

## Prevalence of Subclinical Endometritis and its Effect on Pregnancy in Crossbred Dairy Cows in Gondar

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**Abstract:** A cross sectional study was conducted on Cross breed dairy cows at small, medium and large farm scales based on questionnaire survey and regular follow up in and around Gondar town from October 2011 to April 2012. The objectives of this study were to determine the prevalence of subclinical endometritis in clinically normal postpartum dairy cows and to measure the effect of subclinical endometritis on Pregnancy. Apparently healthy 3<sup>rd</sup> trimester pregnant cows were selected based on the absence of abnormal vaginal and uterine discharges through taking history from owners and external inspection. The reproductive tract of selected cows was examined by rectal palpation and Subclinical endometritis was diagnosed by endometrial cytology using uterine lavage on postpartum dairy cows from 30-60 days. The current study revealed that the overall prevalence of subclinical endometritis with  $\leq 5$  neutrophil on endometrial cytology was 68.3%. The prevalence of subclinical endometritis in pregnant heifers, cows with poor hygienic condition, cows used artificial insemination, cows having good body condition score and cows in small scale farms was 100%, 71.4%, 69.8%, 75% and 86.7% respectively without statistically significant difference ( $p > 0.05$ ) among parity, hygienic condition, body condition score, method of service and farm scales respectively. However, cows positive in subclinical endometritis had a relative pregnancy rate of 21.4% with highly significant association ( $p = 0.000$ ) compared to cows free of subclinical endometritis. In conclusion, subclinical endometritis diagnosed by endometrial cytology was associated with reduced rate of pregnancy.

**Key words:** Crossbreed Dairy Cows % Pregnancy % Prevalence % Subclinical Endometritis

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### INTRODUCTION

The major constraints of dairy production in Ethiopia can be grouped in to two. These are technical and non-technical problems. Technical problems are those problems specific to animal production. These are animal diseases, animal genotype, lack of appropriate extension program and shortage of feed and water. Non technical problems are those mostly common to other agricultural sectors; including increased livestock and human populations. Other minor constraints are lack of infrastructures and market, poor education of farmers, seasonality of milk production and consumption pattern of people [1].

The key for an optimal fertility in dairy herd is a healthy uterine environment. A healthy uterus is the basis for high submission and conception rates [2].

Postpartum uterine disease represents a leading cause of reproductive inefficiency Lewis [3] and could mean significant economic loss for the dairy industry [4].

There are different types or manifestations of uterine disease among postpartum dairy cows. Metritis, clinical endometritis, subclinical endometritis and pyometra are the types of uterine diseases most commonly reported [5]. Postpartum subclinical endometritis has a negative effect on reproductive performance as it increases services per conception, the calving to first service interval and the calving to conception interval, reduces pregnancy and decreases the conception rate [6].

Studies on subclinical endometritis (SCE) found prevalence in the ranging between 12 and 94%. The prevalence of subclinical endometritis depends on the occurrence of early post partum uterine diseases, the time of examination and the diagnostic technique [7].

The diagnostic criteria for subclinical endometritis should identify cows at risk of pregnancy failure within an appropriate time. A variety of methods such as uterine palpation, ultrasonographic features of the uterus and endometrial cytology has been reported to identify subclinical endometritis in postpartum cows [8].

Finally, subclinical endometritis is one of the most common causes of reducing pregnancy in high producing dairy cattle, profitability and sustainability of farming [9].

The main objectives of this study are, to determine the prevalence of subclinical endometritis and its effect on pregnancy in cross breed dairy cows.

## MATERIALS AND METHODS

**Study Area:** A cross sectional study was conducted from October 2011 to April 2012 in and around Gondar town, Gondar is located in North Western part of Ethiopia at about 727 km away from Addis Ababa and the altitude is 2200 meter above sea level. The area is located under mid altitude, agro-climatic zone and receives a bimodal rainfall; the average annual rainfall is about 1172 mm with 19.7°C annual temperature. The human population of the town is 2,896,873 of which 1,569,205 are males and 1,327,668 are females and the cattle population is 2,407,544 [10].

**Study Animals:** Study animals were apparently healthy 3<sup>rd</sup> trimester pregnant cross breed dairy cows which were obtained by taking history of pregnancy from the owner by conducting questionnaire survey and personal observation. A total of 82 cows were diagnosed as 3<sup>rd</sup> trimester pregnant cows and examined at postpartum by using uterine lavage.

**Study Design and Sampling:** A cross sectional survey and longitudinal follow up was carried out on cross breed dairy cows. The farm scales were classified small, medium and large farm scales according to ILRI [11] the herd size ranges from 1-2, 3-10 and >10 cows respectively. The study dairy farms were 25, 40 and 6 farms in small, medium and large category respectively.

