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Subclinical Endometritis in Postpartum Buffaloes: An Emerging Threat

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ABSTRACT

Buffalo contributes 12.8 per cent of world milk supply. In India, buffalo accounts for 33 per cent of the milk producing animals and 45 per cent of overall milk production of the country. Optimum fertility of buffaloes is the key to economically successful dairy farming. Postpartum uterine infections have negative impact on reproductive performance leading to drastic reduction in farm return. Endometritis being one of the major postpartum disorder causing heavy losses to dairy industry. Postpartum sub-clinical endometritis is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis is indicated by the presence of purulent/mucopurulent discharge. Routine methods for diagnosing endometritis involve uterine biopsy, lavage and swab but these techniques causes irritation and distortion of cells. Endometrial cytology by cytobrush technique is most efficient and early diagnostic technique when used along with microbial assay for diagnosis of sub-clinical endometritis. Following diagnostic accuracy, one has to use appropriate therapy for management of this condition. An effective treatment is one which eliminates load of pathogenic bacteria and enhances uterine defense and repair mechanisms, and thereby halts and reverses the inflammatory changes that impair fertility. The treatment of endometritis should not be limited to clinical or bacteriological cure but also be economical and should improve the fertility. A wide variety of therapies for endometritis have been used with variable success proving the treatment of this condition to be still challenging.

Keywords: Subclinical endometritis, endometrial cytology, uterine defense mechanism

The current world buffalo *(Bubalus bubalis)* population is 194 million (FAO, 2010) out of which 97.13 per cent are in Asia and the Pacific region, whereas 57 per cent of it is in India (110.58 million). Buffalo contributes 12.8 per cent of world milk supply. In India, buffalo accounts for 33 per cent of the milk producing animals and 45 per cent of overall milk production of the country.

Buffalo, "the black gold of India," originated in the Indo-Gangetic plain, flourished throughout Asia and became a symbol of life, religion and endurance. The riverine breeds of buffalo of the Indian sub-continent are mainly raised for milk production since; they yield six to seven litres of milk daily. Buffalo milk is rich in nutrients (e.g. fat, lactose, protein and minerals) and contains less water than cow milk (FAO, 2010). While, the world cattle population over the last two decades has increased by less than one per cent per year, the buffalo population has gone up by two per cent per year, with higher rise in India (3.5%). This population growth rate of buffalo can be attributed to better feed conversion efficiency as compared to the local cattle.

In many parts of India milk production is apparently the greatest component of economic change of the lower strata of the farming community, while in some parts of the country, income from the sale of milk constituted 25 per cent of the total farm income. This income also corrects the negative balances due to losses from agricultural farming particularly for marginal and small farmers. Hence, milk production is apparently the major component of economic change in the lower section of the farming community.

Optimum fertility of buffaloes is the key to economically successful dairy farming. Postpartum uterine infections have negative impact on reproductive performance leading to drastic reduction in farm return. Postpartum reproductive disorders cause heavy economic losses in livestock sector in India. Endometritis being one of the major postpartum disorder causing heavy losses to dairy industry.

In clinical surveys, incidence of endometritis has been reported to vary from 2.4 to 20 per cent (Narasimha Rao and Sreemannarayana, 1982) and 4.5 to 25 per cent in an abattoir study (Vale *et al.*, 1984) in buffaloes. Studies on clinical and sub-clinical endometritis reported the prevalence of these diseases ranging from 18 to 37 per cent (Drillich *et al*., 2005) and 12 to 94 per cent (Barlund *et al*., 2008), respectively. Endometritis delays ovarian rebound and uterine involution, increase days open and accordingly extend the calving interval. It not only causes infertility but also results in subfertility even after successful clinical resolution of the disease. Pande (2012) in a retrospective study on prevalence of peripartum disorders in buffaloes of Jabalpur (Madhya Pradesh) revealed an incidence of postpartum endometritis as 20.28 per cent between 2005-2010.

The prevalence of postpartum endometritis depends upon the occurrence of early postpartum uterine diseases, the time of examination and the diagnostic technique (Falknberg and Heuwieser, 2005). Routine methods for diagnosing endometritis involve uterine biopsy, lavage and swab but these techniques causes irritation and distortion of cells. Consistency in dealing with endometritis following conventional therapies is not achieved due to lack of diagnostic standards (Kasimanickam *et al.*, 2005). However, more sophisticated novel diagnostic techniques like endometrial cytology and ultrasonography concentrate on endometrial alterations and imaging of uterine changes beyond clinical signs of Endometritis and play an important role for early diagnosis of sub-clinical endometritis.

