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# CYTOLOGICAL STUDY OF SUBCLINICAL ENDOMETRITIS WITH RESPECT TO AGE, PARITY, FARM SCALE AND BODY CONDITION SCORE IN DAIRY COWS

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Supporting Information

**ABSTRACT**: The objective of this study was to investigate the incidence of subclinical endometritis with respect of age, parity, farm scale and body condition score in dairy cows. A total n= 147 of apparently healthy 3rd trimester pregnant cows were selected with no signs of clinical endometritis were examined from January 2016 to September 2017. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Age and parity were statistically significant (P<0.05) for subclinical endometritis. Older cows greater than 6 years were more affected sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) ( $\chi^2$ =51.97; P<0.05), the difference was statistically significant. The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) ( $\chi^2$ =14.48; P<0.05), the difference is statistically significant. However, body condition score and farm scale showed no significant variation with regard to subclinical endometritis.

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# INTRODUCTION

A high level of reproductive efficiency requires each cow to be bred successfully, and calve with a calving interval that maximizes the output of milk within the herd. Under normal circumstances, however, microbial contamination of the uterus is a frequent finding in postpartum dairy cows (Groenendaal et al., 2004).

Interestingly, multiparous cows have increased bacterial contamination ~50 days after calving compared to primiparous cows (Galvão et al., 2009a). Milk production has a detrimental effect on leukocyte function; therefore, leukocytes from multiparous cows are expected to be more severely affected because of greater milk yields (Sheldon et al., 2009). In fact, phagocytic activity of neutrophils in older cows is more markedly reduced after calving compared to younger cows. Therefore, increased levels of pro-inflammatory cytokine production in the uterine endometrium might help to prevent subclinical endometritis; however, because ultiparous cows have greater demands for milk yield, they might be less able to clear an infection completely and, therefore, might be more likely to have subclinical endometritis. Another important factor that might be involved in the susceptibility to subclinical endometritis is the circulating levels of immunoglobulins. Immunoglobulins work as opsonins, which greatly enhance phagocytic capacity. Primiparous cows have lower immunoglobulin content in colostrums, which indicates lower circulating immunoglobulin levels; therefore, phagocytosis might not be optimal in early lactation in primiparous cows (Sheldon et al., 2009).

New techniques have been described for the diagnosis of subclinical endometritis. The inflammation of the endometrium is characterized by the proportion of polymorphonuclear (PMN) cells in a cytological sample taken from clinically healthy cows. Cytological samples obtained by flushing the uterine lumen (Gilbert et al., 2005; Mateus et al., 2002; Sheldon et al., 2006).

Cows with a higher condition score at calving were less prone to subclinical endometritis and conceived more successfully to first service. Cows calving in a higher body condition score produced more milk, fat and protein in the first 90 days of lactation. Body condition score represents a subjective assessment of the tissue reserves of dry and lactating cows (Sheldon et al., 2009). Therefore, the objective of the study was to identify the effect of age, parity, farm scale and body condition score in dairy cows in and around Gondar.

## MATERIALS AND METHOD

# 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 meters above sea level, 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 woyna dega, 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 (CSA, 2008).

# Sample size

A sampling frame i.e. the list of the dairy farms was acquired from the urban agricultural development office at the beginning of the study. Dairy farms / cows were selected from this list using a stratified sampling procedure to ensure the selection of proportional and representative sampling of dairy farms and cows.

#### **Questionnaire survey**

A systematic question was designed and instituted to obtain relevant and reliable information about their animals. The questionnaire were checked for clarity of the questions prior the interview, respondents were briefed to the objective of the study. Following that, the actual questionnaires were presented.

#### **Regular follow up**

About 147 pregnant cows were randomly selected in and around Gondar that were expected to give birth within the study period. These cows were subjected to different clinical and gynecological examinations including rectal palpation and findings were recorded once a week.

#### **Body condition scoring**

The Body Condition Scoring (BCS) was determined according to Richard (Barlund et al., 2008; Richard, 1993). For all cows under the study their body condition were grouped from 0-5. Body condition score 0 stands for cows with the poorest body condition while score 5 for cows with the best condition.

