RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 2: 274-280 (February 2025)

Genital Changes, Haemato-biochemical Profile and Therapeutic Management of Endometritis in Crossbred Dairy Cows

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10.18805/IJAR.B-4737

ABSTRACT

Background: Reproductive performance is one of the major determinants for the economic improvement of a dairy farm. Endometritis and other uterine complications cause decrease the pregnancy rate. In this study, the genital changes and haemato-biochemical profile of crossbred dairy cows affected with endometritis was studied to evolve a suitable treatment protocol in order to improve reproductive efficiency.

Methods: Crossbred cow affected with endometritis were selected based on mucopurulent vaginal discharge at estrus and tested positive for white side test. Animals were divided randomly into 8 groups with 24 animals in each group. Different therapeutic regimens fortified with supportive treatment were opted. The therapeutic regimens were supportive therapy having bypass fat, vitamins and minerals, Lugol's iodine, Lugol's iodine with supportive therapy, intrauterine (IU) antibiotic, IU antibiotic with supportive therapy, *E. coli*. LPS, *E. coli*. LPS with supportive therapy and control group respectively. Efficacy of each treatment regimen was based on first service conception rate (FSCR).

Result: Of all the therapeutic regimens, fortification of Lugol's iodine with supportive therapy resulted in higher FSCR (83.33%) indicating better applicability as a treatment tool for endometritis in crossbred cows.

Key words: Conception rate, Crossbred dairy cows, Endometritis, Lugol's iodine.

INTRODUCTION

High reproductive efficiency of the dairy herd is one of the most essential components to maintain the profitability of the farm. Reproductive disorders in cows reduce their breeding efficiency making them incapable of producing a calf a year. Common reproductive disorders in cattle are anoestrus, repeat breeding, uterine infection, dystocia, ovulatory defects *etc*. which vary in occurrence with type of animal, breed, parity and level of nutrition and production. Uterine infection is one of the major factors for causing infertility in bovine and inducing irreparable economic losses to the farmers (Drackley *et al*., 2005). Uterine infections may result in prolonged uterine involution and delayed resumption of post partum ovarian activity. Prevention and treatment of uterine infection is essential for ensuring the profit of the dairy industry.

Uterine infection had been classified as puerperal metritis, clinical endometritis, sub clinical endometritis and pyometra. Clinical endometritis is characterized by purulent (>50% pus) or mucopurulent (approximately 50% pus and 50% mucus) uterine discharge detectable in vagina within 21 days or more post partum without systemic illness (Sheldon *et al*., 2006). The severity of disease depends on the types of pathogens, genetic factors and immune response of animals (Sheldon *et al*., 2008). Several Risk factors are associated with the clinical endometritis such as hygiene of the perineum at the time of calving (Schuenemann *et al*., 2011), peripartum metabolic status (Konyves *et al*., 2009), retained fetal membranes (Sheldon *et al*., 2009), delivery of twins and dystocia (Foldi *et al*., 2006). Different therapeutic techniques are in practice for treatment of clinical endometritis such as systemic antibiotic administration (Brick *et al*., 2012) or local antibiotics (Galvao *et al*., 2019), Lugol's iodine (Ahmed and Elseikh, 2014), $PGF_2\alpha$ and intrauterine dextrose (Kaufmann *et al*., 2010) or immunomodulators like *E. coli* Lipopolysaccharide (Bhuyan *et al*., 2015). However, success rate of these treatment regimens have been found to vary and availability of a cost effective therapeutic managemental protocol for overcoming the endometritis problem in cattle is very much scanty. Hence, the present investigation was conducted to study the genital changes, blood biochemical profile of crossbred dairy cows affected with endometritis and its therapeutic management.

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How to cite this article: Dutta, L.J., Deka, R., Nath, K.C., Bhuyan, M., Baruti, M. and Bora, D.P. (2025). Genital Changes, Haemato-biochemical Profile and Therapeutic Management of Endometritis in Crossbred Dairy Cows. Indian Journal of Animal Research. 59(2): 274-280. doi: 10.18805/IJAR.B-4737.

