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Research Article

SUBCLINICAL ENDOMETRITIS IN CROSS BRED COWS WITH SPECIAL REFERENCE TO BLOOD-BIOCHEMISTRY AND REPRODUCTIVE PARAMETERS

R.J. CHAUDHARI*, S.U. GULAVANE, M.N. RANGNEKAR, R.R. SHELAR, S.M. GAIKWAD and R.S. JAWALE[1]
Department of Animal Reproduction, Gynaecology & Obstetrics,
Maharashtara Animal and Fishery Sciences University, Nagpur-440001
[1]Department of Zoology, Veer Vajekar A.S.C. College, Phunde, Uran, Navi Mumbai-400702

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ABSTRACT

Present study comprised of 72 crossbred cows (group I= 60 endometritic and group II=12 healthy) at 30±2days postpartum. The polymorphonuclear neutrophils (PMN) cell count was assessed by cytology of endometrial samples through cytobrush technique. The cut-off point for the diagnosis of subclinical endometritis was taken at 5% PMN cells in cytosmear. The PMN cell numbers in the uterine cytosmear were significantly higher (P≤0.5) in the subclinical endometritis affected cows than the healthy cows. The mean glucose, urea, albumin, Aspartate Aminotransferase (AST), calcium and phosphorus values were 43.60±1.10 (mg/dl), 35.29±0.91(mg/dl), 116.50±2.92(IU/L) and 2.78±0.05 (g/dl), 7.48±0.08 (g/dl) and 3.81±0.05 (g/dl), respectively in Group I, cows. Whereas in group II, the values were 56.83±2.33(mg/dl), 22.96±1.09(mg/dl), 87.68±2.28(IU/L) and 3.25±0.03 (g/dl), 8.36±0.13 (g/dl) and 4.16±0.11 (g/dl), respectively. The mean values of serum glucose, urea, albumin and AST showed significant (P<0.05) difference in post-partum subclinical endometritis and healthy cows. The mean days required for first AI, number of insemination per conception, first service conception rate and days open where significantly higher in Group I. Thus, there was significant difference in the blood and reproductive parameters in subclinical endometritic cows compared to healthy cows and cytobrush technique can be used as a tool for diagnosis of subclinical endometritis.

Keywords: Biochemical, Cross-bred cows, Cytobrush, PMN cells, Reproductive parameters, Subclinical endomteritis

Endometritis can be diagnosed by cytobrush technique through increased percentage of polymorphonu clear cells (PMN) in endometrial cytology samples (Kasimanickam *et al.*, 2004, Dubuc *et al*., 2010 and Dutt *et al.*, 2017). Many authors reported that uterine infection increases the service period and calving interval of the dairy cows, which indirectly causes major economic impact on dairy production. Annual losses due to uterine infection is estimated around Rs. 2,902.32 to Rs. 3,101.70 per animal under Indian conditions (Jeyakumari *et al.*, 2003). Blood tests from individual animals are regularly used to assess the herd nutrition status which helps to diagnose metabolic diseases in dairy cattle at earliest. However, the linkage between nutrition and reproduction is complex and manipulation of diet shows inconsistent results (Boland and Lonergan, 2003).

Various diagnostic techniques have been studied to diagnose subclinical endometritis in cows and out of these technique, cytobrush technique is the most suitable to monitor the uterine health of cows, which do not express clinical signs (Kasimanickam *et al*., 2004 and Dutt *et al.*, 2017). The present study was planned to observe the relationship between cytological subclinical endometritis diagnosed by PMN cells and biochemical metabolites as well as reproductive parameters in postpartum crossbred cows.

MATERIALS AND METHODS

The cows at 30±2 days postpartum stage were screened from co-operative dairy societies in and around Nasik and Pune districts of Maharashtra by Cytobrush technique for cytology and out of screened cows, a total of 72 crossbred cows were included in the present study. The subclinical endometritis was diagnosed by endometrial samples which were collected by using disposable cytobrush designed for humans (SMB Corporation of India, Cyto Brush Kit) after modification for use in cows (Chaudhari, 2018). Endometrial cytology samples were collected by rotating the cytobrush in a clockwise direction while in contact with the uterine wall from the mid uterine horn through cervix. Cytobrush was rolled on agrease-freeglass slide to prepare a smear. Air-dried endometrial smears were stained by Field stain A and B (Himedia, India). Slides were washed again, air-dried and evaluated at 400× magnification under a microscope (Labomed LX-200, Binocular Microscope, India). A minimum of 100 cells were counted in each smear, and percentage of PMN count was carried out. The cows having <5% PMN cells in cytobrush cytology were considered as normal cows included in Group I, while those with >5% PMN count in cytosmear were considered as positive for subclinical endometritis and included in Group II as proposed by Kasimanickam *et al.* (2004) and Honparkhe *et al.*(2014).