#### Data management and statistical methods

Data collected from the longitudinal follow up study were entered in Microsoft excel. For analysis of the data statistical package for social science (SPSS) (version 18) was used. In this chi- square test, confidence interval and logistic regression were calculated. The Generalized Linear Model was utilized to analyse the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analyse the relationship between the amount of neutrophils with age, parity and body condition score. A probability of P < 0.05 was set as the significance level. The Confidence Interval (CI) was set at 95%. The Receiver Operating Characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

## RESULTS

In the present study endometrial cytology revealed that the polymorpho neutrophils 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.

Age and parity were statistically significant (P<0.05) for subclinical endometritis. Older cows greater than 6 years were more affected by sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) ( $\chi^2$ =51.97; P<0.05), the difference was statistically significant (Table 1). The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) ( $\chi^2$ =14.48; P<0.05), the difference is statistically significant. Body condition score was also statistically significant (Table 1). However, farm scale showed no significant variation with regard to subclinical endometritis (Table 1).

#### DISCUSSION

In the present study, multiparous cows (48.42%), emaciated cows (40.40%), and cows above 6 years of age (71.70%) were the most affected by subclinical endometritis which is higher than the previous finding (19.23%) by Takele et al. (2005). This could be due to the repeated exposure of the genital tract of multiparous cows to environmental pathogenic microorganisms causing gradual decrease in the efficiency of immune mechanism.

The current study revealed an overall incidence of 46% (67/147) of subclinical endometritis infection in dairy cows with a Polymorph nuclear cells (PMN) level of  $\geq$ 3 neutrophils in the uterine sample set as a threshold value indicative for Subclinical endometritis (SCE). This incidence of Subclinical endometritis (SCE) was similar with Belachew and Fekadu (2009) in Debre zeit which was 47.5% and 30.5%, respectively. However, in other studies samples were taken at 4 and 8 weeks postpartum period separately, whereas samples in this study were taken 4 to 8 weeks postpartum together. An explanation for the higher prevalence of SCE in this study could be the difference in the time arrangement. Gilbert (2005) in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Dubuc et al. (2010) in

Québec which was the prevalence of subclinical endometritis of 56%. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk (DIM) 45% for cows between 34 and 47 DIM (Gilbert, 2006). The variation of subclinical endometritis in the current study as compared to the other cited studies could be due to the difference in the management system of dairy cows.

The incidence of SCE in this study was 46% which was higher than the incidence of 13.4% as reported by Singh et al. (2008) in Germany. However, samples in this study were taken between 4 to 8 weeks postpartum period, whereas samples in the cited study were taken four hours after breeding. The higher incidence of SCE in this study as compared to the above cited study could be the difference in the time of sampling.

The incidence of subclinical endometritis in cows having body condition score 1 and 2 was 40.40% which is lower than cows having body condition score 3 and 4 (56.25%) significant (P=0.03) difference in the body condition score was also recorded. The prevalence of subclinical endometritis in cows with body condition score (BCS)-2 was 73% which was not statistically significant (P=0.554) and was higher than cows with BCS-3 of 62.6% which is in agreement with Belachew and Fekadu (2009).

40-60 days postpartum for subclinical endometritis by uterine cytology.								
Variables	No of examined	Positive for subclinical endometritis	Incidence %	Chi-square	p-value			
Age								
2-3	44	13	29.55					
4-6	50	16	32.00	51.97	0.000			
>6	53	38	71.70					
Parity								
Primiparous	52	21	40.38	14.48	0.01			
Multiparous	95	46	48.42					
Farm scale								
Small	54	25	46.30					
Medium	66	29	43.94	0.30	0.85			
Large	27	13	48.15					
Body condition score								
1&2	99	40	40.40	10.24	0.03			
3&4	48	27	56.25					
Pregnancy status								
Pregnant	72	7	9.72	70.63	0.000			
non pregnant	75	60	80.00					

# Table 1 - Analysis of results of cow's age, parity, farm scale, body condition score and pregnancy status examined 40-60 days postpartum for subclinical endometritis by uterine cytology.

# **CONCLUSION and RECOMMENDATION**

A herd health program is critical in maintaining uterine health and identifying potential problem areas. Routine postpartum examinations will help to identify problems early so that effective therapy can be administered in problem situations. The study showed that direct association of subclinical endometritis with age and parity. Therefore, proper herd health management and proper feeding is very important and also detailed studies should be conducted to identify their etiology, distribution and prevalence.

# DECLARATIONS

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I would like to thank dairy cattle holders for giving their animal.

#### **Conflict of interest**

The author declares that there is no conflict of interest.

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