Submitted: 28-07-2021 **Accepted:** 08-12-2021 **Online:** 07-01-2022

MATERIALS AND METHODS

Selection of animals

The study was conducted in crossbred cows maintained in 100 private dairy farms of North East India during August, 2017 to August, 2020. All the animals were of 60-90 days postpartum at 2nd to 5th lactation having body condition score of 3.5 to 4.5 (Mishra *et al*., 2016), maintained under semi intensive system of rearing and fed mostly straw, green grasses with small amount of concentrate mixture comprising wheat bran and rice polish.

The animals were considered positive for endometritis on the basis of the following conditions-

a) Mucopurulent vaginal discharge observed at the time of examination.
b) Reported mucopurulent discharge in preceding or present estrus.
c) Vaginal discharge testing positive for white side test (Popov, 1969).

Therapeutic management of endometritic cows

To study the efficacy of different treatment protocol for addressing endometritis, a total of 192 cows were selected and divided randomly into 8 groups with 24 animals in each group. The treatment protocols were:

Group A-supportive treatment

supportive treatment was started from day of estrus comprising bypass fat (Fatomax, Intas Pharmaceuticals Ltd.) @ 150 g/day/ animal for 15 days, Mineral mixture (Minfa Gold, Intas Pharmaceuticals Ltd.) @ 30 g/day/animal for 15 days, injectable phosphorus (Tonophosphan Vet, Intervet India Pvt. Ltd.) @ 2 g intramuscularly alternate days for three occasions and Vitamin A (Intavita H, Intas Pharmaceutical Ltd.) @ 25,0000 I.U. intramuscularly for three occasions at alternate days.

Group B-Lugol's Iodine (0.25%)

Lugol's Iodine, 0.25% solution given intrauterinely with the help of 50 ml syringe and metal uterine catheter on the day of estrus. Lugol's iodine solution was infused into the uterus till the uterus become fully turgid as detected per rectum and the infused solution was expelled out by gentle massage keeping the metal uterine catheter *in-situ*. After evacuation of the uterus 2/3rd of the amount of the solution infused earlier was reinfused into the uterus and kept.

Group C- Lugol's Iodine + supportive

Lugol's Iodine, 0.25% as mentioned under Group B with supportive treatments as mentioned under group A.

Group D- Intrauterine (IU) antibiotic

Lenovo AP IU (Levofloxacin hemihydrates 20 mg+ Ornidazole 40 mg+ Alpha Tocopherol acetate 5 mg), 60 ml infused intrauterinely for 3 consecutive days from the day of estrus.

Group E- IU Antibiotic + supportive

Lenovo AP as mentioned in Group D along with supportive treatment as mentioned under group A.

Group F-LPS

Single infusion of *E. coli*. LPS 100 µg in 20 ml phosphate buffered saline solution intrauterinely.

Group G- LPS+ supportive

E. coli. LPS and supportive treatment as mentioned under group A.

Group H

Control group without any treatment.

Clinico-gynaecological examination

All the animals were examined per rectum on day 0 (on the day of estrus), and day of subsequent estrus after initiating treatment protocol. On each day of examination, characteristics of vaginal discharge, uterine tone and presence of ovarian structures *viz*. palpable follicle and palpable corpus luteum (CL) were recorded.

Blood biochemical constituents

Blood samples were collected on day 0 and day of subsequent estrus after initiating treatment protocol, serum was separated. The blood biochemical constituents like calcium, phosphorus, iron, zinc, level of hormones *viz*. estrogen and progesterone as well as metabolic hormones like leptin, ghrelin as well as insulin like growth factor-I (IGF-I) were estimated using commercially available kits using manufacturers' instructions.

Post treatment FSCR

All the cows under treatment groups were subjected to artificial insemination (AI) in the next detected estrus following treatment. Pregnancy diagnosis was confirmed by per rectal examination on 60 days post AI.

