Journal of Animal Research: v.12 n.06, p. 897-902. December 2022

DOI: 10.30954/2277-940X.06.2022.11

Effects of Post -Insemination Intrauterine Antibiotics on Conception Rate in Crossbred Cows

Revathy, V.J., Souvik Dhara*, Bimmy Painuly, Deepa Madhwal and Mridula Sharma

Department of Veterinary Gynaecology & Obstetrics, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, INDIA

**Corresponding author: S Dhara; E-mail: souvikdhara2016@gmail.com*

Received: 02 Oct., 2022 **Revised:** 24 Nov., 2022 **Accepted:** 29 Nov., 2022

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 post insemination) Group II (Cephapirin given 6 hrs post insemination) and Group III (control cows, no IU antibiotic treatment has been given). The cows in estrus were inseminated twice (12 hours apart) 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 in different group (I to III) of cows was 60, 20 and 10 %, respectively. On the basis of recovery rate as well as conception rate Gentamicin was found to be the best compared to Cephapirin.

HIGHLIGHTS

- Getamicin provided effective recovery rate of repeat breeder cows.
- Subclinical endometritis in cows can effectively treat with Gentamicin IU therapy.

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

Incidence of reproductive diseases in high yielding cattle is highly variable. In India 25% and 30%, incidence of metritis and endometritis were reported respectively (Parmer, 2021). Interruption of several factors such as uterine microbial balance, host immunity, environmental and other animal factors may lead to uterine infections. An optimal uterine environment is one of the basic requirements for the viability of spermatozoa and further embryonic development within the female reproductive tract (Adnane, 2017). Intra-uterine infusion of Cephapirin, a first generation Cephalosporin, had improved reproductive performance of cows with subclinical endometritis (Kasimanickam *et al*., 2005), clinical endometritis (Denis-Robichaud and Dubuc, 2015), retained fetal membranes and stillbirths (Skuja and Antane, 2017). Gentamicin, an aminoglycoside is powerful against most microorganisms associated with bovine reproductive tract infection (Shafique *et al*., 2022). *In-vitro* tests showed that Gentamicin was effective against all microorganisms isolated from the uterus of post-partum cows especially against E. *coli* (Sharma *et al*., 2017). Antibiotics are assumed to reduce bacterial load in the uterus and, indirectly, diminish inflammation in the endometrium (Singh *et al.,* 2018).

The objectives of present study were to study the effects of post insemination intra uterine 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 and on recovery and conception rate in subclinical endometritis affected crossbred cows.

How to cite this article: Revathy, V.J. Dhara, S., Painuly, B., Madhwal, D. and Sharma, M. (2022). Effects of Post -Insemination Intrauterine Antibiotics on Conception Rate in Crossbred Cows. *J. Anim. Res.,* **12**(06): 897-902.

Source of Support: None; **Conflict of Interest:** None

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, geographically at temperate region located at 29 ℃N latitude, 79.3 ℃E longitude, in the Tarai belt of Uttarakhand. The repeat breeder cross bred endometritic cows (N = 30) were divided into three groups, as Group I (Gentamicin IU given 6 hrs post insemination, 200 mg in 500 ml of PBS solution), Group II (Cephapirin given post insemination, 500 mg) and Group III (control- no IU antibiotic treatment). Cows were subjected for two inseminations at 12 hours interval.

Evaluation of Cervico-vaginal mucus

Cervico- vaginal mucus (CVM) of cows was visually screened for appearance (clear/turbid), consistency (thin/thick) and presence of any purulent materials. 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). The colour changed 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) =

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

Serum amyloid-A

Blood samples (3 ml volume) were collected before and after (24^{th} hr) treatment for Estimation of serum amyloid-A (SAA) using Bioassay bovine serum amyloid-A ELISA kit (Bioassay Technology Laboratory, Shanghai, China) 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 substrate. Therefore, target molecule can be estimated using ELISA reader.

STATISTICAL ANALYSIS

The data were analyzed by analysis of variance (two way ANOVA) in SPSS software (Version 16.0) (Snedecor and Cochran, 1994). Statistically significant means were separated by multiple range test.

