Research article

DOI: 10.48165/ijar.2022.43.2.5

The Indian Journal of Animal Reproduction

The official journal of the Indian Society
for Study of Animal Reproduction

ACS Publisher
www.acspublisher.com

Year 2022, Volume-43, Issue-2 (Dec)

ISSN 0970-2997 ***(Print)***

ISSN 2583-7583 ***(Online)***

Association Between Bacterial Count and Polymorphonuclear Cells in Uterine Lavage of Postpartum Subclinical Endometritic Cows

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ABSTRACT

This study was conducted to find out the total bacterial count and percentage of polymorphoneutrophils (PMN) cells in uterine lavage samples obtained at a weekly interval from postpartum dairy cows and also to find out the correlation between total bacterial count and PMN cells recovered from the uterus of postpartum dairy cows. Twenty-one postpartum dairy cows were divided into two groups viz. group A (without postpartum complication; n=16) and group B (retained placenta/postpartum metritis; n=5) calving. Cows belonging to the group B were administered antibiotics for 5 days after parturition. Samples for endometrial cytology and total bacterial count were collected aseptically by uterine lavage method at weekly intervals from day 21 to 42 postpartum. Data were statistically analysed by using one-way ANOVA, correlation using Statistical Analysis Software, SAS® 9.2 TS Level version 2M2 for Windows (USA). Based on endometrial cytology, the incidence of sub-clinical endometritis (SCE) recorded was 18.75% in group A and 100% in cows with postpartum complications (Group B). Total bacterial count in the group A animals differed significantly ($P<0.05$) with the group-B animals. However, the difference between the total bacterial counts in group-A animals and diagnosed negative or positive for SCE did not differ significantly. A highly significant ($P<0.05$ and $P<0.01$) correlation was found between bacterial load and PMN cells obtained by uterine lavage technique.

Key words: Postpartum dairy cows, sub-clinical endometritis, total bacterial count, uterine lavage.

How to cite: Rana, A., & Singh, M. (2022). Association Between Bacterial Count and Polymorphonuclear Cells in Uterine Lavage of Postpartum Subclinical Endometritic Cows.
The Indian Journal of Animal Reproduction, 43(2), 20–24. 10.48165/ijar.2022.43.2.5

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Received 01-06-2023; Accepted 10-06-2023

INTRODUCTION

Bovine genital tract infections (specific or non-specific) are considered as main cause of conception failure (Singh *et al.* 2017). The postpartum uterus is usually contaminated with a variety of bacteria as compared to other stages of the reproductive phases (Sheldon *et al.* 2002). Postpartum uterine infections lead to puerperal metritis, clinical endometritis and sub-clinical endometritis (Sheldon *et al.* 2006). Sub-clinical endometritis is marked by inflammation of the endometrium that results in a significant reduction in reproductive performance without clinical signs (Parikh *et al.* 2022). For the recovery from clinical or subclinical endometritis following parturition, immune system plays a very important role in uterine defence mechanism against invading microorganisms. Polymorphonuclear cells in genital discharge is directly related to pregnancy outcomes in cows (Sood and Bhavna 2014). Alterations in the normal inflammatory response could be the origin of sub-clinical endometritis (Singh *et al.* 2008). The present study was conducted to find the proportion of polymorphonuclear (PMN) cells and bacterial load in uterine lavage samples obtained from postpartum dairy cows.

MATERIALS AND METHODS

A total of 21 normal calved dairy cows from Livestock Instructional Farm Complex, CSKHPKV Palampur (32.6^0N, 76.3^0E, altitude 1290.8m), were selected for the study. Cows were randomly divided into two groups i.e group-A (without postpartum complications) and group-B (with postpartum complications like retained placenta and or metritis). Group-B cows were treated with enrofloxacin @ 5 mg/kg body weight intramuscular for 5 days. Uterine lavage samples were collected aseptically via uterine lavage technique to evaluate total bacterial count and endometrial cytology to diagnose sub-clinical endometritis (Dini *et al.* 2015) at weekly intervals from day 21 to 42 postpartum. Briefly in this technique, Foley's catheter (18" French gauze) was introduced and fixed into body of the uterus after cleaning the vulvar area. Then, 30-40 ml of sterile normal saline (Sodium Chloride 0.9%, Abbott Laboratories Ltd.) was infused into uterus through Foley's catheter. After trans-rectal massage of uterus for 5-10 seconds, fluid was aspirated and collected into a sterile tube. The uterine lavage samples were used for bacterial count estimation and endometrial cytology.

