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Original Research Article

Effect of Uterine Defense Modulation on Recovery and Conception Rate in Endometritic Repeat Breeding Crossbred Cows

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A B S T R A C T

A study was carried out to evaluate the effect of immunomodulators on recovery and conception rate in endometritic repeat breeding crossbred cows. The endometritic cows (n=60) were randomly divided into 6 groups (10 cows/group). In group A, *E. coli* LPS (100 µg in 30 ml PBS once), in group B, oyster glycogen (500 mg in 30 ml PBS once), in group C, whole colostrum (30 ml once), in group D, lyophilized colostrum (reconstituted in 30 ml PBS once), in group E, Cflox-Tz (60 ml for three consecutive days) and in group F (positive control), PBS (30 ml once) was infused through intrauterine route. Normal cyclic fertile animals (n=10) were served as negative control (Group G). Efficacy of these immunomodulators was assessed by thin, clear CVM with negative colour reaction to white side test and drastic decline in the pH and bacterial load of CVM at subsequent estrus. The results indicated that administration of *E. coli* LPS, oyster glycogen, whole colostrum and lyophilized colostrum showed negative colour reaction to white test and a significant ($p<0.05$) reduction in pH and bacterial load of CVM at subsequent estrus. A significantly ($p<0.05$) higher recovery and conception rate was observed in group A (80 and 70%), B (80 and 50%), C (70 and 50%), D (60 and 40%) and E (70 and 50%) as compared to group F cows (20 and 10%). It was concluded that *E. coli* LPS showed better immunomodulatory and therapeutic efficacy in endometritic cows as highest recovery and conception was achieved in this group.

Keywords

Cows, Conception rate, Endometritis, Immuno modulators, Recovery rate, Repeat breeding

Introduction

Uterine infection is the most common reproductive disorder in dairy cows. Among them, endometritis is one of the serious and common problems that may lead to repeat breeding condition in cattle causing heavy economic loss to dairy industry. In India, the incidence of endometritis has been reported to vary from 11.8 to 34.67% among repeat breeder bovines (Prasad, 2006). Bacterial endometritis is a major cause of infertility (56.6%) in crossbred cows (Agarwal *et al.*, 2002). An optimal environment is one of the basic requirements for the viability of the spermatozoa and development of the embryo within the female reproductive tract (Sreenan and Bechan, 1974). Under normal circumstances, the uterine defense mechanisms prevent entry of bacteria inside reproductive tract. If uterine defense mechanism (UDM) is impaired or weakened, bacteria may colonize the uterus and lead to development of uterine infection and endometritis.

As far as therapeutic approach of endometritis is concerned, various types of therapeutic agents, viz. hormones (Mohanty *et al.,* 2017), intrauterine antibacterials (Tison *et al.,* 2017), antiseptics (Ahmed and Elsheikh, 2014), homeopathic drugs (Hemmelchen, 2002) have been used for the treatment of endometritis. It is obvious from most of the reports that parental and intrauterine treatments with various antibacterial drugs gave inconsistent results.

High cost of treatment, compulsory milk disposal, development of resistance to antibiotics, inhibition of uterine defense mechanism, all led to find an alternative therapy for the treatment of uterine infections. On the other side, hormonal therapy has good success, but its use is limited due to inconsistent results, high cost of treatment, milk disposal and several side effects (Dhaliwal *et al.,* 2001). Hence, there is an urgent need to develop an ecofriendly, effective, non-antibiotic and safer drug for the treatment of endometritis.

