



Effect of Administration of Autologous Plasma along with Leucocytes on Hormonal Changes in Relation to Recovery Rate and Conception in Endometritic Cows

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Received: 07 Aug, 2018

Revised: 25 Nov., 2018

Accepted: 04 Dec., 2018

ABSTRACT

The effect of autologous plasma along with leucocytes in the treatment of endometritis and its relation with hormonal changes associated with recovery was evaluated in present study. A total of 16 endometritic crossbred cows were randomly allocated into two groups, Group I (n=10) received single intra uterine infusion of autologous plasma along with leucocytes whereas, the animals of Group II (n=6) served as control. Blood samples (5ml) were collected twice from all the experimental animals, first at the time of diagnosis of endometritis (pretreatment) and second at subsequent estrus (post-treatment). The clinical recovery was assessed by negative reaction in white side test and reduction of total bacterial count in cervico-vaginal mucus (CVM) at subsequent estrus and breeding was done through artificial insemination. Plasma thyroxin (T_4), tri-iodo thyronine (T_3) and cortisol concentration were evaluated in both the groups at pre and post treatment. Pregnancy was confirmed after 60 days post-insemination per rectally. The results revealed that plasma T_3 (1.41 ± 0.20 vs. 1.98 ± 0.17 nM) and T_4 concentration (27.11 ± 5.97 vs. 35.98 ± 4.73 nM, $p \leq 0.05$) increased, whereas the cortisol level declined (6.77 ± 1.38 vs. 2.12 ± 0.63 ng/ml) following treatment with autologous plasma in the endometritic cows. The overall pregnancy rate was significantly higher in the treated group (70%) compared to no pregnancy in control. Thus, it can be concluded that treatment autologous plasma could be associated with restoration of thyroid hormones level and reduction of stress, besides improving the conception rate in the endometritic cows.

Keywords: Autologous plasma, Leucocytes, Thyroid hormones, Cortisol, Endometritis, Cow

Higher reproductive performance is essential for maintaining optimum production in dairy enterprises. Reproductive disorders play as major stumbling block in achieving desired reproductive efficiency of cow. Among the major reproductive disorders, the overall prevalence of clinical endometritis and sub clinical endometritis to be 18.7% and 12.4%, respectively (Kaufmann *et al.*, 2010). Endometritis in clinical or subclinical forms, caused by the pathogenic bacteria affects the ovarian cyclicality, precludes fertilization; prevent successful implantation and development of viable embryo, thus enhances days open and accordingly extends the calving interval (Sheldon *et al.*, 2006). It not only causes infertility at

the time of infection but also results in sub-fertility even after successful clinical resolution of the disease. Though parental or intra uterine treatment with antibacterial agent and antibiotic have varying degree of success, the inconsistent recovery rate, high cost of treatment, milk disposal after antibiotic therapy and immergence of microbial resistance are obvious disadvantage of their use. Therefore, alternative therapies for the treatment of endometritis using natural substances as or means of activation of natural defense mechanism in uterus have been attempted in recent past (Sarkar *et al.*, 2016). Intra uterine use of autologous plasma along with leucocytes has been made to stimulate the uterine defense mechanism



(UDM) by increasing opsonising capacity which enhances the phagocytic activity of polymorpho nuclear (PMNs) cells (Asbusy, 1984; Methai, 1999). 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). The concentration of thyroxine (T_4) and tri-iodothyronine (T_3) in the peripheral circulation have been found to be associated in persistent infections as well as in the subclinical condition (David *et al.*, 1998). A decrease in plasma T_4 and T_3 level occurred due to constant caloric deprivation or to enhance endogenous cortisol production (Peterson and Ferguson, 1989). 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 autologous plasma along with leucocytes on the thyroid and cortisol profile in endometritic cows in association with the recovery and conception rate.

MATERIALS AND METHODS

The present study was carried out on crossbred (Freisian \times Shahiwal) cows (n=16), diagnosed positive for endometritis at Military Dairy Farm, Bareilly, U.P., India. The cervico-vaginal mucus (CVM) was collected at estrus from the suspected cows and examined for the presence of white flakes or pus and subjected to colour reaction to white side test (Popov, 1969). The collected CVM was mucolysed by vortexing for 10-15 minutes after mixing with sterile distilled water and used for total bacterial count.

