Strategic treatment with immunomodulators to resolve endometritis in cow: A review

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ABSTRACT

Postpartum uterine infections result from uterine contamination with bacteria during parturition. The uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and release of bacterial toxins that lead to establishment of uterine diseases. Treatment of endometritis with parenteral or intra-uterine infusion of the antibacterial agents and antibiotics have met with varying degree of success but inconsistence of recovery rate, high cost of treatment, milk disposal after antibiotic therapy, immergence of microbial resistance are obvious disadvantage of their use. They also markedly destroy the phagocytic activity of polymorphonuclear leucocytes, which are responsible for maintaining non-specific uterine defence. So, there is urgent need to find out an alternative therapy for treatment of uterine infections by activation of natural defence mechanism in the uterus. Prospects in use of certain non-specific immunomodulators in routine therapeutic protocol for endometritis and other uterine disorders is reviewed in present studies.

Key words: Cow, Endometritis, Immunomodulators, Treatment.

INTRODUCTION

High reproductive performance is required for successful management of dairy farms. Reproductive disorder is one of the main impediments in success of dairy husbandry and causes considerable magnitude of economic losses in livestock sector in India. The annual incidence of uterine infections in postpartum animals ranges from 10-50% in dairy cattle (Lewis, 1997) and 20-75% in dairy buffaloes (Usmani et al., 2001). Uterine abnormalities include endometritis, metritis, pyometra, mucormetra, uterine abscess and tumors. Among all the above affections of uterus, endometritis is most commonly encountered under field or farm conditions in cattle and buffaloes (Agarwal and Tomer, 2003). Endometritis is defined as mild inflammation of the endometrium that commonly occurs without any systemic or generalized disturbances following calving, coitus or artificial insemination in cattle (Hussain and Danniel, 1991; Maurya et al., 1992; Saini, 1993). Among the periparturient diseases of the dairy cattle, endometritis alone is reported to have greatest impact on fertility (Borsberry and Dobson, 1989). Under normal circumstances, the uterine defence mechanism (UDM) prevents invading bacteria from colonizing in the uterus but when this mechanism gets impaired or weakened, bacteria may colonize in the uterus and lead to endometritis (Hussain and Daniel, 1991). Predisposing factors that are associated with the development of endometritis includes retained placenta, dystocia, multiple births, abortion, high milk yield, season of the year of calving etc. (Noakes et al., 2001). Endometritis is often self limiting with recovery occurring after subsequent estrous cycle as infections are eliminated either by contractions of the myometrium, which forces the lochia out through the cervix or by phagocytic activity of leucocytes in the uterine fluids and endometrium (Vandeplassche and Bouters, 1983). Although the treatment of endometritis with parenteral or intra-uterine infusion of the antibacterial agents and antibiotics have meet with varying degree of success (Shukla and Pandit, 1989), the inconsistence of recovery rate, high cost of treatment, milk disposal after antibiotic therapy, immergence of microbial resistance are obvious disadvantage of their use. Moreover, antimicrobial drugs have also been shown to markedly destroy the phagocytic activity of polymorphonuclear leucocytes (PMN cells), which are responsible for maintaining non-specific uterine defense (Massera et al., 1980).

Incidence of endometritis: The incidence of endometritis is very high during first two weeks of postpartum and the rate varied 85 to 93% but reduces to 5 - 9 % in 46 to 60 days postpartum (Elliot et al., 1968). In overseas, the overall incidence of endometritis varied from 34 - 44% (Heuwieser et al., 2000). The incidence of clinical endometritis has been found to be 27% in lactating cattle of which has most significant risk factor being retention of foetal membrane...
(Timothy et al., 2010). The incidence of clinical endometritis was recorded as 16.9% (Sheldon et al., 2006) whereas, in another study it has been seen that the incidence of subclinical endometritis was 53% at 40 to 60 days post-partum and found to be associated with delayed conception (Gilbert et al., 2005). The rate of endometritis is reduced in progress of lactation in dairy cattle. The prevalence of endometritis during early (15-20d), intermediate (21-28d) and late (29-60d) post-partum period was recorded as 67.8%, 40.5% and 14.4%, respectively in two Japanese dairy herds (Gautam et al., 2009).

