



Research on Bovine Endometritis: Current Insights and Future Directions – A Review

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ABSTRACT

The post-partum reproductive performance of cows is identified as a pivotal factor influencing the success or failure of the dairy industry. Achieving timely uterine involution, complete endometrial regeneration, and the resumption of ovarian cyclic activity are crucial for conception within the desired timeframe. However, uterine contamination with pathogens during parturition remains an unavoidable challenge, leading to suboptimal productivity and fertility. Metritis and clinical endometritis are recognized post-partum conditions, while sub-clinical endometritis has emerged as a significant threat to achieving desired fertility in dairy cows. This review summarizes a comprehensive assessment of factors contributing to delayed clearance and persistence of uterine infections in dairy cows, particularly sub-clinical endometritis (SCE). Diagnostic techniques, including bacterial culture, the Whiteside test, endometrial cytology, and trans-rectal ultrasonography (TRUS), are discussed, each with advantages and limitations. The paper explores the association between SCE and various risk factors, such as negative energy balance (NEB), metabolic indicators, and hormonal changes during the post-partum period. The significance of parameters like body condition score (BCS), backfat thickness (BFT), and metabolic indicators (leptin, NEFA, BHBA) in assessing energy status and predicting reproductive efficiency is highlighted. In addition, the underexplored area of fungal infections in dairy cows' uteri, emphasizing fungi's opportunistic nature and their potential to cause reproductive failure has also been reviewed. The immune system's role in uterine defense against fungal infections and the diagnostic challenges, including mycological cultures and cytological examinations, the therapeutic challenges, and the poor prognosis associated with fungal endometritis have also been discussed.

Key words: Subclinical endometritis, Fertility, Bovines, Pathogens

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INTRODUCTION

Reproductive failure in dairy animals is one of the most significant issues that are responsible for losses of millions

annually. Inefficient reproduction in cattle, primarily due to conception failures, poses a substantial challenge to profitable dairy production. This is especially evident in delayed age at first calving in heifers and extended inter-calving

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intervals, ultimately reducing calf production (Singh *et al.*, 2017). The causes of infertility encompass various factors, including abnormalities in the reproductive tract, endocrine disorders, infectious agents, management errors, nutritional deficiencies, and issues with artificial insemination procedures (Singh and Pant, 1998; 1999).

Unceasing research represents an essential facet of progress. Advancements in the field of reproductive sciences have been made possible through the curiosity of scientists. The dedication of animal reproduction researchers has consistently led to the development of management strategies aimed at optimizing reproductive efficiency and minimizing economic losses.

The post-partum reproductive performance of cows plays a pivotal role in the success or failure of the dairy industry. Factors such as timely uterine involution, complete endometrial regeneration, and the resumption of ovarian cyclic activity are key to achieving conception within the desired timeframe (Sheldon and Owens, 2017). Nevertheless, uterine contamination with pathogens during parturition remains an unavoidable challenge, resulting in suboptimal productivity and fertility (Singh *et al.*, 2017). Up to half of dairy cows may develop one or more types of reproductive tract inflammatory disease within 5 weeks after calving (Pascottini *et al.*, 2023).

Metritis and clinical endometritis are two common post-partum conditions. In contrast, another uterine infection, known as sub-clinical endometritis, has emerged as a significant threat to achieving desired fertility in dairy cows. Clinical endometritis and subclinical endometritis are two of the main uterine diseases in dairy cows during the puerperium period that can directly affect fertility in dairy herds and milk production (Paiano *et al.*, 2023).

SUB-CLINICAL ENDOMETRITIS

Several factors, including dystocia, retained placenta, abortions, and stillbirth, have been identified as underlying causes leading to delayed clearance and persistence of uterine infections, including sub-clinical endometritis (Lee *et al.*, 2018). Consequently, the prompt diagnosis and treatment of sub-clinical endometritis (SCE) are of utmost importance, as they play a significant role in mitigating the risk of further fertility.

Diagnosis of sub-clinical endometritis

According to its definition, sub-clinical endometritis (SCE) in cows lacks overt clinical symptoms for diagnostic

purposes, yet it has had a significant and sustained economic impact on the dairy industry over the past decade. Various diagnostic techniques have been employed to identify SCE, including endometrial cytology, uterine biopsy, biochemical analysis of uterine fluid, uterine lavage sample optical density test (ULSOD), bacterial culture, the Whiteside test, leukocyte esterase test, and the measurement of acute-phase proteins and inflammatory markers. Additionally, Doppler and B-mode ultrasonography of the middle uterine arteries and the uterus have been used for SCE diagnosis (Sharma and Singh, 2021).

