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Endometrial cytology and transrectal ultrasonography in diagnosis of subclinical endometritis in postpartum crossbred cattle#

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Abstract

Postpartum diseases of uterus represent a foremost predicament in cattle farms affecting reproductive efficiency. Postpartum (PP) uterine diseases include metritis, endometritis and subclinical endometritis (SCE). Barring SCE, all other diseases project the clinical signs. Diagnosis of SCE at the early stage of PP has been a challenging task for many veterinarians. The present study aimed to validate and correlate the proficiency of transitional changes in uterus and cervix assessed by transrectal ultrasonography (TRUS) and endometrial cytology (EC) in the diagnosis of SCE in PP cattle. The research was carried out in two phases i.e, 30th day postpartum (DPP) (n=74) and first exhibited oestrus after 45th DPP (n=24) (54.63 ± 1.13 d) to emphasise the proficiency of both the diagnostic modalities. Endometrial cytology could discriminate between cows positive and negative for SCE significantly at 30th DPP (20.54 ± 0.48 vs 5.55 ± 0.41). It was potent in diagnosing SCE after 45th DPP also, as a significant reduction in the per cent of PMN cells (20.54 ± 0.48 to 7.56 ± 0.32) was noticed. Uterine parameters assessed by TRUS like cervical and uterine diameter and endometrial thickness in both the phases of assessment did not correlate to the EC changes and also could not discriminate between SCE positive and negative animals. The results of the study signify that EC is efficacious in the diagnosis of SCE in cows, while TRUS is impractical as a sole diagnostic modality for SCE.

Keywords: *Subclinical endometritis, endometrial cytology, transrectal ultrasonography*

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The postpartum period consists of uterine involution, endometrial regeneration, resumption of ovarian cyclicity and purging of opportunistic bacteria that have gained the advantage to enter and colonise the immune-suppressed uterus (Sheldon *et al*., 2017). Depending on the endocrine status of the animal, postpartum (PP) period can be classified into three stages *viz*., puerperal period (10-12 days PP) in which pituitary is responsive to GnRH and formation of the first dominant follicle (DF), intermediate stage (20-30 days PP) consisting of ovulation of the first DF and post ovulatory period (40-45 PP) that denotes complete involution of uterus under the influence of progesterone produced by active corpus luteum (CL) that developed after ovulation (Elmetwally, 2018). Delay in uterine involution can be attributed to factors like dystocia, retention of foetal membranes, hypocalcaemia, ketosis and other postpartum diseases.

Postpartum uterine diseases play a greater role in predicting the future fertility of animals and act as determining elements in projecting the economics of dairy cattle farms. Postpartum uterine diseases include metritis, endometritis and subclinical endometritis (SCE). All these diseases project definitive clinical signs for the diagnosis except SCE, a condition characterised by inflammation of endometrium and infiltration of polymorphonuclear (PMN) cells into the endometrium with the absence of purulent discharge from vagina (Sheldon *et al*., 2009).

Because of its asymptomatic nature and deleterious effects on reproduction, early diagnosis and treatment of SCE pose the greatest tasks in reproductive management of dairy herds. In addition, the lack of proper diagnostic methods for SCE makes it more challenging. Techniques like transrectal palpation of uterus, uterine fluid culture, vaginoscopy, uterine cytology, uterine biopsy and sonographic changes of uterus are advocated for the diagnosis of SCE with variable success rates (Arias *et al*., 2018). An early line of defence against the pathogenic organisms is instituted by neutrophils resulting in an increased PMN cell within the uterine lumen. Endometrial cytology (EC) is a technique used for the interpretation of the different cells by acquiring a sample from the superficial layer of endometrium (Gilbert *et al.,* 1998). Transrectal ultrasonography (TRUS) could associate the parameters like uterine horn diameter and intra uterine fluid volume with uterine infection and inflammation (Mateus *et al.,* 2002). The present study aimed to validate and correlate the competence of EC and uterine changes assessed by TRUS in the diagnosis of SCE in PP dairy cows.

Materials and methods

Experimental animals

The investigation was performed in University Livestock Farm and Fodder Research and Development Scheme (ULF and FRDS), Mannuthy, Kerala Veterinary and Animal Sciences University. Postpartum dairy cows without any parturition difficulties and postpartum complications and clinically normal were selected for the study. The crossbred cows were maintained under uniform management and a balanced feeding condition as per the recommendations by Nutrient Requirements of Animals – Cattle and Buffalo (ICAR-NIANP, 2013). In first phase of the study (V1), a total of 74 animals were screened on the 30th day postpartum (DPP) for SCE by EC and TRUS.