**Etiology:** Bacteria are, more or less always, present in the reproductive tract of a cow, which become pathogenic under favourable conditions and cause clinical or subclinical signs of endometritis. A wide range of bacterial species contaminate the uterus, but clinical disease is only associated with particular uterine pathogens, including *E. coli*, *A. pyogenes*, *Prevotella sp.*, *Fusobacterium sp.* and *P. melaninogenicus* (Sheldon et al., 2002 and Williams et al., 2005). *E. coli* is mainly present in uterus during the first few days postpartum whereas, *A. pyogenes* and other Gram negative anaerobes are present in the uterus for a longer period (Dohmen et al., 1995). Persistent bacterial infections with *Arcanobacter pyogenes* and *Bacteroides sp.* apparently reduce chemotactic and phagocytic activity of neutrophilic cells (BonDurant, 1999). Some of these bacteria act synergistically to enhance the likelihood and severity of uterine diseases (Olson et al., 1984). Studies to evaluate the appearance and odor of vaginal mucus have shown that *A. pyogenes*, *Proteus sp.* and *F. necrophorum* are associated with purulent or mucopurulent discharge evident in the vaginal mucus while *A. pyogenes*, *E. coli*, and non-hemolytic *Streptococcus sp.* are associated with foul smelling exudates (Williams et al., 2005). It has been reported that persistent infection with *A. pyogenes* after 21 days postpartum will reduce conception rate at first postpartum service (Singh et al., 2008). Apart from bacterial agents, several fungi viz. *Candida albicans*, *C. tropicalis*, *C. pseudotropicalis*, *C. guillermondii*, *Mucor* and *Aspergillus sp.* were also isolated from cows with endometritis and endo-cervicitis (Decan and Rosu, 1973).

In another study, Singla et al. (2004) reported that the microorganisms that were isolated from uterine swabs of cows with subclinical endometritis were mainly *E. coli*, *Staphylococcus aureus* and *Proteus spp.* whereas the incidence of *Strep. spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, *Corynebacterium spp.* and *Bacillus spp.* were relatively less. On the other hand, Barman et al. (2013) in a recent study reported that non-lactose fermenting organisms belonging to family *Enterobacteriaceae* were the most common isolates (25%), followed by *E. coli* (20%), *Streptococcus spp.* (15%), *Corynebacterium spp.* (15%), *Staphylococcal spp.*, *Pseudomonas spp.* (10% each), and *Bacillus spp.* (5%).

**Predisposing factors:** Under normal circumstances, the process of uterine involution effectively expels debris and encourages endometrial regeneration, so that the percentage of cows in which bacterial infection remains present at 3 weeks postpartum should decline to 40%; however, in approximately 10 to 17% of postpartum cows, conditions favouring bacterial growth persist and eventually cause endometritis (Sheldon and Dobson, 2004). Specific factors that may delay the elimination of bacteria from the postpartum uterus include the level and nature of the bacterial contamination, the degree of uterine involution, retention of fetal membranes, and the cow’s immune status (Sheldon et al., 2006). Besides that the occurrence of abortion, premature birth, dystocia, induction of parturition, bacterial contamination of the uterus, laceration or lesion of the uterus, cervix, vagina and vulva, uterine inertia, lack of exercise, injury during insemination, unhygienic conditions at the time of calving, lactational stress etc (Correa et al., 1993, Noakes et al., 2001). Furthermore, some of metabolic diseases like ketosis, hypocalcaemia and overfeeding during the dry period also lead to the development of endometritis in cows (Arthur et al., 2009).

