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Effect of Endometritis Diagnosed by Cytology on Reproductive Parameter in Postpartum Crossbred Cows

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A B S T R A C T

In dairy cattle, endometritis is one of the conditions of highest impact on dairy cows. It reduces fertility and increases the production costs. The aim of this study was to observe the effect of endometritis in crossbred cows at 32 ± 2 days postpartum on the reproductive performance of crossbred dairy cows. In this study 24 cows were selected based on polymorphnuclear cells (PMN) scoring by cytobrush and divided into 2 groups. In group I- the 12 cows positive for endometritis were included, whereas in group- II, the cows which were found negative on cytosmear or showing lower than 5 % PMN cells in uterine cytology were included and kept as control group. Statistically significant difference was found ($p<0.05$) between all reproductive parameters of endometritis affected and healthy cows. It was found that the mean days required for exhibition of first postpartum estrus, number services per conception, first service conception rate and days open for first group were 76.67 ± 5.16, 4.16 ± 0.42, 8.33 % and 162.67 ± 8.07 days, respectively. The corresponding values for group II, were 57.83 ± 3.38 days, 2.17 ± 0.34, 33.33 % and 100.67 ± 8.42 days, respectively. In conclusion, we determined the effect of endometritis average measured in days, affecting the reproductive parameter and increases the cost of raising the cows. Therefore efforts should be made in prevention, early identification and control programs to prevent the losses to dairy industry.

Introduction

It is well documented that diseases like sub-clinical endometritis, clinical endometritis, metritis and pyometra are associated with subfertility and infertility in dairy cows. During 4 weeks after parturition, the cows immune system is extremely challenged, most of the cows in the early postpartum period develop a non-pathological endometritis, and the lochia and placenta are usually eliminated by the second week postpartum (Thatcher *et al.*, 2006). Postpartum endometritis is associated with high economic losses due to the prolonged days open, multiple services and longer inter-calving interval s, which can result in high involuntary culling (LeBlanc *et al.*, 2002; Azawi, 2008; Yavari *et al.*, 2009).

Recent reports showed that the postpartum period is often lengthened and pregnancy rate is reduced due to endometritis. (Kasimanickam *et al.*, 2005 and Gilbert *et al.*, 2005). Therefore it is necessary to diagnose endometritis with appropriate tool to treat it simultaneously to control economic losses.

Endometrial cytology is done to diagnose endometritis in the present experiment. The sample was collected by uterine lavage method or cytobrush technique. Subsequently, the effect of endometritis on different reproductive parameters was also studied at 32 ± 2 days postpartum in crossbred cows.

Materials and Methods

Present study was undertaken after getting approval from institute ethical committee resolution No.BVC/IEC-VCI/2014/127/2013. The postpartum crossbred cows were screened from Villages of co-operative dairy societies and interested dairy owners in and around Nasik districts of Maharashtra state. The Gir and its crosses with Holstein and Jersey cows were included in the present study. They were fed with concentrates, greens, dry fodder and the mineral mixture as per the milk yield and lactation. The routine deworming and Vaccination schedule was also followed. The heat detection was carried out daily by the farmer in the morning, afternoon and night hours.

The endometrial sample was collected by cytobrush technique as described by Kasimanickam *et al.,* (2005) with little modification and fabrication in the cytobrush assembly. A new cytobrush assembly for bovine was fabricated (fig 1). Immediately after removing the cytobrush from uterine horn, the cytobrush was rolled on clean glass slide.

The sample was also collected by lavage technique. The slide was fixed with methyl alcohol and slides were air dried. After fixing the smear on slide, all smears obtained by cytobrush and lavage techniques were stained with Leishman's stain. The slides were washed in running water, air dried and observed under microscope with 100 X magnification under oil immersion.

A total of 100 cells were counted in each specimen and classified into endometrial cells, polymorpho nuclear cells (PMN), eosinophils, lymphocytes and macrophage like cells. Out of 100 cells the number of PMN cells were calculated and recorded. Specimens with a PMNs percentage exceeding 5% at first examination in both the techniques were concluded as positive for subclinical endometritis. While those slides below the 5 % of PMNs cells were taken as negative for subclinical endometritis (fig. 2 and 3).

