# STUDIES ON OZONE AND AUTOLOGOUS PLASMA INTRA-UTERINE THERAPY IN INFECTIOUS REPEAT BREEDER COWS

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Received: 19-12-2021 Accepted: 12-11-2022

**ABSTRACT**

The study was undertaken to evaluate the therapeutic efficacy of Ozone and Autologous plasma in 40 infectious repeat breeder cows. The animals were divided in 2 different groups viz. Group-I 20 and Group-II 20 treated with Ozone @55µg/ml in 60ml distilled water and Autologous plasma @ 30ml in intrauterine route, respectively. The diagnosis was confirmed by estimation of pH, PMN cell count, White Side Test and Spinnbarkeit test, which indicated 93.00% cows having alkaline pH, PMN cell count >5% and all cases positive for White Side Test. It was observed that pH of CVM in infectious cows before treatment was 8.00±0.07 and 8.13±0.08 which was reduced after treatment 7.32±0.07 and 7.49±0.08, while PMN cells before treatment revealed as 9.35±0.37 and 9.80±0.44 which was changed to 4.90±0.40 and 4.80±0.65 after treatment and Spinnbarkeit value before treatment was 9.00±0.32 and 9.15±0.27 changed to 15.15±0.59 and 14.95±0.61 after treatment under Groups-I and II, respectively. Recovery rate after treatment were found as 75.00 and 65.00 per cent, respectively. The conception rate was found as 68.42 and 55.00 per cent in cows in Group-I and II, respectively. On conclusion, cows treated with Ozone showed higher recovery rate.

**Keywords:** Ozone, Autologous plasma, Intra-uterine therapy, Recovery rate, Repeat Breeder cows.

## INTRODUCTION

A repeat breeder is usually defined as a female cow or buffalo that has not conceived after three or more successful services or artificial insemination, showing normal estrus cycle with apparently healthy genitalia. The key to economically effective dairy farming is good dairy cow fertility (Amiridis *et al*., 2009). Puerperal diseases are thought to have a deleterious impact on postpartum reproductive success. Repeat breeding is also regarded as one of the most serious reproductive problems in cattle (Yusuf *et al*., 2010).

Diagnosis of uterine infection in infectious repeat breeder cows is based on clinical testing and turbid vaginal discharge observation including pH evaluation, White Side test, PMNL cell count and antibiotic sensitivity test (Gilbert et al., 2005). The uterine defense mechanism (UDM) stops invading organisms from colonizing the uterus under normal circumstances, but when this mechanism is disrupted or weakened, bacteria can colonise the uterus and cause endometritis. Antibiotics and antiseptics as well as hormonal therapy are used to treat endometritic cows (Vijayarajan *et al*., 2007).

Recently, the alternative therapy proposed for the treatment of infection includes ozone preparations in the form of boluses, injections, foams, pearls, cream, palettes etc. (Djuricic *et al*., 2015). According to recent publications, intrauterine ozone therapy may also be carried out in animals (Duricic *et al*., 2014; Polat *et al*., 2015).

Intra uterine use of autologous plasma along with leucocytes has been made to stimulate the uterine defense mechanism (UDM) by increasing opsonizing capacity which enhances the phagocytic activity of polymorpho- nuclear (PMNs) cells (Asbusy, 1984). We have also observed a significant reduction in bacterial load and decline in pH of CVM in endometritic cows following treatment with autologous plasma (Sarkar et al., 2015). In stressful animals, an increase in plasma cortisol level impairs neutrophil functions and thus increases susceptibility to bacterial infection. Hence, the present study was aimed to determine the effect of therapies with Ozone and autologous plasma in Repeat breeding cows in association with the recovery, conception rate and pregnancy rate.

## MATERIAL AND METHODS

In this research work, a total of 40 repeat breeder cows with a history of at least 100 days of calving and daily cyclicity but failure of conception within three inseminations or natural services were selected.

