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Prophylactic effect of intrauterine ozonated oil on uterine involution in postpartum crossbred dairy cows

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Abstract
The objective of present study was to evaluate the prophylactic efficacy of intrauterine ozonated oil (TRIOMET^{TM}, Trieen Health care, Bengaluru), on uterine involution period as well as uterine involution pattern by assessing the reduction rate in previously gravid uterine horn diameter, cervical diameter, previously gravid uterine horn length and intrauterine fluid (IUF) in postpartum(pp) 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. Uterine involution pattern assessment was carried out by employing USG and per rectal examination on day 7, 14, 21, 28 and 35 pp. The intrauterine administration of 100 mL of ozonated oil either one-time or two- times prophylactically, did not hasten either the rate of reduction in previously gravid uterine horn diameter and length or cervical diameter compared to the control group. The involution period recorded for all the cows in different study groups did not show significant difference (Group I vs II vs III; 35.83 ± 0.43 vs 35.50 ± 0.37 vs 34.83 ± 0.84). Ultrasonographic and per rectal examination of postpartum uterus in normally calved cows during the study period revealed that intrauterine ozonated oil used in the study did not have significant effect in hastening the uterine involution rate.

Keywords: intrauterine ozonated oil, dairy cows, uterine involution, prophylactic effect

Introduction
The percentage of people engaging in dairying has been increased over last two decades making, the country largest milk producer, accounting for more than 13.00 per cent of world's total milk production (Belhekar and Dash, 2016) [3]. But dairy farmers are facing set back nowadays due to increased days open period because of delayed uterine involution caused by increased uterine bacterial load so that "calf a year" motto is not being achieved by the dairy farmers. Uterine involution is one of the puerperium stages, wherein it involves return of gravid uterus to normal cyclical non-gravid uterine state through physiological and histological uterine modification (Elmetwally, 2016) [11]. Uterine involution mainly involves processes such as myometrial contractions to eliminate the bacterial contamination and organic debris in the form of lochia and histological regeneration of the endometrium (Elmetwally, 2018) [12].
Uterine involution process gets delayed due to various factors such as negative energy balance, high bacterial load and hormonal imbalance (Sheldon *et al.*, 2004; Gilbert *et al.*, 2005; Hammon *et al.*, 2006; Le Blanc *et al.*, 2012) [28, 15, 16, 17]. It also depends on various factors such as age, breed, season, parity, myometrial contraction during early puerperium, nutrition, milk yield, suckling effect and postpartum complications like dystocia, retained fetal membrane, postpartum uterine infections (Peter *et al.*, 2009; Elmetwally, 2018) [24, 12]. Uterine involution process assessment in terms of uterine size reduction is usually carried using trans-rectal ultrasonography (Okano and Tomizuka, 1987) [23] and per rectal examination (Morrow *et al.*, 1966; Gier and Marion, 1968) [20, 14].
Increasing the uterine defense mechanism as well as reducing the uterine bacterial load is the primary option available to hasten the uterine involution process. This can be achieved by using either intrauterine or systemic antimicrobials (Zobel *et al.*, 2012; Zobel *et al.*, 2014) [31, 32], antiseptics or hormonal therapy (Roberts, 1986) [25]. 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) [6, 7, 32], alternative prophylactic therapies are gaining importance.

Ozone molecule in one such option wherein it not only act as antimicrobials but also an immunomodulator through its oxidative properties (Ducusin *et al.*, 2003; Ohtsuka *et al.*, 2006) [8, 22]. Ozone molecule in various forms such as gas, spray and vegetable oils are gaining popularity as a prophylactic or therapeutic option for postpartum uterine infections as it does not produce unwanted effects of antimicrobials (Duricic *et al.*, 2015) [9]. Vegetable oils like olive and sunflower oils possess antibacterial and immunomodulative properties (Carata, 2018) [4] which can be easily blend with unstable ozone molecule. Few studies reported that along with antimicrobial property ozonated oil aids in regeneration of tissue through fibroblast growth factor (FGF) which might help in uterine size reduction thereby causing macroscopic and microscopic involution (Scrollavezza *et al.*, 1997; Đuričić *et al.*, 2016; Carata, 2018 and Escandón *et al.*, 2020) [27, 10, 4, 13]. The available data on effect of ozonated oil on uterine involution pattern is limited, hence the present study was designed to document the prophylactic effect of intrauterine ozonated oil on uterine involution in postpartum crossbred 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. Uterine involution pattern was assessed using ultrasonography (Easi-Scan™, BCF TECHNOLOGY LIMITED, UK)) by measuring previously gravid uterine horn diameter, cervical diameter and by visualizing intrauterine fluids on day 7, 14, 21, 28 and 35 pp. Cows were restrained in a trevis, perineum was cleaned with soap water following which, transrectal palpation and ultrasonography of the post gravid uterus was performed. Uterus was assessed for its position in the pelvic cavity, tonicity and consistency on day 5-7, 14, 21, 28 and 35-day postpartum. Post gravid uterine horn diameter was recorded by measuring the two-end point of perimetrium in transverse axis from the freezed cross-sectional images. The box in the easy scan monitor was set, equivalent to 1 cm, based upon which measurement was done. Similarly, Diameter of the cervix was measured in longitudinal axis between two hyperechoic lines carefully avoiding rectal wall.