Blood samples were taken from jugular vein using vacuum tubes with anticoagulant. The clotted blood samples were centrifuged at 1500 rpm for 20 minutes, and serum was harvested and stored at –20 °C until analysis. Estimation of serum glucose (mg/dl), urea (mg/dl),

Corresponding author: rjchaudhari@yahoo.com

aspartate aminotransferase (AST) (I/U) and albumin (g/l) was carried out by commercially available kits with the Robonik biochemical autoanalyzer. Mean values and standard error of the mean (SEM) were calculated for all the biochemical and reproductive parameters. Data obtained were analysed statistically using Student's t-test (two sample assuming unequal variance) in MS-excel-2007 software.

RESULTS AND DISCUSSION

The PMN cell count ranged from 6.00 to 87.00% in the present study (Fig. 1 and 2). Significant (P≤0.5) alteration was observed in reproductive parameters of endometritic cows as compared to the healthy cows (Table 1). The PMN cell count in the uterine cytosmear was significantly higher in the endometritis affected cows than the healthy cows which demonstrated the influx of PMN cells in to the uterus to remove the infection.

There was significant difference in mean glucose (mg/dl), urea (mg/dl), AST (IU/L) albumin (mg/dl), calcium (mg/dl) and phosphorus (mg/dl) and reproductive parameters between postpartum endometritic and healthy control cows. Baranski *et al*. (2012) reported higher percentages of cytologically diagnosed subclinical endometritis as 75.40% at 4th week postpartum in cows.

The present data indicated that glucose level and PMN cell counts were inversely proportional to each other and that glucose level varies within normal range (42.00 to 68.00 mg/dl). The level of blood glucose gives an animal's carbohydrate status and is very important in maintaining the hormonal synchrony which in turn regulates reproductive cycle. High blood glucose levels in cows with low PMN count may stimulate the hypothalamic centre

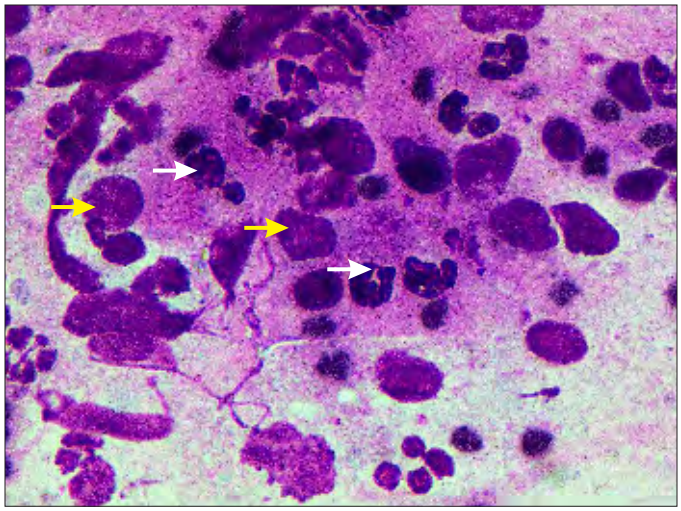

Fig. 1. Cytological sample taken from endometritis affected cow
Neutrophils: White arrows, Endometrial cells : Yellow arrows

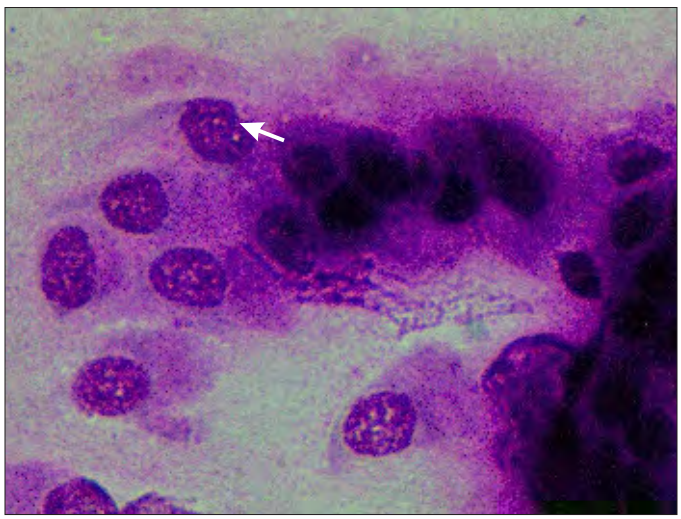

Fig. 2. Cytological sample taken from healthy cow
Uterine epithelium : White arrow

Table 1

PMN cells, biochemical and reproductive parameters (Mean±SE)

Sr. No	Parameters		Group I (n=60)	Group II(n=12)	t- stat
1.	PMN Score by Cytobrush		29.03 ± 02.40^{a}	02.67 ± 0.48^{b}	4.76
2.	Reproductive parameters	Days required to first A.I.	77.78 ± 1.36^{a}	69.50 ± 3.04^{b}	2.48
		Number of inseminations	3.15 ± 0.19^{a}	2.17± 0.34^{b}	2.12
		per conception (NIPC) Days open	133.80 ± 4.32^{a}	100.67± 8.42^{b}	3.19
		First service conception rate % (FSCR)	25.00	33.33	-
3.	Biochemical parameters	Glucose (mg/dl)	43.60±1.10^{a}	56.83±2.33^{b}	5.61
		Urea (mg/dl)	35.29±0.91^{a}	22.96±1.09^{b}	6.30
		AST (IU/L)	116.50±2.92^{a}	87.68±2.28^{b}	4.33
		Albumin (g/dl)	2.78±0.05^{a}	3.25±0.03^{b}	4.26
		Calcium (mg/dl)	7.48±0.08^{a}	8.36±0.13^{b}	4.66
		Phosphorus (mg/dl)	3.81±0.05^{a}	4.16±0.11^{b}	2.94

