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# Comparison of endometrial cytology and transrectal ultrasonography for the diagnosis of subclinical endometritis*

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**Abstract**

*The objective of the present study was to compare the efficacy of endometrial cytology (EC) and uterine echobiometry by transrectal ultrasonography (TRUS) for the diagnosis of subclinical endometritis (SCE) in crossbred dairy. A total of 20 crossbred cows, at 30 and 40 days postpartum (DPP), without any clinical signs or abnormal discharge were subjected to transrectal ultrasonography (TRUS) and endometrial cytology (EC) examination. Among the TRUS parameters, uterine horn diameter (UD) was found to be least useful in the diagnosis of SCE. Cervical diameter (CD) also had poor to moderate sensitivity when compared to EC and less accurate in diagnosing SCE. Fluid in uterus (FIU) had more sensitivity than UD and CD for the diagnosis of SCE but its presence is dependent on the stage of oestrous cycle. Hence, when TRUS is used as a supporting diagnostic aid along with EC, it has promising value in the diagnosis of SCE.*

**Keywords:** *Subclinical endometritis (SCE), transrectal ultrasonography (TRUS), Endometrial cytology (EC).*

Successful reproduction is one of the crucial elements for a profitable dairy industry. For maintenance of high level of reproductive efficiency, healthy uterine environment, timely detection of oestrus, successful breeding and a calving interval that maximizes the milk production within the herd is required. The major economic losses in a herd attributed to infertility are reduced milk production, uterine diseases, treatment cost and increased culling rate due to failure of conception.

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Postpartum period is a time when the uterine health will be compromised. It is normal to find microbial contamination of the uterus following parturition. The uterus gets contaminated with a wide range of bacterial organisms, which are normally eliminated during the process of uterine involution. However, some animals may be immunocompromised causing the persistence of the microorganisms leading to an infection. Postparturient complications like dystocia, retention of foetal membranes, uterine prolapse, metabolic diseases, nutritional and hormonal imbalance can aggravate the condition. When the postpartum uterine defence mechanisms are disrupted the endometrial lining of the uterus is affected resulting in growth of microbes and poor fertility **(**Purohit *et al*., 2015).

Endometritis can be classified as clinical (CE) and subclinical endometritis (SCE). Subclinical endometritis is difficult to diagnose as there are no visible symptoms (Foldi *et al*., 2006; Barlund *et al.,* 2008). The diagnosis of CE has been based on rectal palpation, vaginoscopy, Metricheck or ultrasonography. Diagnosis of SCE has been presumptive in the past and repeat breeder cows were considered to have SCE since transrectal palpation revealed no abnormality (Purohit, 2008). The diagnosis of SCE is based on uterine cytology where the percentage of Polymorphoneutrophils (PMN) was counted (Kasimanickam *et al.,* 2004 and Gilbert *et al.,* 2005). Brodzki *et al*. (2015) had stated that endometrial cytology could be used as an invaluable aid for diagnosing SCE in those animals without any overt manifestations. Many authors had suggested different cut off levels for PMN cells to diagnose the SCE positive animals at different days postpartum.

More recently transrectal ultrasonography (TRUS) and endometrial cytology (EC) have been suggested for the definitive diagnosis of SCE (Lenz *et al.,* 2007; Oral *et al*., 2009). Ultrasonographic features helpful in the diagnosis of SCE include accumulation of fluid, increased endometrial thickness, uterine horn diameter (UD) and cervical diameter (CD) (Lenz *et al.,* 2007; Oral *et al*., 2009; Purohit *et al*., 2015).

The presence of fluid in uterus (FIU) at three weeks postpartum in cows could be considered as a reliable indicator of endometritis, with good sensitivity and specificity (Mateus *et al.* 2002; Kasimanickam *et al.,* 2004; Barlund *et al.,* 2008 and Oral *et al.,* 2009). LeBlanc *et al*. (2002) concluded that an increase in the size of the uterus with more than 8 cm in diameter and cervix with more than 7.5 cm in diameter between 20 and 33 days postpartum (DPP) in cows with suffering from clinical endometritis were associated with a decrease in pregnancy rate. Kasimanickam *et al*. (2004) classified animals with UD and CD greater than three cm to be positive for endometritis at 33 to 47 DPP.

