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Research Paper

Effect of Pre-insemination Intrauterine Antibiotics on Conception Rate in Crossbred Cows

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ABSTRACT

The repeat breeder crossbred endometritic cows (N = 30) were divided into three groups (n= 10, each), as Group I (Gentamicin IU given 6 hrs before AI) Group II (Cephapirin given 6 hrs before AI) and Group III (control cows, no IU antibiotic treatment). The cows in estrus were inseminated twice (12 hours apart, if not conceived at first, again inseminated at second heat) with good quality frozen thawed semen. Cervico- vaginal mucus and blood sample was collected and physic-biochemical properties (appearance, consistency, Whiteside test, pH, Total bacterial load) and Serum amyloid- A, were evaluated respectively. The overall conception rate of different groups (I to III) of cows were 60, 20 and 10 %, respectively. On the basis of recovery rate as well as conception rate Gentamicin found to be the best when compared to Cephapirin.

Keywords: Endometritis, Antibiotics, Conception rate, Repeat breeder cows

Incidences of reproductive diseases in cattle are highly variable in high yielding cows. In India, Rao and Sreemannarayana. (1983); Sar *et al.* (1996) and Rao, (1982) have reported 25% and 30%, metritis and endometritis, respectively. It is important to have high reproductive efficiency for successful dairy enterprises (Olynk, 2008). Interruption on several factors such as uterine microbial balance, host immunity, environmental and other animal factors may lead to uterine infections (Potter *et al.* 2010). An optimal environment is one of the basic requirements for the viability of spermatozoa and further embryonic development within the female reproductive tract (Sreenan and Bechan, 1974). Intra-uterine infusion of Cephapirin, a first generation Cephalosporin, a rational antibiotic of choice, had improved reproductive performance of cows with subclinical endometritis (Kasimanickam *et al.* 2005) or clinical endometritis (LeBlanc *et al.* 2002), with retained fetal membranes and stillbirths (McDougall, 2001). Gentamicin, an aminoglycoside is powerful against most microorganisms associated with bovine reproductive tract infection (Hennessey *et al.* 1971). *In-vitro* tests showed that Gentamicin was 77.4% effective against all microorganisms isolated from the uterus of post-partum cows and 100% against E. *coli* (Bretzlaff *et al.* 1983; Sharma, 2017). Antibiotics are assumed to reduce bacterial load in the uterus and, indirectly, diminish inflammation in the endometrium (Bretzlaff, 1987).

The objectives of present study were to study the effect of pre- AI, IU antibiotics (Gentamicin and Cephapirin) treatment on serum acute phase proteins (SAA) and physico-biochemical characteristics of cervico- vaginal mucus (CVM) in subclinical endometritis affected crossbred cows on recovery and conception rate in subclinical endometritis affected crossbred cows.

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MATERIALS AND METHODS

The present study was carried out on thirty crossbred cows of Instructional Dairy Farm (IDF), Nagla, G. B. Pant University of Agriculture and technology, Pantnagar, Udham Singh Nagar, geographically at temperate region located at 29 °N latitude, 79.3 °E longitude, in the Tarai belt of Uttarakhand. The research was conducted at the month of January to March with temperature ranges from 14.8 – 22.4 °C, and relative humidity 41- 82% to assess effect of pre-artificial insemination intrauterine antibiotics (Gentamicin and Cephapirin as confirmed by *in-vitro* antibiotic sensitivity test) on conception rate in subclinical endometritic, crossbred cows. The repeat breeder cross bred endometritic cows (N = 30) were divided into three groups, as Group I (Gentamicin IU given 6 hrs before AI), Group II (Cephapirin given 6 hrs before AI), Group III (control- no IU antibiotic treatment). Cows were subjected for two AI at, 12 hours apart.

