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Research Article

EFFICACY OF INTRAUTERINE OZONE THERAPY IN REPEAT BREEDER COWS WITH SUBCLINICAL UTERINE INFECTION

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ABSTRACT

The present study was undertaken to observe the effect of intrauterine ozone therapy on reproductive performance of cows with subclinical uterine infection. A total of 40 cows were selected and divided into three treatment and one control group. In group I (n=10), normal cyclical animals were inseminated twice at their natural estrus with good quality semen. In group II (n=10), 3300 mcg (55 mcg/ml, 60 ml) ozone was administered in normal cyclical animals through intrauterine route on day 1 and 2 of the oestrous cycle via glass syringe. In group III (n=10), 3300 mcg (55 mcg/ml, 60 ml) ozone was administered in repeat breeder cows with subclinical uterine infection aseptically through intrauterine route on day 1 and 2 of the oestrous cycle via glass syringe. In group IV (n=10), 4.0 g (60 ml) cephalexin was administered in repeat breeder cows with subclinical uterine infection by intrauterine route on day 1 of oestrous cycle via glass syringe. The bacterial load was reduced in group II, III and IV after intrauterine treatment. The first AI conception rate in group I, II, III and IV was found to be 40, 60, 50 and 40%, respectively.

Keywords: Cow, Intrauterine, Ozone therapy, Repeat breeder, Subclinical, Uterine infection

Subclinical uterine infection is one of the most frustrating causative factors in repeat breeder cows, which leads to reduced fertility at field level. It is characterized by inflammation of the endometrium in absence of clinical signs. The therapeutic approach towards uterine infection involves either antibiotics and antiseptics or hormonal therapy. However, new strains of bacteria can develop due to indiscriminate use of antibiotics along with certain problems like inconsistent recovery rate, high cost of treatment, milk disposal and emergence of microbial resistance and reduced phagocytic activity of polymorpho nuclear (PMN) cells. To overcome these problems, the alternative therapy is proposed recently for the treatment of uterine infection, which includes various forms of ozone preparations (Duricic *et al.*, 2014; Polat *et al.*, 2015). Ozone (O_3) is a gaseous molecule, which consists of three nascent oxygen atoms. It is potent antioxidant, instable and it quickly transforms into oxygen (Di Paolo *et al.*, 2004). The oxidative process in peroxidase activity may destroy bacterial capsules and cell membranes and block DNA replication. Ozone has germicidal, fungicidal, virucidal (Bocci, 1996), immunomodulatory (Korzun *et al.*, 2008) and anti-inflammatory (Guennadi *et al.*, 2008) properties. It stimulates lymphocytes and monocytes in the tissue to release cytokines, which accelerate the tissue regenerations and granulation process (Ohtsuka *et al.*, 2006). Thus, ozone is very useful in every form to treat infection in the body and to the best of our knowledge, no scientific efforts have been made in India to evaluate the efficacy of ozone gas in uterine infection. Therefore, the present study was undertaken to observe the effect of intrauterine ozone therapy on reproductive performance of normal cyclical cows and repeat breeder cows with subclinical uterine infection.

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MATERIALS AND METHODS

The study was carried out at Teaching Veterinary Clinical Complex (TVCC), Krantisinh Nana Patil College of Veterinary Science, Shirwal. Based on white side test and endometrial cytology, a total of 40 cows ranging between 3-9 years of age (20 normal cyclic and 20 repeat breeder cows with subclinical uterine infection) were selected and divided into three treatments and one control group. A total ten cows were included in each group. The diagnosis of repeat breeders with subclinical uterine infection was done on the basis of white side test, which was subsequently confirmed by uterine cytology by adopting cytobrush technique as described by Dutt *et al.* (2017). Slides were brought to the laboratory within 1 hour of slide preparation and stained with modified Geimsa stain as per Kenide *et al.* (2016). The percentage of polymorpho nucelar cells was determined by counting a minimum of 100 cells under microscope at 400x magnification in order to predict subclinical uterine infection. The sample with more than 5 per cent PMNs was considered as positive for subclinical uterine infection as per Oral *et al.* (2009). The bacterial load or colony forming unit (CFU) in uterine sample was counted before and after treatment by adopting total plate count technique as described by Malik (1967). In group I (n=10), normal cyclical animals were inseminated twice at their natural estrus with good quality semen. Whereas, in group II

