

Prevalence and Diagnostic Evaluation of Endometritis in Infertile Mares: Bacteriological, Cytological, and Haematological Correlations

Dhruv B. Chaudhari^{1*}, Dinesh V. Chaudhari¹, Munja J. Bharai², Jalendra K. Mahla²

ABSTRACT

The study was conducted on infertile mares from stud farms around Anand and those presented to the Veterinary Clinical Complex, Kamdhenu University, Anand, to assess the prevalence of infertility and endometritis, identify major uterine pathogens, and evaluate age-related haematological and cytological changes. Thirty mares with a history of infertility were examined. The incidence of infertility and endometritis increased notably with age, as mares above eight years showed a higher prevalence of uterine infection, suggesting age-related degeneration of uterine defense mechanisms. Bacteriological examination revealed *Escherichia coli* as the predominant isolate, followed by *Streptococcus* spp., *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp., and *Klebsiella* spp. β -haemolytic *Streptococcus* was associated with elevated uterine pH, while *E. coli* infection showed no pH correlation. Cytological examination showed increased polymorphonuclear neutrophils (PMNs), confirming endometrial inflammation. Haematological analysis indicated mild immunosuppression, reflected by slightly reduced leukocyte and lymphocyte counts, while erythrocytic and platelet values remained largely within normal limits, with a minor decrease in platelet mass. In conclusion, endometritis was identified as a major cause of infertility in mares, with higher susceptibility in older animals. The predominance of *E. coli* and *Streptococcus* spp., coupled with supportive cytological and haematological changes, highlights the importance of integrated diagnostic approaches for effective detection and management of uterine infections in mares.

Key words: Age-related infertility, Bacteriology, Cytology, Equine endometritis, Haematology, *Escherichia coli*, Infertility.

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INTRODUCTION

Endometritis is one of the most prevalent reproductive disorders in mares and a leading cause of infertility, early embryonic loss, and reduced conception rates (Card, 2005; Rasmussen *et al.*, 2015). It is characterized by inflammation of the endometrium, primarily resulting from bacterial infection, impaired uterine clearance, or age-related degeneration of uterine defence mechanisms. Factors such as defective myometrial contractions, poor lymphatic drainage, anatomical abnormalities, and post-breeding fluid retention contribute to the persistence of infection and inflammation (LeBlanc and Causey, 2009). Based on etiology and pathophysiology, endometritis is classified as endometrosis, sexually transmitted, persistent mating-induced (PMIE), or chronic infectious forms (Varadin, 1975). The most frequently isolated pathogens include *Escherichia coli*, *Streptococcus equisubsp zoepidemicus*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas aeruginosa*, and *Klebsiella* spp. (Troedsson *et al.*, 2008). Among these, *E. coli* and *Streptococcus* spp. are predominant, with *E. coli* often adhering tightly to the endometrial surface, complicating detection and treatment (LeBlanc, 2010).

Endometrial cytology serves as a rapid and reliable diagnostic tool for uterine inflammation. The presence of increased polymorphonuclear neutrophils (PMNs) exceeding

¹Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat-388001, Gujarat, India

²Private Equine Practitioner, Anand-388001, Gujarat, India

¹Department of Veterinary surgery and Radiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India

Corresponding Author: Dr. Dhruv B. Chaudhari, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand-388001, India. e-mail: chaudharydhruv844@gmail.com

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2-10% of total cells is considered diagnostic of endometritis (Crickman and Pugh, 1986; Baranski *et al.*, 2003). Cytological examination, when combined with bacteriological culture, enhances diagnostic accuracy, especially in subclinical cases (Card, 2005). Haematological evaluation in endometritic mares often reveals mild leucopenia and lymphopenia, indicating

systemic inflammatory stress or immunosuppression, while erythrocytic and platelet values generally remain within normal physiological limits. Therefore, integrating bacteriological, cytological, and haematological assessments provides a comprehensive understanding of uterine pathology, helping in accurate diagnosis and better reproductive management of endometritis in mares.

MATERIALS AND METHODS

The present study was conducted on mares exhibiting a history of infertility. The research involved randomly selected infertile mares presented to the Veterinary Clinical Complex, College of Veterinary Science, Kamdhenu University, Anand, as well as stud farms located in the Anand and Kheda districts of Gujarat, India, during the year 2024-25.