Cervico- vaginal mucus of cows was visually screened for appearance (clear/turbid), consistency (thin/thick), presence of any purulent materials etc. The pH of CVM was assessed by pH indicator strips (pH range of 6.5 to 9 supplied by Hi Media Laboratories Pvt. Ltd., Mumbai) as described by Tsiligianni *et al.* (2001). CVM was subjected to Whiteside test (Popov, (1969). (Total 1 ml of mucus will be mixed with 1ml of 5% NaOH solution (1:1) in a test tube and boiled in flame of a spirit lamp). Colour change to yellow or light yellow, was considered positive and if no colour change, it was categorized as negative for endometritis. Bacterial load was determined by using following formula:

Bacterial count (colony forming unit) per ml (Bacteriological analytical Manual of US, FDA, 2015)

$$= \frac{\text{Average number of colonies counted} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

Blood samples (3 ml volume) were collected before and after (24th hr) treatment for Estimation of serum amyloid a (SAA) using Bioassay bovine serum amyloid A kit in which tested antigen and enzyme labeled antigen competitively bind to immobile antibody. The higher concentration of antigen in test sample and the enzyme labeled antigen binds to immobile antibody, so the lower intensity of colour develops after addition of subsrate. Therefore, target molecule can be estimated using ELISA reader.

RESULTS AND DISCUSSION

Appearance and consistency (%) - Appearance and consistency of CVM is presented in table and figs. 1 and 2. After treatment, number of cows with clear discharge were significantly ($p< 0.05$) higher in group I.

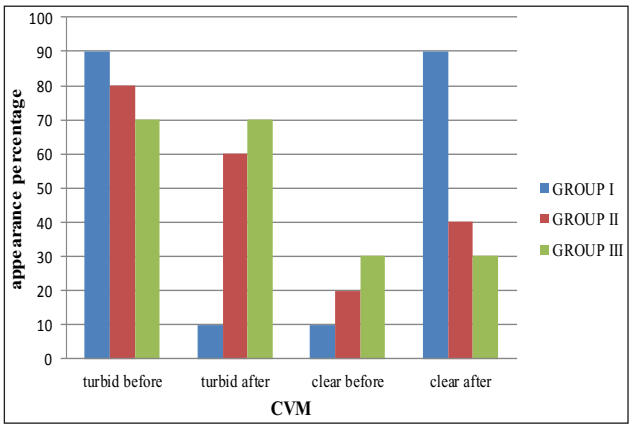

Fig. 1: Appearance of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows

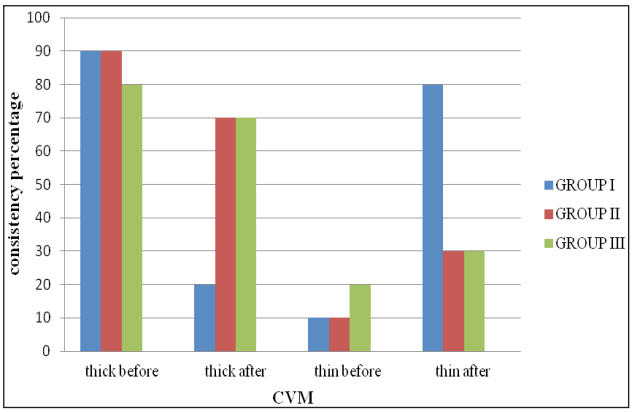

Fig. 2: Consistency of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows

In group I, there was significant ($p< 0.05$) reduction in cows which showed thick mucus discharge following IU treatment, compared to Cephapirin and untreated cows.

pH of CVM

The mean pH of different groups is present in table and fig. 3. After the IU antibiotic treatment, pH significantly reduced ($p< 0.05$) in group I .

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Table 1: Appearance (%) of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Appearance	Treatment	Group I (n = 10)	Group II (n = 10)	Group III Control (n = 10)
Turbid	Before	90.00^{Aa} (9)	80.00^{Aa} (8)	70.00^{Aa} (7)
	After	10.00^{Bb} (1)	60.00^{Aab} (6)	70.00^{Aa} (7)
Clear	Before	10.00^{Bb} (1)	20.00^{Bb} (2)	30.00^{Bb} (3)
	After	90.00^{Aa} (9)	40.00^{Bab} (4)	30.00^{Bb} (3)

Values bearing different superscripts (A, B, C) in rows (between groups) and (a, b) in columns (within groups) differ significantly (p <0.05). Figures in parenthesis indicate number of cows.