(n=10), 3300 mcg (55 mcg/ml, 60 ml) ozone was administered in normal cyclic animals through intrauterine route on day 1 and 2 of the oestrous cycle via glass syringe. In group III (n=10), 3300 mcg (55 mcg/ml, 60 ml) ozone was administered in repeat breeder cows with subclinical uterine infection aseptically through intrauterine route on day 1 and 2 of the oestrous cycle via glass syringe. Ozone was generated by using commercially available medical ozone generator (Fig. 1). In group IV (n=10), 4.0 g (60 ml) cephalexin was administered in repeat breeder cows with subclinical uterine infection by intrauterine route on day 1 of oestrous cycle via glass syringe. The pH of cervical mucus was evaluated before and after treatment. Furthermore, uterine cytology for PMN count (Fig. 2), total viable count and bacterial isolation was done before insemination in group I whereas, these parameters were studied before and after treatment at subsequent estrus in groups II, III and IV. Cows in group I were inseminated during first estrus with good quality frozen semen. The animals of group II, III and IV were bred by artificial insemination during subsequent standing heat after treatment with ozone and cephalexin. These cows were negative for white side test and PMN count was less than 5 per cent at subsequent estrus. The non-returning cows from all four groups were subjected to pregnancy diagnosis on day 25-30 post insemination by ultrasonography. The data was analyzed statistically by applying paired 't' test at 5 per cent and 1 per cent level of significance as per Snedecor and Cochran (1994). The permission for the present research work was obtained from the Institutional Ethics Committee for Veterinary Clinical Research with resolution no. IAEC/15/KNPCVS/02/2019.

RESULTS AND DISCUSSION

Table 1 showed that, the mean pH of cervical mucus in cows suffering from subclinical uterine infection in group III and group IV before treatment was found to be 8.1±0.057 and 8.0±0.049, respectively, whereas in healthy cows from group I and group II, it was found to be 7.4±0.053 and 7.5±0.048 before treatment. The mean pH of group III and group IV was more alkaline than that of group I and group II. Kumar *et al.* (2009) reported more alkaline pH in uterine infection due to bacterial metabolites and inflammatory exudates. The pH has reduced significantly (p>0.01) in groups II (7.3±0.042), III (7.5±0.052) and IV (7.5±0.048) after intrauterine

Table 1

Mean pH of cervical mucus in different experimental groups

Group	No. of Cows	Mean pH of cervical mucus before treatment	Mean pH of cervical mucus after treatment	t Value
I	10	7.4±0.053	----	----
II	10	7.5±0.048	7.3±0.042	05.28 **
III	10	8.1±0.057	7.5±0.052	16.10**
IV	10	8.0±0.049	7.5±0.048	20.35**

**(p<0.01)

Fig. 1. Ozone Generator

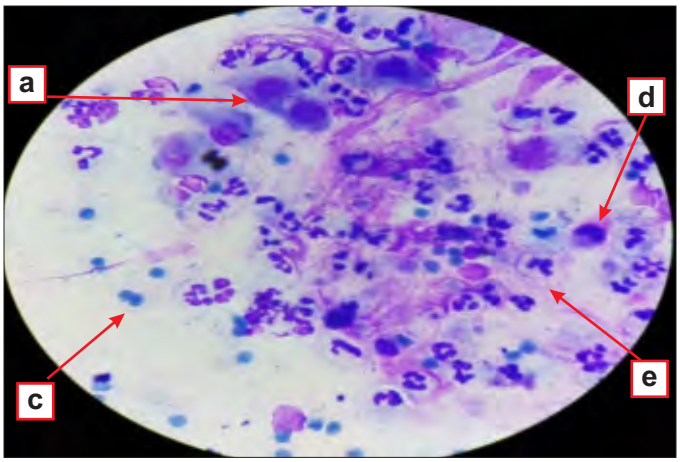

Fig. 2. Endometrial Cytology in Cows with Subclinical Endometritis (a-Endometrial cells; b- PMN cell; c- RBC, d- Lymphocyte)

Fig. 3. Early pregnancy showing embryo

Table 2

Mean PMN (%) in different experimental groups

Group	Mean PMN (%) before treatment	Mean PMN (%) after treatment	t value
I	0.7±0.26	----	----
II	1.4±0.30	0.7±0.26	4.5**
III	7.4±0.68	2.5±0.34	5.8**
IV	9.5±0.54	3.3±0.66	8.3**

**($p<0.01$)

treatment. Salphale *et al.* (1993) also reported that there was drop in pH of cervical mucus towards neutral end after treatment, similar to the findings of present study. Singh *et al.* (2018) observed physico-chemical properties of cervical mucus of endometritic cows and stated that the mean pH of cervical mucus of endometritic cows varied from 8.20±0.08 to 8.4±0.12, which is slightly higher than the present findings. There is no data available which shows the effect of ozone on pH of cervical mucus in cyclical and endometritic cows, hence cannot be compared.

As depicted in Table 2, the mean PMN % in Group I and Group II which were cyclical cows was found to be 0.7±0.26 and 1.4±0.30%, respectively. The mean PMN% in group III and group IV repeat breeder cows with subclinical uterine infection were recorded as 7.4±0.68 and 9.5±0.54, respectively. The higher PMN cell count in group III and IV compared to group I and II indicated that the subclinical uterine infection was present in these cows. There was reduction in the PMN % in Group II (0.7±0.26), III (2.5±0.34) and IV (3.3±0.66) after intrauterine treatment with ozone and cephalexin. The reduction in the PMN count in these groups indicated reduction in the uterine infection because of the treatment. Singh *et al.* (2016) also reported reduction in the PMN count after intrauterine treatment, which is in agreement with the findings of the present study.