Selection and Clinical Examination of Animals

A total of thirty mares with a history of infertility were included in the study irrespective of breed and parity. Detailed breeding, health, and reproductive histories were recorded. Mares were examined during the estrus phase based on behavioural signs and rectal palpation findings. Animals exhibiting anatomical abnormalities, systemic illness, or pregnancy were excluded. All mares underwent thorough physical and gynaecological examinations. Per-vaginal and per-rectal examinations were carried out to assess the condition of the cervix, uterus, and ovaries.

Cytobrush Technique and Endometrial Cytology

A customized stainless-steel cytobrush assembly (51×5 mm) developed for mares, consisting of a barrel and stylette designed to hold a human Pap smear brush (Mayfair Endocervical Brush, Care India Surgicals, Ludhiana, India) was used. All units were sterilized before use. During sampling, mares were restrained, the tail was bandaged, and the external genital area was thoroughly cleaned. The lubricated cytobrush assembly was guided manually per vaginum and passed through the cervix into the uterine lumen, where the brush was rotated gently in both directions to obtain endometrial cells. Two samples were collected from each mare: one brush was immersed in sterile saline for bacteriological culture, and the second was rolled onto a clean, grease-free glass slide for cytological evaluation. Smears were fixed in methanol, air-dried, and stained using Field's stain A and B, then examined under 100× oil immersion. A minimum of ten microscopic fields were evaluated for epithelial cells, debris, bacteria, and inflammatory cells. Inflammation was diagnosed according to the criteria described by daDel Prete *et al.* (2024). A smear was considered positive when the mean neutrophil count was ≥ 2 per field at 100× magnification or when PMNs represented $\geq 2\%$ of the total cell population. Mares exhibiting mucopurulent or cloudy cervico-vaginal mucus and PMN $\geq 2\%$ during estrus were diagnosed as endometritic and included in the study.

Blood and Uterine Sample Collection

Blood was drawn aseptically from each mare using a sterile 5 mL syringe fitted with a 21-gauge needle and immediately transferred into EDTA vials for haematological assessment, which was done within 24 h using auto-haemato-analyser (Abacus Junior Vet s5, Diatron NL Ltd, Budapest, Hungary).

Uterine samples were collected aseptically using a double-guarded sterile swab inserted through the cervix into the uterine lumen. The swabs were immediately transported to the microbiology laboratory under cold conditions for bacteriological analysis, while a portion of the sample was used for cytological examination.

Bacterial Isolation

Under aseptic conditions in a laminar flow hood, the sterilized culture media prepared in advanced were poured into Petri dishes, which were gently rotated in both directions to ensure even distribution. To verify sterility, the plates were incubated at 37 °C for 48 h before use. Cytobrush samples collected for bacteriological investigation were transported to the laboratory and maintained at 37 °C for 6-8 h prior to inoculation. Samples were streaked onto blood agar containing 5% sheep blood, MacConkey agar, Sabouraud dextrose agar, and Eosin-methylene blue (EMB) agar in the Department of Microbiology. The plates were incubated aerobically at 37 °C and examined for growth after 24 h, with a second examination at 48 h to detect bacteria or yeast. Bacterial isolates were identified based on colony morphology, Gram staining, and basic biochemical tests. After 48 h, cultures were classified as (i) substantial growth of uterine pathogenic bacteria in monoculture or (ii) no growth/contamination. A culture was considered monoculture if more than 90% of colonies on blood agar were of a single species

Statistical Analysis

All observations were recorded, tabulated, and analyzed descriptively to determine the prevalence of infertility and endometritis, distribution of bacterial isolates, and age-related variations in haematological and cytological findings. Results were expressed as mean \pm standard error (SE), and appropriate statistical tests (t-test) were applied wherever necessary to compare differences between age groups.

RESULTS AND DISCUSSION

Prospective Analysis of Infertile Mares

A prospective analysis was conducted on 30 infertile mares of varying ages and reproductive histories to identify underlying causes of subfertility. The study revealed multiple contributing factors, including early embryonic death, post-breeding endometritis, chronic endometritis, abortion, and subclinical endometritis, with confirmatory diagnosis made by endometrial cytology and bacterial isolation.