Involution pattern was also assessed by measuring previously gravid uterine horn length per rectally using precalibrated hand on the same study days. Intra-luminal fluid (IUF) was identified by the presence of anechoic and hypoechoic pockets in the uterine horns. The number of animals showing detectable amount of IUF was recorded and expressed in percentage. The time series data on the dependent variables such as cervical diameter, previously gravid uterine horn diameter and length were analysed by two-way repeat measures ANOVA. Level of significance was set at 95%. GraphPad Prism 5.01 was used for data analysis and preparation of line and bar charts. Consent from the college level institutional Animal Ethics Committee (IAEC) was obtained to conduct the study (IAEC No.VCH/IAEC/2019/123; dated 03.12.2019)

Results and Discussion

The data regarding the prophylactic effect of intrauterine infusion of ozonated oil on uterine involution is unavailable. So present study data is compared with data recorded by various authors while studying involution pattern of cattle with normal puerperium.

1. Previously gravid uterine horn diameter

Overall reduction of previously gravid uterine horn diameter between study groups did not show significant variation except on day 7 pp between control and two-time ozone infused group (Table 1, fig 1) which could be attributed to parity of the cow (Elmetwally, 2018) [12]. The diameters recorded in the present study were almost comparable with recordings of Des Coteaux *et al.* (2010) [5] but higher than measurements recorded by Saut *et al.* (2011) [26]. Okano and Tomizuka (1987) [23] have reported that difference between both previously gravid and non-gravid horn diameter will usually be unrecognizable after fourth week postpartum which is in accordance with present study observations.

Fig 1: Uterine horn diameter on day 14pp

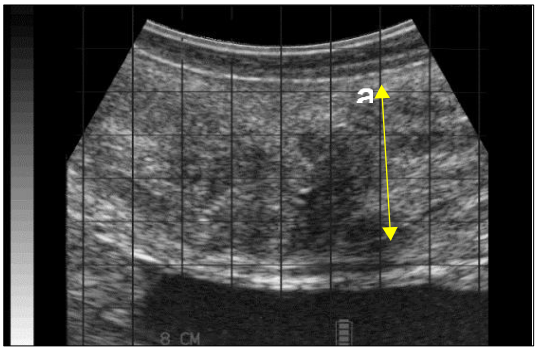

Fig 2: Cervical diameter on day 14pp

Table 1: Previously gravid uterine horn diameter (mm) in postpartum dairy cows (Mean ± SE)

Group	7th day	14th day	21st day	28th day	35th day
Group I (n=12)	70.50 ± 1.07	50.00 ± 0.62	31.58 ± 0.46	26.25 ± 0.27	24.66 ± 0.28
Group II (n=12)	69.33 ± 1.26	49.25 ± 0.76	31.00 ± 0.50	25.83 ± 0.47	24.16 ± 0.38
Group III (n=12)	67.91 ± 1.56	48.5 ± 0.89	29.33 ± 0.54	25.83 ± 0.29	23.75 ± 0.39

Note: Mean ± SE bearing different superscripts are statistically different ($p< 0.05$)

2. Cervical diameter

The mean cervical diameter of cows in all the three groups under study measured at different postpartum days did not differ significantly, except for day 7 pp. Trans rectal ultrasonographic measurements of cervical diameter recorded in the present study (Table 2, fig 2) is in accordance with the measurements recorded by López-Helguera (2012) [19]. Okano and Tomizuka (1996) [23] have recorded a higher cervical diameter compared to present study measurements. Many authors have reported that the cervical diameter in postpartum dairy cows on day 21-25 onwards exceeds the uterine horn diameter (Roberts, 1986 and Noakes, 2018) [25, 21] which is in agreement with the present study observation. The significant difference noted among different study groups on day 7 pp could be due to parity of the cows (Bastidas *et al.*, 1984 and Elmetwally, 2018) [2, 12].

