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Diagnosis of Subclinical Endometritis During Postpartum Period on Subsequent Pregnancy in Small, Medium and Large Scale Dairy Farms in and Around Gondar, North West Ethiopia

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Abstract: During the study period 147 apparently healthy 3rd trimester pregnant cows were selected in smallholder, medium and large scale dairy farms in and around Gondar, North Western Ethiopia from January 2012 to September 2013. After calving, endometrial samples were collected from the uterus of apparently normal cows by using uterine lavage technique on postpartum dairy cows from 40-60 days. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with giemsa. The percentage of polymorphonuclear cells (neutrophils) was calculated. It was found that increase in the number ofneutrophils correlated with decrease in pregnancy. In conclusion, subclinical endometritis diagnosed by endometrial cytology was associated with reduced rate of pregnancy.

Key words: Subclinical Endometritis · Endometrial Cytology · Pregnancy · Neutrophils

INTRODUCTION

Subclinical endometritis is a chronic, unapparent inflammatory process of endometrium with a relatively high proportion of polymorphonuclear cells (Neutrophils) cells in the uterus, which suppresses the fertility of affected cows. Proportion of polymorphonuclear cells (Neutrophils) cells considered to be "relatively high" depends on sampling technique as well as on the time from parturition [1]. The key for excellent fertility in dairy herds is a healthy uterine environment, optimal estrus detection efficiency and ideal timing for breeding [2].Subclinical endometritis in dairy cows and has a profound negative impact on pregnancy [3].

Recent studies have focused on a more sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. New techniques have been described for the diagnosis of subclinical endometritis (SE). The inflammation of the endometrium is characterized by the proportion of polymorph nuclear cells (PMN) in a cytological sample taken from clinically healthy cows. Cytological samples can be obtained by flushing the uterine lumen or by using the cytobrush technique [1]. This latter technique uses a small brush that is inserted into the uterus to collect endometrial cells and to determine the proportions of PMN in the sample. Studies on SE found a prevalence of SE in the range between 12 % and 94 %. Different study designs and inconsistent definitions for SE hinder a valid comparison of the results of these studies. The time of examination in these studies varied from 21 to 60 day of postpartum [4].

Subclinical endometritis causes considerable infertility problems in the presence of uterine bacterial contaminations of disrupt the delicate hormonal milieu of the hypothalmia-pitutary ovarian axis and disrupt follicular growth and development of uterine infections have been reported to be associated with an increased incidence of cystic ovarian disease [5].

Postpartum endometritis in cattle is a multifactorial disease with high economic impact. Inflammation of the bovine uterus has been demonstrated to decrease fertility. Both clinical and subclinical endometritis were associated with increased days to first service as well as decreased conception and pregnancy rates resulting in an increased risk of culling [6]. The objective of this study was to diagnose subclinical endometritis and its effects on pregnancy in dairy cows.

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MATERIALS AND METHODS

Study Area: The study was conducted in urban and peri urban areas of Gondar town dairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital city Addis Ababa. It is located at latitude, longitude, altitude of 12.3-13.8°N, 35.3-35.7°E and 2200 m.s.l, respectively. The annual mean minimum and maximum temperature of the area vary between 12-17°C and 22-30 °C, respectively. The area is located under woynadega, agro-climatic zone and receives a bimodal rainfall the average annual precipitation rate being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June through September [7].

Study Farms: The dairy farms considered for this study were categorized into defined strata based on cow herd size; these were small scale dairy farm (SSDF), medium scale dairy farm (MSDF) and large scale dairy farm (LSDF) having 1or 2, 3 to 10 and 11 to above as described by ILRI [8], respectively. During the study period 147 apparently healthy 3rd trimester pregnant cows were selected.

Study Design: Cows were selected in smallholder, medium and large scale dairy farms and the study was conducted from January 2012 to September 2013. The owners of the farms were informed about the relevant characteristics of the study and agreed with the design. The dairy farms were visited every 15 days. Enrolment of cows, clinical examination and evaluation were performed by the same investigator. The cows were examined by using lavage between 40 and 60 days postpartum for the presence of sub clinical endometritis. Endometrial cells were collected by uterine lavage technique.

Clinical Examination: In each cow a clinical examination ofthe reproductive tract was performed by vaginalexamination and transrectal palpation of the uterus and the ovaries. Cows with vaginal discharge were diagnosed as affected by clinical endometritis and excluded from the study. In addition, cows which had received systemic or intrauterine antibiotic therapy within 6 days prior to enrollment were not selected for the study. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents post insemination.

Cytological Samples: The cows were examined between 40 and 60 days after calving for the presence of subclinical endometritis by using the lavage technique. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with Giemsa.

Subclinical endometritis was determined using endometrial cytology [4, 9]. To minimize contamination of the sample, the vulva and perineum were cleaned with water and soap properly. The uterus was lavaged by infusing 50 ml of 0.9% sterile sodium chloride solution with 50 ml syringe attached to a 52 cm sterile plastic infusion rod. The uterus was then manipulated and massaged through rectum for about 10 seconds and some of the infused fluid was aspirated into the syringe via the same sterile plastic infusion rod by negative pressure aspiration and retracted to recover the fluid. No special effort was made to retrieve the fluid if it did not flow freely.