Purposive sampling conduct to select study animals which were cross breed dairy cows and simple random sampling posses for selecting dairy farms and 3<sup>rd</sup> trimester pregnant cross breed dairy cows in the selected farms taken as a sample.

The sampling was determined by the formula given by Thrusfield [12]. Since there was no similar study done previously on the study area the expected prevalence was 50% with the confidence interval of 95%.

$$n = \frac{1.96^2 \times P_{\text{exp}}(1 - P_{\text{exp}})}{d^2}$$

n = Required sample size

P<sub>exp</sub> = Expected prevalence

d = Desired absolute precision

**Sample Collection Technique:** The vulva of the animal was cleaned by water and 50 ml of 0.9% sterile sodium chloride solution was taken by a 50 ml syringe. A 52 cm sterile plastic infusion rod was inserted into the uterus through rectum and the uterus was lavaged by infusion of 0.9% sterile sodium chloride solution with 50 ml syringe attached to a 52 cm sterile plastic rod. The uterus was then massaged through rectum and then the fluid was recovered by negative pressure aspiration into the syringe and transferred to the 10 ml test tube without preservative. The uterine samples were put into the icebox and brought to faculty of veterinary medicine microbiology laboratory within 2 hours of collection.

**Subclinical Endometritis Determination:** Subclinical endometritis was determined using endometrial cytology. A total of 82 apparently healthy cows on week 4-8 post partum were examined by uterine lavage and the uterine samples were centrifuged at 800 rpm for 5 min. Then after centrifugation a drop of sediments were streaked onto a clean microscope slide and air-dried. Then the slides were fixed with methanol and stained with Geimsa for 45 minutes and the amount of neutrophil were observed under light microscope at 40x and finally samples with greater than or equal 5 neutrophil were categorized as subclinical endometritis whereas amount of neutrophil less than 5 considered as normal [13].

**Data Management and Analysis:** The data obtained from the questionnaire, the findings of pregnancy diagnosis and uterine lavage technique were recorded on Microsoft excel work sheet, then data analyzed by SPSS version 18 statistical soft ware and chi-square were employed to analyze the occurrence of subclinical endometritis based on parity, hygienic condition, body condition score, method of service and farm scales.

Pregnancy problems were determined using Descriptive statistics; Chi-Square test ( $P^2$ ) and the confidence interval was set at 95% and  $P < 0.05$  is considered as statistically significant.

## RESULTS

The uterine lavage examination was conducted on 82 cross breed dairy cows from 4 - 8 weeks postpartum. Uterine samples revealed an overall prevalence of subclinical endometritis infection of 68.3% (56/82). The prevalence pregnant animals in subclinical endometritis positive cows was 21.4% (12/56). Variation of the prevalence of subclinical endometritis had been observed on parity, hygienic condition, method of service, body condition score and farm scales.

The cows were grouped as pregnant heifer, primiparous and multiparous based on parity. Of 82 cross breed dairy cows the prevalence of subclinical endometritis were 100%, 52.4% and 71.4% in pregnant heifer, primiparous and multiparous cows respectively (Table 3).

The cows were grouped as pregnant heifer, primiparous and multiparous based on parity. Of 82 cross breed dairy cows the prevalence of subclinical endometritis were 100%, 52.4% and 71.4% in pregnant heifer, primiparous and multiparous cows respectively (Table 3).

The method of services used for these cows were AI, Bull and both. The prevalence of subclinical endometritis was 69.8%, 63.6% and 66.7% in AI, Bull and Both respectively.

The hygienic condition of the cows was grouped into three categories. The three categories were Poor hygienic condition, Good hygienic condition and Very good hygienic condition. From a total of 82 cross breed dairy cows 71.4%, 67.4% and 63.6% were the prevalence of subclinical endometritis in poor, good and very good hygienic condition cows respectively.

The cows were grouped as cows having body condition score 2, 3 and 4. The prevalence of subclinical endometritis was 73%, 62.2% and 75% in BCS 2, 3 and 4 respectively.

The study farms were classified into small, medium and large farm scales according to IRLI [11] the herd size ranges from 1-2, 3-10 and >10 cows respectively. The prevalence of subclinical endometritis was 86.7%, 66.7% and 61.8% in small, medium and large scale farms respectively (Table 7).

