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Prophylactic effect of intrauterine ozonated oils in augmenting the reproductive performances of postpartum crossbred dairy cows

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Abstract

The objective of present study was to document the prophylactic effect of intrauterine ozonated oil on reproductive performances in postpartum crossbred dairy cows. Thirty-six normally calved crossbred dairy cows were randomly divided into 3 groups with 12 each. Group I served as control and Group II and III received one time and two time intrauterine ozonated oil (100 mL) on day 5-7 and 14, respectively. Reproductive performance of all the cows in the study group was assessed based on parameters such as, calving-first service interval (CFSI), number of inseminations per conception, calving-conception interval (CCI) and first service conception rate (FSCR). The mean values of all the reproductive parameters did not differ between control and preventive ozone infused groups and also between the preventive ozone infused groups. In conclusion, intrauterine ozonated oil used in the present study did not have significant effect in improving the reproductive performances of studied animals.

Keywords: Intrauterine ozonated oil, dairy cows, reproductive performances, prophylactic effect

Introduction

Post-partum uterine infections stand first among the reasons causing infertility in bovines (Lee and Kim, 2007) ^[16]. In addition to causing, decreased milk production (Dubuc *et al.*, 2011) ^[8] and poor reproductive performances, postpartum uterine infections also demand high cost of diagnosis and treatment (Ganaie *et al.*, 2018) ^[12] thereby affecting economic profitability of the farmers. Uterine infections occur more in postpartum period due to compromised uterine environment, high bacterial load and suppressed immune system (Gilbert *et al.*, 2005; Sheldon *et al.*, 2006) ^[14, 20], thereby causing poor reproductive performances.

The most common way to treat postpartum uterine infections prophylactically is by using intrauterine or systemic antimicrobials (Zobel *et al.*, 2014) ^[22], intrauterine antiseptics, and hormonal therapy (Roberts, 1986) ^[19]. However, due to limitations of intrauterine antimicrobials such as, ineffectiveness in the presence of purulent lochia, antimicrobial resistance, residues in milk and meat and impairment in uterine defense mechanism (Dinsmore *et al.*, 1996; Djuricic *et al.*, 2012a; Zobel *et al.*, 2014) ^[4, 6, 22], alternative prophylactic therapies are gaining importance. Ozone molecule in various forms such as gas, spray and vegetable oils is gaining popularity as a prophylactic or therapeutic option for postpartum uterine infections as it does not produce antimicrobial resistance and residual effect in food, unlike antimicrobials. Ozone can be used as antibacterial (Duricic *et al.*, 2015) ^[10], fungicidal, viricidal (Korzun *et al.*, 2008) ^[15] and immunomodulators (Ducusin *et al.*, 2003; Ohtsuka *et al.*, 2006) ^[9, 18] using its oxidative properties. Studies on prophylactic effect of intrauterine ozone therapy on postpartum dairy cows showed that ozone has positive impact on improving reproductive performance in postpartum dairy cows by decreasing the days open (Djuricic *et al.*, 2011; Djuricic *et al.*, 2012; Zobel *et al.*, 2014; Duricic *et al.*, 2015; Escandón *et al.*, 2020) ^[5, 6, 22, 11]. Though the ozone in different forms is used for therapeutic management of various uterine disorders, limited information is available regarding the use of intrauterine ozonated oil as a prophylactic approach for postpartum uterine infections and

in augmenting reproductive performances in bovines. Hence the present study documented the effect of intrauterine Ozonated oil on reproductive performances of postpartum dairy cows.

