

## COMPARATIVE STUDY OF OZONE, HERBAL DRUG AND LUGOL'S IODINE IN ENDOMETRIC BUFFALOES

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### SUMMARY

The present study was undertaken to observe the effect of intrauterine ozone and other medicinal therapies on reproductive performance of endometritic buffaloes. Cases were diagnosed by White side test, Cervical pH, Spinnbarkeit test and PMN cell count. A total of 60 buffaloes were selected and divided into three treatment groups. In group I (n=20), 110 mcg/ml ozone in 30 ml distilled water; in group II (n=20), Neem oil @ 10mcg/ml in 30 ml distilled water and in group III (n=10), Lugol's iodine @ 1:25 in 30 ml normal saline by intrauterine route on the day of estrus was administered. The bacterial load was reduced in all three groups after the intrauterine treatment. The first AI conception rate in group I, II and III was found to be 70, 40 and 45%, respectively.

**Keywords:** Buffaloes, Endometritis, Intrauterine Ozone therapy, Pregnancy rate, Recovery rate

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In developing countries buffalo plays an important role in maintaining milk and meat production (Pasha and Hayat, 2012). Indian buffaloes are probably four times more productive than average indigenous cows but according to Modi *et al.* (2011) the infertility problems are also high in buffaloes. A group of sub-fertile buffaloes without any anatomical deformities or presence of infection, shows reproductive problems with regular estrus pattern over three or more consecutive oestrus cycles having normal oestral interval (17-25 days) are categorised as repeat breeder animals.

The micro-organisms during the coitus/insemination, parturition, handling of dystocia and retention of placentas cases, likely to cause the uterine contamination resulting into uterine infection. According to Bretzlaff (1968), animals with uterine infection show presence of anaerobic and aerobic bacteria in uterus leading to reduced fertility. Pathogenic organism into uterine mucosa colonize or penetrate the epithelium and causes toxemia during uterine infection (Azawi, 2008). Buffalo having uterine infection shows, slow growth of dominant follicle, reduce peripheral plasma progesterone and estrogen and reduce ability to ovulate (Williams *et al.*, 2007). Uterus has its own defence mechanism but it is not sufficient to prevent the entry and colonization of micro-organisms.

For controlling infection of uterus in endometritic buffaloes different therapeutic strategies can be attempted like antiseptic, herbal ecobolic and ozone treatment. Ozone has more antimicrobial properties that is responsible for inactivation of bacteria, spores and viruses in minimum

time period. Ozone has bactericidal property (Silva *et al.*, 2009), immune stimulating property (Zimran *et al.*, 2000) and anti-inflammatory properties (Guennadi *et al.*, 2008) which eliminate infection.

In present research, a total of 60 repeat breeder buffaloes were examined. Buffaloes were selected for study from various breeds, age and parity. Post-partum buffaloes in late lactation and already in dry stage were examined and such buffaloes in good herd with good AI practices must be pregnant for next calving, but here we have selected those buffaloes having the history of repeat breeding, were subjected to per-rectal and per-vaginal examination for any abnormality. Appropriate body condition score (BCS > 2.5) with turbid, yellowish, smoky, whitish, and flakes vaginal discharge was considered for present study.

### EXAMINATION OF CLINICAL SAMPLES

**Spinnbarkeit test:** For measuring the Spinnbarkeit value, 2-3 drops of collected mucus sample was taken on a grease free glass slide then another grease free glass slide was placed over it. The mucus was stretched between two slides by moving the second slide away from the first one, until the mucus breaks. The distance between the two slides just before the breakage of the mucus string was measured through a scale (cm scale).

**pH measurement:** Collected mucus was immediately checked with the help of digital pH meter for cervical mucus pH.

**White Side Test:** Aseptically collected cervical mucus sample was mixed with equal volume of 5% NaOH in a test

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Table 1

**Comparison of cervical mucus sample for white side test.**

Color	Test results	Degree of endometritis	Grading by number
No color	Negative	Nil	0
Light yellow	Positive	Mild	1
Yellow	Positive	Moderate	2
Dark Yellow	Positive	Severe	3

tube and mixture thoroughly and heated up to the boiling of mixture. Change in color of sample mixture shows degree of endometrial infection (Table 1).

**PMN cell count:** With the help of cytobrush uterine cytology was carried out on day of estrus. After removal of the cytobrush from the genital tract, smear was immediately prepared by rolling a brush on a glass slide. Slide was fixed with methanol solution and stained with Geimsa (1/10) for 30 minutes. The slide was viewed under a microscope at 40X and 100X magnification. Samples containing more than five percent polymorpho-nuclear cells per 100 endometrial cells were diagnosed as positive cases of infectious repeat breeding buffaloes.