Recently, besides traditional procedures of treatment of endometritis in cows, some new preparations have been used, which stimulate different mechanisms of uterine immune system. These preparations are designated as biologically active immunomodulators. In the recent years, the treatment of endometritis with various immunomodulators, viz. *E. coli* lipopolysachharide (Sahoo *et al.,* 2014; Bhuyan *et al.,* 2015), Oyster glycogen (Biswal *et al.,* 2014; Krishnan *et al.,* 2015), Leukotriene B_4 (Krishnan *et al.,* 2015), Serum, plasma or hyper immune serum (Sarkar *et al.,* 2015), PMN extracts and its components, bacteria free filtrate, granulocyte macrophase colony stimulating factor, herbal extracts like neem (Kumar *et al.,* 2013), *Tinospora cordifolia*, garlic (Kumar *et al.,* 2011), tulsi (Kumar *et al.,* 2011), tumeric etc. have been studied with varying degree of success. These substances stimulate UDM in endometritic cows and thereby help in removal of bacteria from the uterus. Colostrum has antimicrobial and immunological properties. Little attempts have been made to test the antibacterial and immunomodulatory properties of whole colostrum and lyophilized colostrum inspite of easy availability at relatively lower cost. Therefore, the present study was under taken to assess the effect of immunomodulators on recovery and conception rate in endometritic repeat breeding crossbred cows.

Materials and Methods

Experimental design and treatment protocol

Animals were selected on the basis of history and breeding records, per-rectal examination and physico-chemical properties of cervico-vaginal mucus (CVM) at estrus (appearance, consistency, white side test and pH). Animals with the history of repeat breeding were thoroughly examined per-rectally to rule out any anatomical defect of genitalia and ovarian abnormalities. Experimental animals comprised of the cows manifesting signs of estrus regularly after 20-21 days, showing clear/turbid mucus discharge, and positive for white side test were included in the study. The repeat breeder animals with endometritis (n=60) were randomly divided into 6 groups (10 animals/group) as previous as stated in abstract.

Collection of cervico-vaginal mucus and its physico-chemical examination

Estrus cows were properly restrained in the service crate. After proper washing of vulvar and perineal regions with water and mild antiseptic solution (equal volume of lugol's iodine and water) estrual CVM was collected by recto-vaginal technique described by Dabas and Maurya (1988). The uterus was gently massaged per rectum and CVM flowing out was collected in the sterilized test tube. Cervical mucus was collected twice, one before treatment at the time of estrus and second at the subsequent estrus following treatment. The test tube containing CVM was brought to the laboratory after keeping it into thermocol box containing ice packs. This CVM was used for physico-chemical studies (appearance, consistency, pH and white side test) and to estimate bacterial load.

The CVM of repeat breeder cows was visually screened for appearance (clear/turbid), consistency (thin/thick), presence or absence of any purulent materials etc. These properties were identified and classified as per method of Sukhdeo and Roy (1971). The appearance of CVM was classified into two main types as clear or like an egg white and turbid or cloudy appearance. The consistency of CVM was classified as thin or that flows easily on a glass slide when inclined at 45^0 angle and thick, sticky mucus on a glass slide or that does not flow at 45^0 angle.

The pH of CVM was assessed by pH indicator strips as described by Tsiligianni *et al.,* (2001). For determination of pH, pH paper strip was saturated in a drop of CVM placed on a clean slide and the colour was matched with the standard colour provided with the strips and pH was noted. For each sample pH was determined in duplicate.

The estrual CVM was subjected to white side test as described by Popov (1969). Two ml of CVM was mixed with 2 ml of 5% sodium hydroxide (NaOH) solution in a test tube and boiled on the flame of spirit lamp. If colour of mucus turned light yellow or yellow, it was considered positive for endometritis. The samples giving no colour reaction were considered negative for endometritis.

Examination of total bacterial load in cervico-vaginal mucus

Phosphate buffer saline (PBS) and estrual CVM was mixed in the ratio of 1:1 and was mucolysed using a vortex shaker for 5 minutes. One ml of this sample was serially diluted with PBS in 10 fold dilutions and was inoculated by standard "Pour Plate Technique" on nutrient agar plate (Cruickskank *et al.,* 1975). Triplicate plates for each sample were incubated at 37^0C for 24 hours. The plates revealing colonies between 30 to 300 were selected and bacterial load was determined using following formula.

