial gland hyperplasia, reduces myometrial contractility, suppresses uterine immune defence and favours bacterial colonisation in a uterine environment (Schlafer & Gifford, 2008; England et al., 2012; Hagman, 2018; Santana et al., 2020; Xavier et al., 2023). These changes impair normal uterine clearance mechanisms, which predispose the uterus to opportunistic pathogens, most frequently *Escherichia coli* (De Bosschere et al., 2001; Singh, 2017; Xavier et al., 2022; Lopes et al., 2021). This progression from hormonally induced endometrial changes to secondary bacterial infection underlies the pathophysiology of the CEH–pyometra complex.

**Corresponding author : dvija98@gmail.com Ph. 9539812706*

The diagnosis of CEH–pyometra typically involves a combination of clinical evaluation, haemato-biochemical analysis, and imaging modalities. Among these, ultrasonography has been considered a cornerstone diagnostic tool, providing a non-invasive, real-time assessment of uterine health status. Further, it allows detailed visualisation of uterine size, luminal contents, wall thickness, and vascular perfusion, all of which contribute to evaluating disease severity (Hagman, 2022; Xavier et al., 2023). In addition to imaging, serum progesterone concentrations provide critical insight into the hormonal environment that supports disease progression. Recently, many researchers have emphasised the value of molecular investigations, particularly the expression of progesterone receptors in the endometrium, which are crucial for understanding the pathogenesis and molecular regulatory mechanisms underlying the CEH–pyometra complex (Vermeirsch et al., 1999; Prapaiwan et al., 2017; Hagman, 2023). Thus, despite the use of individual tools, a multidimensional diagnostic model may offer greater precision in staging disease progression, evaluating prognosis, and guiding evidence-based treatment decisions.

Therefore, the present study was undertaken to evaluate the diagnostic value of ultrasonographic, hormonal (serum progesterone), and molecular alterations in endometrial progesterone receptor (PR) expression in bitches affected by CEH–pyometra complex, aiming to contribute to a comprehensive understanding of its pathogenesis and clinical management.

Materials and methods

Animal selection and grouping

The study was conducted on twelve intact female dogs presented to the University Veterinary Hospitals at Kokkalai and Mannuthy. Animals with clinical signs suggestive of pyometra, as well as healthy dogs in dioestrus undergoing elective ovariohysterectomy (OHE), were included in the present study.

A detailed clinical history was obtained from each owner, followed by a thorough clinical, gynaecological and ultrasonographical examination in selected animals. Based on exfoliative vaginal cytology and ultrasonography, animals were categorised into two groups: Group I (n = 6): Clinically healthy bitches in the dioestrus phase. Group II (n = 6): Bitches diagnosed with cystic endometrial hyperplasia–pyometra (CEH-Pyometra) complex.

Serum progesterone concentrations were determined on the day of surgery using chemiluminescence immunoassay (CLIA) kits (PROG-CLIA, Mindray, China), following the manufacturer's instructions.

Pyometra-affected bitches were examined using B-mode transabdominal ultrasonography with a 1–8 MHz curvilinear probe and a 4–15 MHz linear probe (MyLabX8 eXP Vet, Esaote, Italy). Two-dimensional imaging was performed to assess the maximum cross-sectional diameter of the uterine horns and uterine wall thickness using electronic calipers. Ultrasonographic evaluation of the uterus was performed to assess uterine horn diameter, uterine wall thickness and Doppler indices. Pulse-wave Doppler ultrasonography was used for the quantitative assessment of blood flow velocity and haemodynamic parameters of the uterine arteries, following the methodology described by Batista et al. (2016). Colour Doppler ultrasonography was initially employed to identify and localise the uterine arteries bilaterally at the level of the uterine body in a longitudinal plane. Once a blood vessel with optimal colour signal was visualised, the Doppler gate was positioned, ensuring that the angle of insonation did not exceed 60°.

Pulsed-wave Doppler was then used to record the flow velocity waveforms. Three consecutive waveforms displaying the highest doppler shifts were selected for analysis. Peak systolic velocity (PSV, cm/s) and end-diastolic velocity (EDV, cm/s) were measured directly, while the resistance index (RI = (PSV - EDV)/PSV) and pulsatility index (PI = (PSV - EDV)/TAV) were automatically computed by the ultrasound system.