Out of screened cows, 24 cows positive for endometritis based on PMN scoring by cytobrush technique. They were divided into 2 groups. In group I- the 12 cows positive for endometritis were included, whereas in group - II, the cows which were found negative on cytosmear or showing lower than 5 % PMN cells in uterine cytology were included and kept as control group.

Pregnancy was confirmed per-rectally on day 45 post-insemination. The different reproductive parameters, viz., the days to exhibit first postpartum estrus, first service conception rate, number of inseminations per pregnancy and days open were calculated calculated as per Parkinson and Barrett (2009).

Mean and standard error of collected data was calculated and analyzed for comparison as per methods suggested by Snedecor and Cochran (1994).

Results and Discussion

Effect of endometritis on reproductive parameters

The data of the reproductive parameters in endometritis affected and healthy cows were presented in table.1. Mean days required for

exhibition of first postpartum estrus, number services per conception, first service conception rate and days open for first group were 76.67 ± 5.16 (range of 41 to 103 days), 4.16 ± 0.42, 8.33 (%) and 162.67 ± 8.07 (range 103 to 205 days) respectively. The corresponding values for group II, were 57.83 ± 3.38 days (range 41 to 80 days), 2.17 ± 0.34, 33.33 % and 100.67 ± 8.42 days (range 67 to 157 days) respectively.

The endometritis significantly ($p<0.05$) affect all the reproductive parameters as compared to the healthy cows.

In the present study endometritis affected cows required 19 days more to come into heat as compared to healthy cows. The delayed estrus exhibition may be due to delayed uterine involution, delayed rebound of ovarian activity or both. Similar finding was reported by Dourey *et al.,* (2011), who found that cows with endometritis have a longer mean interval to ovulation (48.60 ± 3.60 days) than those healthy cows (27.80 ± 1.80 days). This may be due to the fact that during early postpartum period uterine infection suppresses the growth and function of the dominant follicles (Williams *et al.,* 2007) especially the granulosa cells, through inhibition of estradiol production from aromatization of androgens, leading to alterations in the lifespan of the follicle at recruitment and selection, and consequently ovulation (Herath *et al.,* 2007). In addition, uterine infections or the associated inflammation, suppress pituitary LH secretion and perturbs postpartum ovarian follicular growth and function, which disrupts ovulation in cattle (Peter *et al.,* 1989 and Sheldon *et al.,* 2002).

The significant difference in the number of services per conception was observed between the cows with and without endometritis ($P <0.05$). The endometritis affected cows required 2 more services than the healthy cows to remain pregnant. Gilbert *et al.,* (2005) and Barlund *et al.,* (2008) reported similar findings regarding the increase in the number of insemination per conception in endometritis affected cows. Whereas Prakash *et al.,* (2015) also found that, non-infected cows had higher first service conception rates than infected cows. Similarly, Gilbert *et al.,* (2005) reported severe reduction (11.00 % vs. 36.00 %; $P < 0.01$) in first service conception rates in cows affected with endometritis, and these cows required more services (3.00 Is. 2.00; $P < 0.01$) to remain pregnant.

Endometritis significantly impacts the reproductive efficiency have been reported increases ranging from 7-31 days in the calving to conception interval (Borsberry and Dobson, 1989 and Fourichon *et al.,* 2000).

Whereas Fourichon *et al.,* (2000) carried out the meta-analysis and calculated the increase of 15.30 and 27.70 mean days open in cows with endometritis that became pregnant, and all cows with endometritis, respectively, relative to unaffected cows.

Table.1 Effect of endometrtitis on reproductive parameter

Sr no	Reproductive parameter	Group I	Group II	T stat	T critical
1	Days required for first estrus	76.67 ± 5.16^{a}	57.83 ± 3.38^{b}	3.06	2.07
2	Number of inseminations per conception.	4.16 ± 0.42^{a}	2.17 ± 0.34^{b}	3.66	
3	Days open	162.67 ± 8.07^{a}	100.67 ± 8.42^{b}	5.32	
4	First service conception rate (%)	8.33%	33.33%	-	-

Superscript showing different letter in rows differ significantly ($p<0.05$)

Table.2 Cost of days open for per cow in two Groups

Particulars	Group I	Group II
Average Number of Open Days	162.67	100.67
Total maintenance cost for open days (Rs.)	24401	15101
Number of services per conception	4.17	2.17
Cost of AI (Rs.)	625.5	325.5
Total cost rearing (Rs.)	25026	15426
Total cost (Rs.)	**25026**	**15426**