Following diagnostic accuracy, one has to use appropriate therapy for management of this condition. Use of antibiotics have also been tried to combat uterine infections with variable success but this often requires frequent administration and milk disposal for fear of antibiotic residues in milk. Apart from high cost of treatment, there are also chances of development of antibiotic resistance and decrease in phagocytic activity of neutrophils. Intrauterine administration of 0.25-0.5 per cent Lugol's iodine has been reported to be effective in treating endometritis but its inadvertent administration may cause intense irritation of endometrium resulting in coagulative necrosis and ovaro-bursal adhesions. Alternative or additional therapeutic measures including modulation of uterine immunity etc. involving intrauterine administration of immunomodulators in uterus are also playing potential role in resolving endometritis. So, considering above facts this review depicts an overview of sub-clinical endometritis including uterine defence mechanism, pathophysiology, diagnosis and its therapeutic management.

Endometritis and its prevalence

Histologically, endometritis is defined as a disruption of the epithelium with the presence of inflammatory cells (Bodurant, 1999). Subclinical endometritis was first described as cytological endometritis considering the presence of PMNL in the endometrial lumen (Gilbert *et al.*, 1998), and then standardized by Kasimanickam *et al*. (2004) based on the negative effects on reproductive performance (Madoz *et al*., 2014). Postpartum sub-clinical endometritis (SE) is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis (CE) is indicated by the presence of purulent/mucopurulent discharge (Sheldon *et al*., 2006). Postpartum endometritis has a negative effect on reproductive performance, causing an increase in the number of services per pregnancy and in the length of calving-conception interval (Bell and Roberts, 2007).

The prevalence of sub-clinical endometritis varied between 20 to 53 per cent from 20 to 60 days postpartum (Kasimanickam *et al*., 2004 and Gilbert *et al*., 2005). LeBlanc *et al*. (2012) quoted the incidence of metritis between 10 and 20 per cent, clinical endometritis as 15 per cent and sub-clinical or cytological endometritis as 15 per cent in postpartum dairy cattle. While Bajaj (2015) reported an incidence of postpartum endometritis in buffaloes to be 50.67 per cent, out of which incidence of sub-clinical and clinical endometritis was recorded as 26.00 and 24.67 per cent, respectively.

Etiology and predisposing factors of endometritis

Normally in a postpartum animal uterine infection is shed off by rapid uterine involution discharging debris and mobilization of natural host defense mechanism (Hussain and Daniel, 1992). Ability of uterus to combat infections is decreased due to periparturient problems like dropsy of fetus and fetal membrane, traumatization of genital tissues during dystocia or obstetrical manipulation, metabolic disorders, traditional practices like inserting of hand by owners and quacks for stimulation of milk let down and unhygienic managemental conditions during calving period (Paisley *et al*., 1986). Postpartum uterine discharges if not expelled timely provide a medium for bacterial multiplication. If the uterus is severely debilitated, any of a variety of contaminating organisms can cause toxic puerperal metritis followed by its persistence and impaired fertility (Seals *et al*., 2002).

A number of Gram-positive and negative aerobes and anaerobes can be isolated from the early postpartum uterus. Most of these are environmental contaminants and are gradually eliminated during the first 6 weeks postpartum. Even though, numerous bacteria in a variety of combinations have been isolated from infected uterus, *Archanobacterium pyogenes* either alone or in combination with other bacteria such as the anaerobic *Fusobacterium necrophorum*, *Bacteroides* spp. and *Escherichia coli* are usually associated with uterine infection in cattle (Sheldon *et al*., 2004 and Bajaj *et al*., 2015).

There is decrease in intrauterine oxygen reductase potential in the presence of aerobic bacteria infection thereby creating an anaerobic environment. This decrease in intrauterine oxygen reductase potential may be associated with either microorganism metabolism or increase in oxygen consumption by polymorphonuclear (PMN) cells. The presence of *A. pyogenes* in uterine fluid approximately 21 days postpartum indicates severe endometritis and results in conception failure (Dohmen *et al*., 1995).

Uterine defense mechanism

Bacterial contamination of uterus may also occur during natural service or insemination. Persistence of infection in the uterus is determined by the degree of uterine contamination, defense mechanism and the presence of devitalized tissues and fluid for the growth of bacteria (Noakes *et al*., 2002). Oportunistic pathogens are not able to colonize in the genital tract during normal circumstances due to several protective mechanisms, *viz.*, anatomical and functional barriers, specific and nonspecific immune responses (Foldi *et al*., 2006).