Bacterial culture: Bacterial culture has been employed as a diagnostic approach for SCE. However, several researchers have reported mixed findings when comparing it with other diagnostic methods. Consequently, the drawbacks of this method include time consumption, the potential for false-negative results, and the need for laboratory facilities.

Whiteside test (WST): Isolating non-specific organisms from uterine fluids can be a costly and time-consuming process. In response to the need for a more accessible diagnostic method with minimal infrastructural requirements, the Whiteside test (WST) has been utilized for the rapid diagnosis of SCE (Neelam, 2017). Originally developed for mastitis diagnosis, the WST assesses the concentration of leukocytes, which are typically elevated during inflammation, in uterine discharge. The degree of endometritis is determined based on the intensity of colour formation (yellow), enabling a quick diagnosis of uterine infection (Rana, 2019).

Endometrial cytology: Endometrial cytology stands out as one of the most precise and commonly used methods for diagnosing SCE. This method relies on the fact that the proportion of polymorphonuclear cells (PMNCs) increases during uterine inflammation (Pascottini, 2016). It is considered semi-invasive and comparatively cost-effective, making it a preferred choice. Different techniques, such as Cytobrush (CB), Cytotape (CT), and Low volume uterine lavage (LVUL), can be employed for endometrial sampling.

With Cytobrush, a sterile insemination casing loaded with a cytobrush is gently rotated against the uterine wall to obtain an endometrial sample. Results are interpreted by calculating the percentage of PMNCs at different post-partum stages or during estrus, making it a reliable diagnostic method (Rana, 2019). Cytotape is a newer technique for obtaining PMNCs from the uterus. It involves using a piece of paper tape rolled onto the tip of a standardly loaded insemination gun covered with a double guard sheath. The procedure includes introducing the insemination gun into

the vagina and cervix under rectal guidance, releasing the catheter's tip from the double guard sheath upon reaching the uterine lumen, and rolling it twice on the dorsal wall of the uterine body with gentle pressure applied through the rectum. Cytotape works on the same principle of evaluating PMNC proportions to diagnose uterine inflammation without the need for additional cow manipulation during sample collection, providing several advantages over the cytobrush technique (Rana, 2019). Cytotape is preferred over the cytobrush due to the superior quality of cells (less fragmentation) and reduced red blood cell (RBC) contamination, primarily attributed to the rigid bristles of the brush causing cell fragmentation when rolled on a microscope slide (Rana *et al.*, 2020).

Endometrial cytology samples can also be obtained by infusing a sterile sodium chloride solution into the uterus using a sterile, plastic infusion pipette, followed by the recovery of 4-5 mL of the solution. The retrieved solution is centrifuged, and the pellet is smeared onto a clean microscope glass slide, which is then stained with modified Giemsa dye (Rana, 2019). This technique provides a more representative sample of uterine contents due to its collection over a larger surface area. A strong correlation has been observed between bacterial load and PMN cells obtained via the uterine lavage technique (Rana and Singh, 2022). However, the results obtained through LVUL have been inconsistent, mainly due to difficulties in the recovery of lavage fluid.

Endometrial histopathology is considered the gold standard test because it allows the collection of more PMNCs, particularly from the deeper stratum compactum (Pascottini, 2016). Histologically, it provides detailed insights into the stage of inflammation, including disruption of the surface epithelium, leucocytic infiltration, cystic dilatation of endometrial glands, various degrees of glandular degeneration, and endometrial fibrosis. However, its use is debatable due to its time-consuming nature, invasiveness, and potential implications for future fertility (Coto and Lucy, 2018).

Trans-rectal ultrasonography: Trans-rectal ultrasonography (TRUS) has become an integral part of the routine assessment of the reproductive tract in dairy cows, serving as a diagnostic tool for various physiological and pathological changes in the uterus and ovaries. Given the significance of the post-partum period, early non-invasive diagnosis plays a crucial role in predicting the future fertility of dairy cows. Two primary modes of ultrasonography are employed: B-mode and Doppler mode. B-mode focuses on characterizing changes in echotexture, uterine size, fluid accumulation within the uterine lumen, and differentiation of ovarian structures based on

the transmission-reflection of ultrasonic waves. In contrast, the Doppler mode mainly relies on the principle of frequency shifts in moving red blood cells to study the hemodynamic changes in the uterus and ovaries (Sharma *et al.*, 2019b).