Bacterial count (colony forming unit) per ml = Average no. of colonies counted x dilution factor x 2

Assessment of clinical recovery and conception rate

The clinical recovery was assessed by change in appearance, consistency, pH, white side test and bacterial count of CVM at subsequent estrus. At subsequent standing estrus following treatment, all the cows were inseminated twice, 12 hours apart. Cows which returned to estrus after first insemination were inseminated again at second and third subsequent estrus after treatment. Pregnancy was confirmed per-rectally at 45-60 days post insemination.

Statistical analysis

The data so obtained were analyzed statistically by standard method (Snedecor and Cochran, 1994) using two-way analysis of variance, student's t-test and Chi-square test to test the significant differences.

Results and Discussion

In the present study before administration of therapy, most of the endometritic cows had turbid and thick mucus discharge. Prior to treatment, the CVM of all the endometritic cows showed positive colour reaction to white side test. At subsequent estrus, the CVM turned clear in 80, 80, 70, 60 and 70 % of cows that were administered with *E. coli* LPS, oyster glycogen, whole colostrum, lyophilized colostrum and sensitive antibiotic, respectively. However, only 30% of positive control cows (group F) showed clear mucus discharge at subsequent estrus. After treatment, the CVM became thin in 80, 70, 70, 60 and 70% of cows following treatment with *E. coli* LPS, oyster glycogen, whole colostrum, lyophilized colostrum and sensitive antibiotic, respectively. However, only 30% cows of positive control group showed thin mucus at subsequent estrus (Table 1).

Cervical mucus is a viscoelastic secretion of constantly secreting mucus producing cells of endo-cervix and acts as a mechanical barrier against pathogen of the uterus (Lim *et al.,* 2014). Dev *et al.,* (1997) observed that clear estrual cervical mucus is conducive for sperm penetration and conception, whereas turbidity reduces sperm motility in estrual mucus. Clean and transparent cervical mucus shows normal health, while reproductive disorders may results turbid and dirty mucus (Verma *et al.*, 2014). In the present study, maximum cows with endometritis showed thick mucus before treatment, which is in agreement with the finding of Selvraj *et al.,* (2002), who also recorded higher percentage (73.60%) of repeat breeding cows with thick cervical mucus discharge. Sharma and Tripathi (1987) reported that thick cervical mucus retard sperm motility and thus may cause fertilization failure. In dairy cattle, Lim *et al.,* (2014) reported higher conception rate (74.1%) with thin cervical mucus as compared to thick mucus (25.9%).

At subsequent estrus, the CVM showed negative colour reaction to white side test in 80, 70, 70, 60 and 70% cows, which were treated with *E. coli* LPS, oyster glycogen, whole colostrum, lyophilized colostrum and sensitive antibiotic, respectively. However, in positive control group cows, only 20% cows showed negative colour reaction to white side test (Table 1). Positive reaction to white side test could be explained on the basis of number of leukocytes present in the uterine discharge (Popov, 1969). Paterial and Rawal (1990) recorded that the normal uterine discharge has less number of leukocytes to cause any change of colour, whereas in clinical and subclinical cases of endometritis, discharge contains increased number of leukocytes causing a colour reaction. The ribonucleic acid present inside the nucleus of white blood cells reacts with 5% NaOH and produces a colour reaction (Gupta *et al.,* 2011).

Prior to treatment, the pH of CVM in endometritic cows varied from 8.20±0.08 to 8.45±0.12 and the difference in mean pH values of group A, B, C, D, E and F was non-significant. At subsequent estrus following treatment, the pH reduced significantly ($p<0.05$) in all the treatment group cows in comparison to their pre-treatment values and varied from 7.30±0.42 to 7.60±0.13. In positive control group cows (group F), the pH was also reduced post-treatment, but the decline was non-significant as compared to their pre-treatment values (Table 2). The pH of CVM is important to determine the success of pregnancy in livestock because CVM is the transport medium for sperm (Predojevic *et al.,* 2007). Normal pH of CVM ranges from 6.0 to 7.8 (Hamana *et al.,* 1976). Since the pH of CVM turns alkaline in infected cows, it acts as an indicator of endometritis (Kumar *et al.,* 2009). Alkaline CVM of endometritic animals is caused by metabolites of bacteria and inflammatory exudates (Salphale *et al.,* 1993). Once the infection is eliminated from the uterus, the pH drops towards the neutral side (Markusfeld, 1984). In the present study prior to treatment, the mean pH of CVM in all the groups of cows except group G was alkaline indicating infection. During uterine infections or endometritis, an increase in pH of cervical mucus has been reported by several workers (Palanisamy *et al.,* 2014; Krishnan *et al.,* 2015). The drastic reduction in the pH values following treatment in the present study was correlated with decreased levels of metabolites of bacteria and inflammatory exudates as reported by Salphale *et al.,* (1993).

