



Therapeutic Efficacy of Ozone Insufflations in Alleviating Endometritis in Cows

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ABSTRACT

The present investigation was carried out to study the effect of intrauterine ozone insufflation alone or in combination with cephalixin in treating the postpartum clinical endometritis in dairy cows. A total number of fourteen postpartum clinical endometritic cows selected after screening were allotted randomly into 2 Groups (n=7). The Group I & II cows were administered thrice with intrauterine ozone insufflations (21mg) either alone or in combination with cephalixin in two days interval. Seven postpartum normal cyclic animals were taken as control. Following treatment, the overall reduction in combined mucous character and odour in both groups (Group I: 82.04% and Group II: 87.25%) were similar and highly significant ($p < 0.01$). The most predominant bacteria found was *E. coli* (28.57%) followed by *Staphylococcus* spp. (21.42%), *Streptococcus* spp. (21.42%), *Pseudomonas* spp. (14.28%), *Proteus* spp. (7.14%) and *Acinetobacter calcoaceticus* (7.14%). Overall reduction in bacterial count post treatment (day 7) as compared to pre-treatment were highly significant ($p < 0.01$) i.e. 99.60% in Group I and 99.72% in Group II. PMN cell counts of uterine smear were significantly ($p < 0.01$) increased from pre-treatment (25.85 ± 3.28 in Group I and 22.85 ± 2.77 in Group II) to post treatment (77.71 ± 2.86 in Group I and 87.14 ± 1.35 in Group II) which indicated the infiltration of neutrophils due to action of ozone and cephalixin. The conception rates following first AI were recorded as 42.85% in Group I (3/7), 71.42% (5/7) in Group II and 42.85% (3/7) in control group. Thus, intrauterine ozone insufflation has the potential to alleviate postpartum clinical endometritis as an efficacious, cost-effective treatment option and an alternative to antibiotics with an overall improvement of fertility rate in dairy cows.

Key words: Bacterial load, Endometritis, Ozone therapy.

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INTRODUCTION

Endometritis is the inflammation as well as a localized infection of the mucous membrane of the uterus; with mucous, muco-purulent to purulent discharge from uterus into the vagina, that appears three weeks after parturition or later (Turk et al., 2011). Ozone inactivates the bacteria, virus, fungi, yeast and protozoa. It disrupts the integrity of bacterial cell envelope through oxidation of phospholipids and lipoproteins. In fungi, it inhibits the cell growth at certain stages. Ozone administration leads to stimulation of production enzymes which act as free radical scavengers and cell-wall protectors like glutathione peroxidase, catalase and superoxide dismutase. It also causes increase in production of interferon and the greatest output of tumour necrosis factor and interleukin-2 which launches an entire cascade of subsequent immunological reactions. The present investigation was carried out to evaluate the effect of intrauterine ozone insufflation alone or in combination with cephalixin in treating the postpartum clinical endometritis in dairy cows.

MATERIALS AND METHODS

The present experiment was conducted in crossbred cows suffering from endometritis presented in the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar and cases attended at mobile health coverage scheme, OUAT at owner's residence in and around Bhubaneswar. A total number of fourteen postpartum clinical endometritic cows selected after screening were allotted randomly into 2 equal Groups (n=7). The Group I & II cows were administered intrauterine with ozone insufflations thrice in two days interval either alone or in combination with cephalixin (Lixen-IU) and samples were collected just before treatment. A multi-purpose ozone generator producing 21 mg of ozone in 2.5 mins was used for this purpose. Seven postpartum normal cyclic animals were taken as control. The uterine discharge was collected by adopting aseptic procedures, with the help of a sterile glass uterine catheter attached to a twenty (20) ml of syringe with the help of a rubber adopter. A sterilized hollow Aluminum cylindrical tube was introduced to the external os of the cervix and used as protecting device to avoid vaginal contamination. The aspirated uterine content was transferred into a sterile glass vial aseptically. The physical characteristics of uterine discharge in clinical endometritis pertaining to colour, odour and consistency were evaluated as per the scoring pattern laid down by Sheldon et al., (2006) for mucous character and Williams et al., (2005) for scoring odour of uterine fluid. Cytological

slides of endometrial samples were prepared by rolling the modified cytobrush on a clean glass microscope slide and the percentages of PMN cells were determined. The bacterial load or colony forming unit (CFU) in uterine sample was counted by adopting total plate count technique as described by Malik (1967). The data generated was analyzed statistically according to the method suggested by Snedecor and Cochran (1994).