Statistical analysis

Data generated in the present study were analyzed using the SPSS (version 20.0, SPSS, Chicago, IL, USA). Difference with values of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Characteristic genital changes

Characteristic genital changes on day 0 and day of post treatment estrus in crossbred cows affected with endometritis treated using different treatment regimens have been presented in Table 1. All the cows had mucopurulent vaginal discharge on the day of estrus became normal in next subsequent estrus after treating with lugol's iodine + supportive treatment. However, in other treatment groups 16.66 to 37.50% cows and control group (100%) cows showed presence of mucopurulant discharge in subsequent estrus. However, Bhardwaz *et al*. (2018) reported 60.00% clear discharge at subsequent estrus following intrauterine infusion of Lugol's iodine. In case of intra uterine antibiotic + supportive treatment regime frequency of normal vaginal discharge at post treatment

Table 1: Frequency of occurrence (%) of genital changes in endometritic crossbred cows at different days of treatment with different treatment regimens.

Treatment regimens	Days of treatment	Charateristic genital changes					
		Vaginal discharges		Uterine tone		Ovaries structure	
		Mucopurulent	Normal	Good	Moderate	Palpable follicle	Palpable corpusluteum
Supportive treatment (n=24)	Day 0	100.00 (24)	0.00 (0)	33.33 (8)	66.66 (16)	100.00 (24)	0.00 (0)
	post treatment estrus	33.33 (8)	66.66 (16)	66.66 (16)	66.66 (16)	100.00 (24)	0.00 (0)
Lugol's iodine (n=24)	Day 0	100.00 (24)	0.00 (0)	16.66 (4)	83.33 (20)	100.00 (24)	0.00 (0)
	post treatment estrus	16.66 (4)	83.33 (20)	70.83 (17)	29.17 (7)	100.00 (24)	0.00 (0)
Lugol's iodine + supportive (n=24)	Day 0	100.00 (24)	0.00 (0)	41.66 (10)	83.33 (20)	100.00 (24)	0.00 (0)
	post treatment estrus	0.00 (0)	100.00 (24)	58.33 (14)	16.67 (4)	100.00 (24)	0.00 (0)
IU antibiotic (n=24)	Day 0	100.00 (24)	0.00 (0)	62.50 (15)	37.50 (9)	100.00 (24)	0.00 (0)
	post treatment estrus	33.33 (8)	66.66 (16)	79.16 (19)	20.83 (5)	100.00 (24)	0.00 (0)
IU antibiotic + supportive (n=24)	Day 0	100.00 (24)	0.00 (0)	33.33 (8)	66.66 (16)	100.00 (24)	0.00 (0)
	post treatment estrus	16.66 (4)	83.33 (20)	75.00 (18)	25.00 (6)	100.00 (24)	0.00 (0)
E.coli. LPS (n=24)	Day 0	100.00 (24)	0.00 (0)	29.16 (7)	70.83 (17)	100.00 (24)	0.00 (0)
	post treatment estrus	37.50 (9)	62.50 (15)	70.83 (17)	29.16 (7)	100.00 (24)	0.00 (0)
E.coli. LPS + supportive (n=24)	Day 0	100.00 (24)	0.00 (0)	37.50 (9)	62.50 (15)	100.00 (24)	0.00 (0)
	post treatment estrus	20.83 (5)	79.16 (19)	75.00 (18)	25.00 (6)	100.00 (24)	0.00 (0)
Control (n=24)	Day 0	100.00 (24)	0.00 (0)	29.16 (7)	70.83 (17)	100.00 (24)	0.00 (0)
	post treatment estrus	100.00 (24)	0.00 (0)	12.50 (3)	87.50 (21)	100.00 (24)	0.00 (0)

Figures in the parentheses indicate number of observations.