All animals underwent OHE under standardised anaesthetic protocols. Uterine tissues were collected post-operatively for further molecular analyses.

Relative gene expression studies

Following OHE, a part of the middle uterine horn was collected and stored at -80ºC in RNA later (Sigma Lifesciences, Canada) until RNA isolation. Total RNA was extracted using the total RNA Kit (Origin Diagnostics and Research, Kerala) according to the manufacturer's protocol and eluted in 50 µL RNase-free water. RNA yield (approx. 2000 ng/µL) was quantified using a Nanodrop spectrophotometer, and purity was confirmed by OD ratios (A260/280 > 1.8; A260/230 ≈ 2.0). RNA integrity was further verified by 1% agarose gel electrophoresis. RNA concentrations were normalised to 500 ng for cDNA synthesis using a commercial cDNA synthesis kit with RNase inhibitor (Origin Diagnostics, Kerala).

Real-time PCR reactions were conducted by SYBR Green qPCR Master Mix (Thermo Fisher Scientific, USA) and specific primers: PR (5' TGATGACCAAATAACTCTCATCCAG, 3'GTGCAAAATATAGCATCTGCCCA cat no. XM_038429443.1) using Bio-Rad CFX 96™ Touch Real-Time PCR system. Data were normalised to *GAPDH (5'* AAGGCTGAGAACGGGAAACT, 3' TACTCAGCACCAGCATCACC cat.no XM_038586521) and analysed by the comparative CT method. Gradient PCR was performed to optimise annealing temperatures.

Each reaction contained 500 ng cDNA (1 µL), with all samples run in triplicate across three independent biological replicates.

Data on ultrasonographic parameters, serum progesterone concentration, and *PR* gene expression were compared between groups using independent t-tests. Analyses were performed using SPSS software (version 24.0).

Results and discussion

Transabdominal B-mode ultrasonography was found to be a valuable diagnostic tool for assessing uterine structural changes in bitches. A significant increase in both uterine horn diameter and wall thickness was observed in affected animals compared with healthy dioestrus controls (Table 1). The mean uterine horn diameter in CEH-pyometra cases measured 20.65 ± 4.60 mm, notably higher than the 12.40 ± 1.70 mm recorded in healthy animals, indicating pathological luminal distension due to exudate accumulation. These findings are in agreement with previous reports by Shah et al. (2017), Samanta et al. (2018) and Unnikrishnan (2018). Uçmak et al. (2021) further suggested that uterine diameters exceeding 2 cm may be considered diagnostic for CEH–pyometra. Similarly, uterine wall thickness was significantly increased in affected bitches (3.31 ± 0.28 mm) compared to controls (1.70 ± 0.42 mm), supporting observations by Veiga et al. (2012) and Uçmak et al. (2021). These ultrasonographic changes are consistent with underlying histopathological alterations such as glandular hyperplasia, stromal proliferation, and inflammatory infiltration of the endometrium (De Bosschere et al., 2001; Hagman, 2014).

Among the pyometra-affected animals, 33.30 per cent exhibited Grade B changes (moderate glandular hyperplasia with early cyst formation), 50 per cent showed Grade C alterations (pronounced cystic glandular hyperplasia with increased gland diameter and endometrial thickening), and 16.7 per cent presented with Grade D lesions, characterised by advanced cystic changes, marked wall thinning, and areas of necrosis. These findings align with the progressive stages of cystic endometrial hyperplasia (CEH) as classified by Bigliardi (2004). Moreover, in pyometra cases, the intrauterine fluid varied from anechoic to hypoechoic, with the latter being more commonly observed, consistent with reports by Thilak (2025) and Nyland and Mattoon (2002). In contrast, healthy bitches displayed a uniformly thin, echogenic endometrial lining typical of normal dioestrus, with no cystic or abnormal changes. Collectively, these ultrasonographic features highlight the diagnostic and prognostic value of imaging in CEH–pyometra, offering critical non-invasive insights into disease severity and progression.