Table 2: Consistency (%) of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Consistency	Treatment	Group I (n = 10)	Group II (n = 10)	Group III Control (n = 10)
Thick	Before	90.00^{Aa} (9)	90.00^{Aa} (9)	80.00^{Aa} (8)
	After	20.00^{Bb} (2)	70.00^{Aa} (7)	70.00^{Aa} (7)
Thin	Before	10.00^{Bb} (1)	10.00^{Bb} (1)	20.00^{Bb} (2)
	After	80.00^{Aa} (8)	30.00^{Bb} (3)	30.00^{Bb} (3)

Values bearing different superscripts (A, B, C) in rows (between groups) and (a, b) in columns (within groups) differ significantly (p <0.05). Figures in parenthesis indicate number of cows.

Table 3: pH (mean ± SE) of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Sl. No.	Groups	No. of cows	Before treatment	After treatment	Difference in pH
1	Group I (Gentamicin -1st AI -2nd AI)	10	8.85 ± 0.07^{Aa}	7.30 ± 0.11^{Bb}	1.45 ± 0.04^{L}
2	Group II (Cephapirin -1st AI -2nd AI)	10	8.65 ± 0.15^{Aa}	8.60 ± 0.17^{Ab}	0.35 ± 0.02^{M}
3	Goup III (Control)	10	8.50 ± 0.15^{A}	8.50 ± 0.15^{A}	0.09 ± 0.01^{N}

Means bearing different superscripts within group (a,b) and between groups (A, B, L, M) differ significantly (p< 0.05).

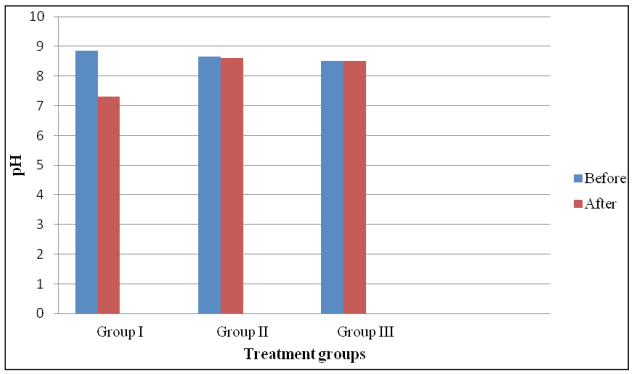

Fig. 3: pH of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows

Whiteside test

Cows with positive (%) Whiteside test indifferent groups is presented in table and fig. 4. In group I, following IU antibiotic treatment, there was a significant reduction ($p < 0.05$) in the percentage of cows, remains positive for Whiteside test. Higher percentage of cows became negative to white side test after IU Gentamicin treatment compared to Cephapirin treated and control group.

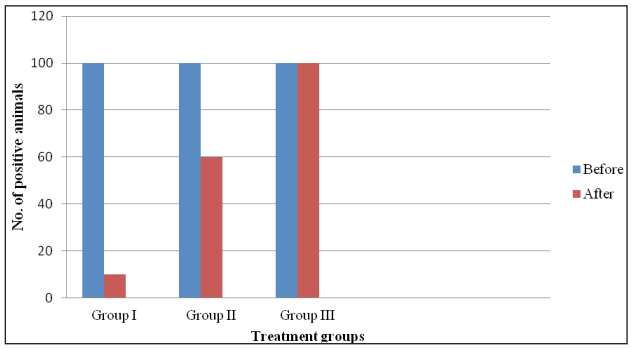

Fig. 4: Whiteside test on CVM (% positive animals) in pre- AI, IU antibiotic treated endometritic crossbred cows

Total Bacterial load (× 10^4/ ml)

The mean bacterial load of group I, II and III is presented in the table and fig. 5. Following treatment, significant ($p < 0.05$) reduction in the bacterial load of group I was observed. Significant difference

($p < 0.05$) in bacterial load after IU treatment was observed in Gentamicin and Cephapirin treated groups.