Table 1: Prevalence of subclinical endometritis in crossbreed dairy cows

| Subclinical endometritis | No. of cows | Percentage | Chi-square | P-value |
|--------------------------|-------------|------------|------------|---------|
| Positive                 | 56          | 68.3       |            |         |
| Negative                 | 26          | 31.7       | 82.000     | 0.000   |
| Total                    | 82          | 100.0      |            |         |

Table 2: Prevalence of pregnancy in subclinical endometritis positive cows

| Pregnancy status | No of cows | %     | Chi-square | P-value |
|------------------|------------|-------|------------|---------|
| Positive         | 12         | 21.4  |            |         |
| Negative         | 44         | 78.6  | 44.083     | 0.000   |
| Total            | 56         | 100.0 |            |         |

Table 3: Prevalence of subclinical endometritis in cows based on parity

| Parity          | No of examined animals |           | Chi-square | P-value |
|-----------------|------------------------|-----------|------------|---------|
|                 | examined               | Positive  |            |         |
| Pregnant heifer | 5                      | 5(100%)   |            |         |
| Primiparous     | 21                     | 11(52.4%) | 5.031      | 0.081   |
| Multiparous     | 56                     | 40(71.4%) |            |         |
| Total           | 82                     | 56(68.3%) |            |         |

Table 4: Prevalence of subclinical endometritis in cows based on method of services

| Method of services | No of examined animals |           | Chi-square | P-value |
|--------------------|------------------------|-----------|------------|---------|
|                    | examined               | Positive  |            |         |
| AI                 | 53                     | 37(69.8%) |            |         |
| Bull               | 11                     | 7(63.6%)  | 0.189      | 0.910   |
| Both               | 18                     | 12(66.7%) |            |         |
| Total              | 82                     | 56(68.3%) |            |         |

Table 5: Prevalence of subclinical endometritis cows based on hygienic condition

| Hygienic conditions | No of examined animals |            | Chi-square | P-value |
|---------------------|------------------------|------------|------------|---------|
|                     | examined               | Positive   |            |         |
| Poor                | 28                     | 20 (71.4%) |            |         |
| Good                | 43                     | 29(67.4%)  | 0.252      | 0.882   |
| Very good           | 11                     | 7(63.6%)   |            |         |
| Total               | 82                     | 56(68.3%)  |            |         |

Table 6: Prevalence of subclinical endometritis in cows based on BCS

| Body condition scores | No of animals examined |           | Chi-square | P-value |
|-----------------------|------------------------|-----------|------------|---------|
|                       | examined               | Positive  |            |         |
| 2                     | 37                     | 27 (73%)  |            |         |
| 3                     | 37                     | 23(62.2%) | 1.183      | 0.554   |
| 4                     | 8                      | 6(75%)    |            |         |
| Total                 | 82                     | 56(68.3%) |            |         |

Table 7: Prevalence of subclinical endometritis in cows based on farm scales

| Farm scales | No of examined animals |           | Chi-square | P-value |
|-------------|------------------------|-----------|------------|---------|
|             | examined               | Positive  |            |         |
| Small       | 15                     | 13(86.7%) |            |         |
| Medium      | 33                     | 22(66.7%) | 0.134      | 0.935   |
| Large       | 34                     | 21(61.8%) |            |         |
| Total       | 82                     | 56(68.3%) |            |         |

## DISCUSSION

The objectives of this study were to determine the prevalence of subclinical endometritis in clinically normal postpartum dairy cows, based on the absence of abnormal discharge on external inspection to measure the effect of subclinical endometritis on pregnancy. The prevalence of the disease is very variable and depends on the diagnosis technique; the days in milk of the genital examination and the statistical method used to determine the cut-off point of the neutrophil ratio obtained from endometrial cytology [14].

The current study revealed an overall prevalence of 68.3% subclinical endometritis infection in cross breed dairy cows with a polymorphonuclear neutrophil level of  $\geq 5$  in the uterine sample set as a threshold value indicative for subclinical endometritis. This prevalence of subclinical endometritis was higher than Belachew and Fekadu [15] in Debrezeit which was a 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 4 up to 8 weeks postpartum cows together. An explanation for the higher prevalence of subclinical endometritis in this study could be the difference in the time arrangement. Gilbert [16] in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Couto *et al.* [17] 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 DIM, 45% for cows between 34 and 47 days in milk [13]. The prevalence of SCE in the current study was 68.3% from 4 up to 8 weeks postpartum period which was higher than the prevalence of the above mentioned cited studies. The reason for the higher prevalence of subclinical endometritis in the current study compared to the above mentioned cited studies could be due to the difference in the management system of dairy cows.

The prevalence of subclinical endometritis in this study was 68.3% also higher than the prevalence of 13.4% subclinical endometritis reported by Kaufmann [18] in Germany. However, samples in this study were taken 4 up to 8 weeks postpartum period, whereas samples in the cited study were taken four hours after breeding. The higher prevalence of subclinical endometritis in this study compared to the above cited study could be the difference in the time of sampling.