As much fluid as possible was recovered by negative pressure aspiration into the syringe and transferred to the 10 ml sterile test tube without any preservative. The uterine samples were put into the icebox and brought to the Faculty of Veterinary Medicine, Microbiology laboratory within 2 hours of collection and centrifuged at 800 rpm for 5 min. A drop of sediment was streaked on to a clean microscope slide and air-dried. Then the slide was fixed with methanol and stained with Geimsa for 45 min and examined under a microscope at 400× magnification. Initially the whole slide was assessed and a representative area was selected to determine the PMN % among all other cells was estimated. The percentage of neutrphils PMN % was determined by counting 80–100 cells on a representative field of vision. The threshold value for the proportion of PMN indicated samples with $\geq$3% neutrophils were categorized as subclinical endometritis and cows were characterized as suffering from subclinical endometritis. The counted cells contained epithelial cells, neutrophils, large mononuclear cells (Presumed to be macrophages) and small mononuclear cells (Presumed to be lymphocytes). The samples that did not contain epithelial cells were considered not taken from uterus and rejected for the study.

Data Management and Statistical Methods: To measure the impact of subclinical endometritis on subsequent pregnancy descriptive statistics for the amount of neutrophils were used. The data was analyzed using

statistical package for social science (SPSS) (Version 18). The Generalized Linear Model was utilized to analysis the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analysis the relationship between the amount of neutrophils and the conception rate in the first insemination after sampling. The Pearson correlation test and the Chi-square correlation test were used to analysis the impact of quantitative factors on each other and the impact of qualitative factors on each other respectively.

The student T test was used to analysis the impact of quantitative factors on qualitative factors and vice versa. A probability of $P < 0.05$ was set as the significance level. The Receiver Operating Characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

RESULTS

The incidence of subclinical endometritis was different in small, medium and large scale farms 25 (37.88%), 29(43.94%) and 13 (18.18%), respectively.

Only 72 (48.98%) of the selected cows became pregnant in AI after sampling and 75(51.02%) did not. The amount of neutrophils was lower in the cows that became pregnant in the first AI after sampling. With an increase in the number of neutrophils the likelihood of pregnancy decreased. The mean number of services per conception as 2.04 for sub clinical endometritis positive cows.

The horizontal line in the box was median (The middle of the entire list of numbers). Down edge of the box was first quartile (The middle number in the first half of the data set) and up edge of the box was third quartile (The middle number in the second half of the data set). Median, first quartile and third quartile of pregnant cows are lower than those of non-pregnant cows.

The descriptive statistics for pregnancy were shown in Table 2, separated by different amount of neutrophils. The Receiver Operating Characteristics (ROC) analysis revealed that the best cut off point(Based on likelihood of pregnancy) was as follows: cowswith < 3% neutrophil considered normal andcows with > or ≥3% neutrophil considered to haveSE. Based on this cutoff point, the overall incidence of SE indairy cows was 46% (67/147) in this study. Sensitivity and specificity are 81.3 and 97.2%, respectively, based on the selected cut off point for like likelihood of pregnancy.SEhad a negative impact on conception rate in the first AIafter samplings, which were 10% for cows with SE and55% for cows without SE.

Table 1: Descriptive statistics for the number of neutrophil

Number of neutrophil	Frequency	[Percentages]
0	81	55.11
1	2	1.36
2	1	0.68
3	8	5.44
4	9	6.12
5	3	2.04
6	5	3.40
7	2	1.36
8	6	4.08
9	4	2.72
10	3	2.04
11	2	1.36
12	5	3.40
13	6	4.08
14	7	4.77
15	3	2.04
Total	147	100

Table 1: The descriptive statistics for pregnancy, separated by neutrophil percentages

	Pregnancy		
Amount of neutrophil	-	+	Total
0	13	68	81
1	1	1	2
2	-	1	1
3	7	1	8
4	8	1	9
5	3	0	3
6	5	0	5
7	2	0	2
8	6	0	6
9	4	0	4
10	3	0	3
11	2	0	2
12	5	0	5
13	6	0	6
14	7	0	7
15	3	0	3
Total	75	72	147

In the present study endometrial cytology revealed that the PMN count of 3% and above was suggestive of subclinical endometritis. The samples which ranged from 3% to 15% of PMN cells could be correlated with subclinical cases of endometritis. Hence, the endometrial samples which contain PMN cells of 3% and above were considered as positive for subclinical endometritis.