The return of ovarian cyclic activity postpartum influences the uterine immune response. During late gestation, the hypothalamic-pituitary axis is under the negative feedback effect of estrogens and progesterone produced by the placenta and ovaries. The first ovulation after calving occurs between 14 to 28 days postpartum (Yavras, 2000). In fact, high concentrations of progesterone during the luteal phase suppress the immune response of the uterus and make the uterus more susceptible to bacterial infection (Lewis, 2003). Early ovulation and elevation of circulating progesterone concentrations before elimination of uterine bacterial contamination has been linked to the establishment of endometritis and pyometra in postpartum cows (Olson et al., 1984; Sheldon and Dobson, 2004). Del Vecchio et al. (1994) showed that intrauterine infusions of *A. pyogenes* and *E. coli* in postpartum beef cows when progesterone concentrations were basal did not cause uterine infection whereas, all cows developed uterine infections when the bacteria were infused after the onset of luteal function and progesterone concentrations had begun to rise. However, the mechanisms underlying these observations are not clear. For instance, Subandrio et al. (2000) showed that blood or uterine neutrophil function in cattle is not consistently up-regulated by exogenous estradiol, nor suppressed by progesterone. Plasma estradiol on the day of calving is also thought to contribute to the overall immune-suppression observed in dairy cows around calving (Goff and Horst, 1997). Galvão et al. (2010) also observed higher estradiol concentration on the day of calving for cows that developed uterine disease.
**Diagnosis of endometritis:** Diagnosis of endometritis by rectal palpation and fortuitous observation of a vaginal discharge, if present in adequate amount is probably the basis for its treatment in the field (Lewis, 1997). Rectal examination (LeBlanc et al., 2002), vaginoscopic examination (Barlund et al., 2008), endometritis clinical score (Williams et al., 2005), white side test (Sarkar et al., 2006), ultrasonography (Mateus et al., 2002a; Barlund et al., 2008) and uterine biopsy (Gonzalez et al., 1985) have been used for efficient detection of endometritis. Uterine palpation per-rectum has been the most frequently used method to diagnose endometritis. Diagnosis of clinical endometritis by palpation per-rectum is a challenge because uterine size and palpable quality of content may vary between individuals and strongly depends on the stage of the postpartum period. It has been reported that diagnosis of clinical endometritis using palpation per-rectum is subjective, not effective and prone to error as it lacks standardization (Gilbert, 1992; Foldi et al., 2006; Palmer, 2008).

It has been shown that the pH of vaginal mucus turns to alkaline in infected cows. In the estrogen dominance, uterine pH becomes higher than in progesterone dominance (Fukusima 1972). White side test is used routinely as cow side test to diagnose the sub-clinical endometritis as cited by Popov (1969); Polyantsev and Popov, (1972), Sarkar et al. (2006). This test depends on the proportion of leucocytes particularly, neutrophil content in the cervico-vaginal mucus. It is considered as positive for endometritis when the colour of mucus turned yellow after boiling with equal amount of 5% sodium hydroxide solution. The intensity of colour depends on the severity of endometritis. Deori (2002) found equal percentage of cows with moderate and slight degree of endometritis by white side test of cervico-vaginal mucus.

Vaginoscopy is another easy tool used to evaluate the vagina and cervix in postpartum cows and it may be employed as a routine diagnostic tool by veterinary practitioners especially if rectal palpation is the only other diagnostic modality being used (LeBlanc et al., 2002). Barlund et al. (2008) reported that vaginoscopy lacked sensitivity when compared to endometrial cytobrush cytology for the diagnosis of both clinical and subclinical endometritis, but did support the use of vaginoscopy for the diagnosis of clinical endometritis in cows > 4 weeks postpartum. Examination of uterine cytology samples provides valid reflection of endometrial health at different stages of postpartum. Various threshold proportions of PMNs have been reported with greater of PMNs being indicative of disease earlier in the postpartum period. Dubuc et al. (2010) reported that ≥6% PMNs or a mucopurulent vaginal discharge were the most appropriate indicators of endometritis in cows 35±3 DIM whereas, ≥4% was the most appropriate in cows 56±3 DIM. Other workers also used an arbitrary >5% PMN threshold in endometrial cytology to diagnose cows having endometritis (Raab, 2004; Gilbert et al., 2005; Fischer et al., 2010). Kasimanickam et al. (2005) have also used the endometrial cytology to diagnose endometritis in absence of any mucopurulent vaginal discharge in cows at 20 to 33 and 34 to 47 days postpartum and the threshold value for PMNs was considered 10 and 18%, respectively. Gilbert et al. (2005) characterized subclinical endometritis as the incidence of >5% PMNs visible on a cytosmear using low volume lavage at 40 to 60 days postpartum. Barlund et al. (2008) reported that the sensitivity of endometrial cytobrush cytology was only 12.9%, while the specificity was 89.9% in cows sampled 28-41 days postpartum. Further, Ghasemi (2011) also considered a threshold value >15% PMN in endometrial cytobrush cytology and found that this was more sensitive and useful diagnostic tool than ultrasonography to diagnose endometritis in postpartum dairy cows.