Eight mares (26.7%), which did not exhibit any clinical signs of endometritis as evidenced by the absence of

vaginal discharge and intrauterine fluid during per-rectal examination, were considered the cases of subclinical endometritis after the result of the endometrial cytology (PMN%). The detection of subclinical cases in apparently healthy mares aligned with earlier reports. Rasmussen *et al.* (2015) observed that 28.6 % of clinically healthy mares presented for AI breeding were diagnosed with subclinical infectious endometritis.

Early embryonic death (EED) and occasional abortion during early gestation were also recorded. The EED was observed in 6 (20.0%) mares. These mares cycled normally but failed to maintain pregnancy beyond the embryonic stage. This clinical pattern aligned with the observations of Pasolini *et al.* (2016), who reported that endometritis is among the most frequent causes of subfertility in mares that cycle normally, but either fail to conceive or, abort after conception.

Chronic endometritis was detected in 6 (20.0%) mares, all of which had remained barren for successive breeding seasons or had repeated unsuccessful conceptions. Vaginal discharge containing flakes or purulent material was observed in these mares. This finding was consistent with previous study by Causey (2006), who reported chronic endometritis in 25.0 to 60.0 % of barren mares, indicating a significant prevalence of this condition among infertile mares. Similarly, Christoffersen *et al.* (2015) reported chronic endometritis in 34.0 % of infertile Thoroughbred mares in the UK, highlighting the widespread occurrence of this disorder.

Post-breeding endometritis was also observed in 8 (26.7%) mares indicating a notable incidence of uterine inflammatory response occurring subsequent to mating or AI. Our observations were in agreement with Ginther *et al.* (1985), who reported that endometritis pathways were linked to an 18.2 % embryonic loss in pony mares between 11- and 15-days post-ovulation, primarily due to endometritis-induced preterm luteolysis.

Abortion history was present in 2 (6.7%) mares, one of which was attributed to twin pregnancy, a well-known cause of early pregnancy loss in mares due to uterine overcrowding and compromised placental development. The second abortion occurred in a mare with a history of uterine inflammation, suggesting that endometrial pathology may have contributed to fetal loss. These findings were consistent with previous studies, that abortion in mares can result from infectious agents, hormonal imbalances, uterine pathology, or management-related factors (LeBlanc and Causey, 2009; Christoffersen *et al.*, 2015). This highlights that, although abortion was less frequent in the present study compared to other reproductive disorders such as endometritis or early embryonic death, it remains an important contributor to reproductive failure, especially when compounded by pre-existing uterine conditions or multiple gestations.

Bacterial Isolation and Endometrial Cytology

In this study, bacterial isolation and endometrial cytology were performed on all 30 mares. Out of these, 20 (66.7%) mares showed bacterial growth, and all of these were

considered positive for endometritis based on endometrial cytology findings. The remaining 10 samples did not show any bacterial growth, which could be attributed to suboptimal temperature maintenance during transport or potential laboratory handling errors, rather than absence of infection. We selected these 20 mares to determine the incidence of endometritis based on the correlation between bacterial culture and endometrial cytology.

In the study, bacteriological examination of uterine samples (n=20) revealed that *Escherichia coli* was the most frequently isolated organism, accounting for 40% of cases, reinforcing its role as the leading cause of endometritis in mares. *Streptococcus* spp. accounted for 20 % of isolates, while mixed infections (*E. coli* + *Streptococcus* spp.) were detected in 10 % of cases. Other opportunistic pathogens included *Staphylococcus* spp. (10%), *Proteus* spp. (10%), *Pseudomonas* spp. (5%), and *Klebsiella* spp. (5%). These findings were consistent with the observations of LeBlanc *et al.* (2007), and earlier reports where β -haemolytic *Streptococci* and *E. coli* were identified as the most common uterine pathogens in mares (Albihn *et al.*, 2003; Nielsen, 2005; LeBlanc *et al.*, 2007; Riddle *et al.*, 2007; Frontoso *et al.*, 2008). Katila (2016) reported that among the colonies identified as potentially pathogenic or non-pathogenic organisms the most common pathogens were β -haemolytic *Streptococcus*, *E. coli*, but also *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella*, fungi and yeasts caused endometritis.