RESULTS & DISCUSSION

The combined rating (score) of mucous character and odour in endometritic cows during pre and post treatment period were displayed in Table 1. The pre-treatment score of uterine mucous character and odour were similar (5.57 ± 0.20) for both the treatment Groups. On day 3rd post treatment, the score was 4.86 ± 0.14 and 4.14 ± 0.70 in Group I and II respectively with respect to mucous character and odour. On day 5th value for the same parameter were 2.71 ± 0.71 and 2.42 ± 0.57 concurrently whereas the day 7th values were 1 ± 0.53 and 0.71 ± 0.42 for Group I and Group II respectively. The percentage reduction was comparatively higher in Group II (25.67%) compared to Group I (12.75 %) on day 3rd post treatment whereas the percentage of reduction was comparatively higher in Group I (44.23 %) compared to Group II (41.54 %). On 5th day the percentage of reduction was comparatively higher in Group II (70.66 %) than in Group I (63.1%). The overall reduction percent were 82.04% and 87.25% for corresponding Group on 7th day. Comparison of pre-treatment value of Group I and Group II against the 7th day rating recorded highly significant difference ($p < 0.01$). Sheldon et al., (2006) and Williams et al. (2005) reported that the character of vaginal mucous as well as odour can be clubbed in order to evaluate the severity of clinical endometritis. The unpleasant smell of cervical mucous may be due to *Escherichia coli*, *Proteus spp.* and *Streptococci spp.* The foetid smell might be due to the breakdown of proteins. The decrease in scoring value of combined character might be due to the effect of ozone insufflation alone or in combination with cephalixin. Both the types of treatment regimens have the ability to reduce inflammation as a result of either bactericidal or bacteriostatic effects. Further it has been reported that ozone therapy augments immunity and favours precipitation of leucocytes into uterine lumen. Hence it can be concluded that both the treatment regimens were equally effective in limiting both mucous character and odour.

The pre-treatment and post treatment bacterial loads of uterine mucous are portrayed in Table 2. The test of significance did not reveal any significant difference between Group I and Group II for this parameter.

There was significant difference ($p < 0.01$) between pre-treatment bacterial load (1st day) and day 3rd value in Group I whereas in Group II, comparison between same days value differ significantly ($p < 0.01$). The overall reduction percent in bacterial load on day 7 post treatment were 99.60 % and 99.72 % for corresponding Group. Comparison of pre-treatment value of Group I and Group II against the 7th day rating recorded highly significant difference ($p < 0.01$). Following parturition, the uterine environment undergoes a physiological necrotic process during involution period both external and internal bacteria multiplies and cause suppuration and inflammation, resulting in proliferation of bacteria in initial stages. In case of endometritis, the bacterial load continues to augment due to compromised uterine immunity. In the present study, the bacterial load counted was at par with the findings of Singla et al. (2004) and Pradhan et al. (2014), where they reported similar count of uterine bacteria both in endometritis and repeat breeding cases of cattle and buffaloes. Significant reduction in post treatment period corroborates the report of Rao et al. (2001), following prostaglandin treatment. Similarly, Kumar et al. (2004) and Singla et al. (2004) recorded significant reduction in

the bacterial load following immunomodulation therapies like autologous plasma and E. coli LPS. In the Group I, the uterine inflammation of ozone might have acted in neutralising the bacterial contamination, by its potent oxidative properties. Induction of ozone in the uterus might have released nascent oxygen causing disruption of the integrity on bacterial cell wall through oxidation of phospholipids and lipoproteins. It inactivates the bacteria, virus, fungi, yeast and protozoa by upsetting the reproductive cycle (Elvis and Ekta, 2001).

The bacterial isolates identified were predominant *Escherichia coli* in four samples (28.57 %) followed by *Streptococcus* spp. in three samples (21.42 %), *Staphylococcus* spp. in three samples (21.42 %), *Pseudomonas* spp. in two samples (14.28 %), *Proteus* spp. in one (7.14 %) and *Acinetobacter calcoaceticus* in one sample (7.14 %). *Escherichia coli* were identified in one case as a single entity (7.14 %) whereas it was present as a mixed infection in three samples (21.57 %). Out of two cases, *Pseudomonas* spp. appeared as a single isolate in one sample (7.14 %) and the other sample was mixed type (7.14 %). *Staphylococcus* spp. was identified in three uterine samples of which its presence was marked in one (7.14

Table 1: Comparison of combined score of mucus character and odour of different groups during treatment.

Groups	Pre treatment		Post treatment				Overall % reduction	“t” value (1 st vs 7 th day)
	Day 1	Day 3	Day 5		Day 7			
	Combined score of mucus character and odour	% Reduction (1 st vs 3 rd)	Combined score of mucus character and odour	% Reduction (3 rd vs 5 th)	Combined score of mucus character and odour	% reduction (5 th vs 7 th)		
I	5.57 ± 0.20	4.86 ± 0.14	12.74	44.23	1 ± 0.53	63.1	82.04	7.999**
II	5.57 ± 0.20	4.14 ± 0.70	25.67	41.54	0.71 ± 0.42	70.66	87.25	10.42**
“t” value	---	0.9937 ^{NS}	---	0.3123 ^{NS}	---	0.4202 ^{NS}	---	---

Table 2: Bacterial load (CFU) in uterine discharge from different groups during treatment.