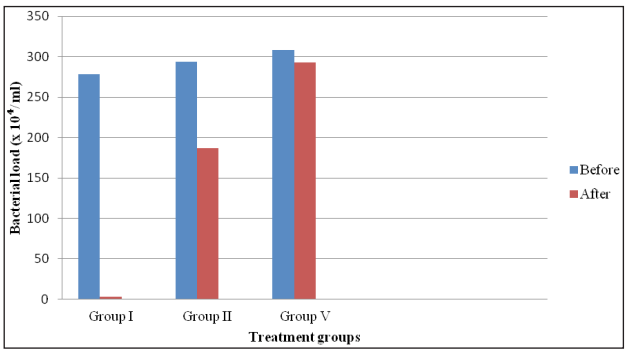

Fig. 5: Bacterial load (× 10^4/ ml) of CVM in post- AI, IU antibiotic treated endometritic crossbred cows

Serum- amyloid A concentration (µg/ ml)

Serum amyloid- A concentration in all the groups (I, II and III) are presented in table 6. Following treatment, serum amyloid- A concentration was significantly ($p< 0.05$) reduced in group I as compared to group II and III. Group II also shows significant ($p < 0.05$) percentage reduction in total SAA estimation compared to group III.

Recovery rate and Conception rate (%)

The recovery rate following intrauterine antibiotic treatment was assessed on the basis of bacterial load of cervico- vaginal mucus and negative colour reaction to Whiteside test. Cows negative for Whiteside test and showed reduction in bacterial load to normal level (16.80 ± 1.62) were considered as recovered (Ceciliani *et al.* 2012). The recovery rate of different groups (I to III) of cows were 80, 30 and 30 %, respectively. The recovery rate was significantly ($p< 0.05$) higher in group I compared to group II and group III cows. Significantly ($p< 0.05$) higher recovery rates of groups I cows were evident by more number of cows, showing significant reduction ($p< 0.05$) in total bacterial load of cervico-vaginal mucus following treatment and negative reaction to Whiteside test following treatment.

In group I and III, first service conception rate was 60% (6 out of 10), 10% (1 out of 10), respectively. Second service conception rate was 20% (2 out of 10) for group II, however no cows were conceived in group I and III in second service. The conception rate of group I (60%) cows was significantly ($p < 0.05$) higher in comparison to group II (20%) and group III (10%) cows. However, cows of group II had higher conception rate than control cows and lower conception rate as compared to group I cows. The higher first service conception rate in Gentamicin groups indicates the effectiveness of antibiotics against the gram-positive and gram-negative bacteria (Warriach *et al.* 2008).

Clean and transparent cervical mucus shows normal health, while reproductive disorders may result in turbid and dirty mucus. This nature of mucus is essential for sperm survival and transport, otherwise there is less chance of fertilization of ovum (Dunson *et al.* 2007). This tissue concentration is above the minimum inhibitory concentration required for most uterine pathogens. Clinically, Cephapirin reduces CVM discharge (clinical cure) by 80%, and bacteria (bacteriological cure) in the uterus by 60% (Tison *et al.* 2016). Reduction in percentage of cows with turbid CVM in control group might be due to natural uterine defence mechanism (Singh, 2018).

In the present study, before IU antibiotic treatment, the pH of cervico- vaginal mucus in all groups was alkaline indicating infection, as alkaline cervico-

Table 4: Whiteside test on CVM (% positive animals) in pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Sl. No.	Groups	No. of cows	Positive for white side test before treatment	Positive for white side test after treatment
1	Group I (Gentamicin -1st AI -2nd AI)	10	100.00^{Aa} (10)	10.00^{Cb} (1)
2	Group II (Cephapirin -1st AI -2nd AI)	10	100.00^{Aa} (10)	60.00^{Bb} (6)
3	Group III (Control)	10	100.00^{A} (10)	100.00^{A} (10)

Means bearing different superscripts within group in rows (a,b) and between groups in columns (A, B, C) differ significantly ($p< 0.05$). Figure in parenthesis indicate no. of cows.