Numerous critical events, such as uterine involution, endometrial regeneration, and the resumption of ovarian activity, occur during the post-partum period, and their progression is significantly impacted by persistent uterine infections, including clinical and SCE (Sharma and Singh, 2019). The B-mode of TRUS detects the quantity of fluid within the uterine lumen, thickening of the endometrial wall, and cervical measurements to assess uterine inflammation in the absence of clinical signs of illness. Several researchers have established a correlation between the volume of intra-luminal uterine fluid (ILUF) and the proportion of polymorphonuclear cells (PMNCs) diagnosed through TRUS and cytology, suggesting ILUF as a potential diagnostic marker for SCE (Marino *et al.*, 2017).

The Doppler mode of ultrasonography remains an underexplored tool for diagnosing endometritis in dairy cows, although recent research has focused on assessing uterine inflammation using various hemodynamic indices, including Pulsatility index (PI), Resistance index (RI), Time-averaged mean, maximum velocity (TAMEAN and TAMAX), blood flow volume to the uterus, and the diameter of the middle uterine artery (Sharma *et al.*, 2019a). During the post-partum period, endometrial regeneration requires a substantial blood supply, extending up to six weeks post-partum (Sheldon and Dobson, 2004). Beyond this period, an increased volume of blood flow to the uterus is primarily due to the vasodilatory effects of inflammatory agents (Sharma *et al.*, 2019b). Reduced PI and RI, along with increased TAMEAN, TAMAX, blood flow volume to the uterus, and middle uterine artery diameter, have been considered important indicators for assessing uterine inflammation and subsequent reproductive performance in post-partum dairy cows (Rawy *et al.*, 2018). However, the lack of standardization and limited familiarity with Doppler ultrasonography among practitioners have made it less popular for diagnosing uterine inflammation in dairy cows.

In conclusion, TRUS alone may not exhibit high sensitivity or specificity for diagnostic purposes, but when combined with cytology or biopsy, the results regarding diagnosis have proven to be accurate and reliable (Salah and Yimer, 2017). Therefore, it is necessary to compare endometrial histopathology, cytology, and ultrasonography to determine the suitability of the methods used for diagnosing SCE.

Energy status and their association with sub-clinical endometritis

Several risk factors, including negative energy balance (NEB), have been linked to the development of sub-clinical endometritis (SCE) after calving. During early lactation, reduced dry matter intake, elevated serum non-esterified fatty acids (NEFA), and β -hydroxybutyrate (BHBA) concentrations can lead to SCE. Serum levels of BHBA, albumin, and urea are related to the incidence of SE, being BHBA a predisposing factor and albumin and urea protective factors. Therefore, these metabolites should be carefully considered during the postpartum period as indicators of SCE (Yanez *et al.*, 2022).

The post-partum period is characterized by heightened energy demands for lactation, resulting in NEB, which can persist for varying durations, depending on the nutritional resources available to dairy cows during this phase. To assess the energy status in dairy cows, both subjective and objective parameters are examined, including body condition score (BCS), backfat thickness (BFT) in the Thurl area, and metabolic indicators such as serum leptin, NEFA, and BHBA (Sharma *et al.*, 2021).

The visual or tactile estimation of subcutaneous fat, which corresponds to the cow's BCS, serves as an indicator of energy reserves and reflects the energy metabolism during lactation. BCS is a reliable criterion for assessing the balance between feed intake and milk production, offering a quick, non-invasive, and cost-effective method that holds several advantages over other techniques. Cows with a BCS lower than 2.50 at calving are more likely to experience prolonged uterine inflammation, while those with very high or low BCS are at greater risk of dystocia, retained fetal membranes, and subsequent SCE (LeBlanc, 2014). There exists an inverse relationship between the polymorphonuclear cell (PMNC) population and BCS during the screening of cows for SCE at 6-8 weeks post-calving (Rana, 2019). The incidence of SCE by day 42 has been reported as 18.75% in cows with uncomplicated postpartum events and 100% in cows with complications such as retained placenta and metritis (Rana and Singh, 2022).