The major anatomical barriers between contamination and the sterile uterus include the muscular sphincters of the vulva, vestibule and cervix. The collagenous rings and cervico-vaginal mucus (especially the scanty, tenacious mucus of the luteal phase) can function as physical barrier for organisms (Sheldon and Dobson, 2004). The circular and longitudinal layers of the uterine musculature provide physical propulsion of particular material, including microbes. Epithelial cells are the first to make contact with potential pathogens that enter the uterus. Epithelial and stromal cells interactions are critically important for the endometrial function, with stromal cells affecting epithelial cells through the release of soluble factors and turning over of extra cellular matrix (Wira *et al*., 2005).

The innate immune system is alerted due to the presence of pathogens by endometrial cell toll-like receptors (TLRs) detecting pathogen–associated molecules such as lipopolysaccharide (LPS), DNA and bacterial lipids. The innate immune system, including toll-like receptors (TLRs), antimicrobial peptides (AMPs) and acute phase proteins (APPs) constitutes an initial defense of the mammalian endometrium against microbes. The endometrial cells secrete cytokines and chemokines to direct the immune response and increase the expression of AMPs. Chemokines attract PMNs and macrophages to eliminate the bacteria, although neutrophil function often gets disturbed in postpartum animal. Persistence of PMNs in the endometrium in the absence of bacteria is thought to be the primary characteristic of sub-clinical endometritis (Zerbe *et al*., 2003). Blood-derived PMNs are the main effector cells for removing bacteria from the uterus after calving. However, endocrine and metabolic changes around the time of parturition in cattle modulate PMN phagocytic function and gene expression (Medsen *et al*., 2002). Furthermore, blood PMNs obtained from endometritic cattle have shown decreased phagocytic activity. The transmigration of PMNs into the uterine lumen also modulates their function. For example, interleukin-8 induced attraction of PMNs into the uterine lumen increased the generation of reactive oxygen species (ROS) by these cells. However, when PMNs are in the uterine lumen, their function is further modulated by soluble factors in lochial secretions. Whereas, lochial secretions of healthy cattle only moderately affect the function of PMNs, the secretions of infected cattle severely depressed the generation of ROS (Zerbe *et al*., 2002).

Hematological alterations during endometritis

Various blood parameters like total leucocytic count, differential leucocytic count and hemoglobin concentration have been used along with other diagnostic techniques to correlate the inflammatory and infectious conditions of uterus.

Duvel *et al*. (2014) in their study on peripheral blood leucocytes of cows with sub-clinical endometritis reported that cows suffering from sub-clinical endometritis showed significantly higher blood leucocyte count and a significantly elevated monocyte and neutrophil count. Whereas, Heidarpour *et al*. (2014) observed a significant increase in leucocytes ($p<0.05$), neutrophils ($p<0.001$) and lymphocyte ($p<0.001$) counts in cows suffering from clinical and sub-clinical endometritis as compared to healthy animals.

Acute phase protein levels

Acute phase proteins (APP) refer to a group of hepatic glycoproteins which are stimulated by inflammatory mediators and respond to initial reaction to infection, inflammation or trauma in animals (Marinkovic *et al*., 1989). The function of APP is to promote immunoglobulin production and tissue repair, prevent further injury and recycle useful molecules and debris (Kent, 1992).

Ruminants are significantly different from other species in their acute phase response in that haptoglobin (Hp) is the major acute phase protein. The other APPs in cattle are serum amyloid A (SAA) and α_1 acid glycoprotein (AGP). In healthy cattle the serum haptoglobin concentration is <20 mg/L but can increase upto >2 g/L in 2 days of infection. Many studies have indicated that cattle and buffaloes with sub-clinical (SE) and clinical endometritis (CE) showed a significant higher haptoglobin concentration as compared with healthy animals (Heidarpour *et al*., 2012; Bajaj *et al*., 2015). Assessment of serum haptoglobin concentrations at different points of time could serve as reliable biomarker for the diagnosis and monitoring of clinical endometritis (Biswal *et al*., 2014).

Diagnosis

Early and efficient diagnosis is essential for proper treatment of condition. Although there are various methods for diagnosis of endometritis in buffaloes, but the diagnosis still remains challenging due to lack of simple and effective diagnostic technique. Different diagnostic methods commonly used are as under:

Per-rectal palpation

Though trans-rectal palpation of uterus is the most commonly practiced diagnostic method but it is not an accurate method for diagnosis of all type of endometritis (Oral *et al*., 2009). However a holistic and systematic approach will be more helpful in providing information for such a multi factorial disease.