Materials and Methods

Thirty-six normally calved crossbred dairy cows with no history of dystocia, retained fetal membrane (RFM) and systemic illness were randomly divided into 3 groups with 12 each. Cows in Group I did not receive any treatment and served as control and cow in Group II and III received one time and two time intrauterine ozonated oil (100 mL) on day 5-7 and 14, respectively. All the study animals were inseminated following a voluntary waiting period of 45 days and their reproductive performances were assessed based on parameters such as, calving-first service interval (CFSI), number of inseminations per conception, calving-conception interval (CCI) and first service conception rate (FSCR). Mean values of reproductive parameters of all the study groups were analysed using one-way ANOVA. When F ratio was significant, Tukey's Multiple Comparison Test was used in order to find which pairs differed significantly. Consent from the college level institutional Animal Ethics Committee (IAEC) was obtained to conduct the study (IAEC No.VCH/IAEC/2019/117; dated 03.12.2019).

Results and Discussion

The mean values of reproductive parameters of all the study group are presented (Table 1). Calving to first service interval (CFSI), Calving to conception interval (CCI) and number of inseminations per conception did not differ between control and preventive ozone infused groups and also between the preventive ozone infused groups.

Table 1: Reproductive performances of postpartum dairy cows (n = 12)

Group	Calving to first service interval (CFSI) in days	Calving to conception interval (CCI) in days	Number of inseminations per conception
Group I	60.58±0.49	113.91±10.09	3.66±0.49
Group II	62.42±0.95	110.75±11.00	3.41±0.54
Group III	61.58±1.89	109.91±10.91	3.41±0.54

None of the values were significant (p>0.05)

1. Calving-first service interval (CFSI): The present study revealed that calving to first service interval in Group I (control), Group II (one-time ozone infusion on day 7) and Group III (ozone infused twice on day 7 and 14 pp) are 60.58±0.49, 62.42±0.95 and 61.58±1.89 days, respectively. The CFSI between control and treated groups recorded in the present study are almost similar to the results recorded by Escandón *et al.* (2020) ^[11] (75.1 vs 66.1 days) but lesser than the values 81 recorded by Djuricic *et al.* (2011) ^[5] (131.11 vs 118.20 days) and Djuricic *et al.* (2012) ^[6] (pearl vs spray vs control; 110.5 vs 105 vs 112 days).

2. Calving-conception interval (CCI): Calving-conception interval (CCI) in the present study for Group 1 (control), Group II (one-time ozone infusion on day 7 pp) and Group III (ozone infused twice on day 7 and 14 pp) are 113.91±10.09, 110.75±11 and 109.91±10.91, respectively. Though the values are not statistically significant, lesser CCI have been observed in preventive ozone infused group compared to control group. The present study also revealed

that CCI value recorded in control and preventive ozone infused group is almost similar to the results recorded by Duricic *et al.* (2015) ^[10] (112 vs 110.5 days) but lesser than the values recorded by Zobel *et al.* (2014) ^[22] (98.03 vs 75.47). Higher values of CCI have been reported than the values recorded in the present study, by Djuricic *et al.* (2012) ^[6] (ozone pearl vs ozone spray vs control group; 145 vs 133 vs 150 days) and Escandón *et al.* (2020) ^[11] (control vs ozonized distilled water; 149.0 vs 126.2).

3. No. of inseminations per conception: Average no. of insemination per conception recorded in present study for Group I (control), Group II (one-time ozone infusion on day 7 pp) and Group III (ozone infused twice on day 7 and 14 pp) are 3.66±0.49, 3.66±0.49 and 3.41±0.54, respectively. The recorded values in the control group are almost similar to the values recorded by Escandón *et al.* (2020) ^[11] (3.1 straws) but higher than the values recorded by Zobel *et al.* (2014) ^[22] (2.42 straws). However, in the same study, the average number of inseminations per conception for preventive intrauterine ozone infused group recorded by Zobel *et al.* (2014) ^[22] (1.68) and Escandón *et al.* (2020) ^[11] (2.1) are much lower than present study observation (ys).