Bacterial count estimation: The total bacterial count was done using the spread plate technique. Briefly, dilution of uterine lavage sample was done in four test tubes in the ratio of 1:10, 1:100 :1000 and 1:10000, respectively. An aliquot of each dilution (0.1 ml) was inoculated on the sterile media in petri dish and was spread evenly using L spreader. Then, the plate was kept at room temperature for 5-10 min in order to get the aliquot absorbed on the plate. Finally, the plates were incubated at 37^0C for 24-48 hrs. The plates revealing colonies between 30 and 300 were selected and the colonies were counted using the colony counter and the results were interpreted as standard plate count/ml as per the following formula.

Calculations: The number of bacteria per ml = No. of colonies (CFU)/dilution X amount plated.

Endometrial cytology: The uterine lavage samples after bacterial count estimation were centrifuged at 1000 rpm for 8 minutes. The supernatant was discarded and a smear was prepared with the pellet and allowed to air dry. Air dried slides were fixed in methanol for 15 minutes and then stained with Giemsa stain for 45 minutes. All slides were evaluated using a light microscope at 40X magnification. Cells were counted in a total of 10 fields and the percentage of epithelial cells and PMNs were assessed. The threshold values of PMN cells as reported by Kasimanickam *et al.* (2004) were used to diagnose SCE. The samples having more than 18 per cent PMN's on day 20-33 postpartum or more than 10 per cent PMN's on day 34-47 days postpartum were considered as subclinical endometritis. Animals having PMN cells percentage less than 18 on day 21 and 28 and more than 10 on day 35 and 42 were diagnosed with SCE.

Statistical Analysis: The statistical analysis was carried out using SAS (Statistical Analysis Software), SAS® 9.2 TS Level version 2M2 for Windows (USA). The mean value of PMN cell count and total viable count were compared by using one-way ANOVA. The correlation between bacterial count and PMN cell count was calculated by using correlation analysis.

RESULTS AND DISCUSSION

Incidence of subclinical endometritis: In the present study, a total of 21 cows were examined at the weekly interval, out of which 16 were in group A and 5 in group B (retained placenta/postpartum metritis). By using the cut-off value of PMN's given by Kasimanickam *et al.* (2004), the incidence of SCE was recorded as 18.75 and 100% in the group A and group B, respectively. The mean PMN % obtained by uterine lavage technique at weekly intervals from day 21 to 42 postpartum in the cows diagnosed negative and positive for SCE has been presented in Table 1.

Table 1: Polymorphonuclear cells (PMN) percentage (mean±SE) in uterine lavage from postpartum dairy cows in relation to sub-clinical endometritis.

Diagnosis of sub-clinical endometritis by uterine lavage technique	Status of sub-clinical endometritis		
	Without postpartum complications (Group A)		With postpartum complications (Group B)
	SCE Negative (n=13)	SCE Positive (n=3)	SCE Positive (n=5)
Days	PMN cells (%)		
21	11.61±0.85^{x} (7.79-17.03)	15.73±0.73^{xy} (14.28-16.66)	18.32±1.95^{y} (14.14-21.42)
28	8.47±0.49^{x} (6.25-11.51)	12.43±0.87^{xy} (11.53-14.18)	14.85±1.86^{y} (11.29-20.4)
35	7.24±0.74^{x} (1.58-10.9)	11.55±0.50^{xy} (10.81-12.51)	14.17±2.14^{y} (9.95-19.76)
42	6.29±0.56^{x} (3.25-9.64)	11.52±0.61^{yz} (10.29-12.23)	10.97±0.36^{yz} (10.20-11.65)

Values with different superscripts within the same row differ significantly (^{x,y}, P<0.01)

Table 2: Total bacterial count in uterine lavage samples from postpartum dairy cows in relation to sub-clinical endometritis.