Ultrasonography has been used extensively as a diagnostic tool to evaluate the presence, volume and nature of uterine luminal fluid. Mateus et al. (2002a) examined postpartum cows by per-rectum ultrasonography and concluded that the volume of intrauterine fluid was significantly associated with impaired uterine involution and the intrauterine fluid volume score was positively correlated with bacterial growth. It seems reasonable that a local inflammatory response within the endometrium would result in some degree of tissue thickening. Barlund et al. (2008) reported that endometrial thickness (≥8mm) measurements were less useful than endometrial cytobrush cytology to diagnose subclinical endometritis between 28 and 41 days postpartum. Although biopsies and cultures of the bovine uterus have been used for diagnosis of endometritis (Dohmen et al., 1995), some others have indicates that these methods are impractical and of limited use (Roberts, 1986; Arthur et al., 2009). The histopathology of uterine biopsy and bacteriological culture of uterine content have been considered the gold standard for diagnosis of endometritis (Gilbert et al., 2005; Sheldon et al., 2006). Although, this procedure depicts existing picture of endometrium during inflammatory conditions, however, it requires more time to depict the result. Several sites should be biopsied and it should be collected on more than one day postpartum that may lead to impair fertility (Bonnett et al., 1991).

Acute phase proteins viz., haptoglobin and serum amyloid A may be used as a marker for diagnosis of endometritis. Serum haptoglobin concentration increases in dairy cows with acute metritis but there is no significant variation observed from normal values in chronic metritis (Skinner et al., 1991; Smith et al., 1998). Serum amyloid A is other substance that can be used for whole herd screening to identify cows with inflammatory diseases (Karreman et al., 2000). According to Zain et al. (1997) increased enzymatic activity of uterine flushing could be a diagnostic indication of endometritis.
The pattern of pro-inflammatory cytokines and other biomolecules involved in clinical or subclinical endometritis in cattle and buffaloes have been explored recently. In our laboratory Loyi et al. (2013) observed a significantly higher expression of various inflammatory mediators viz. CD14, TLR4, IL1β, IL6, IL8 and TNFα mRNA in the endometrium of buffaloes with CE, whereas significant up-regulation of CD14 (1 to 2-fold), IL6 (15 to 36-fold), IL8 (8 to 14-fold) and TNFα (10 to 11-fold) mRNA was observed in SCE. Similarly, in another study on cattle it was observed that the expression of endometrial transcripts of pathogen recognition receptors and major pro-inflammatory cytokines is dependent on the severity of inflammation and stage of cyclicity (Loyi et al., 2015). Enhanced expression of CD14 and IL-8 at the follicular stage and IL-6 and TNF-α in luteal stage could be used as a possible target gene for developing suitable diagnostic markers in cows with SCE in future studies. Furthermore, the up-regulation in expression of IL-1β, IL-8 and TNF-α transcript also observed in peripheral blood mononuclear cells culture of endometritic buffaloes compared to those of healthy buffaloes at the time of diagnosis of endometritis in field condition (Patra et al., 2014). Therefore, the recent cytokine based diagnostic technique might be useful for accurate and early diagnosis of SCE in dairy cattle.