In the present study, the mean $\log_{10}$ CFU/ml in uterine lavage sample in cyclic cows of group I and II before treatment was found to be 5.9±2.00, whereas in repeat breeder cows with subclinical uterine infection of group III and IV, it was found to be 9.2±0.40. Log values of uterine lavage samples obtained from animals of Group I and group II showed CFU count below 3(103), which indicated absence of pathogenic bacteria. While in group III and group IV, there was high CFU count, which indicated high bacterial load in cows with subclinical uterine infection than that of cyclical cows. The low CFU count seen in group I and II could be due to the normal inhabitant bacteria present in uterus. In group II, III and IV, the pre-treatment bacterial load was 8.0±0.84, 8.8±0.39 and 9.6±0.86 which was significantly ($p<0.01$) reduced to 5.8±0.85, 5.1±0.68 and 7.4±0.99, respectively after an intrauterine ozone and cephalexin treatment. The reduction in bacterial load observed in group II and III was more compared to group IV, which may indicate that ozone was more effective compared to cephalexin in terms of bacterial clearance in repeat breeder cows with uterine infection. Similar to the present study, the reduction in the bacterial load in endometritis cases after intrauterine treatment was also reported by Singh *et al.* (2016). Literature on effect of intrauterine ozone gas on bacterial load in uterine secretions was not available for comparison.

Table 3

Bacterial load ($\log_{10}$ CFU/ ml) in different experimental groups before and after treatment

Animal No.	Group I		Group II		Group III		Group IV	
	Before	After	Before	After	Before	After	Before	After
1	3.0	-	9.5	7.5	8.3	3.0	12.0	9.5
2	3.0	-	3.0	3.0	11.1	9.7	8.3	3.0
3	9.5	-	8.3	5.3	8.0	5.0	7.7	4.6
4	3.0	-	11.3	8.1	9.3	4.3	4.6	3.0
5	3.0	-	11.4	10.3	8.3	3.0	11.4	8.3
6	3.0	-	8.4	3.0	7.9	6.0	14.5	12.5
7	3.0	-	5.5	3.0	11.0	7.6	9.0	8.4
8	3.0	-	8.0	7.5	8.0	5.0	7.6	6.5
9	6.3	-	9.2	7.2	8.0	5.0	10.0	8.3
10	3.0	-	5.5	3.0	8.1	3.0	10.4	10.4
Mean	3.9±0.69		8.01±0.84	5.8±0.85	8.8±0.39	5.1±0.68	9.6±0.86	7.4±0.99

In present study, *E. coli*, *Staphylococcus* spp., *Pseudomonas* spp., *Corynebacterium* spp. and mixed bacterial isolates were encountered from the uterine lavage samples obtained from all the experimental cows but their percentage in each group differ significantly. In group I and group II, there was predominance of *E. coli* (45%), *Staphylococcus* spp. (20%) *Pseudomonas* spp. (10%), *Corynebacterium* spp. (5%) and mixed unidentified bacterial isolate (20%). However, for group III and IV, these percentages were 40, 15, 10 and 5 %, respectively. In the present study, the first AI conception rate in group I, II, III and IV was found to be 40, 60, 50 and 40%, respectively. The conception rate in ozone treated normal cyclic animals (Group II) was 20% higher than that of untreated normal cyclic animals (Group I). The conception rate in ozone treated endometritic cows (Group III) was 10 percent higher compared to cephalexin treated group (Group IV, Fig. 3).

The improved conception rate obtained in group II compared to group I might be due to the beneficial effect of ozone gas in improving the uterine environment, tissue oxygenation and blood flow towards uterus. The improved conception rate in ozone treated endometritic cows than cephalexin treated cows might be due to the positive effect of ozone gas in reducing uterine inflammation and spermicidal effect to provide favorable condition for the embryo in the uterus. Five cows from group III and six cows from group IV did not conceive even after reduction in the bacterial load, which showed that the damage repair might have taken long time for complete recovery from the infection or there may be other reasons for conception failure. This suggests that endometrium recovery and conception depend upon the severity of uterine infection and other etiological factors. Some chronic repeat breeder cows with subclinical uterine infection might require longer intrauterine therapy of ozone.

CONCLUSION

The intrauterine ozone gas therapy proved to be efficient in improving conception rate by reducing bacterial load in the uterus. Therefore, this therapy can be considered as an alternative therapy to intrauterine antibiotics for treatment of subclinical uterine infection in repeat breeder cows.

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