4. First service conception rate (FSCR): The present study revealed that first service conception rate for Group 1(control), Group II (one-time ozone infused on day 7 pp) and Group III (ozone infused twice on day 7 and 14 pp) are 25.00, 33.33 and 33.33 percent, respectively (Table2). Escandón *et al.* (2020) ^[11] (ozone vs control; 16.2 vs 50%) recorded highest conception rate for preventive ozone infused group compared to control group. Whereas, the conception rate between preventive ozone infused group and control groups reported by Djuricic *et al.* (2012) ^[6] and Duricic *et al.* (2015) ^[10] are 27.80 vs 20.40 percent and 14.60 vs 14 percent respectively. The values recorded in the present study lies in between the values reported by above authors (25 vs 33.33%).

Table 2: Number of cows found positive for pregnancy at first service (FSCR) (n = 12)

Group	Number of cows found positive for Pregnancy at first service.	First service conception rate (%)
Group I	3	25
Group II	4	33.33
Group III	4	33.33

None of the values were significant (p>0.05)

Observations recorded with respect to the above-mentioned reproductive parameters between preventive ozone infused and control group in the present study, did not show any significant difference. However, many authors have reported significant differences in the above reproductive parameters between preventive ozone infused and control group (Djuricic *et al.*, 2011; Zobel *et al.*, 2014; Constantin and Birtoiu, 2016 and Escandón *et al.*, 2020) ^[5, 22, 3, 11]. Different authors have reported that reproductive performances of the postpartum cows are influenced by various factors such as season, uterine infections, nutrition, management, lactation, bacterial load and endotoxins by interfering with endocrine status of the ovary (Suzuki *et al.*, 2001; Maizon *et al.*, 2004; Gilbert *et al.*, 2005 and Gautam *et al.*, 2009) ^[21, 17, 14, 13]. Similarly, the voluntary waiting period, followed by different authors in their respective study were also varied.

These two facts may be the probable reason for wide variations in the values of reproductive parameters recorded in the present study compared to values reported by other authors.

Djuricic *et al.* (2012) [6] reported that the forms of ozone preparations and the amount of active substance in it are decisive factor to determine its efficacy. The author also reported that ozone can be used in different formulations like pearl, spray, paillets, gas and foam among which gas and spray are having more expanding capacity and less residual effect in uterus. However, ozonated oil used in the present study were having less expanding capacity due to its high viscosity (16.2 cSt) and more residual effect in the uterus which could be the probable reason for the non-significant difference of the reproductive parameters recorded in the present study.

The non-significant results in the present study can be attributed to multiple factors influencing the same. Usually, the peroxide value of the ozonated oil determined its acidic value and viscosity (Carata, 2018) [2]. The ozonated oil used in this study was highly viscous (16.2 cSt) having high peroxide index (1280 mEq/kg) and acidic index (0.561 mgKOH/g) when compared to the formulation of other authors. The peroxide value of ozonated sunflower oil formulated in the study, conducted by Carata (2018) [2] and Campanati *et al.* (2013) [1] were 200 mEq/kg and 75-100 mEq/kg, respectively which is lower than the present study formulation. So, the higher peroxide content of the present study formulation could be the reason for non-significant results. Similarly, volume of ozonated oil infused intrauterine and form of the ozone (ozonised oil having more viscosity) used might have failed to spread the ozone evenly throughout the postpartum uterine lumen thereby failed to exert its effect efficiently.

Many authors have reported beneficial effects of ozone in the form of sprays, gases, pearls and pailletes on reproductive performances in dairy cows (Djuricic *et al.*, 2011 and Zobel *et al.*, 2014) [5, 22]. However, very less work on ozonated oil have been reported and hence, more work needs to be done to standardize the ozonated oil in terms of peroxide values and dosage to be infused so as to use it effectively in bovine reproduction.

Conclusion: The effect of ozonated oil in cows with normal puerperium under study on reproductive performances is non-significant. So, studies using ozonated oil in abnormal puerperium may throw light on its efficacy in improving reproductive performances by combating uterine infections. Further, standardization of ozonated oil in terms of volume, formulation and characterization of oil is needed for better utilisation of ozonated oils in bovine reproduction and also evaluation of the ozonated oil on a larger number of cows is required before adopting it for field use.

Conflict of Interest: Authors have no conflict of interest

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