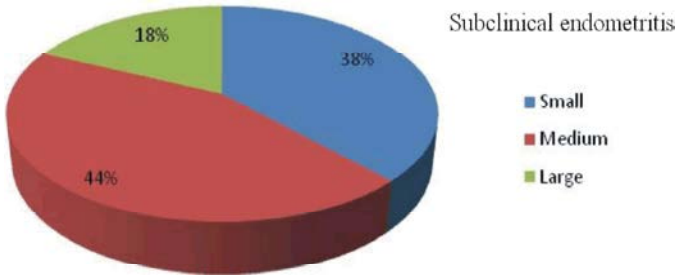

Fig. 1: Percentage of subclinical endometritis in different scale of farms

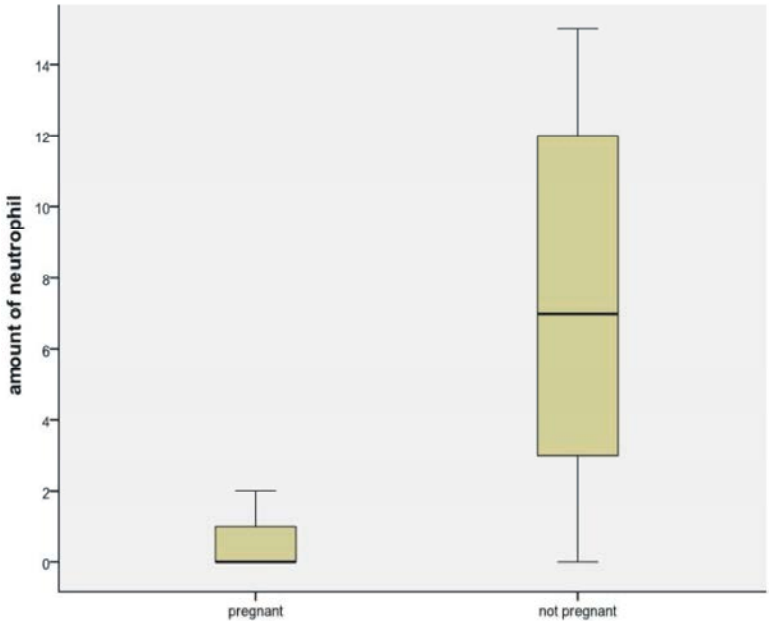

Fig. 2: The correlation between pregnancy and the amount of neutrophils

DISCUSSION

In the present study endometrial cytology shown that the PMN count of 3% and above was indicative of subclinical endometritis. Similarly, in subclinical endometritis, the PMN cells were found to range from 4% to 18% [10]. Gabler *et al.* [11] reported that with ≥5 % PMN cells in the endometrial samples were considered as subclinical endometritis. Those earlier studies clearly indicated that 4% and 5% of PMN cells in endometrial cytological samples might be considered as "cytological marker" or "cytological indicator "for diagnosing subclinical endometritis, while in the present study PMN count of 3% and above may be considered as subclinical endometritis indicated.

The current study revealed an overall incidence of 46% (67/147) of subclinical endometritis infection in dairy cows with a PMN level of ≥3 neutrophiles in the uterine sample which is set as a threshold value indicative for SCE which was in accordance with the earlier [12], in DebreZeit when prevalence of 47.5% and 30.5% respectively. However, in the cited study samples were taken 4 and 8 weeks postpartum period separately whereas samples in this study were taken 40-60 days postpartum cows together. Gilbert [13] in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Couto *et al.* [14] in Québec which was theprevalence of subclinical endometritis of 56%. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk, 45% for cows between 34 and 47 days in milk [9]. The reason for the difference in the prevalence of SCE observed in the current study and earlier studies could be due to the difference in the management system of dairy cows.

The prevalence of subclinical endometritis is very variable and depends on the diagnosis technique; the DIM of the genital examination and the statistical method used to determine the cut-off point of the neutrophils ratio obtained from endometrial cytology [15].

The incidence of SCE in this study was 46% also higher than the prevalence of 13.4% SCE reported by Kaufmann *et al.* [16] in Germany. However, samples in this study were taken 4 up to 8 weeks postpartum period. The higher incidence of SCE in this study compared to the above cited study could be the difference in the time of sampling.

The incidence of SCE in primiparous dairy cows was 40.38% which is lower than multiparous dairy cows of 48.42%. This disagreed with Belachew and Fekadu [12] who have reported that in DebreZeit in which first calf heifers seemed to have a tendency for SCE more often than multiparous cows at week 8 postpartum. Drilich [17] has also reported a higher prevalence in primiparous cows which may be due to less exposure of their uterine environment to microorganism. Kaufmann *et al.* [16] reported that in which the prevalence of SCE was in primiparous cows 7.8% than in multiparous cows 15.2%.

This study also shows a negative effect of subclinical endometritis on pregnancy; out of 67 subclinical endometritis positive dairy cows only 7(9.72%) cows were pregnant. This is in agreement with Belachew and Fekadu [12] who reported 15.3% were pregnant from DebreZeit.

CONCLUSIONS

This study revealed that subclinical endometritis was more prevalent in MSDF followed by LSDF and SSDF. The percentage of neutrophils was lower in the cows that became pregnant in the first AI. An increase in the percentage of neutrophils decreased the likelihood of pregnancy. The best cut off point (based on likelihood of pregnancy) of healthy cows was found to be < 3% neutrophil andcows with > or ≥3% neutrophils hadsubclinical endometritis.

Recommendations: Subclinical endometritis subsequently decrease pregnancy in cows. So. Herds should be managed properly after postpartum. The results of this study indicate that endometrial cytology can be a useful technique in identification of cows with subclinical endometritis.

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