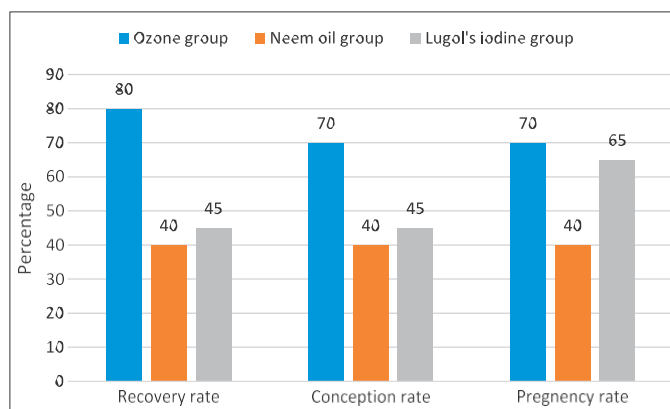
**PREPARATION OF DRUGS AND TREATMENT GROUPS:**

Fig. 1. Comparison of recovery, Conception and pregnancy rate in Ozone, Neem oil and Lugol's iodine groups

**Ozone Treatment (Group I):** Intrauterine ozone water was prepared with the help of ozone generator by treating 30 ml of distilled water with 110 mcg ozone for 30 sec. Prepared ozone solution (30 ml) was infused intrauterine by A.I sheath and 50 ml syringe on day of estrus in Group I (ozone).

**Neem oil (Group II):** Commercially prepared neem oil was used for the present study for treatment of repeat breeding buffalos. 3 ml of neem oil was mixed with 30 ml distil water (10 mcg/ml) and infused by intrauterine route in Group II (Neem oil).

Table 2

**Details of Gynaeco-clinical response in infectious repeat breeder buffaloes to ozonated distil water, Neem oil and Lugol's iodine by intrauterine treatment.**

Sr. No.	Reproductive stage	Parameters	Ozone group	Neem oil group	Lugol's iodine group
1.	Infectious repeat breeding	Clinical cases	20	20	20
	Observations before Treatment (Day-0)	Mean pH of CVM	7.77±0.16 <sup>abc</sup>	7.84±0.17 <sup>ab</sup>	7.06±0.02 <sup>bcd</sup>
		White side test (%)	Positive 100%	Positive 100%	Positive 100%
		Mean PMNs cell	6.9±0.35 <sup>abc</sup>	7.2±0.38 <sup>ab</sup>	7.3±0.33 <sup>a</sup>
		Mean Spinnbarkeit	5.31±0.42 <sup>c</sup>	5.47±0.38 <sup>d</sup>	6.71±0.65 <sup>d</sup>
2.	Observation after first treatment (Day-21)	Mean CVM pH - R	7.31±0.03	7.37±0.05	7.37±0.02
		Mean PMN cell- R	3.25±0.21	3.75±0.31	3.81±0.24
		Mean Spinnbarkeit- R	10.29±0.21	10.53±0.28	11.31±0.41
		Positive white side test (%) -NR	4 (20%)	12 (60%)	11 (55%)
		Mean CVM pH - NR	7.67±0.28	7.68±0.14	7.6±0.10
		Mean PMNs cell- NR	4.5±1.19	5.83±0.47	6.18±0.32
		Mean Spinnbarkeit- NR	6.87±0.39	6.36±0.27	7.86±0.43
		Recovery (%)	16 (80%)	8 (40%)	9 (45%)
		Inseminations	16	08	09
5.	Conceptions	First AI	12	08	08
		Second AI	02	00	01
		Third AI	00	00	00
		Overall (%)	14/20 (70%)	08/20 (40%)	09/20 (45%)
		Service per conceptions	1.33	2.66	2.25
		Pregnancy rate (%)	14 (70%)	8 (40%)	9 (45%)

WST-Indicate white side test, R-recovered cases, NR- non-recovered cases

Mean bearing different superscript differ significantly, superscripts read row-wise.

**Lugol's Iodine (Group III):** Potassium iodide (KI) and iodine crystals (I) were mixed and prepared the stock solution by using 20-30 ml of distilled water. This mixture was then moderately heated and mixed till iodine crystals get dissolved absolutely and it gives amber colour. By using lugol's iodine by 2% concentration working solution by adding of 1 ml lugol's stock solution in 25 ml of distilled water. Infusion of Lugol's iodine @ 1:25 in 30 ml normal saline by intrauterine route on the day of estrus in Group III (Lugol's iodine).

**Statistical analysis:** Data was evaluated by using Completely Randomized Design Test. Details of all treatment group are given in Table 2. Oxidizing property of ozone breaks the microorganism cell membrane and also destroys viruses by diffusing through protein coat (Scrollavezza *et al.*, 1997). By this the mean PMN cell were reduced in current study and similar findings were reported by Escandon *et al.* (2020). In present study, the conception rate of ozone group was 60%, same conception rate was found by Mali *et al.* (2020) by using intrauterine ozone gas. Bhardwaj *et al.* (2018) recorded the use of lugol's iodine in intrauterine treatment in repeat breeder animals. Overall recovery rate was 60.00% and conception rate was 40.00%. In present study, the recovery rate is low (45%) as compared to Bhardwaj *et al.* (2018) but the conception rate was near similar 45%, which supports the present research work. In neem, nimbidin is one of the compound responsible for anti-inflammatory effect, through which neem suppress the functions of microphages and neutrophils involved in inflammation (Dubey and Kashap, 2014). The recovery rate of neem oil group is low, 40% conception rate and pregnancy rate which are similar to Thombre (2017) Kumar (2013) and Rangnekar *et al.* (2005).

In comparison between Ozone, Neem oil and Lugol's iodine groups, Ozone gives good conception and recovery rate.

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