# THE COMPARATIVE EFFICACY OF CLOPROSTENOL AND THERAPEUTIC INTRA UTERINE INFUSION OF POVIDONE IDODINE ON SUBCLINICAL ENDOMETRITIS AND SUBSEQUENT CONCEPTION RATE IN JERSEY CROSS BRED COWS

KAVITHA K.<sup>1</sup>\*, NITHIN A. G.<sup>1</sup>, SARATH T.<sup>2</sup>, KULASEKAR K.<sup>2</sup>, RAJA T.<sup>3</sup>, UMAMAGESWARI J.<sup>2</sup>,

ARUNMOZHI N.<sup>1</sup> and CECILIA JOSEPH<sup>1</sup>

<sup>1</sup>Department of Veterinary Gynaecology and Obstetrics, <sup>2</sup>Department of Clinics,

<sup>3</sup>Department of Animal Husbandry and Economics, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai–600 007 (T.N.), India

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#### ABSTRACT

The present study consists of Jersey cross bred cows brought to Madras Veterinary College-Teaching Hospital, determined to be normal, based on absence of abnormal uterine discharge at the time of estrus. Thorough clinical examination, rectal examination and laboratory evaluations viz. White Side Test (WST), Leucocyte Esterase strip test and Endometrial Cytology (EC) were performed to confirm subclinical endometritis. The cows under study were divided into two groups; Group A cows (n=6) treated with Inj. Streptopenicillin 5g total dose (IM) for three consecutive days followed by Inj. Cloprostenol 500 $\mu$ g (IM) on the seventh day of the cycle. Group B cows (n=6) treated with Inj. Streptopenicillin 5g (IM) along with 20ml Povidone Iodine diluted with 20ml sterile water, intra uterine, for three consecutive days. All the treated cows were subjected to laboratory re-evaluations and inseminated on the following estrum. Improved conception rate was observed in Group A (83.33%) compared to Group B (66.66%) which indicates the immunomodulatory effect of cloprostenol. However, no statistical difference in conception rates were observed between the treatment groups, which may be due to smaller sample size, thus further research is warranted.

Key words: Cloprostenol, Endometrial cytology, Leucocyte esterase test, Subclinical endometritis.

Subclinical endometritis is an inflammation of the uterus in the absence of clinical signs of endometritis and results in a significant reduction of reproductive performance (Sheldon et al., 2006). Bacterial pathogens which contaminate the uterus in postpartum period are recognized by the innate immune system, resulting in chemo-attraction of inflammatory cells, such as neutrophils (Sheldon et al., 2009) which are recruited from the circulation into the uterine lumen to eliminate bacteria (Wade and Lewis, 1996; Subandrio et al., 2000). Inflammation subsides after the elimination of the pathogenic organisms and neutrophils become limited to the fluid in the uterine lumen (Klucinski et al., 1990) which then expelled by uterine contractions. Thus diagnosis of subclinical endometritis can be performed by evaluating the inflammatory process by means of uterine biopsy, ultrasonography, endometrial cytology, bacteriology and leukocyte esterase test (Sheldon et al., 2006, Barlund et al., 2008, Couto et al. 2013, Williams et al., 2005) among which, uterine cytology considered as more accurate method compared to White side test and bacteriological culture (Neelam et al., 2019). Microscopic evaluation of the endometrial surface scrapings or flushed fluid are evaluated to determine the ratio of polymorphonuclear leucocytes (PMNs) to epithelial cells and used to categorize cows as healthy or with subclinical endometritis. The threshold ratio ranges from 4% to 18% depending on the author and the sampling time after parturition (Kasimanickam et al., 2004, Anbhule et al., 2019).

Subclinical endometritis is prevalent in dairy

cows (Hammon *et al.*, 2006) and has a profound negative impact on reproductive performance leading to increased days to first breeding, decreased conception rate and pregnancy rate, and increased culling. The objective of the present study was to evaluate and compare the efficacy of Cloprostenol sodium and Streptopenicilin combination on subclinical endometeritis and intrauterine povidone iodine therapy with respect to the subsequent fertility in Jersey cross bed cows.

### **MATERIALS AND METHODS**

#### **Selection of animals**

Pleuriparous Jersey cross bred cows aged 4 - 7 years, brought to Large Animal Gynaecology ward of Madras Veterinary College Teaching Hospital for artificial insemination with a history of conception failure, previously calved normally followed by regular estrus cycles were selected for this study.

# **Collection of sample**

The estrual discharge was collected by aspiration technique using a sterile AI sheath. After collection, the physical parameters such as colour, consistency and presence of substances (pus and flakes) were recorded.

# Leucocyte esterase strip test

The uterine contents collected by aspiration technique were used for leucocyte esterase strip test. The sample was smeared to test strips on the Multistix 10 SG (Siemen's corporation) reagent strip. Leucocyte Esterase was evaluated after 2 min as per manufacturer's recommendations. The levels of leucocyte esterase activity were scored based on the colour change observed on the strips as the esterase hydrolyzed its yielding a violet

<sup>&</sup>lt;sup>\*</sup>Corresponding author : kavithakannan.vet@gmail.com



Fig. 1: Leucocyte esterase strip test: A- Purple colour change indicates presences of leucocytes and greenish colour change indicates alkaline pH. B-No positive colour changes can me observed

azo-dye (Fig.1) (Kutter *et al.*, 1987; Nazhat *et al.*, 2018). The scores were 0 (negative); + (70 cells/ $\mu$ L); ++ (125 cells/ $\mu$ L); +++ (500 cells/ $\mu$ L).