**Materials and methods**

The study was conducted at University Livestock Farm and Fodder Research Development Scheme, Mannuthy, Thrissur, Kerala from September 2018 and June 2019. The study included 20 crossbred dairy cows of three to 12 years of age in their first to seventh parity, which were clinically normal and without any postpartum complications. The animals were subjected to TRUS and EC.

A balanced feeding protocol was followed consisting of green and dry fodder along with concentrates and mineral mixture according to the Nutrient Requirements of Animals – Cattle and Buffalo (ICAR-NIANP), (2013). Pregnant animals were dried off at seventh month of gestation and maintained in a separate shed. The practice of weaning of calves immediately after parturition was followed and cows were milked twice daily.

Transrectal ultrasonography was performed on 30 and 40 DPP, with a real time colour Doppler ultrasound scanner (MyLab™ Gamma, Esoate SpA, Italy) equipped with liner array, 5-10 MHz frequency transrectal transducer to determine presence of FIU, evaluate the echogenicity of the contents if any and to measure the diameters of uterine horn and cervix.

Cytological studies of the uterus were carried out on 30 and 40 DPP using modified human Pap smear cytobrush, where the handle of brush was shortened to nearly 2cm, heat fixed to stylet of AI gun, withdrawn

to barrel and covered with plastic AI sheath with widened tip. The modified cytobrush was prepared aseptically under laminar air flow. The cows were restrained and vulva and perineum were cleansed to minimize contamination. The cytobrush was introduced into the vagina and by per rectal manoeuvring it traversed the cervix and reached the uterus. Once inside the uterus the stylet was pushed forwards to expose the cytobrush and rolled on to the uterine wall a full 360 degrees clockwise to obtain cellular material from the endometrium. Slides were prepared by rolling the cytobrush onto clean glass microscopic slides which were air-dried and stained using Field Stain.

The EC smear was evaluated using a microscope at 400 X magnification to identify individual cell types, including endometrial epithelial cells and polymorphonuclear (PMN) cells. The PMN cells count were expressed as the per cent of PMN cells counted out of the 100 cells (including PMN cells and endometrial cells). Cows were categorized into two groups *viz*., SCE - positive or negative on the basis of percentage of PMN cells. The animals with more than eighteen per cent PMN cells on days 21- 34 and more than 10 per cent on days 33- 47 were considered positive for subclinical endometritis (Kasimanickam *et al*., 2004).

The data obtained were subjected to statistical analysis using Repeated Measures ANOVA, Cochran's Q test and Receiver Operating Characteristic (ROC) Curve using SPSS 24.

**Results and Discussion**

The objective of the present study was to compare TRUS and EC by cytobrush technique for the diagnosis of subclinical endometritis in postpartum cows. Using cytobrush technique (EC) nine out of the 20 animals were diagnosed as SCE positive with more than 18 per cent PMN cells on 30$^{th}$ day of observation.

The Mean (± SE) percentage of PMN cells in normal and SCE cases are summarized in table 1. In normal animals the values were 1.82 ±0.58 and 1.00 ± 0.56 and in SCE group values were 20.00 ± 0.64 and 11.00 ± 0.62 on 30 and 40 DPP, respectively.

On statistical analysis there was significant difference in the PMN percentage (Fig. 1) between SCE positive and normal groups in both the days of observation and also there was a significant reduction in the PMN percentage from days 30 to 40 in SCE group (Table 1).