The bacterial load was determined by calculating colony forming units on agar plates after 24 hours following standard plate count method. All the 16 cows were diagnosed as positive for endometritis and randomly allocated into two groups, Group I (n=10) received single intra uterine infusion of autologous plasma along with leucocytes and Group II (n=6) as control, received single IU infusion of normal saline. Approximately 250 ml of blood was collected from the animals in group I through jugular venipuncture using the MEDIBAG blood bag (Eastern Medikit Ltd., Gurgaon, India). The plasma and buffy coat portion was separated and stored at refrigeration. The total leucocytes count (TLC) and differential leucocytes count (DLC) was performed following standard haematological

techniques. The viability of leucocytes was assessed by trypan blue dye exclusion test. The therapeutic dose of autologous plasma and WBC was decided based on the total bacterial count in CVM. After 6 hours of uterine lavaging, the treatment was given at the dose rate of 100 ml autologous plasma containing leucocytes at the ratio of 1:10 of total bacterial load in the Group I and in Group II with equal volume of normal saline.

A fraction of blood sample (5 ml) from all the endometritic animals were separated at pre-treatment and another 5 ml blood samples at post treatment in subsequent estrus was collected for hormone assay. The plasma was separated and stored at -20°C for estimation of T_3 , T_4 and cortisol level through solid phase RIA using commercial kit (T_3 and T_4 ; Izotop, Institute of Isotopes com. Ltd., H-1535, Budapest and cortisol, ICN Biomedicals, INC. Diagnostic Division). The clinical recovery of endometritis was assessed by negative reaction to white side test and significant reduction in total bacterial count in cervico-vaginal mucus (CVM) at subsequent estrus. Pregnancy was confirmed by per rectal examination at 60 days post conception.

Data were analyzed using paired T test for comparing pre-treatment and post treatment bacterial load, endocrine profile. The level of significance of two independent propotion was analyzed in Z test. Level of significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

The treatment response of autologous plasma with leucocytes in endometritic cows was assessed based on the white side test of CVM and bacterial count in present study. The nature of the CVM of endometritic cows were either purulent (50-60%) or mucopurulent (40-50%), which showed strong to moderate positive reaction in white side test at the time of treatment. However, at subsequent estrus, the estrual CVM turned clear in 90% animals in treatment group, whereas only 33% animals of control group showed clear mucus.

The recovery rate of cows from endometritis in terms of negative reaction of white side test was higher (80%) in Group I as compared to only 16.7% animals of control group (Table 1). The finding is also correlated with our previous reports where, a significant decline in bacterial

Table 1: Nature and Intensity of colour reaction (White side test) of cervico-vaginal mucus in cows treated (Plasma along with leucocytes) and control groups

Parameter	Group I (Plasma + leucocytes)		Group II (Control)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Nature of CVM (%)				
1. Purulent	50	—	16.7	50
2. Mucopurulent	50	10	83.3	16.7
3. Clear	—	90	—	33.3
White Side Test (%)				
1. Positive	100	20	100	83.3
Moderate	80	10	66.7	33.3
Slight	20	10	33.3	50
2. Negative	—	80 ^a	—	16.7 ^b

Values with different superscripts (a, b) in a row differ significantly ($P < 0.05$).

Table 2: Mean (\pm SE) bacterial load of pregnant and non-pregnant cows

Animals	Bacterial load ($\times 10^6$ /ml)		% Reduction of bacterial load
	Pre-treatment	Post-treatment	
Pregnant (n=7)	88.73 \pm 20.94 ^a	0.82 \pm 0.30 ^b	95.56 \pm 2.66 ^A
Non-pregnant(n=9)	81.707 \pm 30.648 ^a	11.288 \pm 5.582 ^b	61.14 \pm 14.14 ^B

Values within same row (a,b) and within same column (A,B) having different superscripts differ significantly ($P < 0.05$).

load in CVM at subsequent estrus in treated group ($p < 0.05$) and an increase in bacterial count in more than 35% animals in control group at subsequent estrus (Sarkar *et al.*, 2015). This change in white side test could be explained on the basis of the reduced number of leucocytes present in the uterine discharge following recovery of endometritis. In another study on sub-clinical endometritis Murrah buffaloes, treatment with E coli LPS and oyster lycogen resulted in significant reduction of bacterial load (Bajaj *et al.*, 2015). At subsequent estrus, 70% of cows in Group I were conceived as compare to no pregnancy in Group II. Our finding is supported by a similar study of Mattos *et al.* (1999) who also achieved 78.6% pregnancy rate in endometritic mares following treatment with 120 ml plasma along with leucocytes. 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).

The load of bacteria decreased significantly at post treatment estrus in both pregnant and non-pregnant animals though incase of pregnant animals it was more pronounced (Table 2). This could be attributed to the higher endotoxin and intermediary cytokines which could exerts an influence at both the hypothalamic and anterior pituitary levels to influence ovarian function and embryo survival (Williams *et al.*, 2001).