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Table 5: Bacterial load (Mean ± SE × 10^4/ ml) of CVM in pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Sl. No.	Groups	No. of cows	Before treatment	After treatment	Change in Bacterial load	Percentage reduction
1	Group I (Gentamicin -1st AI -2nd AI)	10	278.70 ± 11.51^{Aa}	3.20 ± 0.68^{Bb}	275.50 ± 11.7^{L}	98.85^{L}
3	Group II (Cephapirin -1st AI -2nd AI)	10	294.00 ± 4.55^{Aa}	186.78 ± 5.16^{Ab}	110.00 ± 5.05^{M}	36.47^{M}
5	Group III (Control)	10	308.00 ± 3.56^{Aa}	293.20 ± 7.17^{Aa}	18.6 ± 6.91^{N}	4.81^{N}

Means bearing different superscripts (a, b) in rows (within group) and (A, B, L, M, N) in columns (between groups)differ significantly (p< 0.05).

Table 6: Serum amyloid- A concentrations (µg/ ml) in serum samples of pre- AI, IU antibiotic treated endometritic crossbred cows (N= 30)

Sl. No.	Groups	No. of cows	Before treatment	After treatment	Change in SAA concentration	Percentage reduction
1	Group I (Gentamicin -1st AI -2nd AI)	10	49.05 ± 1.45^{Aa}	16.23 ± 0.94^{Bb}	33.97 ± 0.60^{L}	61.11^{L}
2	Group II (Cephapirin -1st AI -2nd AI)	10	46.38 ± 1.45^{Aa}	37.05 ± 0.72^{Ab}	8.45 ± 0.35^{M}	20.12^{M}
3	Group III (Control)	10	42.07 ± 1.49^{Aa}	36.89 ± 7.04^{Ab}	7.17 ± 1.23^{M}	12.28^{M}

Means bearing different superscripts within group (a,b) in rows and between groups (A, B, L,M) in columns differ significantly (p< 0.05).

vaginal mucus of endometritic animals may be due to metabolites of bacteria and inflammatory exudates in estrual cervical mucus (Salphale *et al.* 1993) causing conception failure. Once the infection is eliminated, the pH of cervical mucus returns shifts towards the neutral side (Markusfeld, 1984).

Gentamicin is a broad spectrum bactericidal drug to which Gram positive as well as Gram negative organisms are highly succeptible, in this way it might have increased the percentage of the cows with negative colour reaction to Whiteside test (Parikh *et al.* 2017). The findings were in close agreement with Sharma *et al.* (2013) and Verma *et al.* (2014). They reported only 20% and 10% Whiteside positive animals after treatment with Gentamicin, respectively. The results were contrary to Pluta *et al.* (2011), as they observed a higher percentage (40.32%) of Whiteside positive cows even after intrauterine treatment of Gentamicin.

The reduction in the bacterial load of CVM in group I is because Gentamicin had worked effectively against the uterine microflora and resulted in a significant (98.85%) reduction of bacterial load of cervico-vaginal mucus following IU treatment (Singh, 2018). Cephapirin treated cows of group II (36.47%) had no significant reduction in the bacterial load because of failure of complete action against uterine microflora. Slight reduction in bacterial load of untreated control group might be due to natural uterine defense mechanism (Dhaliwal *et al.* 2001).

In the present study, the Gentamicin IU treatment reduced the bacteria from the uterus, which might have resulted into decreased inflammatory response in the animals (Vangroenweghe *et al.* 2005; Wagener, 2014). Due to decreased inflammatory response, the concentration of serum amyloid-A was decreased following the antibiotic treatment. However in Cephapirin treated groups, there was no significant reduction in bacterial load which might be the reason for decreased inflammatory response which was evident in SAA concentration following the treatment (Runciman, 2008).

CONCLUSION

Gentamicin was found to be the best antibiotic for the treatment of subclinical infection in repeat breeding crossbred cows and showed its best therapeutic efficacy than Cephapirin. In subclinically endometritic crossbred cows, use of Gentamicin as an intrauterine antibiotic has shown promising results in terms of therapeutic efficacy and conception rate following recovery of cows.

Looking into the cost of standard drugs used for the treatment of endometritis, Gentamicin would be cheaper under field condition. Further, study proved the success of intrauterine infusions to treat subclinical endometritis.

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