# White side test

White side test was performed with 1 mL of estrual mucus collected aseptically and heated with equal volume of 5% sodium hydroxide, up to boiling point.

evenly for cytologic examination. Leishmann – Giemsa staining method was used (Figure 3A, 3B). Cytological assessment was done based on the presence of polymorphonuclear cells (PMN). Presence of more than more than 18% of PMN cells in animals after 8 weeks of postpartum were considered as positive for sub clinical endometritis (Kasimanickam *et al.*, 2004). Animals positive for white side test, esterase strip test and showing



Fig. 2: Intensity of colour change in White Side Test

After cooling the intensity of color changes were studied and graded as normal (turbid or no color), mild infection (light yellow color), moderate infection (yellow color) and severe infection (dark yellow color) (Fig. 2).

### **Preparation of Smear**

A drop of the collected uterine discharge was placed on a clean glass microscopic slide and spread



Fig. 3A: Endometrial cytology pre treatment, showing the presence of PMN cells (indicated by arrow marks); Figure 3B: Endometrial cytology post treatment showing the presence epithelial cells (indicated by arrow marks)

presence more than 18% of PMN cells were selected and divided into two treatment groups consisting of six animals under each group.

### Treatment

Twelve Jersey cross bred cows diagnosed positive for subclinical endomeritis were randomly divided into two treatment groups as follows;

**Group A:** The cows (n=6) were treated with Inj. Streptopenicillin (Dicrysticin-S, Zydus Animal Health Ltd.) 5g total dose intramuscularly for three consecutive days followed by Inj. Cloprostenol sodium (Pragma, Intas) 500 $\mu$ g intramuscularly on the seventh day of the cycle. The timeline of treatments and sample collection are in Fig. 4. Fixed time insemination was performed in all the treated animals 72 hours after injection of Cloprostenol sodium.

All the treated cows were subjected to laboratory re-evaluations to rule out endometritis on the following estrum and artificial insemination was performed. Pregnancy diagnosis of the treated cows was carried out 45 to 60 days post insemination.

Statistical analysis was performed using SPSS software and the data pertaining to conception rate were



SP - Streptopenicillin; PI - Povidon iodine; AI - Artificial insemination; RI - Repeat insemination; PD-Pregnancy diagnosis Fig. 4: Timeline of treatments and sample collection for group A cows

analyzed by using chi-square test and Independent t-test was used for analysis of pre and post- treatment PMN cell percentage.

#### **RESULTS AND DISCUSSION**

The present study was designed to evaluate the effect of  $PGF_{2\alpha}$  on for the treatment of subclinical endometritis and also to compare the effect with intrauterine povidone iodine therapy with respect to subsequent fertility in the treated cows. A positive colour reaction (Purple) with Esterase strip test indicating 125 or more leukocytes per ul in the vaginal discharge along with pH raging from 7.5 to 8.5 was observed in all the study cows (Figure 1). The degree of subclinical endometritis was categorized based on intensity of the colour developed in white side test. Out of the 12 cows tested positive with white side test, light yellow colour was seen in 6 cows indicating mild degree, yellow colour was seen in 4 cow indicating moderate degree and 2 cows showed dark yellow colour, indicating severe degree of infection (Figure 2). Leucoyte esterate strip test and presence of PMN cells eliminate the chances of false positive results

Table 1: Percentage of PMN cells pre and post treatment

	Pre treatment	Post treatment
Group A	22	2
	26	3
	20	4
	19	1
	30	2
	22	3
t value = 11.910 **		
Group B	26	3
	25	4
	23	3
	22	2
	19	2
	20	3
t value = 16.961 **		



Fig. 5: Timeline of treatments and sample collection for group B cows obtained with White side test.

The assessment of pre-and post-treatment bacterial load is important to determine the efficiency of the treatment (Dhaliwal et al., 2001). All the six cows of Group A where negative for white side test (no discoloration) post treatment whereas mild yellow discoloration was noticed in one of the five treated cows of Group B. Percentage of PMN cells (more than 18%) was observed in the first examination with a significant (P < 0.01) reduction of these cells to less than 5% in both the treatment groups at the time of insemination (second examination), irrespective of the treatment protocol (Table 1 and Fig. 3A). The reduction in percentage of Leucocyte count and PMN cells post treatment (Figure 1B, Figure 3B) indicate that both the treatment protocols were effective in treating subclinical endometritis. In cows treated with cloprostenol sodium, luteolysis of the corpus luteum, results in estrus and thereby enhance the immediate uterine clearance and it also improve the immune response by removing the immunosuppressive effect of progesterone (Lewis, 1997). The pregnancy rate in cow treated with streptopenicillin and cloprostenol combination was found to be 83% which was higher compared to 66% obtained with intrauterine therapy. Increase in the overall pregnancy rate after first AI in subclinical endometric cows treated with  $PGF_{2\alpha}$  have been reposted by Kasimanickam et al. (2005) and Galvao et al. (2009). Even though a slight increase in conception rate was observed in Group A, statistically there was no significance which may be due to the smaller sample size. Thus further studies may be warranted with a larger sample size.

Intrauterine administration of antiseptics or antibiotics has little penetration to deeper layers of the uterus and the presence of pus and/or organic debris in the uterine fluids decreases the efficacy of intrauterine medications (Paisley et al., 1986). Taking into account of the density of pathogen growth in affected cows, a combination of systemic antibiotic along with hormone therapy helps in decreasing the duration of treatment for successful conception and also found to be economically feasible. Therefore Cloprostenol sodium in combination with systemic antibiotics can be used as an alternative to intrauterine antibiotic/antiseptic therapy for the treatment of subclinical endometritis in Jersey cross bred cows as it improves the immune response of the host by the

induction of estrus by the lysis of CL thereby decreasing progesterone levels which accounts for the immunomodulatory effect.

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