Before treatment, the mean bacterial load ($x10^4$/ml) of CVM in endometritic cows varied from 303.20±7.97 to 317.40±7.82 and the mean values differed non-significantly between A, B, C, D, E and F groups. At subsequent estrus, a significant ($p<0.05$) reduction in bacterial load of the entire treatment group cows was observed in comparison to their pre-treatment values. In positive control group cows (group F), the bacterial load was also declined after treatment, but the reduction was non-significant as compared to their pre-treatment values (Table 2). The percent reduction in bacterial load was highest in *E. coli* LPS group (99.85%) followed by oyster glycogen (99.78%), whole colostrum (99.66%), lyophilized colostrum (99.36%) and antibiotic group (99.33%). The bacterial load was used to assess the level of infection and acts as a diagnostic indicator of uterine health status (Dhaliwal *et al.,* 2001). In our study, the bacterial load in CVM of endometritic cows corroborates with the findings of Bindrawan (2001) and Deori *et al.,* (2004). In contrast to this, Kumar *et al.,* (2013) and Palanisamy *et al.,* (2015) recorded higher bacterial load than the present study. Lower bacterial load than the present study was recorded by Singh *et al.,* (2001). The difference in the bacterial load of CVM with different workers may be due to difference in the degree of endometritis in cows under study.

Further, 80% cows treated with 100 µg of *E. coli* LPS by i.u. route discharged thin and clear CVM with negative colour reaction to white side test. The pH and bacterial load of CVM was reduced from 8.35±0.24 to 7.30±0.42 and 306.80±6.45 to 0.44±0.08, respectively. The reduction in pH and bacterial load of CVM following *E. coli* LPS treatment might be due to increase in phagocytosis leading to elimination of infection by stimulating uterine defense mechanism. *E. coli* LPS might have stimulated macrophages, which in turn produced interleukin-1 and interleukin-8 that increased the production of granulocyte macrophage-colony stimulating factor (GM-CSF) for rapid recruitment of PMN cells into uterus and thus cleared the bacteria from the uterus by phagocytosis (Methai and Rajasundaram, 2003). LPS at low dose activate monocyte and macrophage to produce TNF, which helps in synthesis of IL-1. Interleukin-1 and TNF both act on endothelial cells to release IL-6 and IL-8. Thus, the initial exposure to LPS results in a cytokine cascade event intended to increase local inflammatory response.

Table.1 Effect of immunomodulators on diagnostic characteristics of cervico-vaginal mucus

S. No.	Treatment groups	Pre-treatment			Post-treatment		
		Turbid discharge (%)	Thick discharge (%)	White side test positive animals (%)	Turbid discharge (%)	Thick discharge (%)	White side test positive animals (%)
1.	Group A (n=10) (*E.coli* LPS)	70.00 (7)	90.00 (9)	100.00 (10)	20.00 (2)	20.00 (2)	20.00 (2)
2.	Group B (n=10) (Oyster glycogen)	70.00 (7)	90.00 (9)	100.00 (10)	20.00 (2)	30.00 (3)	30.00 (3)
3.	Group C (n=10) (Whole colostrum)	80.00 (8)	70.00 (7)	100.00 (10)	30.00 (3)	30.00 (3)	30.00 (3)
4.	Group D (n=10) (Lyophilized colostrum)	80.00 (8)	80.00 (8)	100.00 (10)	40.00 (4)	40.00 (4)	40.00 (4)
5.	Group E (n=10) (Sensitive antibiotic)	60.00 (6)	70.00 (7)	100.00 (10)	30.00 (3)	30.00 (3)	30.00 (3)
6.	Group F (n=10) (Positive control)	80.00 (8)	70.00 (7)	100.00 (10)	70.00 (7)	60.00 (6)	80.00 (8)
7.	Group G (n=10) (Negative control)	00.00 (0)	20.00 (2)	00.00 (0)	00.00 (0)	20.00 (2)	00.00 (0)