Table 2: Cervical diameter (mm) in postpartum dairy cows (Mean ± SE)

Group	7th day	14th day	21st day	28th day	35th day
Group I (n=12)	51.67 ± 0.93^{a}	39.08 ± 0.48	33.16 ± 0.32	30.08 ± 0.19	29.00 ± 0.12
Group II (n=12)	54.17 ± 1.03^{a}	38.75 ± 0.44	33.33 ± 0.30	30.08 ± 0.31	28.91 ± 0.22
Group III (n=12)	57.92 ± 0.96^{b}	39.91 ± 0.83	33.66 ± 0.37	30.33 ± 0.22	29.08 ± 0.14

Note: Mean ± SE bearing different superscripts are statistically different ($p< 0.05$)

3. Previously gravid uterine horn length

In the present study, the previously gravid uterine horn length recorded at different days postpartum in control group and preventive ozone infused groups did not vary significantly till day 21 pp. Whereas, cows in the Group III (ozone infused twice on day 7 and 14 pp) showed faster reduction in horn length compared to Group I (control) and Group II (ozone infusion once on day 7 pp) after day 21 pp. However, previously gravid uterine horn length of most of the cows under study reached 20-40 cm between 28-35 days, which is in agreement with the observations of Roberts (1986) [25]. Adnane *et al.* (2017) [1] reported that cows with increased parity have decreased uterine elasticity leading to slower reduction in the uterine size. So, the probable reason for the faster reduction of horn length in Group III cows could be due to parity of the cows.

Table 3: Previously gravid uterine horn length (cm) in postpartum dairy cows (n=12)

Group	7th day	14th day	21st day	28th day	35th day
Group I (n=12)	62.50 ± 0.94	50.75 ± 0.78	41.08 ± 0.65^{a}	30.75 ± 0.62^{a}	20.41 ± 0.46^{a}
Group II (n=12)	63.25 ± 1.17	49.58 ± 1.19	40.00 ± 0.66^{a}	30.58 ± 0.69^{a}	19.66 ± 0.71^{ab}
Group III (n=12)	61.75 ± 1.04	48.00 ± 1.00	29.83 ± 0.89^{b}	19.41 ± 0.43^{b}	16.41 ± 0.52^{b}

Note: Mean ± SE bearing different superscripts are statistically different ($p< 0.05$)

4. Intrauterine fluid (IUF)

Almost all cows showed undetectable fluids in the uterus after day 28 and 35 pp except, 1 cow out of 12 (8%) in control group showed detectable number of fluids on day 28 pp which is in accordance with Shwetha (2016) [29]. The probable reason for persistence of IUF on day 28 pp in cow of control group compared to other groups could be due to parity and endocrine status of that cow. However, the process of reduction in the IUF of study cows in the present study was faster in preventive ozone infused groups compared to control group which could be due to two-time infusion of the intrauterine ozonated oil prophylactically, at seven-day interval

5. Involution period

Involution periods obtained in the present study were almost in the physiological range and were comparable with observations of Leslie (1983) [18] and Sukareksi *et al.* (2019) [30]. They have reported that the involution completes by day 25 pp when uterine horn diameter reaches 20- 40 mm and both horns are approximately same size in normally calved cows. Similar observations are recorded in all the study groups on day 34-35 pp. However, the involution period recorded for all the study groups did not show significant difference though the recorded involution period was normal. These observations have suggested that multiple application with precise dose and formulations of ozonated oils may be useful to decrease the involution period in cows with abnormal puerperium rather in normal puerperium.

Table 4: Involution period (days) in postpartum dairy cows (n=12)

Group	Involution period
Group I	35.83 ± 0.43
Group II	35.50 ± 0.37
Group III	34.83 ± 0.84

Note: Mean ± SE bearing different superscripts are statistically different ($p< 0.05$)

Conclusion

Though few studies have shown that ozonated oil aids in regeneration of tissue through fibroblast growth factor (FGF) thereby causing macroscopic and microscopic involution, the present study results revealed that the infusion of ozonated oil prophylactically produced similar reduction rate of uterus both in preventive ozone infused and control group except for IUF reduction rate. This may be due to the fact that, single infusion and two-time infusion at an interval of 7 days and the volume infused may not be effective in hastening the involution rate in normal calved healthy cows. Further, the involution process in the present study is also not delayed much, as compared to abnormal puerperium, since selected cows were normally calved without evidence of RFM. So, the ozonated oil used in the present study may be useful for cows with abnormal puerperium rather than normal puerperium for hastening the involution rate thereby reducing the involution period. For doing so, multiple application with precise dose and formulation is suggested.

Conflict of interest: Authors have no conflict of interest

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