Backfat thickness (BFT) is considered a more accurate and reliable indicator of a cow's metabolic status, as subcutaneous fat reserves are directly correlated with energy status and influence post-calving reproductive activity (Galindo *et al.*, 2013). Therefore, an objective assessment of dairy cow energy status through BFT is crucial in predicting reproductive efficiency after calving.

Numerous hormones, including leptin, play essential roles in regulating metabolic and reproductive activities in

dairy cows. Leptin, synthesized by adipose tissue, actively participates in fertility and immune function regulation. Leptin receptors are present on the bovine endometrium, granulosa cells, and corpus luteum, making them pivotal in resuming ovarian activity post-calving (Colakoglu *et al.*, 2017). A significant decrease in leptin concentrations due to energy reserve mobilization, BCS loss, and an increase in NEFA and BHBA levels during the immediate post-partum period can lead to persistent uterine inflammation by inhibiting neutrophil phagocytic activity (Tanaka *et al.*, 2008). However, the direct role of leptin in predisposing cows to SCE remains unclear and requires further investigation.

Metabolic profile indicators, such as non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA), are of great importance in the early diagnosis of energy metabolism disorders in dairy cows (Bhadaniya *et al.*, 2019). Primiparous cows tend to have higher NEFA and BHBA concentrations immediately after calving, making them more susceptible to SCE (Colakoglu *et al.*, 2017).

Further research is essential to enhance our understanding of SCE, particularly its association with the energy status of animals.

FUNGAL ENDOMETRITIS

While there have been numerous studies on endometritis caused by pathogenic bacteria, there is a noticeable dearth of research on fungal infections in the uteri of dairy cows. It's important to note that fungi, in addition to bacteria, can cause infections in the uteri of cows (Saini *et al.*, 2019a).

Fungi are opportunistic pathogens, taking hold in uterine or vaginal environments that have been chronically disturbed (Stout, 2008). They can induce reproductive failure in animals either by directly infecting the reproductive system or by producing toxic metabolites (mycotoxins) *in vitro*, which are later ingested and absorbed (Laing *et al.*, 1988). Fungal infections of the genital tract can lead to endometritis, abortions, and vulvovaginitis. The genera *Aspergillus* and *Penicillium* can thrive under suitable conditions, producing toxins responsible for abortions and metritis in cows (Verma *et al.*, 1999).

Persistent endometritis and repeated intra-uterine antibiotic therapy are predisposing factors for fungal uterine infections. Since the precise conditions that facilitate fungal colonization of the uterus remain unclear, and effective treatment methods for fungal endometritis are currently limited, recurrence is common (Stout, 2008). It is believed that fungi or yeasts that colonize the uterus usually originate from the vaginal and external genitalia areas

and are introduced into the uterus during insemination or intra-uterine treatment (Dascanio *et al.*, 2001).

For a disease to manifest, a susceptible host, a virulent pathogen, and a conducive environment must all be present. Dairy cows face significant metabolic and physical challenges during the transition to lactation. In terms of metabolic challenges, this transition period is characterized by negative energy, mineral, and vitamin imbalances, marked by a decline in dry-matter intake immediately after calving, resulting in a sharp drop in glucose, minerals, and vitamins. This leads to increased mobilization of body fat in the form of non-esterified fatty acids (Galvao, 2013). This state of negative energy balance can result in immune suppression and heightened susceptibility to diseases (Cai *et al.*, 1994).

The pathogenicity of a fungus hinges on its ability to adapt to the host tissue environment and withstand the host's defence mechanisms. Similar to other microbial pathogens, fungal infections involve various stages, including entry and adherence to host tissue, tissue invasion, multiplication, colonization, dissemination within tissues, evasion of the host's immune system, and tissue damage (Khan *et al.*, 2010). Fungi in the genital tract primarily come from the caudal reproductive tract, where they exist as commensals. The genital system usually becomes contaminated by these microorganisms from the environment during breeding, parturition, or genital manipulation. Disease can occur when fungi breach host barriers, or when immunological deficiencies or other debilitating conditions favour fungal entry and growth. The cervico-vaginal fungal community changes in cows according to parity order (multiparous cows vs. nulliparous cows and non-sexually active heifers). Hence, multiparous cows exhibited divergent taxonomic profiles as well as less richness and evenness than nulliparous cows and non-sexually active heifers (De Carli *et al.*, 2022).