estrus was as high as 83.33% against 66.66% following treatment with antibiotic alone. Similar result (70.00%) was also recorded by Singh *et al*. (2018a) in endometritic cows treated with intrauterine Ciprofloxacin and tinidazole. *E. coli* LPS treatment regimen fortified with supportive treatment had beneficial effect in respect of percentage frequency of clear vaginal discharge (79.16%) as compared to *E.coli* LPS therapy (62.50%). However, Singh *et al*. (2018a) recorded 80.00% clear vaginal discharge at subsequent estrus after treatment with *E. coli* LPS in endometritic cows. The clear vaginal mucus following treatment may be due to stimulation of uterine defense mechanism, increased phagocytosis and finally elimination of infection. In control group, all the animals showed mucopurulent vaginal discharge in the subsequent estrus. Similar result was also recorded by Sheldon *et al*. (2008) in endometritic cows. Presence of mucopurulent vaginal discharge in control group might be due to delay in elimination of infection by natural uterine defense mechanism.

In respect of uterine tonicity, Lugol's iodine and supportive group of animals showed highest (83.33%) and control group showed lowest (12.50%) uterine tone in post treatment estrus. This might be due to reduced inflammation of endometrium and elimination of pus from the uterus. All the cows had palpable follicle on the day of estrus and subsequent estrus.

Blood biochemical constituents

Mean serum calcium, phosphorus, iron and zinc at day 0 and post treatment estrus with different treatment regimen in endometritic cows have been presented in Table 2. The mean values for estrogen, progesterone, leptin, ghrelin and IGF-1 are presented on Table 3. Mean value of serum calcium, phosphorus and iron in endometritic cows varied significantly between different treatment regimens on the day of estrus and post treatment estrus, but did not vary between days of treatment. However, serum zinc level did not vary between different treatments regimens on day of estrus and post treatment estrus. These variations were not taken into consideration for comparing the efficacy of different treatment regimens as great individual variations existed among the animals.

The mean value of serum estrogen, progesterone, ghrelin and IGF-1 did not differ significantly between on the day of estrus and post treatment estrus in all the treatment regimens. There is a significant difference between levels of leptin in endometric cows on the day of estrus and post treatment estrus in all the treatment groups except in control group. The value of leptin was found to be increased after treatment in all the treatment groups in the present study. The serum leptin level in fertile and repeat breeder cows was 5.12 ±0.43 and 3.63 ±0.25 mg/dl as reported by Guzel and Tanriverdi, (2014). All the endometritic cows in the present study having 2.08 to 2.92 ng/ml leptin on day 0. The post partum reproductive cycle abnormalities were associated with reduced plasma concentration of leptin (Mann *et al*., 2005). Lower level of leptin resulted negative

Table 2: Level of serum calcium, phosphorus, iron and zinc in endometritis crossbred cows at different days of treatment with different treatment regimes.

Treatment regimen	Calcium (mg/dl)		Phosphorus (mg/dl)		Iron (µg/dl)		Zinc (µg/dl)	
	Day 0	Post treatment estrus	Day 0	Post treatment estrus	Day 0	Post treatment estrus	Day 0	Post treatment estrus
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean±S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Supportive	13.99^{a} ±0.68	12.40^{a} ±0.62	3.13^{a}±0.88	2.98^{a}±0.91	214.60^{a}±3.51	244.90^{a} ±4.87	104.20±3.04	103.60±2.06
Lugol's Iodine	11.17^{bc} ±0.56	9.83^{b} ±1.32	1.20^{c}±0.62	1.27^{d}±0.17	215.50^{a}±4.10	197.50^{c}±2.90	100.10 ±2.17	99.63±2.33
Lugol's Iodine + Supportive	10.88^{bc}±0.77	9.65^{b} ±1.40	2.26^{ab}±0.06	3.42^{ab}±0.46	243.70^{b} ±6.38	266.20^{d} ±3.50	101.60±2.30	103.10±3.19
I.U. antibiotic	10.04^{bc} ±0.47	11.40^{a} ±1.48	1.78^{abc} ±0.10	2.89^{b}±0.24	258.00^{c}±5.41	245.10^{a}±3.39	97.53±2.70	98.80±3.30
I.U. antibiotic + Supportive	10.96^{bc}±0.71	11.80^{a} ±1.04	1.85^{abc}±0.43	2.25^{c}±0.15	237.90^{b} ±2.50	222.70^{b} ±3.64	98.13 ±2.29	99.00±2.60
E. coli. LPS	11.29^{b} ±1.30	11.70^{a} ±0.62	1.37^{bc}±0.29	1.39^{d} ±0.26	216.90^{a}±5.10	234.00^{ab} ±3.63	101.20±2.80	102.13±3.70
E. coli LPS+ Supportive	10.57^{bc}±0.83	9.88^{b} ±1.12	1.44^{bc}±0.23	1.95^{c} ±0.13	235.30^{b} ±3.70	242.60^{a} ±3.05	99.20 ±1.20	101.90±2.28
Control	10.43^{bc} ±0.54	11.66^{a}±1.24	1.82^{abc}± 0.13	2.22^{c}± 0.10	213.32^{a} ±2.48	241.72^{a}±1.43	99.32±1.43	103.14±3.43