Hormonal evaluation demonstrated that progesterone levels were significantly lower in pyometra-affected bitches (2.65 ± 0.79 ng/mL) compared with healthy dioestrus bitches (24.12 ± 5.27 ng/mL), which suggested impaired luteal function (Table 2). In our study, five of the cases were of the open-cervix type and one was closed-

Table 1. Uterine horn diameter, uterine wall thickness and Doppler parameters in normal dioestrus and pyometra-affected bitches

Group (n=6)	Uterine horn diameter (mm) (Mean ± SE)	Uterine wall thickness (mm) (Mean ± SE)	Doppler indices (Mean ± SE)			
			PSV (cm/s)	EDV (cm/s)	RI	PI
Group I	12.40 ± 1.70	1.70 ± 0.42	50.70 ± 4.49	9.92 ± 0.87	0.79 ± 0.02	2.82 ± 0.26
Group II	20.65 ± 4.60	3.31 ± 0.28	52.9 ± 28.93	21.88 ± 2.85	0.57 ± 0.02	1.63 ± 0.17
t-value	3.150*	2.969*	0.22^{NS}	4.012**	7.90**	6.45*
p-value	0.010	0.016	0.831	0.002	0.001	0.02

***Significant at 0.01 level; * Significant at 0.05 level;NS- non significant*

Table 2 Serum progesterone concentration (ng/mL) in and normal bitches and pyometra affected

Parameter (Mean ± SE)	Group I	Group II	t-value	p value
Serum progesterone (ng/mL)	24.12 ± 5.27	2.65 ± 0.79	4.024**	0.002

***Significant at 0.01 level*

Table 3 Relative Quantification of PR gene expression between healthy dioestrus (Group I) and pyometra affected (Group II) bitches

Group (n=6)	Mean C_T ± SE		ΔC_T ± SE	$\Delta\Delta C_T$ ± SE	Fold change ($2^{-\Delta\Delta C_T}$)	p value
	PR	*GAPDH*				
Group I (Dioestrus)	33.95 ± 0.41	27.03 ± 0.37	6.97 ± 0.55	0.00 ± 0.55	1	0.03*
Group II (Pyometra)	31.56 ± 0.74	27.23 ± 0.47	4.32 ± 0.88	-2.59 ± 0.88	6.02	

** Significant at 0.05 level*

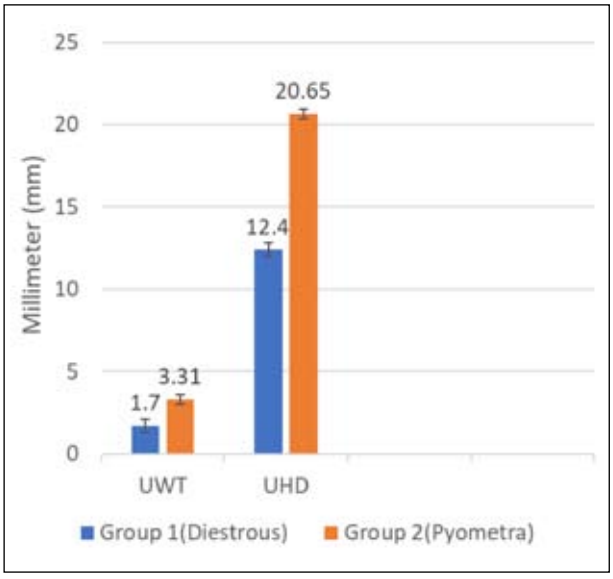

Fig 1. Relative Quantification of *PR* gene expression between healthy dioestrus (Group I) and pyometra affected (Group II) bitches

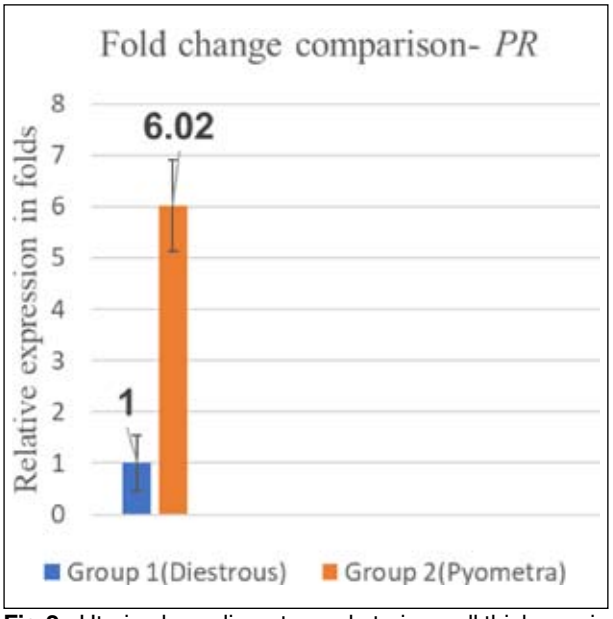

Fig 2. Uterine horn diameter and uterine wall thickness in normal dioestrus and pyometra-affected bitches