Vaginoscopy

Vaginoscopy is considered as a more accurate method than rectal palpation for diagnosis of uterine infections. Observation of vaginal discharges are useful for the diagnosis of clinical endometritis but not for sub-clinical endometritis (Oral *et al*., 2009) as discharges may not always be collected due to closure of os, although these animals may harbour infection in uterus. A single vaginoscopic examination therefore lacks accuracy and hence not a true indicator of absence of uterine inflammation.

Whiteside test

W.H. Whiteside (1939) first used this test for diagnosis of mastitis. In this test, cervical mucus is collected aseptically from suspected animals and is boiled with equal amount of 5 per cent sodium hydroxide. The test is considered positive if the colour turns yellow. This test is based on correlation between the number of leucocytes present in the mucus and intensity of yellow colour (Pateria and Rawal, 1990). However this test may be considered as a test for diagnosis of genital infections but not for endometritis always.

Endometrial biopsy

Microscopic examination of endometrial biopsy samples of endometritic reveal changes in endometrium like disruption of surface epithelium, leucocytic infiltration, varying degrees of glandular degeneration and periglandular fibrosis (Bajaj, 2002).

The drawbacks with this method is that it is time consuming, expensive, invasive, has detrimental effect on future fertility and is seldom useful on the spot for planning therapeutic regimen (Sheldon *et al*., 2006).

Trans-rectal ultrasonography

Trans-rectal ultrasonography is a useful diagnostic tool in determining uterine size, echotexture and fluid accumulation in endometritis (Honparkhe *et al*., 2007). Monitoring endometrial echotexture alterations, especially homogeneity and contrast changed dependency on the cellular density and inflammation status may be potential diagnostic markers for sub-clinical endometritis in cows (Polat *et al*., 2015). Ultrasonography though a practical cow-side test, but when used alone is not specific enough to accurately diagnose sub-endometritis in animals (Barlund *et al*., 2008 and Bajaj, 2015).

Uterine/endometrial cytology

Recent researches have indicated that uterine cytology and endometrial biopsy in postpartum animals might be useful and accurate procedures for detecting existence and severity of endometritis (Honparkhe *et al*., 2014). Endometrial cytology is considered superior to endometrial biopsy as the uterine biopsy outputs are influenced by oestrous cycle whereas there is no significant change in superficial endometrial layer (Madoz *et al*., 2014).

Two methods are commonly used for uterine cytology- low volume uterine lavage and cytobrush technique. Both the techniques are based on percentage of neutrophils present in samples obtained. Cytobrush technique is simple, non-invasive, reliable, efficient and easy to master, provides sufficient uterine cells to perform both cytology and gene expression analysis in single sampling (Ghasemi *et al*., 2012; Baranski *et al.*, 2013). Various research workers have used different threshold values of PMN cells for the identification of sub-clinical endometritis. Kasimanickam *et al*. (2004) found >18 per cent neutrophils at 20-33 days postpartum or >10 per cent neutrophils at 34-47 days postpartum in uterine samples as an indicative of sub-clinical endometritis whereas, Gilbert *et al*. (2005) found 5 per cent neutrophils at 40 to 60 days postpartum as an indicator of sub-clinical endometritis in cattle, while Barlund *et al*. (2008) used a neutrophil threshold value of 8 per cent at 28-41 days postpartum in cattle to declare endometritis. Various research studies have supported that endometrial cytology by cytobrush technique is most efficient and early diagnostic technique when used along with microbial assay for diagnosis of sub-clinical endometritis (Honparkhe *et al*., 2014; Bajaj *et al*., 2015).

Therapeutic management

Endometritis is often self-limiting with recovery occurring after subsequent oestrous cycles (Arthur *et al*., 1989). If Uterine Defence Mechanism (UDM) is impaired or weakened, bacteria may colonize the uterus and lead to development of uterine infection and endometritis. Thus an effective treatment is one which eliminates load of pathogenic bacteria and enhances uterine defense and repair mechanisms, and thereby halts and reverses the inflammatory changes that impair fertility. So, an ideal therapy for uterine infections comprises of elimination of pathogenic bacteria from uterus without inhibiting normal UDM and at the same time should not cause adulteration of milk or meat for human consumption (Agarwal *et al*., 2013).