Repeat breeder animals were evaluated on estrous phase for endometritis by Gynaeco-clinical analysis with pH estimation, White side test (Bhat *et al*., 2014), PMN cell count and Spinnbarkeit test. Thus, on diagnosis, 40 cows were found to be suffering from infectious endometritis.

In addition, clear cervical mucus and its specific characteristics, such as colour, consistency, viscosity, odour, appearance, through which female reproductive health were evaluated. pH of cervico-vaginal discharge was studied immediately after collection of the sample, with digital pH meter.

Total 40 animals were divided equally in 2 different groups viz. Group-I (20 cow) and Group-II (20 cow) treated with Ozone @55µg/ml in 60ml distilled water and Autologous plasma @ 30ml in intrauterine route, respectively.

Ozone was generated using the medical ozone generator which uses pure quartz dielectrics, high frequency electrical corona discharge technology and latest electronic components to deliver 05 to 65 micrograms per ml ozone using pure oxygen supply that is free from any metallic particles. The other important components of ozone generator machines are oxygen pressure flow controller and silicone rubber tubing.

In a sterile conical glass flask, 80 ml of blood was collected from healthy cows via jugular vein puncture, with EDTA at a rate of 1.5 mg/ml of blood as an anticoagulant. To avoid coagulation of the collected blood, it was carefully mixed with anticoagulant. The plasma was separated by centrifuging the sample for fifteen minutes at 3000 rpm. The plasma was collected and stored in a sterile glass vial at -20°C until it was utilized.

**RESULT AND DISCUSSION**

The pH observations in selected infectious repeat breeder cows were recorded as per normal value i.e., mildly acidic (6.5-6.9), normal (7.0-7.5), mildly alkaline (7.6-8.0), moderately alkaline (8.1-8.5) and highly alkaline (8.6 and above). It was noted that 13 (32.50%), 22 (55.00%) and 5 (12.50%) cows showed slightly, moderately and highly alkaline pH, respectively.

In present research work (Group I) use of Ozone @ 55 mcg/ml in 60 ml distilled water was utilised for intrauterine therapy two times at 24 hr interval on the day of estrus. The average pH, PMN cell count, Spinnbarkeit value and white side test of repeat breeder cows was 8.08±0.07, 9.35±0.37, 09.00±0.32 cm and all positive for white side test, respectively. After treatment the (Group I) pH, PMN cells count and Spinnbarkeit value were found to be 7.26±0.04, 3.4±0.28, and 15.13±0.60 cm. These findings were agreement with (Mali *et al*., 2020) ozone therapy used in intrauterine treatment in repeat breeder cows. The PH value were 7.30±0.04 alkaline and the PMN cell value were 2.5±0.34 respectively and (Escandon *et al.,* 2020) the intrauterine ozone treatment lowered ($p<0.01$) PMN (3.7±1.4 per cent) and first service conception rate increased (50.00%). This value was lower than present trial, because of might be due to different physiology or nutritional factor. Although $O_3$ is one of the most powerful oxidants, it also encourages the production of antioxidant enzymes. In addition, it activates some immune system cells and inactivates pathogens such as bacteria, fungus, yeasts, protozoa, and viruses. $O_3$ is used to treat a variety of disorders and in veterinary medicine as a result of these activities (Sciorsci *et al.,* 2020).

White side test was shown to be negative in 15 (75.00 per cent) recovery in (groups I) cow. Over all recovered cases next estrous artificial insemination was done. There was13 (68.42%) (group I) cow concived and 13 (65.00%) was pregnant, respectively. This finding is in agreement with (Duricic *et al.,* 2014) higher conception rate in endometritic cows than control group after intrauterine ozone spray treatment, respectively and (Durrani *et al*. 2017) in cross-bred dairy cows with bacterial infections, intrauterine treatment with ozone was more responsive (38/50, 76 per cent) recovery rate respectively. (Deori and Phookan 2015) ozone foam was administered to cows with metritis and endometritis, and found that the fertility of the cows improved. As a result, it may be an effective and alternative treatment for cows with metritis and endometritis.