Means bearing different superscript in a column within a specific blood biochemical constituent differ significantly.
* P<0.05.

energy balance and hamper fertility (Jayaprakash *et al*., 2016).

From the results obtained in the present study it was difficult to correlate the beneficial effect of supportive treatment on blood biochemical constituents when used for fortification with conventional methods used for treatment endometritis in cows.

Post treatment FSCR

The post treatments FSCR in endometrtic crossbred cows are presented in Table 4. There was significant difference in the number of animals treated and number of animals conceived in all the treatment regimens. The post treatment FSCR in endometritic cows recorded highest when the cows were treated with lugol's iodine+ supportive (83.33%) and lowest in the control group (0.00%). Perusal of available literature revealed little information on fortification need of therapeutic techniques used for treatment of endometritis in animals for effective comparison of results obtained in the present study. However, role of nutrition on reproductive performance in animals is well established. The FSCR in cows with endometritis treated with 0.25% Lugol's iodine in the present study was higher (66.66%) to Singh *et al*. (2018b). Conception rate obtained in endometritic cows using different antibiotic was found to vary in wider range. In the present study, 45.83% conception rate was recorded after treated with IU antibiotics. Similar result (48.28%) with regards to conception rate has been reported in earlier studies (Singh *et al*., 2018b) following treatment with intrauterine ciprofloxacin. Moreover, higher post treatment conception rate (66.67%) in endometritic cows after treatment with levofloxacin, ornidazole and alpha tocopherol combination was in record (Mishra *et al*., 2018). The variations in the post treatment conception rate in infectious repeat breeder cows might be due to variation in type of antibiotic, route of administration, dose, degree of sensitivity of antibiotic, severity of uterine infection and time of breeding following treatment (Mosaferi *et al*., 2013). Intrauterine *E coli* LPS therapy resulted in 54.16% conception rate in endometritic cows in the present study. Other reports on use of LPS for treatment of uterine infection in cattle indicated much higher conception rate following treatment ranging from 70.00 to 90.00% (Singh *et al*., 2018b). However, lower FSCR (40.00%) was recorded by Bhardwaz *et al*. (2018). In infectious repeat breeder crossbred cows using intrauterine *E. coli.* LPS. This might have stimulated macrophages and produced interleukin-1 and interleukin-8 that increased the production of granulocyte macrophage-colony stimulating factor for rapid recruitment of polymorphonuclear cells into the uterus and thus cleared the bacteria by phagocytosis process (Methai and Rajasundaram, 2002).

The comparison of results showed fortifications of supportive treatment with lugol's iodine was found to be the best treatment for improving highest FSCR in endometritic cows. Lugol's iodine has benefits like zero withdrawal period, antibacterial, antiviral, antifungal and

Table 3: Level of serum estrogen, progesterone, leptin, ghrelin and IGF-1 in endometritis crossbred cows at different days of treatment with different treatment regimes.