RESULTS AND DISCUSSION

Appearance and consistency of CVM is presented in table 1, 2 respectively. After treatment, number of cows with clear discharge were significantly ($p< 0.05$) higher in group I. 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. The mean pH of different groups is present in table 3. After the IU antibiotic treatment, pH significantly reduced ($p< 0.05$) in group I. In the present study, before IU antibiotic treatment, the pH of cervico- vaginal mucus in all groups was alkaline indicating infection, as alkaline cervico-vaginal mucus of endometritic animals may be due to metabolites of bacteria and inflammatory exudates in estrual cervical mucus (Kumar *et al*., 2015) causing conception failure. Once the infection is eliminated, the pH of cervical mucus returns shifts towards the neutral side (Bhat *et al.,* 2015)

Cows with positive (%) Whiteside test indifferent groups is presented in table 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. 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

Table 1: Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on appearance of cervico-vaginal mucous (CVM, %)

Appearance of CVM	Treatment	GROUP I (n = 10)	GROUP II (n = 10)	GROUP III (n = 10)
Turbid	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)
Clear	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) (between groups) and (a, b, c) (within groups) differ.

Table 2: Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on consistency of cervico-vaginal mucous (CVM, %)

Consistency of CVM	Treatment	GROUP I (n = 10)	GROUP II (n = 10)	GROUP III (n = 10)
Thick	Before	90.00^{Aa} (9)	80.00^{Aa} (8)	80.00^{Aa} (8)
	After	20.00^{Bb} (2)	60.00^{Aab} (6)	70.00^{Aa} (7)
Thin	Before	10.00^{Bb} (1)	20.00^{Bb} (2)	20.00^{Bb} (2)
	After	80.00^{Aa} (8)	40.00^{Bab} (4)	30.00^{Bb} (3)

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

Table 3: Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on pH of cervico-vaginal mucous (CVM) (mean ± SE)

Groups	No. of cows	Before treatment	After treatment	Difference in pH
Group I	10	8.55 ± 0.16^{Aa}	7.20 ± 0.08^{Bb}	1.35 ± 0.08^{L}
Group II	10	8.75 ± 0.08^{Aa}	8.50 ± 0.13^{Ab}	0.30 ± 0.05^{M}
Group III	10	8.60 ± 0.10^{A}	8.60 ± 0.10^{A}	0.1 ± 0.00^{N}

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

T**able 4:** Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on White side test of cervico-vaginal mucous (%)

Groups	No. of cows	Positive for white side test before treatment	Positive for white side test after treatment
Group I	10	100.00^{Aa} (10)	20.00^{Cb} (2)
Group II	10	100.00^{A} (10)	70.00^{Bb} (5)
Group III	10	100.00^{A} (10)	100.00^{A} (10)

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

higher percentage (40.32%) of Whiteside positive cows even after intrauterine treatment of Gentamicin.

The mean bacterial load of group I, II and III is presented in the table 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. 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,

Table 5: Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on bacterial load (Mean ± SE x 10µ/ ml) of cervico-vaginal mucous

Groups	No. of cows	Before treatment	After treatment	Change in Bacterial load	Percentage reduction
Group I	10	293.50 ± 6.48^{Aa}	3.50 ± 0.45^{Bb}	290 ± 6.44^{L}	98.81^{L}
Group II	10	301.40 ± 7.82^{Aa}	187.5 ± 11.12^{Ab}	113.9 ± 8.28^{M}	37.79^{M}
Group III	10	296.00 ± 4.24^{Aa}	289.44 ± 11.10^{Ab}	27.2 ± 1.68^{N}	2.22^{N}

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

Table 6: Effects of post-insemination intrauterine antibiotic treatment in endometritic crossbred cows (N= 30) on Serum amyloid- A concentrations (µg/ ml) in serum samples (Mean ± SE)

Groups	No. of cows	Before treatment	After treatment	Change in SAA concentration	Percentage reduction
Group I	10	36.06 ± 2.49^{Aa}	14.84 ± 0.86^{Bb}	21.05 ± 1.43^{L}	58.84^{L}
Group II	10	54.80 ± 4.38^{Aa}	40.40 ± 1.18^{Ab}	10.23 ± 2.36^{M}	26.27^{M}
Group III	10	44.57 ± 1.49^{Aa}	37.79 ± 7.04^{Ab}	7.36 ± 1.56^{N}	15.21^{N}

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

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 (Sarkar *et al*., 2016).

Serum amyloid- A concentration (µg/ ml) 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. 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).