Ozone showed remarkable recovery of infection in repeat breeder cows, suggesting its antimicrobial effect (Polat, 2015) with satisfactory result in terms of conception and pregnancy rate at a very cheap cost. Ozone disrupts the cell membrane of the micro-organisms and diffuse through the protein coat of the nucleic acid of the viruses and kill them (Duricic *et al.,* 2012[a]).

Intrauterine ozone administration as an antiseptic treatment approach in cases of intrauterine infections, which provided potent antimicrobial activity for a wide range of microorganisms and a high oxidation potential that led to rapid transformation into free oxygen (Duricic *et al*., 2014). Ozone also boosts host immunity by stimulating erythrocyte metabolism and the immunological response in local tissues and promotes microenvironmental repair (Guennadi *et al*., 2008) and even cure undetected metritis (Calderon *et al.,* 2016).

**Table no 1: showing pH and PMN of CVM in infectious repeat breeder cows before and after treatmenta**

| Treat-ment groups | No. of Obs. | pH Before Treatment | pH After Treatment | PMN Before Treatment | PMN After Treatment | Spinnbarkeit Before Treatment | Spinnbarkeit After Treatment |
|---|---|---|---|---|---|---|---|
| I | 20 | 8.04±0.05[a] | 7.22±0.04[b] | 8.83±0.37[a] | 4.42±0.39[b] | 9.15±0.27[a] | 16.33±0.44[b] |
| II | 20 | 8.11±0.06[a] | 7.27±0.03[b] | 9.79±0.50[a] | 3.55±0.22[b] | 09.00±0.32[a] | 15.13±0.60[b] |

Infectious repeat breeding cases in Group-II were treated with Autologous plasma @ 30 ml was utilised for intrauterine therapy two times at 24 hr interval on the day of estrus. The average pH, PMN cell count, Spinnbarkeit value showed 8.13±0.08, 9.80±0.44, 9.15±0.27 cm and all tested positive for white side test, respectively. After treatment the (groups II) pH, PMN cells count and Spinnbarkeit value were found to be 7.25±0.06, 3.25±0.41 and 16.33±0.44 cm (table no 1). These finding is in agreement with Sahadev *et al.,* (2007) investigated the impact of immune-modulators on biochemical changes in the uterine defence mechanism in cows with endometritis. Autologous plasma (30 ml) and (*E. coli* LPS +10 ml autologous plasma) were used to treat cows. They observed that before therapy for endometritis, the pH of uterine flushing was 8.11 ± 0.06 and 8.30 ± 0.11, respectively. They were 7.69 ± 0.11 and 7.77 ± 0.18 changes after therapy, respectively. Similar to the present findings, Sarkar *et al.,* (2015) reported the cervical vaginal mucus pH 8.05 ± 0.11 at pre-treatment of estrus and dropped significantly (P <0.05) at subsequent estrus to 7.25 ± 0.11.

**Table No 2: Comparative details of efficiencies of different therapeutic protocols in Infectious repeat breeding cows**

| S. No | Treatment groups | Recovery rate % | Conception rate % | Pregnancy rate % |
|---|---|---|---|---|
| 1. | OZ | 75.00 | 68.42 | 65.00 |
| 2. | AP | 65.00 | 55.00 | 50.00 |

There was 55.00% conception and 50.00% pregnancy rate and these finding is in agreement with Sarma *et al*. (2013) who reported 60.00 per cent recovery and 50.00 per cent conception rate in endometritic cows, respectively. This could be due to the significant increase in opsonising capacity and phagocytic ability of PMN cells, besides activation of the complement pathway which led to reduction of total bacterial load following treatment with leucocytes enriched plasma in cows (Sarkar *et al*., 2015).

In conclusion, Ozone used in repeat breeder cows for recovery of infectious repeat breeder cows was found to be just superior to Autologous plasma therapy.

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