Isolation of β -haemolytic *Streptococcus* from the efflux was highly associated with a rise in pH (8.0), whereas, recovery of *E. coli* was not associated with pH (6.5). The uterine efflux with the presence and absence of bacteria had the pH of 6.0 and 7.0, respectively. These observations were in agreement with those made by LeBlanc *et al.* (2007).

The incidence of infertility and endometritis in relation to the age of barren mares is presented in Table 1. Among the 30 infertile mares examined, 20 % were below eight years of age, while 80 % were above eight years. Among 20 endometritic cases, again 20 % were below eight years of age, while 80 % were above eight years. The results clearly indicated that infertility and endometritis were markedly higher in mares aged more than eight years compared to younger ones (Table 1), suggesting a strong relationship between increasing age and infertility. The reduction in reproductive efficiency with age can be attributed to progressive degenerative changes in the endometrium, including glandular fibrosis, lymphatic lacunae formation, and impaired uterine clearance. These pathological alterations hinder normal uterine function, thereby reducing conception rates.

Table 1: Relationship between age and incidence of infertility and endometritis in barren mares

Age of barren mares	Incidence of Infertility (%)	Incidence of endometritis (%)
<8 years	20 % (n=6)	20 % (n=4)
>8 years	80 % (n=24)	80 % (n=16)
Total	100 % (n=30)	100 % (n=20)



Similar findings were reported by LeBlanc *et al.* (2007), who noted that older mares exhibit delayed uterine clearance and increased susceptibility to endometritis due to impaired myometrial contractility and weakened uterine defence mechanisms. Moreover, Christoffersen *et al.* (2015) and Troedsson *et al.* (2008) observed that repeated breeding, multiple parturitions, and chronic inflammatory exposure over successive reproductive cycles lead to cumulative uterine damage and fibrosis in aged mares. Ali *et al.* (2021) reported that among 219 Arabian mares in Saudi Arabia, moderate and severe endometritis were diagnosed in 60 (27.4%) and 39 (17.8%) mares, respectively. All affected mares were either older than 10 years or had a low body condition score (2.1 ± 0.3), emphasizing the role of both age and physical condition as critical risk factors. Furthermore, Derbala *et al.* (2018) observed an age-related increase in endometritis, with the incidence rising from 57 % in mares with an average age of 6.7 years to 75 % in mares with an average age of 14.4 years.

Cytological and Haematological Findings in Endometritic Mares

Endometritic mares showed total leukocyte and lymphocyte counts at the lower end of the normal range (Table 2), consistent with Satué *et al.* (2012), who observed reduced immune cell levels. These haematological findings suggest a mild suppression of systemic immune response in affected mares. However, unlike their report of elevated erythrocytic parameters (haemoglobin, haematocrit, MCV, MCHC), values in this study remained within physiological limits, showing no signs of anaemia or erythrocytosis. Monocyte, neutrophil, eosinophil and basophil counts along with their respective percentages, remained within normal limits, although the monocyte percentage (5.57%) approached the upper limit. Neutrophil counts and percentages were normal, and no significant neutrophilia was noted, which differs from the elevated segmented neutrophils observed by Satué *et al.* (2012). Platelet count ($153.75 \times 10^3/\mu\text{L}$) and platelet crit (0.09%) were slightly below the reference values, suggesting marginally reduced platelet mass. Mean platelet volume (7.36 fl) and platelet distribution width (28.81 fl/%) remained normal. This shift in platelet count was not described in Satué *et al.* (2012) work. Virendra *et al.* (2022) demonstrated that barren mares had a significantly higher number of neutrophils and an increased neutrophil to epithelial cell ratio in endometrial cytology compared to fertile mares, with uterine fluid detected in most barren animals. These findings underline that cytological evidence of inflammation is a key marker of endometritis-associated infertility, even in the absence of systemic alterations.