The prevalence of subclinical endometritis in primiparous dairy cows was 100% which is higher than multiparous dairy cows of 71.4 but not significant ( $p = 0.081$ ). This is agree with Belachew and Fekadu [15] in DebreZeit in which first calf heifers seemed to have had a tendency ( $p = 0.08$ ) for being diagnosed positive for subclinical endometritis more often than multiparous ones at week 8 postpartum and Drilich[19] also reported a higher prevalence in primiparous cows and this may be due to higher pregnancy and/or parturition stress and first exposure of their uterine environment to microorganism. On the other hand this result is disagree with Kaufmann [18] in which the prevalence of subclinical endometritis in primiparous cows of 7.8% which is lower than multiparous cows of 15.2%.

This study also shows that effect of subclinical endometritis on pregnancy, from a total of 56 subclinical endometritis positive cross breed dairy cows only 12 cows with a prevalence of 21.4% were pregnant which was highly significant ( $p = 0.000$ ). This is agreeing with Belachew and Fekadu [15] in DebreZeit which was 15.3%. The prevalence of subclinical endometritis in cows used AI was 69.8% which was higher than cows used Bull with a prevalence of 63.6% and 66.7% of both bull and AI but which was not statistically significant ( $p = 0.910$ ). The reason for the higher prevalence of subclinical endometritis in AI used cows could be due to exposure of the cows to bacterial contamination during artificial insemination.

The prevalence of subclinical endometritis in cows having poor calving hygiene was 71.4% but the association is not significant ( $p = 0.882$ ) which is higher than cows having good and very good calving hygiene with a prevalence of 67.4% and 63.6% respectively. This is agreeing with Belachew and Fekadu [15] in DebreZeit with a prevalence of 58.6% and 40.0% in cows having poor and good calving hygiene. The reason for the occurrence of subclinical endometritis in cows having poor calving hygiene was due to exposure of the cows to bacterial contamination at parturition.

The prevalence of subclinical endometritis in cows having body condition score 4 was 75% which is higher than cows having body condition score 2 (73%) and body condition score 3 (62.2%) which was not significant ( $p = 0.554$ ). The prevalence of subclinical endometritis in cows having body condition score (BCS) 2 was 73% which was not statistically significant ( $p = 0.554$ ) was higher than cows having BCS 3 of 62.6% which is agree to Belachew and Fekadu [15] in DebreZeit which was the prevalence of 64.5% in BCS $>2.5$  and 28.6% of in BCS $>2.5$  which was significant ( $p = 0.035$ ).

The prevalence of subclinical endometritis in small scale farms was 86.7% which was not statistically significant ( $p = 0.935$ ) higher than medium (66.7%) and large (61.8%) farm scales. The reason for the higher prevalence of subclinical endometritis in small scale cross breed dairy cows could be due to poor sanitation management and handling of cows at the time of parturition and early post partum period.

### CONCLUSION

Subclinical endometritis is an inflammation of the uterus that results in significant reduction in reproductive performance in the absence of overt clinical signs. The diagnostic techniques used to confirm subclinical endometritis in cross breed dairy cows was uterine lavage. The present study indicated that the prevalence of subclinical endometritis was high and it has a great impact on pregnancy of cross breed dairy cows in and around Gondar town. Variation of the prevalence of subclinical endometritis had been observed on parity, hygienic condition, method of services, body condition score and farm scales. Cows' especially first calf heifers were more commonly affected by subclinical endometritis with high prevalence. The prevalence of subclinical endometritis also high in cows having poor calving hygiene, poor body condition, cows inseminated by artificial insemination. An improvement of the managerial systems such as increased calving and post calving hygiene, improve the hygienic condition of pregnant heifers and its housing at parturition and after calving are taking in to account to reduce prevalence of subclinical endometritis and increases the pregnancy rate of cows in the study area.

Based on the above conclusion the following recommendations are forwarded:

- C The owners should be aware to improve the managerial systems of their cows by veterinarians to minimize prevalence of subclinical endometritis and pregnancy rate as much as possible.
- C The owners should be responsible to keep sanitation of dairy cows through improved housing systems and nutrition to reduce bacterial exposure of cows at parturition and increase the pregnancy rate after calving.
- C Gloves should be wearing appropriately and calving ropes should be cleaned in order to reduce subclinical endometritis during pregnancy diagnosis and delivery problems.

- C Semen evaluation should be taken in order to inseminate healthy bull semen to cows during artificial insemination to reduce subclinical endometritis.
- C Further studies should be conducted on subclinical endometritis to overcome problem of Pregnancy.

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