The mean concentration of T_3 , T_4 and cortisol were estimated at pre and post treatment estrus and the mean value are presented in Table 3. The T_3 concentration was significantly higher at post treatment estrus in both treatment (1.41 \pm 0.20 vs. 1.98 \pm 0.17 nM) and control group (1.57 \pm 0.19 vs. 2.09 \pm 0.22 nM), respectively. Similarly, the T_4 hormones increase significantly (27.11 \pm 5.97 vs. 35.98 \pm 4.73 nM, $p \leq 0.05$) at subsequent estrus in the treatment group whereas, its concentration remain at similar level in control cows. The lower level of plasma T_3 and T_4 in the endometritic cows of the present study are in agreement with the previous observations (Abede and Eley, 1992;

Table 3: Effect of autologous plasma along with leucocytes treatment on plasma thyroxine (T₄), tri-iodothyronine (T₃) and cortisol level in endometritis cows

Groups	Hormonal profile (Mean±S.E.)					
	T ₄ (nM)		T ₃ (nM)		Cortisol (ng/ml)	
	Pre-treatment estrus	Post-treatment estrus	Pre-treatment estrus	Post-treatment estrus	Pre-treatment estrus	Post-treatment estrus
Group I (n=10)	27.11±5.97 ^a	35.98±4.73 ^b	1.41±0.20 ^{aA}	1.98±0.17 ^b	6.77±1.38 ^{aA}	2.12±0.63 ^b
Group II (n= 6)	29.86±2.89 ^a	31.97±5.38 ^a	1.57±0.19 ^{aB}	2.09±0.22 ^b	2.04±0.63 ^{aB}	3.45±1.32 ^a

Values within same row (a,b) and within same column (A,B) having different superscripts differ significantly (P<0.05).

Table 4: Mean (±SE) plasma thyroxine (T₄), tri-iodothyronine (T₃) and cortisol concentrations of pregnant and non-pregnant cows

Animals	T ₄ (nM)	T ₃ (nM)	Cortisol (ng/mL)
Pregnant (n= 7)	44.50±3.23 ^A	2.37±0.21	1.97±0.60
Non-pregnant(n= 9)	34.28±2.05 ^B	2.07±0.19	3.00±0.57

Values within same row (a,b) and within same column (A,B) having different superscripts differ significantly (P<0.05).

Sarkar *et al.*, 2006) in cows with endometritis, might be due to the physiologic, pharmacologic and systemic illness or stress of the animals that affect thyroid hormone levels. At post treatment, the significant higher level of T₃ and T₄, which might be due to the elimination of bacterial infection and stress in these animals.

Further, the concentration of cortisol level was also decreased significantly in treated group at post treatment estrus (6.77 ±1.38 vs. 2.12 ±0.63 ng/ml) whereas; it was tended to increase in control animals (3.45 ± 1.32 vs. 2.04 ± 0.63 ng/ml). The result of higher plasma cortisol concentration at pre-treatment estrus is in accordance with the earlier report in endometritic cows (Bindrawan, 2001). The peripheral concentration of cortisol indicate as stress index and the levels of T₄ and T₃ hormone concentration represent the basal metabolic rate of animals (Garg *et al.*, 2001). Shalaby (1997) suggested that the increase plasma cortisol level in infected animal depressed the lymphocytes blastogenesis and decreased iodination with an enhanced random migration of neutrophils that compromised the most potent bacteriocidal mechanisms of PMNs cells in endometritic cows. In another study, impaired neutrophils function based on lowered production of superoxide and hydrogen peroxide was reported at the early postpartum period in buffaloes with impending metristis and endometritis (Patra *et al.*, 2013).

In our study, the animals that recovered and conceived at post treatment estrus had higher T₄ values (44.50±3.23 vs. 34.28±2.05 nM) than those failed to conceive that indicated a positive effect of thyroid hormones on conception rate (Table 4). Moreover, there was higher plasma cortisol level in the cows remain non-pregnant (3.00±0.57 vs. 1.97±0.60 ng/mL) compared to the pregnant cows (Table 4). It can be viewed as increased level of cortisol may inhibit the ovulation process by suppressing the LH surge (Einarsson *et al.*, 2008) or hindering the implantation process of the embryo (Einarsson *et al.*, 2008) as a result there was lowered pregnancy rate. So, it can be concluded that the administration of plasma along with leucocytes treatment in endometritic cows could be an effective alternative therapies as it reduced bacterial load, ameliorate stress by reducing cortisol level and improves metabolic status by enhancing thyroxin level at post treatment.

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