In the present study, endometritic mares exhibited a markedly elevated percentage of polymorphonuclear cells (PMN%) in endometrial cytology (12.92 ± 1.93) compared to the reference value for healthy mares (< 2%). This

substantial increase in PMN percentage clearly differentiates affected mares from normal ones. This observation was consistent with the report of Virendra *et al.* (2022), confirming that increased neutrophil infiltration is a hallmark cytological feature of endometritis. Moreover, while systemic haematological parameters in our study remained largely within physiological ranges, the pronounced rise in PMN percentage strongly indicates localized uterine inflammation as the predominant factor affecting reproductive efficiency.

Table 2: Endometrial cytology and blood haematological parameters (Mean \pm SE) in twelve endometritic mares

Parameter	Endometritic mares	Referral value normal mares
Endometrial cytology (PMN %)	12.92 \pm 1.93	< 2.00
WBC count ($\times 10^3/\mu\text{L}$)	6.43 \pm 0.80	5.5 - 12.5
Lymphocytes ($\times 10^3/\mu\text{L}$)	1.98 \pm 0.30	1.5 - 5.0
Monocytes ($\times 10^3/\mu\text{L}$)	0.33 \pm 0.05	0 - 0.8
Neutrophils ($\times 10^3/\mu\text{L}$)	4.23 \pm 0.76	3.0 - 7.0
Eosinophils ($\times 10^3/\mu\text{L}$)	0.04 \pm 0.02	0 - 1.0
Basophils ($\times 10^3/\mu\text{L}$)	0.00 \pm 0.00	0 - 0.2
Lymphocytes (%)	34.43 \pm 5.37	20 - 55
Monocytes (%)	5.57 \pm 0.98	0 - 6
Neutrophils (%)	62.16 \pm 4.57	35 - 75
Eosinophils (%)	0.41 \pm 0.22	0 - 10
Basophils (%)	0.02 \pm 0.02	0 - 1
RBC count ($\times 10^6/\mu\text{L}$)	7.15 \pm 0.42	6.5 - 12.9
Hb (g/dL)	12.22 \pm 0.76	10 - 17
MCV (fl)	50.74 \pm 1.53	37 - 58
MCH (pg)	17.08 \pm 0.68	13 - 19
MCHC (g/dL)	31.45 \pm 1.62	31 - 38
RDW (%)	19.97 \pm 0.53	18 - 22
PLT count ($\times 10^3/\mu\text{L}$)	153.75 \pm 14.96	100 - 350
PCT (%)	0.09 \pm 0.01	0.1 - 0.4
MPV (fl)	7.36 \pm 0.36	5 - 8
PWDC (fl/%)	28.81 \pm 2.91	25 - 50

Overall, endometritic mares exhibited slightly depressed leukocyte and lymphocyte counts similar to the observations of Satué *et al.* (2012), along with reduced platelet values, while other haematological parameters were comparable to those in healthy mares. Thus, while both studies support the view that haematology alone cannot confirm endometritis, the consistent finding of reduced leukocytes and lymphocytes strengthens their role as potential indicators of systemic changes in affected mares.

Taken together, the findings consistently indicate that mare with advancing age are at significantly higher risk of uterine infections, and factors such as body condition and reproductive tract conformation further influence susceptibility. The study further highlights that endometrial cytology is a highly sensitive tool for diagnosing endometritis in mares, and that increased neutrophil presence, rather than systemic haematological deviations, provides the most reliable evidence of compromised uterine health and fertility.

CONCLUSIONS

The present study demonstrated that endometritis is a major reproductive disorder contributing to infertility in mares, with a markedly higher prevalence among animals above eight years of age. *Escherichia coli* emerged as the predominant uterine pathogen, followed by *Streptococcus* spp., either as single or mixed infections. The association between β -haemolytic *Streptococcus* and elevated uterine pH further confirmed its pathogenic significance. Endometrial cytology proved to be a sensitive and reliable diagnostic tool, as affected mares exhibited markedly increased polymorphonuclear neutrophil percentages, clearly distinguishing them from healthy individuals. Although haematological parameters remained largely within normal limits, a mild reduction in leukocyte and lymphocyte counts indicated subtle systemic immune suppression. Collectively, the findings emphasize that a combined diagnostic approach incorporating bacteriological, cytological, and haematological evaluations is essential for accurate detection and effective management of endometritis in mares, thereby improving fertility outcomes and reproductive efficiency.

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