Early treatment of endometritis results in better conception rates and shorter calving intervals in herds suffering from endometritis. The treatment of endometritis should not be limited to clinical or bacteriological cure but also be economical and should improve the fertility. A wide variety of therapies for endometritis have been used with variable success proving the treatment of this condition to be still challenging. A veterinarian has to consider many aspects like type of drug, its dose and route of administration, economics and its residual effect before initiating the treatment. The commonly used intrauterine and systemic therapies includes:

Lugol's Iodine

Dilute solution of Lugol's iodine has been commonly used by vets for treatment of endometritis as it causes irritation of endometrium, stimulates uterine tone and mobilizes neutrophils to uterine lumen (Roberts, 1971).

Comparative study on concentration of Lugol's iodine for management of endometritis has revealed that 0.5 per cent Lugol's iodine was more effective as compared to 0.25 per cent and 0.1 per cent concentration in clearing CVM (83% vs 33% and 0%, respectively) with increasing conception rates (66.6% vs 16.6% and 0%, respectively) (Singh *et al*., 2010)

Antibiotics

Antibiotics have been widely used for the treatment of uterine infections but the success is highly variable in terms of clearing infection and conception rate. Treatment of endometritis by antibiotics should be directed towards improving fertility. Antibiotic should be active against the main uterine pathogens and should maintain its activity in the environment of the uterus. The treatment also should not inhibit the normal uterine defence mechanism and should be well tolerated and not induce irritation in the endometrium (Azawi, 2008).

Indiscriminate use of antibiotics in producing animals is under critical debate as far as their residue concentrations in milk and meat and resistance in humans against antibiotics used is concerned.

Prostaglandins

Prostaglandin $F_2\alpha$ and its analogues are also being used as treatment of choice (Drillich *et al*., 2005). They decrease progesterone concentration, increase estrogen concentration associated with luteolysis, increase uterine contractions, enhance phagocytic activity of uterine PMNs. This luteolysis and follicular growth results in increased resistance of uterus to bacterial infection. Prostaglandin $F_2\alpha$ and its analogues have been found to be more effective than oestradiol for treatment of first degree endometritis in cows (Akhtar *et al*., 2009).

Immunomodulators

In present scenario parental and intrauterine treatments with various antibiotics give inconsistent results. Higher cost of treatment, compulsory milk disposal, antibiotic resistance and inhibition of uterine defence mechanism (UDM) all led to find an alternative therapy for treatment of uterine infections. Immunomodulators are considered as an alternative approach for treating uterine infections. These substances when infused into uterus activate UDM and initiate local immune system (Dhaliwal *et al*., 2001). The different immunomodulators used in treatment of endometritis include:

E. coli Lipopolysaccharide (E. coli LPS)

Administration of 100 µg *E. coli* lipopolysaccharide (LPS) as single intrauterine infusion stimulate uterine defense mechanism by influx of neutrophils in uterine lumen within 6 hrs, of administration and clears the infection thereby restoring the fertility (Singh *et al*., 2000, Dadarwal *et al*., 2007; Bajaj *et al*., 2015).

E. coli LPS has been found to be most effective in controlling uterine infections as compared to other immunomodulators like lysozyme and oyster glycogen (Palanisamy *et al*., 2014)

Oyster glycogen

Intrauterine administration of oyster glycogen leads to PMN migration into uterine lumen. Variable concentration of oyster glycogen (OG) 1.0 or 10 per cent leads to leucocytosis in uterine lumen by 20-30 folds.

Proteolytic enzymes

Trypsin, chymotrypsin and papain are hydrolytic enzymes that have the capacity to split proteins and fat bonds. The immunomodulatory effect of proteolytic enzymes occurs both directly and indirectly. Papain works directly as a cystein-protease, similar to bacterial cystein-proteases from gram-negative anaerobes, on the CD14 molecule of macrophages and monocytes and raises up their level of efficacy as the instigator of acute phase reaction.

Administration of proteolytic enzymes results in better pregnancy rates as compared to E. coli LPS and levamisol (Honparkhe *et al*., 2014; Singh, 2014)

Herbal drugs

Herbal ingredients like *Aristolochia bactreata, Rubia cardifolia, Peganum harmala, Camiphora molmol* and *Lepadenia reticulata* have emmenagogue, uterotonic, anti-inflammatory and antibacterial actions and can be used as an alternative approach for treatment of endometritis (Bajaj, 2002).

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