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 nearly 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 90, 40 and 20 %, respectively. The recovery rate was significantly ($p < 0.05$) higher in group I compared to group II and group III. Significantly ($p < 0.05$) higher recovery rates of groups I cows were evident by a greater 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, II and III, first service conception rate was 60% (6 out of 10), 20% (2 out of 10) and 10% (1 out of 10), respectively. The conception rate of group I (60%) cows was significantly ($p < 0.05$) higher compared 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*., 2009).

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 indicated 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.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, College of Veterinary and Animal sciences and Director of Research and Extension, G.B. Pant University of Agriculture and Technology, Uttarakhand for providing all facilities and financial assistance during the entire study.

REFERENCES

Adnane, M., Kaidi, R., Hanzen, C. and England, G.C. 2017. Risk factors of clinical and subclinical endometritis in cattle: a review. *Turkish J. Vet. Anim. Sci.*, **41**(1): 1-11.

Ahmadi, M.R. and Dehghan, S.A. 2007. Evaluation of the treatment of repeat breeder dairy cows with uterine lavage plus PGF2α, with and without cephapirin. *Turk. J. Vet. Anim. Sci.*, **31**: 125–129.

Bhat, F.A., Bhattacharyya, H.K., Fazili, M.R., Hussain, S.A. and Khan, M.Z. 2015. Studies on estrual cervical mucus of repeat breeding cows with special reference to ovulatory disturbances and genital infection. *Theriogenol. Ins.*, **5**(2): 113-116.

Ceciliani, F., Ceron, J.J., Eckersall, P.D. and Sauerwein, H. 2012. Acute phase proteins in ruminants. *J. Proteom.,* **75**(14): 4207-4231.

Denis-Robichaud, J. and Dubuc, J. 2015. Randomized clinical trial of intrauterine cephapirin infusion in dairy cows for the treatment of purulent vaginal discharge and cytological endometritis. *J. Dairy Sci.*, **98**(10): 6856-6864.

Dunson, D.B., Barid, D.D., Wilcox, A.J. and Weinberg, C.R. 2007. Day specific probabilities of clinical pregnancy based on two studies with imperfect measures of ovulation. *Hum. Reprod*., **14**(7): 1835-1839.

Jeong, G.Y., Park, S.J., Kim, N.H., Baek, K.S., Lim, H.J., Herty, K, Kang, K.S., Lee, H.J., Chang, W.K. and Kim, H.S. 2010. Factors effecting on artificial insemination in multi-parturition cattle. *J. Emb. Trans*., **25**(3): 155-159.

Kasimanickam, R., Duffield, T. F., Foster, R. A., Gartley, C. J., Leslie, K. E., Walton, J. S. and Johnson, W. H. 2005. The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. *Theriogenol.*, **63**(3): 818-830.

Kumar, S., Bhardwaz, A., Srivastava, A.K., Rao, M. and Kumar, N. 2015. White Side Test-A Field Test on the Cervical Mucus of Cows for Diagnosis of Endometritis. *Intas Polivet*, **16**(2): 207-213.

Pandey, S., Pandit, P.K. and Choudhry, R.A. 1983. Repeat breeding cows in relation to physical characteristic of cervical mucus, fertility and treatment. *Indian Vet. J*., **60**(11): 946-947.

Parikh, S.S., Savaliya, B.D., Makwana, R.B., Patbandha, T.K. and. Gajbhiye P.U. 2017. Therapeutic efficacy of various intrauterine drugs on repeat breeder Gir cows. *Int. J. Sci. Env. Techol.*, **6**(3): 2107 – 2111.

Parmar, K.H., 2021. Endometritis in bovine: A review. *Agric. Rev.*, **42**(3): 342-347.

Pateria, A.K. and Rawal, C.V.S. 1990. White side test for subclinical metritis in buffaloes. *Ind. J. Anim. Reprod.,* **11**(2): 142-144.

Pluta, K., Irwin, J.A., Dolphin, C., Richardson, L., Fitz-patrick, E. and Gallagher, M.E. 2011. Glycoproteins and glycosidases of the cervix during perioestrous period in cattle. *J. Anim. Sci.,* **89**(12): 4032-4042.

Popov, Y.N. 1969. Diagnosis of occult endometritis in cows using white side test on cervical mucus. *Veterinaria,* **46**(4): 85-87.

Rao, A.V.N. 1982. Causes and incidence of reproductive disorders among *Zebu cross Taurus cross* breed cows in Andhra Pradesh. *Theriogenol.,* **17**:189-191.