This was in par with the studies by Kasimanickam *et al*. (2004) who used ROC analysis to identify the PMN per cent above which fertility was significantly reduced and

**Table1.** Mean (± SE) of PMN cells on day 30 and 40 postpartum

| Group | Per cent of PMN cells | |
|---|---|---|
| | Normal (n = 11) | SCE (n = 9) |
| Day 30 | 1.82±0.58$^{Ab}$ | 20±0.64$^{Aa}$ |
| Day 40 | 1±0.56$^{Ab}$ | 11±0.62$^{Ba}$ |

*(a,b Means bearing different superscripts within rows differ significantly at 5% level A,B Means bearing different superscripts within columns differ significantly at 5% level)*

**Table 2.** Relationship between fluid in uterus and subclinical endometritis on 30 and 40 DPP

| Group | Normal (n = 11) | | SCE (n = 9) | |
|---|---|---|---|---|
| | Day 30 | Day 40 | Day 30 | Day 40 |
| FIU | 5 (45.45) | 6 (54.54) | 6 (66.67) | 6 (66.67) |

**Table 3.** Mean (± SE) of Cervical diameter (mm) and Uterine horn diameter (mm) in normal and sub clinical endometritis positive animals

| Group | Cervical diameter (mm) | | Uterine horn diameter (mm) | |
|---|---|---|---|---|
| | Normal (n= 11) | SCE (n = 9) | Normal (n= 11) | SCE (n = 9) |
| Day 30 | 29.62±0.77 | 28.96±0.84 | 14.77±0.52 | 15.23±0.74 |
| Day 40 | 28.68±0.85 | 29.56±0.67 | 14.83±0.55 | 15.35±0.69 |

*Measured at 5% level of significance*

arrived at 18 per cent for 20–33 days postpartum and 10 per cent for 34–47 days postpartum.

By performing TRUS, on day 30, six animals in SCE group and five animals in normal group had FIU and on day 40, six animals in SCE group and six animals in normal group had FIU. The percentage of animals with FIU was similar in both groups. This was in contrast to Dourey *et al*. (2011) who reported that there was a positive correlation between the quantity of uterine fluid and PMN percentage on four weeks postpartum, but it did not affect the interval from calving to first ovulation (Table 2).