Fig.1 Showing cytobrush assembly contains outer catheter (A), inner stylette (B), cytobrush along with adopter (C) and AI plastic sheath

Fig.2 Cytosmear showing endometritis affected cow PMN cell and endometrial cells. (100X)

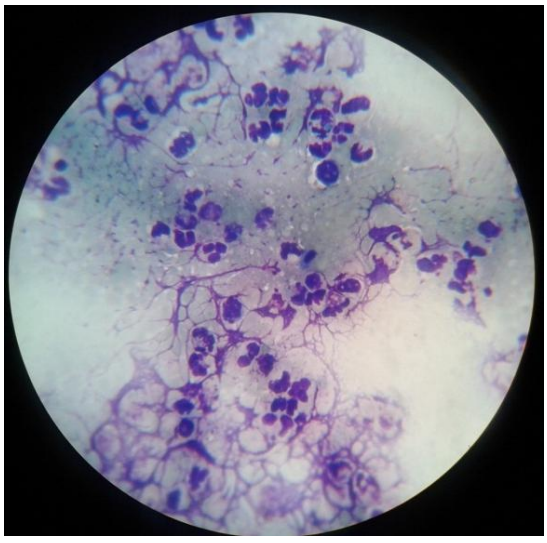
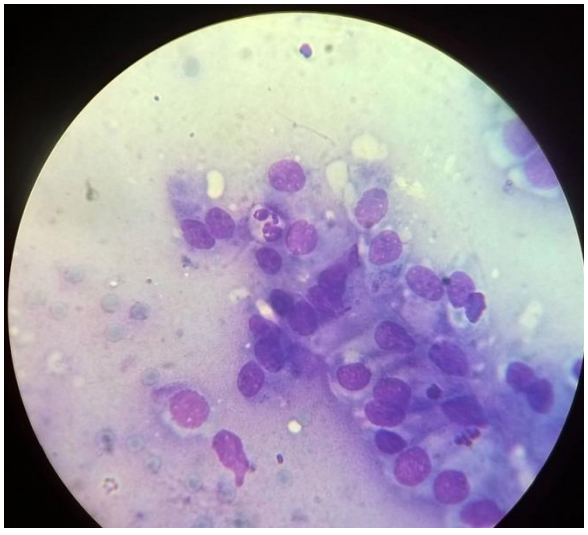


Fig.3 Cytosmear showing endometrium of healthy cow PMN cell and endometrial cells. (100X)



The endometritis affected cows in present study required 26 days more to become pregnant than the healthy cows. Similar observation was reported by Kasimanickam *et al.,* (2004); Gilbert *et al.,* (2005) and Dubuc *et al.,* (2010).

The first service conception rate was significantly higher (33.33 %) in healthy cows than endometritis cows (8.33%).

The reduction in the reproductive efficiency of endometritis affected cows may be associated with uterine tissue damage, delayed uterine involution (Shrestha *et al.*, 2004), disruption of endometrial function, reduction of steroid concentrations of ovarian follicles (Green *et al.,* 2011), and perturbation of ovarian cycles (Opsomer *et al.,* 2000; and Herath *et al.,* 2007).

Economic losses due to endometritis in postpartum cows

The cost of rearing one cow per day on the field, where the present study was undertaken, was Rs. 150/- per day at farmers' door.

The cost of one insemination on the farm was Rs. 150/- which was derived by taking into account the cost of semen straw, cost of LN_2 and inseminator charges.

The results of the calculations of the cost effectiveness of all three groups are presented in Table 3.

From the perusal of the Table 2, it was observed that the overall cost calculated for number of days open was lowest for Group II (Rs. 15426.00) than Group I (Rs. 25026.00).

Overton and Fetrow (2008) reported that the cost of each case of metritis is approximately US$329–386, due to antibiotic treatment and the detrimental effects of metritis on reproductive performance, milk production, and survivability.

The present study showed that the endometritis causes major economic losses in the dairy industry. Similar findings were also reported by Dubuc *et al.,* (2010).

Sheldon and Dobson (2004) reported a huge loss (16 million Euros per year) in dairy due to uterine infection.

It may be concluded that endometritis is responsible major economic loses in cows due to delayed uterine involution, delayed resumption of ovarian activity and overall conception rate.

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