EFFECT OF INTRAUTERINE IMMUNOTHERAPY ON UTERINE CELLULAR DYNAMICS AND CONCEPTION RATE IN ENDOMETRITIC COWS

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Received : 26.09.2013 Accepted : 26.05.2014

ABSTRACT

The efficacy of three intrauterine (IU) immunomodulators was investigated in endometritis of cross bred cows. Forty endometritic cows were divided into 4 groups (n=10) and marked as T_1, T_2, T_3 and T_4. T_1 cows received *E. coli* lipopolysaccharide (LPS). Oyster Glycogen (OG) and Autologus Plasma (AP) were used in group T_2 and T_3, respectively. T_4 was kept as control with single infusion of 60ml Phosphate Buffer Solution (PBS, pH-7.2). After 24 hours of treatments significant ($P<0.01$) increase of neutrophils and lymphocytes in uterine flushing were observed with significant ($P<0.01$) reduction in epithelial denudation in all treatment groups. Conception rate increased significantly ($P<0.01$) in groups T_1, T_2, and T_3. The results indicated that intrauterine immunomodulators can be used as alternative to antibiotic in resolving endometritis in cows.

Key words: Cows, Endometritis, Intrauterine Immunotherapy

INTRODUCTION

Among the post parturient diseases endometritis alone appeared to be a major cause of infertility in cows. (Agarawal *et al.*, 2002). The antiseptic, antibiotics, hormones etc have been used since long back for its treatment but all are with conflicting and inconsistent results (Roberts, 1971; Hussain and Daniel, 1991). Moreover, the indiscriminate use of antibiotics warns the danger of various side effects and human health hazard problems (Kaneene *et al.*, 1986; Arora *et al.*, 2000). Hence, the present study was designed to ascertain the efficacy of certain IU immunomodulators as alternative to conventional therapy for endometrtis in cows.

MATERIALS AND METHODS

Total 40 crossbred cows with the history of conception failure were diagnosed to have endometritis with the help of gynaecological examination (LeBlance, 2002) and on the basis of the results (development of yellow to brown colour) of white side test in uterine discharge (Popov, 1969).The cows were randomly allocated to 4 groups (n=10), namely T_1, T_2, T_3 and T_4. T_1 group was treated with single dose of 100µg *E. coli* LPS IU in 60 ml PBS. T_2 cows received OG @ 500mg/cow as single dose IU in 60 ml PBS. The cows of T_3 group were infused with 50 ml AP daily for 3 consecutive days. T_4 (control) group was treated only with 60 ml PBS for single time. In all the cows uterine flushing was done before and 24 hours after treatment while at estrus with 60 ml PBS following the procedure of Promod *et al.* (2002). Flushing sediment was used

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for total nucleated cell count (TNC) and differential leucocyte count (DLC).The recovery was assessed at post treatment subsequent estrus by gynaecological examination and white side test in uterine discharge. Recovered cows were inseminated artificially and pregnancy had been diagnosed after 50 days insemination.

Paired-t test was performed between pre and post treatment values and one way analysis of variance was performed between groups. Moreover, Normal Deviate test of proportion was performed on conception and recovery rate as per the method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The post treatment absolute (Table 1) and per cent neutrophil count (Table 2) increased significantly ($P<0.01$) in all the treatment groups except control. Maximum increase in absolute (15.62+0.85 10^6/ml) and per cent (68.00+1.52%) neutrophil count was observed in T_2 group with OG treatment followed by *E. coli* LPS. Singh *et al.* (2001) and Sahadev *et al.* (2007a) also reported similar results after LPS infusion. In contrast Deroi *et al.* (2004) reported very high neutrophil per cent at post treatment assay. This variation in neutrophil per cent count might be due to the rate of degeneration of neutrophil in exposure to an unhealthy environment (Weiser 2004). Almost similar result on percent and absolute neutrophil count was reported by Anderson *et al.* (1985) after OG treatment. The present finding of post treatment neutrophil per cent was in consonant with Waelchli *et al.* (1987) and Sahadev *et al.* (2007) after AP infusion. LPS at low dose activated monocyte and macrophage to produce TNF, which cause synthesis of IL-1. Then IL-1 and TNF both acted on endothelial cell to release IL-6 and IL-8. Thus, the initial exposure to LPS results in a cytokine cascade event intended to enhance local inflammatory response. Under which more influx of leucocytes, immunoglobulin and complement took place into the uterine lumen. OG also acted similar way to combat infections (Prasad *et al.*, 2006).The AP also produced mild local inflammatory reaction on endometrium to recruit PMN cells into the uterus (Waelchli *et al.*, 1987). In addition to this, plasma derived complement and antibodies helped in opsonization and phagocytosis (Asbury, 1984). Plasma or serum has got direct bactericidal property (Taylor, 1987) and the C_{5a} component of complement acted as strong chemoattractant. Absolute and per cent lymphocyte count after treatment increased significantly ($P<0.01$) in group T_1, T_2, T_3. In contrast, Sahadev *et al.* (2007) reported decreased lymphocyte per cent after immunomodulators infusion which might be a relative one resulting in mononuclear cell infiltration into the endometrium following AP treatment. In the present study highest absolute (4.98+0.65 x 10^6/ml) and per cent (33.20+2.48%) lymphocyte count were recorded after *E. coli* LPS treatment substantiating it as the most mitogenic and chemoattractive for lymphocyte than OG and AP.

Cytokines released from the *E. coli* LPS activated macrophages such as IFN-ã, TNF or IL-1 cause expression of adhesion molecules like CD_{54}, CD_{11a}, CD_{18} and CD_{43} (IG super family) on endothelial cell and permits lymphocytes and monocytes to adhere and move into inflammed tissue and also migration of T-lymphocytes towards the inflammed tissue (Tizard, 1996). LPS also activated complement and triggered lymphocyte proliferations with local antibody production leading to better opsonization and phagocytosis. (Tizard, 1996; Vegad and Katiyar, 2001). Further, protein contaminants in OG might play a role in chemoattraction, infiltration and proliferation of lymphocytes into the uterus leading to significant ($P<0.01$) raise in lymphocytes population 24 hours after treatment. (Anderson *et al.*, 1985 and Tizard, 1996).

Showing significant ($P<0.01$) effect of treatment the post treatment absolute (1.16+0.29 x 10^6/ml) and per cent (5.30+1.58%) epithelial cells decreased to maximum in T_2 group followed by T_1, T_3 and T_4 which was in close agreement with Prasad *et al.* (2006). The immunomodulators enhanced uterine defense mechanism resulting in enhanced phagocytosis of pathogen and natural healing with reduced denuded epithelium (Prasad *et al.*,2006). The recovery rate was 100% in group T_1 followed by T_2 (80.00%), T_3

(60.00%) and T_4 (20.00%). The conception rate on the basis of total numbers of cow treated was 90.00 (T_1), 80.00 (T_2), 50.00 (T_3) and 20.00% (T_4). Similar results were reported by Singh *et al.* (2000) and Deori *et al.* (2004) after *E. coli* LPS treatment and Devaraj *et al.* (2006) with OG treatment. The conception rate after AP treatment by Methai *et al.* (2005) and Devaraj *et al.* (2006) were also in accordance with the present findings. However, the recovery and conception in control group might be due to uterine defense mechanism leading to spontaneous recovery (Hoedemaker, 1998). 100% recovery in any group could not be achieved probably because of the differences in bacteria species involved that could evade and escape the UDM (Tizard, 1996). The conception failure in some recovered cows may be due to the pathological condition of the uterus like fibrotic changes of endometrium making animal helplessly sterile (Roberts, 1971).

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