Groups	Pre treatment (1 st day) Bacterial load	Post treatment Day 3		Post treatment Day 5		Day 7		Overall reduction (%)	“t” value (1 st vs 7 th day)
		Bacterial load	% Reduction (1 st vs 3 rd)	Bacterial load	% Reduction (3 rd vs 5 th)	Bacterial load	% Reduction (5 th vs 7 th)		
I	79.95 ± 21.77	8.60 ± 3.15	89.24	2.64 ± 0.93	69.31	0.32 ± 0.04	87.92	99.6012	3.6571**
II	106.04 ± 23.38	9.20 ± 4.55	91.32	1.01 ± 0.29	89.02	0.30 ± 0.03	70.61	99.72	4.5213**
“t” value	0.8272 ^{NS}	0.1084 ^{NS}	---	1.6749 ^{NS}	---	0.3997 ^{NS}	---	---	---

** $p < 0.01$ level of significance

%) and two samples (14.28 %) in form of single and mixed isolates respectively. Streptococcus spp. was found in three samples of which one sample showed single infection (7.14 %) and the remaining samples were of mixed variety (14.28 %). Proteus spp. was found in a single case (7.14 %) with other bacteria (Mixed type). Similarly, Acinetobacter calcoaceticus (Micrococcus spp.) was found only as a mixed isolate in one case (7.14%). Very high percentage of Escherichia coli, Streptococcus spp. and Staphylococcus spp. have been isolated from postpartum endometritis of dairy cows subjected to ozone therapy (Constantin and Birtoiu, 2016). While studying the dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows, Prunner et al. (2014) identified various types of bacteria by using cytobrush technique and their findings corroborates the present report on bacterial isolation.

The PMN cell count during pre and post treatment period was presented in Table 3. The PMN cell count (Per cent) at pre-treatment (day 1) were 25.85 ± 3.28 and 22.85 ± 2.77 in Group I and Group II respectively. Whereas in normal cows the PMN cell per cent in uterine fluid at estrous was counted as 3.28 ± 0.68 . Test of significance did not reveal any significant difference between pre-treatment value of Group I and Group II. On the contrary, both the Group I (25.85 ± 3.25) and Group II (22.85 ± 2.77) cows had significantly higher PMN cell count ($p < 0.01$) compared to normal estrous cows (3.28 ± 0.68). On 7th day sampling, the per cent of PMN cells were 77.71 ± 2.86 and 87.14 ± 1.35 for Group I and Group II respectively and the PMN value of Group II was significantly higher ($p < 0.01$) than Group I. The percentage increase in PMN cell count on 7th day from 1st day was 200.61 % and 281.35 % in Group I and Group II respectively. The post treatment PMN cell count in Group I and Group II cows were significantly higher ($p < 0.01$) compared to pre-treatment value. During uterine infection chemotactic molecules are released by the bacteria which stimulate the influx of neutrophils from blood vessels to uterus. The uterine immune response to microbes leads to an influx of neutrophils from the peripheral

circulation into the endometrium and uterine lumen (Zerbe et al., 2000; Dhaliwal et al., 2001). Increase in PMN cell has been reported in E. coli and Actinomyces pyogenes infection of uterus (Zerbe et al., 2002). Ozone has also been reported to be a good uterine immunomodulator (Elvis and Ekta, 2001).

Clinical recovery of both Group I and Group II were cent percent when equal number of cows in each Group treated with ozone alone ($n=7$) or in combination with Cephalexin ($n=7$). The conception rate was 42.85% ($n=3$) following insemination of seven cows in Group I. The combined therapy of ozone and Cephalexin (Group II) recorded a conception rate of 71.42% ($n=5$) out of cows inseminated artificially. The control Group registered a conception rate of 42.85% ($n=3$) out of seven cows. Comparison of conception rate between ozone treated and control groups did not reveal any significant difference as evidenced from chi-square analysis. Both the therapeutic regimens might have neutralised the infections and the conception rate was moderate in Group I whereas Group II animals were numerically superior for conception rate because it might be the effect of ozone which enhances the efficacy of other drugs (Scrollavezza et al., 1997). In veterinary practice, the widespread use of ozone has been occurred for treatment of various pathological conditions of genital system in domestic ruminants, such as RFM, clinical metritis or endometritis as well as for improvement of fertility (Zobel et al. 2014 and Đjuričić et al., 2015). Constantin and Birtoiu (2016) acclaimed curative properties of ozone in puerperal endometritis as a uterine treatment. Further they reported improved first service conception rate and 50% of pregnancy rate following first service.

CONCLUSIONS

Intra-uterine ozone therapy can be successfully used as an immunostimulant and anti-inflammatory drug which can act as a novel treatment option for treatment of postpartum clinical endometritis.

Table 3: Polymorphonuclear cell (%) in endometrial smear obtained with modified cytobrush assembly.

Groups	1 st day	“t” value (between groups on day 1)			7 th day	“t” value (between groups on 7 th day)	% Increase	“t” value
		I vs II	II vs III	I vs III				
I	25.85 ± 3.28				77.71 ± 2.86		200.61	11.89**
II	22.85 ± 2.77	0.69 ^{NS}	6.85**	6.73**	87.14 ± 1.35	2.97**	281.35	20.83**
III	3.28 ± 0.68				-		-	-

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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