Days	CFU/ ml		
	Without postpartum complications (Group A)		With postpartum complications (Group B)
	SCE Negative (n=13)	SCE Positive (n=3)	SCE Positive (n=5)
21	31324.62±12552.24 (3220-158000)	79200±43508.31 (12600-161000)	92700±28023 (20800-142000)
28	10110.77±1482.37^{a} (2740-18900)	11033.33±1068.22^{a} (8900-12200)	85740±35979.52^{b} (11300-212000)
35	5630.77±1622.70^{a} (1020-22400)	8033.33±1431.00^{ab} (5800-10700)	40950±20314.92^{b} (11800-101000)
42	1317.692±375.39^{a} (340-4700)	5466.66±2069.89^{a} (3200-9600)	21350±3770.389^{b} (10600-28200)

Values with different superscripts within the same row differ significantly (P<0.05)

In this study, the difference in the PMN percentage was significant (P<0.01) for the cows diagnosed positive or negative for SCE. As the days postpartum increased, the PMN percentage decreased, which was similar to the findings of Kasimanickam *et al.* (2004). In group B cows, PMN percentage still remained high even after treatment and they all were diagnosed as SCE positive. Gilbert and Santos (2016) compared the proportion of cells in cytology samples after calving and it was reported that the PMN proportion was unchanged at 1 and 3 weeks postpartum but decreased by 5 and 7 weeks postpartum. The high uterine PMN population in the later postpartum period is well known to be associated with poor reproductive outcomes (Vieira-Neto *et al.* 2014).

Total bacterial count in uterine lavage sample: The results of the present study clearly showed the difference in total bacteria count between the animals of group A and B and diagnosed positive for SCE. Total bacterial count in the group-A animals differed significantly (P<0.05) with the Group B animals. However, the difference between the bacterial load in group A cows and diagnosed negative or positive for SCE did not differ significantly (Table 2).

Bajaj *et al.* (2018) reported 186000 CFU/ml total bacterial counts in SCE-positive animals between days 28 to 42 postpartum, which were higher than the present study findings. Also, 425000 and 0.00 CFU/ml bacterial load was reported in endometritis and non-endometritis cows, respectively (Bajaj *et al.* 2018), whereas in the present study, less bacterial load was present in the group A cows and diagnosed negative for SCE as compared to cows diagnosed positive for SCE. In our study, it was recorded that bacterial load decreased as the days postpartum increased which may be due to the clearance of uterine bacterial contamination via a natural defence mechanism (Prasad 2006). Almost 80-100% of cows have been reported to have bacterial contamination of the uterus in the first two weeks postpartum (Foldi *et al.* 2006) and those cows which are unable to clear the infection, subsequently develop uterine disease (Sheldon and Dobson 2004).

Correlation between total bacterial count and PMN cells obtained from the uterus of postpartum dairy cows: The correlation procedure performed via SAS to find out the relationship between total bacterial count and PMN cells.

Table 3: Inter-relationship between total bacterial count and PMN cells recovered from cows evaluated for sub-clinical endometritis

Sr.No.	Relationship between	Group	Number of samples	R^2
1.	Total Bacterial count and PMN cells obtained by uterine lavage samples	A	80	0.1873**
		B	24	0.3798**
		Overall	**104**	**0.3173****

* (P<0.05) ** (P<0.01)

A highly significant (P<0.01) correlation was found between bacterial count and PMN cells (table 3), which clearly indicated that the inflammatory process in the uterus caused intensive migration of large numbers of PMNs into the uterine lumen. A variety of bacteria contaminate the uterus of cows shortly after calving, but the relationship between individual bacterial species or groups of bacteria and the development of uterine infection is poorly understood (Knudsen *et al.* 2016). Prunner *et al.* (2014) studied the dynamics of bacteriologic and cytologic changes during the first month after parturition in the uterus of postpartum dairy cows and concluded that the proportion of PMN strongly depended on bacterial counts.

CONCLUSIONS

On the basis of present study, it was concluded that the incidence of sub-clinical endometritis by day 42 was recorded as 18.75% in cows with uncomplicated postpartum and 100% in cows with postpartum complications like retained placenta and metritis.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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