The mean (± SE) of CD (mm) in the normal group was 29.62 ± 0.77 and 28 ± 0.85

Fig. 1 Microphotograph of endometrial cytology (PMN cells marked by arrow), 400X

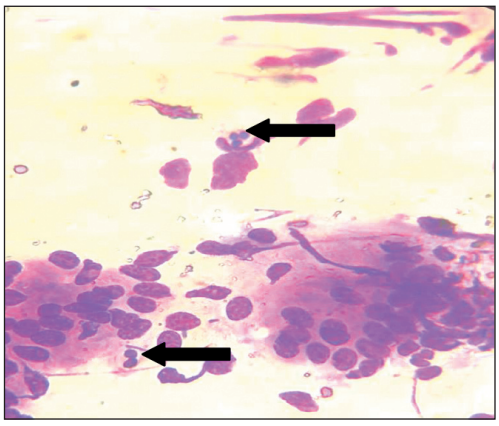


Fig. 2 Cervical Diameter (CD) at 30 and 40 DPP

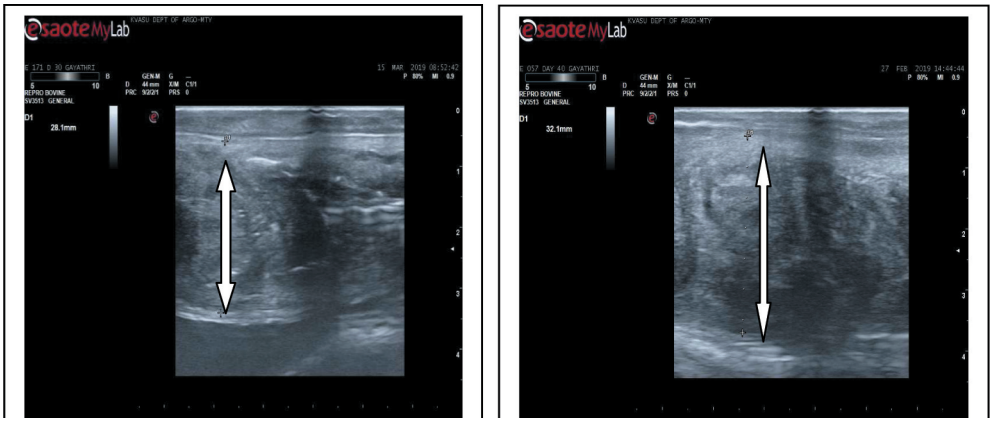
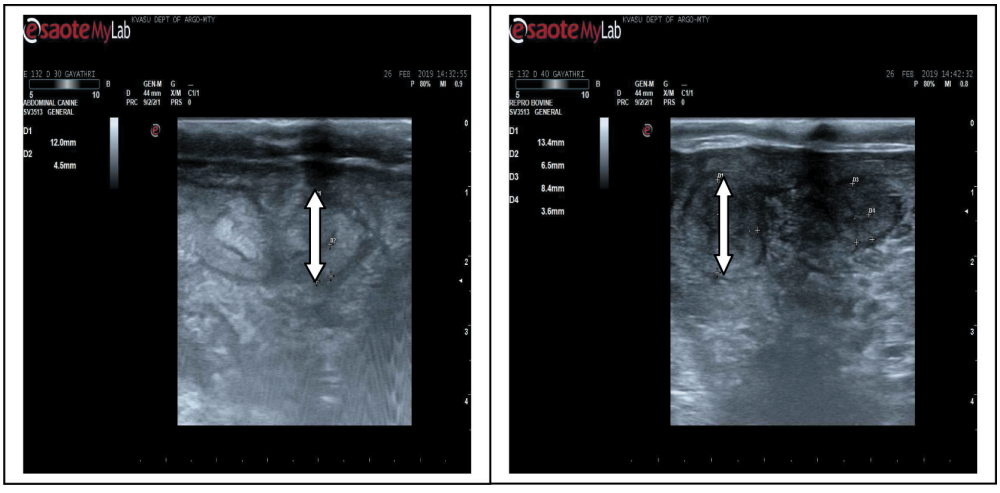



Fig. 3 Uterine horn diameter (UD) at 30 and 40 DPP



and in SCE group was 28 ± 0.96 and 29.56 ± 0.67 on 30 and 40 DPP, respectively (Table 3, Fig. 2).

On statistical analysis there was no significant difference between SCE and normal groups in both the days of observation. The cut off point for cytological endometritis positive was greater than three cm. All the animals had a CD less than three cm by day 30 irrespective of the group. Similar works were done in pure bred cows where the size of cervix in normal condition was considerably large (Kasimanickam *et al*., 2004).

The mean (± SE) of uterine horn diameter (UD in mm) in the normal group was 14.77 ± 0.52 and 14.83 ± 0.55 and in SCE group was 15.23 ± 0.74 and 15.35 ± 0.69 on 30 and 40 DPP, respectively (Table 3, Fig. 3). On statistical analysis there was no significant difference between SCE and normal groups on both the days of observation. Similarly, Mateus *et al*. (2002) also reported that changes in uterine diameter are identifiable only in severe endometritis and such changes are negligible in SCE.

Statistical analysis of the different parameters was performed using Cochran's Q test and Receiver Operating Characteristic (ROC) curve. On using Cochran's Q test for comparing the efficacy of FIU, CD and EC in the diagnosis of SCE, there was no significant difference on both 30 and 40 DPP.

However, on using Cochran's Q test for analysing the efficiency of FIU, UD and EC, the diagnosis based on UD was found to be significantly different from that of EC on both 30 and 40 DPP. Hence, its efficiency in diagnosing SCE is inferior in the present study.