cervix, which might have contributed to the relatively low mean progesterone values recorded, since closed-cervix pyometra has often been associated with comparatively higher circulating progesterone (Dineshkumar, 2005). Similar reductions in progesterone value were reported by Vidya (2019), supporting the association between pyometra and diminished luteal activity. In contrast to the present findings, elevated progesterone concentrations were recorded in pyometra-affected bitches (Chinnu, 2016; Unnikrishnan, 2018; Krishnan, 2022). Such variability was likely attributed to several factors, including differences in the timing of sample collection within the dioestrus phase, variations in assay techniques (radioimmunoassay vs ELISA vs CLIA), and heterogeneity in the stage or severity of the disease (Volpato et al., 2013; Dineshkumar, 2005). As noted by Austad et al. (1979) and Colombo et al. (1988), progesterone levels in pyometra cases frequently overlapped with those observed during normal dioestrus, thereby limiting their diagnostic reliability when considered as the sole tool. Instead of considering absolute circulating progesterone concentrations, sensitivity and the number of progesterone receptors were reported to be more relevant in the pathogenesis of pyometra (Gobello et al., 2003; Fieni, 2006).

A statistically significant six-fold increase in progesterone receptor (PR) expression was detected in the uterine tissues of pyometra-affected bitches compared with healthy dioestrus controls ($p < 0.05$; Fig. 1, Table 3). This pronounced upregulation reflected a dysregulation of the normal reproductive and immunomodulatory mechanisms underpinning CEH–pyometra pathogenesis. Typically, *PR* expression decreased during dioestrus to reduce uterine sensitivity to progesterone; however, the failure of this downregulation observed here suggested a persistent progesterone responsiveness (Vermeirsch et al., 1999). In this context, the marked receptor upregulation indicated that the pathogenic drive of progesterone was not solely dependent upon serum progesterone concentrations but was strongly mediated through receptor sensitivity, in agreement with the observations of Gobello et al. (2003) and Fieni (2006).

Such aberrant *PR* expression was likely to have exacerbated progesterone-driven processes, including enhanced glandular secretions, suppression of local immune defences, and promotion of endometrial hyperplasia, key features of the CEH–pyometra complex (De Bosschere et al., 2001; Marinković et al., 2018). These findings were consistent with those of Vidya (2019), who reported elevated *PR* levels particularly in cases with comparatively low serum progesterone, thereby further supporting the view that receptor dynamics, rather than circulating hormone concentrations, were central to disease development. Nevertheless, some studies recorded unchanged or reduced *PR* expression in cervix of animals affected with pyometra (Kunkitti et al., 2011; Volpato et al., 2013), which suggested that receptor levels may have varied according to cervical status, sampling site, or disease chronicity.

The results of this study clearly demonstrated an inverse relationship between circulating progesterone and uterine *PR* expression in pyometra. Although serum progesterone levels were lower in pyometra-affected bitches than in dioestrus controls, *PR* expression was significantly upregulated, indicating that defective downregulation of the receptor enhanced progesterone sensitivity and contributed to the pathological endometrial changes observed in CEH–pyometra (Gobello et al., 2003; Fieni, 2006).

Conclusion

This study highlights that canine CEH–pyometra presents with clear ultrasonographic alterations, including uterine enlargement and luminal fluid accumulation, together with significant hormonal and molecular changes in endometrial progesterone receptor (PR) expression, localisation, and regulation. The reduction in circulating progesterone, combined with molecular evidence of persistent progesterone-driven activity within the endometrium, indicates that prolonged hormonal stimulation plays a central role in disease development and progression. Integrating ultrasonographic assessment with hormonal and molecular profiling demonstrates strong potential for improving diagnostic accuracy and prognostic evaluation. Such a multidimensional approach enhances understanding of the pathogenesis and regulatory mechanisms underlying the condition and supports the formulation of more targeted diagnostic and therapeutic strategies.

Conflict of interest

The authors declare that they have no conflict of interest.

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