Treatment regimen	Estrogen (pg/ml)		Progesterone (ng/ml)		Leptin (ng/ml)		Ghrelin (pg/ml)		IGF-1 (ng/ml)	
	Day 0	Post treatment estrus	Day 0	Post treatment estrus	Day 0	Post treatment estrus	Day 0	Post treatment estrus	Day 0	Post treatment estrus
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
Supportive	14.35±3.00	14.94±1.08	1.93±0.61	2.24±0.54	2.32_{A}±0.69	4.61_{B}±0.96	244.20±8.40	220.70±5.90	35.83±2.46	42.38±3.04
Lugol's Iodine	15.77±0.82	14.87±1.96	1.42±0.49	1.50±0.09	2.08_{A}±0.49	4.38_{B}±0.39	279.07±6.64	310.90±7.70	35.27±2.16	41.27±1.63
Lugol's Iodine+ Supportive	16.85±2.12	15.47±0.53	1.85±0.57	1.70±0.91	2.18_{A}±0.13	3.64_{B}±1.11	267.09±4.40	286.20±54.50	33.35±3.21	43.68±1.50
I.U. antibiotic	16.13±2.03	14.63 ±2.82	1.66±0.06	1.80 ±0.34	2.73_{A}±0.72	3.89_{B} ±0.62	260.90±8.70	233.30±7.20	39.84±2.09	41.05±2.11
I.U. antibiotic+ Supportive	14.82±2.23	14.42±2.56	1.14±0.20	1.57±0.80	2.92_{A}±0.56	4.09_{B}±1.04	242.40±3.50	225.20±1.10	33.75±2.14	40.07±2.38
E. coli. LPS	14.08±1.66	14.62 ±2.02	1.88±0.52	1.80±0.31	2.29_{A}±0.34	3.11_{B}±0.43	239.30±3.80	264.05±1.90	38.71±1.91	35.20±2.28
E. coli LPS+ Supportive	16.44±4.79	16.15 ±0.52	1.86±0.15	1.85±0.16	2.77_{A}±0.33	4.15_{B}±0.44	257.90±3.10	263.90±7.40	32.44 ±2.85	34.02 ±1.76
Control	15.32±1.94	14.32±1.45	1.39±0.28	1.56±0.45	2.63±0.45	2.67± 0.21	234.82±3.24	222.45±1.94	36.43±2.29	38.98±3.13

Means bearing different subscript in a row within a specific blood biochemical constituent differ significantly, *P<0.05.

Table 4: First service conception rate in endometritic cross bred cows with different treatment regimens.

Treatment regimen	No. of animal treated	No of animal conceived	FSCR (%)	χ^2 value
Supportive	24	11	45.83	
Lugol's Iodine	24	16	66.66	
Lugol'sIodine + Supportive	24	20	83.33	
IU antibiotic	24	11	45.83	
IU antibiotic + Supportive	24	15	62.5	
E. coli. LPS	24	13	54.16	
E. coli LPS + Supportive	24	16	66.66	
Control	24	0	0	16.283*

*P<0.05.

antiprotozoal properties (Sekhon, 2012). Mineral mixture and by pass fat supplementation increased growth and reproductive performances of cross bred cattle (Sahoo *et al*., 2016). Deficiency of Vitamin delays phagocytosis and favors for multiplication of pathogenic micro-organisms in uterus during postpartum period leading to endometritis (Mounir *et al*., 2017). Therefore fortification of fat, vitamin and minerals along with Lugol's iodine had beneficial effect for treatment of endometritis in cows in relation to FSCR.

CONCLUSION

It can be concluded that intrauterine Lugol's iodine fortified with supportive treatment used for treating endometritis in crossbred cows resulted in higher FSCR (83.33%) in comparison to other treatment regimen and all the cows showed normal vaginal discharge at post treatment estrus.

Ethical approval

Approved by Institutional Animal ethical committee (IAEC), Faculty of Veterinary Science, AAU, Khanapara Campus, Guwahati vide approval number 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/291 dated 10/04/2017.

Conflict of interest

All authors decalre that they have no conflict of interest.

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