**Current therapy:** Ideal therapy for uterine infection should involve in eliminating bacteria from the uterus without inhibiting the normal uterine defense mechanisms and reducing the chances of adulteration of milk or meat for human consumption. Current therapies of endometritis and uterine infections have been classified into four major categories which are antibiotics, sulphonamides, anticeptics and hormones (Hussain and Danniel, 1991). There are several drawbacks regarding the uses of these therapeutic protocols. These situations have posed a great challenge in the management of endometritis. Hence, there is urgent need to find out an alternative therapy for treatment of uterine infections by using natural substances as a means of activation of natural defense mechanism in the uterus. Much of the original research associated with immunomodulators of UDM and their application as treatment for endometritis or metritis has been carried out in recent times in cattle and buffaloes (Subandrio and Noakes, 1997; Methai, 1999; Singh et al., 2000; Brindaban, 2001; Kumar et al., 2004; Deori, 2002; Sarkar, 2004; Krishnan, 2011). The immune-modulators used in treatment of endometritis includes (i) endotoxins (Lipopolysaccharides of *E. coli*) (ii) serum, plasma or hyper immune serum (iii) collostral whey (iv) PMN extracts and its components (v) bacteria free filtrate (vi) oyster glycogen (vii) leukotriene B4 (viii) granulocyte macrophase colony stimulating factor (ix) herbal extracts like *Tinospora cordifolia*, Neem oil and others.

**Antibacterial substance:** The success of antibiotic and antibacterial treatment varies from beneficial (Roberts, 1986) to no benefit (Dowlen et al., 1983). It has been seen that the post partum bovine uterus is in anaerobic environment, where amino glycosides group of antibiotics are ineffective because they require oxygen for their activity (Sand et Mandell, 1980). In case of tetracyclines, the need of large systemic dose to get effective tissue concentration against *A. pyogenes* could be toxic to the animal. Ziv (1984) also reported that the uterine secretions severely inhibited the antimicrobial activity of penicillin G, ampicillin, neomycin, di-hydrostreptomycyn, gentamycin, oxytetracycline and chormophenicol in both aerobic and anaerobic conditions due to bacterial penicillinase production. Jayappa and Luken (1983) indicated that intra uterine and systemic treatment with antibiotics either inhibited or destroyed leukocytic activity in bovine uterine secretions and overuse of antibiotics in the bovine uterus could lead to the development of antibiotic resistant strains Whitemore and Anderson (1985). One of other drawback of antibiotic uses is that the residues remain detectable in milk for longer time (36-120 hrs) after intra uterine infusion (Hualand et al., 1984). Olsen et al. (1985) reported that their bactericidal activity is greatly reduced in presence of blood, pus, necrotic tissues and product of leucocytes and thus not used for treatment of uterine infection. In a recent study, Singh et al. (2011) obtained a higher conception rate by using Levofloxacin and α-tocopherol in repeat breeder crossbred cows. By that study they also concluded that the bactericidal activity of Levofloxacin and immune stimulant activity of α tocopherol is the basis behind higher efficacy.

**Hormones:** Though estrogen plays an important role in increasing the natural UDM through increasing the blood circulation, leucocytic infiltration into the uterus, uterine contraction and mucus flow (Roberts, 1986), it is currently seldomly used in the treatment of endometritis in lactating cows as because their appreciable levels have been detected in milk that may make it unfit for human consumption (Roberts, 1986). Furthermore, the use of estrogen in higher doses could lead to the development of cyst in the ovaries (Arthur et al., 2009).