Figure in parenthesis indicate number of animals

Table.2 Effect of immunomodulators on recovery and conception rate in endometritic repeat breeding crossbred cows

S. No.	Treatment groups	pH		Bacterial load ($x10^4$/ml)		Recovery Rate (%)	Conception Rate (%)
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment		
1.	Group A (n=10) (*E.coli* LPS)	8.35 ± 0.24^{Aa}	7.30 ± 0.42^{BCb}	306.80 ± 6.45^{Aa}	0.44 ± 0.08^{Bb}	80.00^{A} (8)	70.00^{A} (7)
2.	Group B (n=10) (Oyster glycogen)	8.45 ± 0.12^{Aa}	7.30 ± 0.13^{BCb}	308.60 ± 5.74^{Aa}	0.67 ± 0.07^{Bb}	80.00^{A} (8)	50.00^{AB} (5)
3.	Group C (n=10) (Whole colostrum)	8.25 ± 0.08^{Aa}	7.35 ± 0.13^{BCb}	306.30 ± 7.57^{Aa}	1.04 ± 0.10^{Bb}	70.00^{AB} (7)	50.00^{AB} (5)
4.	Group D (n=10) (Lyophilized colostrum)	8.38 ± 0.08^{Aa}	7.60 ± 0.13^{Bb}	310.40 ± 9.29^{Aa}	1.98 ± 0.23^{Bb}	60.00^{B} (6)	40.00^{B} (4)
5.	Group E (n=10) (Sensitive antibiotic)	8.20 ± 0.08^{Aa}	7.45 ± 0.16^{BCb}	303.20 ± 7.97^{Aa}	2.03 ± 0.16^{Bb}	70.00^{AB} (7)	50.00^{AB} (5)
6.	Group F (n=10) (Positive control)	8.35 ± 0.08^{Aa}	8.05 ± 0.16^{Aa}	317.40 ± 7.82^{Aa}	129.00 ± 7.18^{Ab}	20.00^{C} (2)	10.00^{C} (1)
7.	Group G (n=10) (Negative control)	7.30 ± 0.08^{Ba}	7.20 ± 0.08^{Ca}	0.32 ± 0.03^{Ba}	0.29 ± 0.03^{Ba}	100.00^{A} (10)	80.00^{A} (8)

Figure in parenthesis indicate number of animals; Means with different superscripts for a parameter within group (a, b) and between groups (A, B, C) differ significantly ($p<0.05$)

Under which more influx of leukocytes, immunoglobulin and complement took place into the uterine lumen (Prasad *et al.,* 2009). LPS also activated complement and triggered lymphocyte proliferations with local antibody production leading to better opsonization and phagocytosis (Vegad and Katiyar, 2001). A similar trend in reduction of pH and bacterial load of CVM was observed by several workers following treatment with *E. coli* LPS (Palanisamy *et al.,* 2014; Palanisamy *et al.,* 2015; Krishnan *et al.,* 2015). Further, the conception rate was significantly ($p<0.05$) higher in the cows treated with *E. coli* LPS (70%) as compared to positive control animals (10%). The findings of the present study regarding effect of *E. coli* LPS therapy on recovery rate of endometritic cows are in accordance with the findings of Shaktawat *et al.,* (2006) and Prasad *et al.,* (2009), who also reported 85.71% and 83.33% recovery rate, respectively following treatment with *E. coli* LPS. Higher recovery rate than the present study was reported by Sarma *et al.,* (2013) and Bhuyan *et al.,* (2015), who observed 100% recovery rate after treatment with *E. coli* LPS. The findings of the present study regarding effect of *E. coli* LPS therapy on conception rate of endometritic cows are similar to the findings of Shaktawat *et al.,* (2006) and Prasad *et al.,* (2009), who reported 78%, 71.43% and 75% conception rate, respectively following treatment with *E. coli* LPS. Higher conception rate than the present study was reported by Sarma *et al.,* (2013) and Bhuyan *et al.,* (2015), who observed 83.33%, 90% and 83.33% conception rate after treatment with *E. coli LPS.*