On ROC curve analysis by keeping EC as gold standard, on day 30, FIU 30 has a sensitivity of 66.7 per cent and a specificity of 54.5 per cent. Area under curve (AUC) was 60.6 per cent which indicates that this model has less to moderate predictability.

CD has poor sensitivity (44.4%) and specificity (54.5%). AUC is small and near to random (49.5%) and has poor predictability.

UD has poor sensitivity (0%) and high specificity (100%). AUC is small and near to random (50%). Hence, this criterion is least valuable for the diagnosis of SCE.

On ROC curve analysis keeping EC as standard on day 40, FIU has a sensitivity of 71.4 per cent and a specificity of 46.2 per cent. AUC was 58.8 per cent which indicates that this model has low to moderate predictability.

CD has poor sensitivity (28.6%) but moderate specificity (69.2%). AUC is small and near to random (48.9%) and has poor predictability.

UD has poor sensitivity (0%) and high specificity (100%). AUC is small and near to random (50%). Hence, this criterion has low value for the diagnosis of SCE.

In the present study, endometrial cytology (EC) was kept as gold standard and TRUS parameters like UD, CD and FIU were compared. It was found that FIU could be used for the diagnosis of SCE with a sensitivity of 66.7 per cent and specificity of 54.5 per cent, when compared to EC, on day 30 and a sensitivity of 71.4 per cent and specificity of 46.2 per cent for day 40. However, FIU was influenced by the stage of oestrous cycle. The sensitivity and specificity obtained for FIU was greater than that reported by Drillich *et al*. (2004) (sensitivity of 57.7% and a specificity of 40.5%) where the presence of ultrasonographically detectable uterine fluid and endometrial cytology using cytobrush (EC) were compared using EC as the gold-standard in animals 21 -27 DPP.

This was also in accordance with Arias *et al*. (2018) who reported that the ultrasonographic detection of intrauterine fluid and evaluation of uterine diameter could be applied as a method to diagnose SCE and that a small amount of FIU or thickened uterine walls could be considered signs of endometrial inflammation.

In the present study there was no significant difference in cervical and uterine

horn diameter between the normal and SCE groups during both the days of study. Diagnosis using CD at 30 and 40 DPP had a sensitivity of 44.4 per cent and 28.6 per cent and a specificity of 54.5 per cent and 69.2 per cent respectively with EC as gold standard. Uterine horn diameter had zero sensitivity on both the days of observation.

The cut-off point for diagnosing an animal as SCE positive was taken according to the study by Kasimanickam *et al.* (2004) where a uterine horn diameter and cervical diameter greater than three cm was considered positive for diagnosing cytological endometritis in apparently normal animals. However, all the animals in the present study had a cervical diameter and uterine horn diameter less than 3 cm by day 30. This could be attributed to the fact that the present study was done in crossbred dairy cows which had a notably smaller uterine and cervical size when compared to the purebred cows used in the reference studies. Hence, the diagnostic parameters involving TRUS are of inferior value for the precise diagnosis of SCE in cross bred dairy cows when compared to EC in the present study.

## Conclusion

The present study suggested that the TRUS parameters like uterine horn diameter, cervical diameter and fluid in uterus was found to be less sensitive and specific for the diagnosis of subclinical endometritis when compared to endometrial cytology. Further, in order to fix a cut off value for CD and UD in crossbred dairy cows detailed studies involving more number of animals may be required so as to achieve a statistically significant value in this regard. In SCE the changes in the uterus are minute and its detection by ultrasonography is subjective to error and requires special techniques for diagnosis such as endometrial cytology. In the present study, the cytological samples were collected using modified cytobrush and the PMN percentage was significantly different in animals with SCE. Such precise changes may not be detected in terms of uterine horn diameter and cervical diameter using TRUS. However, FIU had more sensitivity than cervical diameter and uterine horn width. Therefore, when ultrasonography is used as a supporting diagnostic aid along with cytology, it is of greater value in the accurate diagnosis of subclinical endometritis.

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