The immune system plays a significant role in the uterine defence against fungal infections. Fungal pathogens are mostly opportunistic, causing infections when host defences are compromised. Typically, the uterus is a sterile environment, in contrast to the vagina, which hosts numerous microorganisms. In the immediate postpartum period, the uteri of cows are often contaminated with various organisms. Opportunistic pathogens from the normal vaginal flora or the environment may occasionally invade the uterus. However, a healthy uterus can efficiently eliminate these transient infections. In most cases, a sterile uterine environment should be re-established within eight weeks postpartum (Foldi *et al.*, 2006). Nevertheless, in some instances, microbes may persist and lead to endometritis.

The overall incidence of fungal endometritis was found to be 10.39% in cows and 5.55% in buffaloes in different agro-climatic zones of Himachal Pradesh, with varying rates (Saini *et al.*, 2019b). The isolates included *Rhizopus* spp., *Aspergillus niger*, *Blastomyces* spp., *Mucor* spp., *Aspergillus* spp., *Penicillium* spp., *Rhodotorula* spp., *Candida* spp., *Penicilliumvermiculatum*, *Aspergillus versicolor*, *Murogenella* spp., *Curvularia* spp., *Nigrospora* spp., *Alternaria* spp., and *Pithomyces* spp. (Saini *et al.*, 2019b). Itraconazole was highly sensitive against all the yeast isolates, followed by Miconazole and Clotrimazole in 75%, and Ketoconazole in 37.5% of the isolates (Saini, 2018).

Diagnosis

Cows afflicted with fungal endometritis typically exhibit a protracted history of conception difficulties. To diagnose this condition, uterine discharge collected from an animal during estrus undergoes fungal pathogen isolation. Sabouraud's Dextrose Agar (SDA) is the most commonly utilized medium for this purpose. The fungal pathogens are isolated through the spot inoculation technique, similar to the method employed for bacterial swabs. These samples are then inoculated onto Sabouraud's dextrose agar. It's essential to incubate mycological cultures for a minimum of five days before confirming a negative result due to the often-slow growth of fungal colonies. Evaluation of visual characteristics of the fungal colonies, such as texture, pigment, and growth rate on the medium, along with microscopic examination under a light microscope, is used to identify the morphological structures of the fungal species on the slide.

An alternative method for diagnosing fungal endometritis is cytological examination of uterine secretions, employing Modified Wright's stain for staining and subsequent cytological analysis. This examination helps reveal signs of an ongoing inflammatory reaction, typically marked by the presence of neutrophils in most cases. It may also aid in the detection of yeasts or elongated fungal hyphae (Stout, 2008). In cases where cytology yields inconclusive results, an endometrial biopsy can be performed to diagnose fungal endometritis (Dascanio *et al.*, 2001).

The selection of antifungal agents for treating fungal endometritis should be guided by the in-vitro susceptibility of specific fungal isolates to available drugs. However, this selection is complicated because fungi require special culture media and extended incubation periods (Beltaire *et al.*, 2012).

Uterine infection with fungal organisms poses a significant therapeutic challenge, with a poor prognosis in terms of recovery speed and future breeding potential.

This is because these organisms invade deep into the endometrium, leading to fibrotic degeneration. While the presence of fungi in the uterine lumen of post-partum cows has been reported, it has received limited attention, as most studies of the bovine post-partum uterus have primarily focused on bacteria. Consequently, there is a pressing need for further investigations to comprehend the role of opportunistic fungal pathogens in various theriogenological processes.

CONCLUSIONS

While the literature has explored various causes of conception failure, a significant contributor to reduced fertility in dairy cows is post-partum endometritis. Metritis and clinical endometritis are commonly observed post-partum conditions, and sub-clinical endometritis has emerged as an additional challenge to achieving desired fertility in dairy cows. SCE's impact on dairy cow fertility is well-recognized, primarily due to the absence of clinical signs, making diagnosis and treatment more challenging.

Moreover, despite establishing the presence of fungi in the uterine lumen of post-partum cows, most studies on the post-partum uterus of bovines have been centered on bacterial infections. Therefore, there is a pressing need for more comprehensive investigations into the roles and management of SCE and fungal endometritis in bovine reproduction.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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