The infusion of 500 mg oyster glycogen (OG) by i.u. route in endometritic cows caused 80% recovery rate with thin and clear CVM, which showed negative colour reaction to white side test. The pH and bacterial load of CVM was reduced from 8.45±0.12 to 7.30±0.13 and 308.60±5.74 to 0.67±0.07, respectively at subsequent estrus. The reduction in pH and bacterial load of CVM following OG treatment might be due to increase in phagocytosis leading to elimination of infection by stimulating uterine defense mechanism. Protein contaminants in OG might play a role in chemoattraction, infiltration and proliferation of lymphocytes into the uterus (Tizard, 1996). During uterine infection, chemotactic molecules are released by the bacteria, which stimulate the influx of neutrophils from the blood vessels into the uterine lumen. The findings of the present study regarding effect of OG therapy on pH and bacterial load of CVM are in agreement with the findings of Palanisamy *et al.,* (2014), Palanisamy *et al.,* (2015) and Krishnan *et al.,* (2015), who also reported decreased pH and bacterial load in CVM of endometritic cows after treatment with oyster glycogen.

Further, 50% conception rate was achieved in OG group, which was significantly ($p<0.05$) higher than the positive control group (10%). Findings of the present study regarding effect of OG therapy on recovery rate of endometritic cows are in confirmity with the findings of Prasad *et al.,* (2009) and Sarma *et al.,* (2013), who reported recovery rate that varied between 66.67-80%. Higher recovery rate than the present study was reported by Bhuyan *et al.,* (2015), who recorded 100% recovery rate after treatment with OG. Findings of the present study regarding effect of OG therapy on conception rate of endometritic cows are in agreement with the findings of Biswal *et al.,* (2014), Krishnan *et al.,* (2014) and Bhuyan *et al.,* (2015), who recorded 57.14%, 50% and 50% conception rate, respectively following OG infusion. In contrary to this, Prasad *et al.,* (2009) and Sarma *et al.,* (2013) observed higher conception rate (75% and 80%, respectively).

Similarly, the infusion of whole colostrum caused 70% recovery rate with thin and clear CVM that showed negative colour reaction to white side test. The pH and bacterial load of CVM was reduced from 8.25±0.08 to 7.35±0.13 and 306.30±7.57 to 1.04±0.10, respectively at subsequent estrus. The reduction in pH and bacterial count of CVM at subsequent estrus might be due to enhanced phagocytosis. Colostrum contains high concentration of immunoglobulins (IgG, IgA, IgA_2 and IgM), which might have enhanced the migration of phagocytic cells through various chemotactic factors like interleukins (Hogarth, 1982) and thereby helps in removal of bacteria from the uterus.

It also contains lactoferrin (Groves, 1960), which has antibacterial property that might have killed the bacteria in the uterus. A number of cytokines are also found in colostrum such as interleukins (Hagiwara *et al.*, 2000), tumor necrosis factor (Rudloff *et al.*, 1992), chemokines (Maheshwari *et al.*, 2003) and others, which might have stimulated phagocytosis and cleared bacteria from the uterus. The immunoglobulins in colostrum help in phagocytosis by opsonizing bacteria in the uterus. The findings of the present study regarding effect of whole colostrum therapy on pH and bacterial load of CVM in endometritic cows are in agreement with the findings of Bindrawan (2001), who also reported reduction in pH and bacterial load of CVM at subsequent estrus following treatment with colostral whey. Further, 50% conception rate was also achieved in whole colostrum group, which was significantly ($p<0.05$) higher than the positive control group (10%). Perusal of literature revealed no work on recovery and conception rate of endometritic cows following treatment with whole colostrum. Therefore, our results could not be compared.