Rao, A.V.N and Sreemannarayana, O. 1983. Clinical analysis of reproductive failure among female buffaloes (*Bubalus bubalis*) under village management in Andhra Pradesh. *Theriogenol.,* **18**: 403-411.

Reddy, N.C.S., Brahmhiah, K.V., Naidu, K.S., Babu, A.J. and Kumar, R.V.S. 2012. Effect of uterine lavage on bacterial count and conception rate in repeat breeder cross bred cows. *Indian J. Anim. Reprod.,* **33**(1): 59-62

Robert, S.J. 1986. Veterinary Obstetrics and Genital Diseases, 3rd Edi., S J Roberts Woodstock. pp. 359-381.

Runciman, D.J., Anderson, G.A., Malmo, J. and Davis, G.M. 2008. Effect of intrauterine treatment with cephapirin on the reproductive performance of seasonally calving dairy cows at risk of endometritis following periparturient disease. *Austr. Vet. J.*, **86**(7): 250–258.

Sarkar, P., Patra, M.K. and Kumar, H. 2016. Strategic treatment with immunomodulators to resolve endometritis in cow: A review. *Agricultural Rev.*, **37**(3): 186-195.

Shafique, L., Aqib, A.I., Liang, Q., Qin, C., Ali, M.M., Adil, M., Sarwar, Z., Saleem, A., Ajmal, M., Khan, A. and Pan, H. 2022. Genomic and therapeutic analyses of *Staphylococcus aureus* isolated from cattle reproductive Tract. *Bio. Med. Res. Int.,* **2022**: 6240711.

Sharma, A., Singh, M., Kumar, P., Sharma, A., Kashyap, A., Neelam, I.B., Sharma, A., Chaudhary, N. and Sharma, P. 2017. Bacterial isolation, culture sensitivity test, endometrial cytology of postpartum cows and assessment of their reproductive performance. *Int. J. Curr. Microbiol. App. Sci*., **6**(9): 519-527.

Sharma, V., Prasad, S. and Gupta, H.P. 2013. Studies on physical and rheological properties of cervico-vaginal mucus during early pregnancy in buffaloes (*Bubalus bubalis). Vet. World,* **6**(8): 508-511.

Skuja, S. and Antane, V. 2017. Effects of the Treatment Method of Reproductive Performance in Cows with Retention of Fetal Membranes. *Rur. Sustain. Res.*, **38**(333): 14-23.

Singh, M., Sharma, A., Kumar, P., Bhardwaj, N., Sharma, A. and Bala, I. 2018. Studies on clinical efficacy of some therapeutic regimens for the management of endometritis in cows. *Explor. Anim. Med. Res.,* **8**(1): 110-112.

Tison, N., Bouchard, E., DesCôteaux, L. and Lefebvre, R. C. 2017. Effectiveness of intrauterine treatment with cephapirin in dairy cows with purulent vaginal discharge. *Theriogenol.*, **89:** 305-317.

Tsiligianni, T., Karagiannidis, A., Brikas, P. and Saratsis, P. 2001. Chemical properties of bovine cervical mucus during normal estrus induced by progesterone and/or PGF2α. *Theriogenol.,* **56**(1): 41-50.

Vangroenweghe, F., Lamote, I. and Burvenich C. 2005. Physiology of the periparturient period and its relation to severity of clinical mastitis. *Domest. Anim. Endocrinol*., **29**(2): 283-293.

Verma, K.K., Prasad, Shiv, Kumaresan, A., Mohanty, T.K., layek, S.S., Patbanha, T.K. and Chand, S. 2014. Characterization of physico- chemical properties of cervico-vaginal mucus in relation to parity and conception rate in Murrah buffaloes. *Vet. World,* **7**(7): 467-471.

Wagener, K., Grunert, T., Prunner, I., Ehling-Schulz, M. and Drillich, M. 2014. Dynamics of uterine infections with *Escherichia coli*, *Streptococcus uberis* and *Trueperella pyogenes* in post-partum dairy cows and their association with clinical endometritis. *Vet. J.,* **202**(3): 527–532.

Warriach, H.M., Ahmad, N., Ahmad, G., Khan, M.S., Rabbani, M. and Ahmad, I. 2009. Effect of antibiotic treatment on pregnancy rate of repeat breeder dairy cross bred cows with sub-clinical uterine infection. *Pak. Vet. J.,* **29**(1): 40-42.