^{a,b} Means with different superscript within a row differ significantly at P<0.05

which increases the gonadotropin release and subsequent ovarian activity (Howland *et al.*, 1966). Whereas, there may be suppression of gonadotropic hormone release leading to ovarian inactivity in low level of glucose in high PMN cells showing cows (Howland *et al.*, 1966). Low blood glucose could potentially compromise a variety of essential metabolic processes in ovarian cells including the oocyte that depends on glucose for energy (Berlinguer *et al.*, 2012). Therefore, low levels of glucose may have deleterious effect on follicular development and oocytes quality, which increases the days to first heat and reduces the number of inseminations per conception in cows with high PMN cell count as recorded by Dourey *et al.* (2011).

Many authors reported the linkages between inflammation, altered gene expression, in liver and adipose tissues, subclinical endometritis and reproductive performance (Akbar *et al.*, 2014). Serum albumin is a negative acute phase protein which decreases during acute inflammations. Krause *et al.* (2014) found that the early resumption of postpartum ovarian activity was associated with an increased serum albumin concentration and also observed that anovulatory cows had a higher average percentage of PMN cell count in the uterine lumen, which was associated with a reduced hepatic synthesis of albumin. In the current study, the albumin concentrations were lower in cows with high PMN cell count in uterine cytosmears than those having low PMN cells.

When proteins breakdown, the end product is urea, which spreads in many body fluids including blood and milk and to other parts of the body including reproductive tissues. Increased serum urea causes impaired fertility and embryo growth and survival in dairy cattle due to low uterine pH and a suboptimal environment for embryo growth and development (Rhoads *et al.*, 2006). However, the level of urea in the present study was within the normal range. Cheng *et al.* (2015) reported negative relationship between urea concentrations and gene expression associated with innate immunity and cow uterus inflammation. The innate immune system plays an important role in uterine infection. This may contribute to decrease in conception rate and increase in the days open in cows with high PMN cell count in cytosmears as compared to low PMN cells which are also recorded in the present study.

In present study, serum AST values were significantly higher ($P<0.05$) in endometritic cows than the healthy cows indicating uterine damage and the development of fatty infiltration of the liver cells, damage to hepatocytes and release of the intracellular enzymes into the circulation. Similar observations were recorded by Kaneko *et al.*(1997).

It is known that uterine disease causes impaired reproductive performance. In present study, days required to first artificial insemination, Number of insemination per conception (NIPC) and days open were significantly more in cows with high PMN cells than low PMN, whereas first service conception rate (FSCR) percentage was significantly lower ($P<0.05$) in cow with high PMN cell count in uterine cytosmears. This may be attributed to high PMN cell count that may affect/alter the functions of sperm and also the oocyte quality. PMN cells can produce reactive oxygen species (Aloe *et al.*, 2012) and may damage sperms. Uterine bacterial infections may impair hypothalamic, pituitary functions and directly perturb steroidogenesis by ovarian granulose cells (Sheldon *et al.*, 2009). Cows with higher uterine PMN cell count are more likely to have a delay in resumption of ovarian activity (Krause *et al.*, 2014).

In the present study, it was observed that serum calcium concentrations were significantly ($P<0.05$) lower in the high PMN cell group of cows in comparison to the low PMN cell cows. The observations are in agreement with the previous findings of Kumar and Sharma (1993). A low profile of plasma calcium in the repeat breeder cows could be due to some metabolic disturbance causing poor absorption of calcium from gut (Kaneko *et al.*, 1997). Since calcium is required for neuromuscular excitability, muscle contractions and transmission of nerve impulse at cellular level, its deficiency may result in reduced tone and contractions of uterine muscle, which ultimately may prevent forward movement of sperm and ovum in the opposite direction resulting in inhibition of fertilization/ zygote formation. Mohanty *et al.* (1994) opined that probably the less availability of glucose and calcium to the uterine tissues results into the uterine atony and hence there may be no effective clearance of infection or exudate. Phosphorus is essential for transfer of biological energy especially through ATP (Du Plessis *et al.*, 1999) and deficiency of phosphorus in particular influences at the level of pituitary and ovary and may interfere with fertilization or may cause early embryonic death thereby producing aberrations in the normal reproductive rhythm.

Polymorphonuclear cell count may be used as uterine health indicator in post partum period which affects the biochemical as well as the reproductive parameters in crossbred cows. The cows should be screened for subclinical

endometritis and biochemical aberrations before initiation of hormonal therapy in post partum period so as to optimize reproductive performance in crossbred cattle.

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