**E. coli lipopolysaccharides:** There are lot of research work which confirmed that the *E. coli* lipopolysaccharide (LPS) can be used as an alternating therapy against the traditional use of antibiotics and also prostaglandins. The intra-uterine use of LPS has been reported in mare by Mollett et al. (1985) and Williamson et al. (1987). It acts as a chemo attractants to the PMN and causes influx of serum protein and also immunoglobulin to the uterus. Husain and Daniel (1992) also got similar result after LPS treatment in both healthy cows and those where endometritis were experimentally induced. Saini et al. (1999) observed that *E. coli* LPS effectively improved the stimulation of non-specific cellular immunity to eliminate the endometritis in the cows and found high pregnancy rate following intra-uterine infusion of 100μg *E. coli* LPS in cows with endometritis and repeat breeding.
Singh et al. (2000) reported 100 fold increases in total leucocyte count (TLC) with more than 80% neutrophil having phagocytic activity of five bacteria per neutrophil in uterine flushing collected after LPS treatment in cows. Further, a single intra-uterine infusion of 100 µg E. coli LPS at estrus in repeat breeding cows was effective in clearing the bacterial infection from uterine lumen within one estrous cycle (Singh et al., 2000). In another experiment, Deori (2002) observed that influx of PMN cells increased about 77%, 63% and 68% and along with conception rate of 83%, 67% and 50% following intra-uterine infusion of standard LPS, Indigenous LPS and Bacteria free filtrate, respectively in endometritic cows.

**Serum, plasma or hyper-immune serum:** Addition of a small amount of serum to uterine secretions can increase the opsonising capacity and enhance the phagocytic ability of PMNs significantly (Asbury et al., 1984). It is reported that 90% mares with subclinical endometritis and 52% with clinical endometritis became pregnant following intra uterine infusion of blood plasma (Ward, 1985). In another experiment on mare, Jochle (1998) reported that 5 uterine lavage with saline solution, followed by an instillation of 120 ml autologous plasma with or without oxytocin took 2.7 days to cure when the mares were experimentally infected with 1x10⁹/ml Strep. zooepidemicus during estrus. In cattle use of autologous plasma has been tried to cure endometritis. Venogopal (1995), observed 52.85% conception rate in endometritis cows treated with IU infusion of homologous plasma. Methai (1999) observed 75% recovery rate and 50% overall conception rate after administrating autologous plasma (50 ml IU at the dose rate of 1:10 of total bacterial load and found 70% overall conception rate.

**PMN extracts and their components:** Bovine neutrophil extract has significant bactericidal activity against both gram positive and negative bacteria (Gennaro et al., 1978). The extract of PMN cell contains three small antibiotic peptides called defensins (Ganz et al., 1985). These defensins killed many bacteria and viruses in-vitro and could therefore, possibly be used as a therapy of bovine endometritis. In an in-vitro bacterial assay, Hughes and Couto (1988) showed that PMN extracts of rabbit origin had 100% killing of different bacterial isolates from equine uterus. They suggested that these PMNs could be utilized as non-antibiotic therapy for endometritis.

**Mycobacterial Cell Wall Extracts (MCWE):** Mycolic acid, which is a component of MCWE, has been shown to cause a rapid and widespread influx of PMNs, which is chiefly responsible for the clearance of uterine infection, by stimulating TNFα, interferon γ, IL-6, IL-2 and myeloperoxidase production and by suppressing IL-10 production (Korf et al., 2005). Mycobacterial cell wall extracts (MCWE) was also used in endometritic mares that were experimentally induced with S. zooepidemicus, which resulted in the elimination of endometritis in 35% of the mares by the time of ovulation and 70% by 7 days post ovulation. They have also reported that MCWE are effective in clearing endometrial infection by intravenous or intra-uterine administration (Rogan et al., 2010).

**Granulocyte macrophase colony stimulating factor (GM-CSF):** Granulocyte macrophase colony stimulating factor (GM-CSF), which had been genetically engineered, increased the PMNs in circulations by 12-fold and there was good scope for exploring the use of GM-CSF for the treatment of uterine infections in cows (Hughes and Couto, 1988). Zerbe et al. (2003) have studied the endometrial PMNs response after experimental challenge with S. zooepidemicus in mares and the effect of subsequent treatment with recombinant human IL-8. It was found that PMNs were attracted and translocated in the uterus within 6 hrs of administration of IL-8. This intra-uterine PMNs increased expression of the intra-cellular adhesion molecule of CD11α, in addition to an increased ability to generate reactive oxygen species (ROS).