After infusion of lyophilized colostrum, 60% recovery rate was recorded with thin and clear CVM, which showed negative colour creation to white side test. The pH and bacterial load of CVM was reduced from 8.38±0.08 to 7.60±0.13 and 310.40±9.29 to 1.98±0.23, respectively at subsequent estrus. The reduction in pH and bacterial count of CVM at subsequent estrus might be due to enhanced phagocytosis. The lyophilization process preserves the quality and unique features of colostrum, like antioxidant, immunological and antimicrobial proprieties (Cruz *et al.*, 2017). The immunoglobulins in colostrum help in phagocytosis by opsonizing bacteria in the uterus. In this group, 40% conception rate was achieved, which was significantly ($p<0.05$) higher than the positive control group (10%). No citation in the literature is available regarding effect of lyophilized colostrum on pH and bacterial load of CVM and on recovery and conception rate of endometritic cows following treatment with lyophilized colostrum. Therefore, our results could not be compared.

After infusion of sensitive antibiotic (Cflox-Tz), 70% recovery rate was recorded with thin and clear CVM, which showed negative colour creation to white side test. The pH and bacterial load of CVM was reduced from 8.20±0.08 to 7.45±0.16 and 303.20±7.97 to 2.03±0.16, respectively at subsequent estrus. Ciprofloxacin is one of the new generations of fluorinated quinolones. By inhibiting bacterial DNA gyrase and preventing cell division, it might have killed bacteria effectively and reduced the pH and bacterial load of CVM at subsequent estrus. The findings of this study regarding effect of most sensitive antibiotic (Cflox-Tz) therapy on pH of CVM of endometritic cows supports the findings of Ramsingh *et al.,* (2013) and Kumar *et al.,* (2014), who also observed reduction in pH values of CVM after treatment with most sensitive antibiotic. Similar trend in decline of bacterial load following antibiotic therapy was also reported by Xiang (2009). In this group, 50% conception rate was achieved, which was significantly ($p<0.05$) higher than the positive control group (10%). Findings of the present study regarding effect of sensitive antibiotic therapy on recovery rate of endometritic cows are in agreement with the findings of Shaktawat *et al.*, (2006) and Prasad *et al.*, (2009), who reported 71.43% and 75% recovery rate, respectively following treatment with sensitive antibiotic. However, higher recovery rate than the present study was recorded by Kumar *et al.*, (2014) and Bhuyan *et al.*, (2015), who observed 100% recovery rate after treatment with sensitive antibiotic. Findings of the present study regarding effect of sensitive antibiotic therapy on conception rate of endometritic cows are in agreement with the findings of Shaktawat *et al.*, (2006), Prasad *et al.*, (2009) and Bhuyan *et al.*, (2015), who also reported 57.14%, 58.33% and 50% conception rate, respectively following treatment with sensitive antibiotic. However, higher conception rate than the present study was recorded by Kumar *et al.,* (2014), who observed 62.5% conception rate after treatment with sensitive antibiotic. In contrast to our study, Bindrawan (2001) found lower conception rate (37.50%) following intrauterine infusion of antibiotic.

On the basis of results obtained after experimentation in endometritic repeat breeding crossbred cows, use of *E. coli* LPS has shown promising results in terms of immunomodulation, therapeutic efficacy and conception rate following recovery of cows. Presently this therapy may not be economical under field conditions, but after further experimentation, its use may result in cheaper mode of application in near future as it will curtail down the number of applications to once only instead of several applications in other therapeutic regimens.

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