**Leucotriene B₄ (LTB₄):** Leucotriene B₄ (LTB₄) is an arachidonic acid metabolite known to be physiologically produced by stimulated granulocytes, macrophages and mast cells and plays an important role in neutrophil accumulation during inflammation (Zerbe et al., 1996). A single intra-uterine treatment of 50 ml of 30 nmol/L solution increased the intra-uterine leucocyte count 5-10 times within 24 hrs (Zerbe et al., 1996; Dhaliwal et al., 2001). It stimulates the phagocytic capacity of PMNs particularly, in bovine (Hoedemaker et al., 1992) and may also stimulate the activation, proliferation and release of cytokines by B and T lymphocytes, monocytes and natural killer cells (Claesson et al., 1992). Injections of PGF₂α increase the uterine production of LTB4 seem to promote uterine immune defense and uterine health (Lewis, 2004). In a recent study, Krishnan (2011) assessed the recovery rate by using 50 ml of LTB4 at the concentration of 30 nmol/L on the basis of negative to colour reaction to the white side test, normal pH of CVM and the presence of<5 % PMN cells in uterine cytology smears and observed a significant increase in recovery rate (83.33%) of endometritic cows.

**Oyster Glycogen (OG):** Intra-uterine administration of OG in healthy cows stimulated large no. of PMN cells migration into the uterine lumen and up to 90% of the cells identified in uterine secretion being neutrophils (Subandrio and Noakes, 1997). A single infusion of 500 mg OG intra-uterine increases the uterine neutrophilic influx, cleared bacterial infection and improved conception rate by 66.67% (Singh et al., 2003). A similar studies done by Prasad et al. (2009) in cross bred cows with bacterial endometritis and he got 75% overall conception rate. Recently Krishnan (2011) found 75% recovery rate along with increase in conception rate (41.67%) by using OG 500mg single infusion intrauterine.
**Other immunomodulatory agents:** Herbal extracts provide safe and cheap means of treating any diseases. Borowieck (1989) observed about 84% conception rate in cows treated with alcoholic extracts of 5 plants (chamomyle, marigold, comfrey, salvia and yarrow) for endometritis. Neem (*Azadircta indica*) has been reported to possess non-specific immune stimulant, antimicrobial, antiviral and antifungal properties (Talwar et al., 1997) and has been used in treating endometritic cows with satisfactory results (Brindrawan, 2001). In terms of immunomodulatory and antibacterial properties, Singh (2005) subsequently studied the therapeutic efficacy of neem oil and reported drastic reduction of uterine bacterial load and higher conception rate in endometritic cows. This was further supported by the findings of Kumar et al. (2013), who reported that all the cows received treatment with neem oil extract were recovered from endometritis, caused decline in bacterial load (96.02 ± 2.02 %) and higher conception rate (71.4%).

Another plant species *Tinospora cordifolia* was used extensively for the treatment of endometritis. Kumar et al. (2004) administered 50ml (3000 mg total dose) of aqueous extracts of *Tinospora cordifolia* to the endometritic cows for 3 consecutive days and he obtained recovery rate and conception rate of 66.67% and 27.27%, respectively. Sarkar (2004) used garlic extracts as non-specific immunomodulators and observed 98.21% reduction in bacterial load at subsequent estrus. Hanafi et al. (2010) reported new antifungal compounds i.e. *Rosemarinus officinalis* and *Thymus vulgaris* against micotic endometritis induced by *candida albicans* and have observed that they have good curative effect, immuno-stimulant and free radical scavenger properties.

**CONCLUSIONS**

The incidence of endometritis remained at very significant higher level in commercial dairy farm as well as in farmers’ field. However, the advancement in diagnostic techniques and therapeutic strategies has been emerged to deal with the uterine disorders in general and endometritis in particular. Diagnosis of sub-clinical endometritis at early stages is remained a challenge till today. Therefore, use of certain immunomodulators in early stages of postpartum, following a standard set of diagnostic protocol along with standard package of practices in managing herd hygiene, the problems of endometritis could be minimized and the resultant economic loss of dairy enterprises could be curtailed.

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