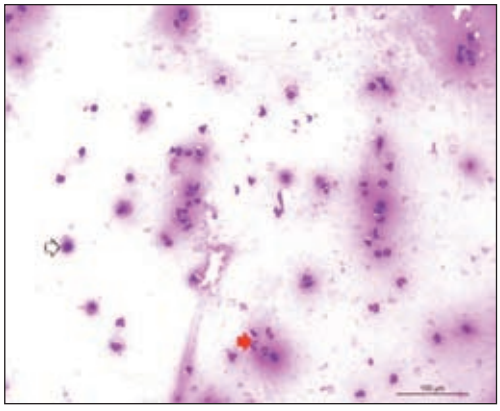
Sample collection

In primiparous and pluriparous cows, endometrial samples for cytological examination were collected using an endocervical cytobrush (Medigold pap smear kit), modified for use in large animals. The normal endocervical cytobrush handle was cut to approximately 2 cm in length and heat fixed onto a 45-cm solid stainless steel artificial insemination (AI) stylet. The instrument was prepared in a laminar airflow chamber and sterilised by ultraviolet radiation for 45 to 60 min before being used for sample collection. The brush with the fixed handle was then covered with a sanitary plastic sleeve to avoid vaginal contamination (Madoz *et al*., 2013; Robert *et al.,* 2017). The vulva was cleansed with cotton, scrubbed with povidone-iodine and the instrument was passed up to the external os of the cervix; the sanitary sleeve was punctured and the instrument was navigated

through the cervix and into the uterine body. After inserting into the body of uterus, AI stylet was pushed forward to expose the cytobrush to endometrial layer. The cytobrush was then rotated in full circle either in clockwise or anticlockwise direction to acquire cellular material from the endometrium. The cytobrush was retracted into the artificial insemination sheath prior to removal from the uterus. Care was taken to ensure that the cytobrush did not damage the endometrial surface.

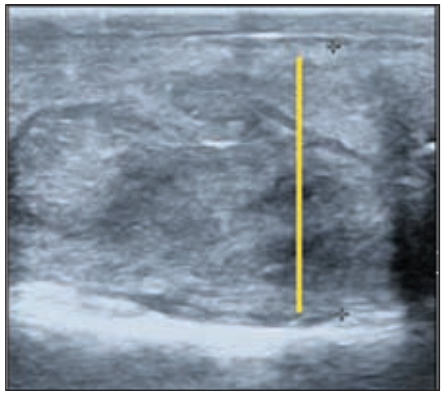
The slides were air dried and fixed with methanol for one to three minutes. The slides were stained using Field stains. After methanol fixation, the slide was flooded with Field stain B for 45 sec to one minute and Field stain A for 15 to 30 sec. Further, slides were washed in running tap water, dried and examined under the microscope (Gahlot *et al.,* 2017). The cytological evaluation was carried out at 100× magnification using a binocular microscope to categorise the different cell types, including PMN cells, endometrial epithelial cells (Fig. 1). The proportion of PMN cells counted out of the total cells was expressed as the per cent of PMN cells in endometrial cytology. A total of 100 cells were counted and based on the percentage of PMN cells, cows were categorised into two groups *viz*., SCE - positive or negative. The ratio of PMN cells to the endometrial epithelial cells was evaluated and animals with more than 18 per cent PMN on 30 DPP were considered SCE positive (Kasimanickam *et al.,* 2004). All the 74 cows were subjected to TRUS using a real time colour Doppler ultrasound scanner (MyLab™ Gamma, Esaote SpA, Italy) equipped with transrectal transducer of 5-10 MHz frequency on 30th DPP to record the diameter of the cervix (Fig. 2a) and uterine horn (Fig. 2b) as well as endometrial thickness (Fig. 2c). The gain, brightness and contrast were set in optimal range for each examination.

Fig. 1 Endometrial cytology in dairy cows (100X)

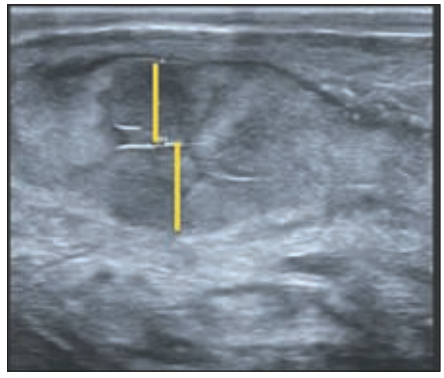


Black arrow- Endometrial cell
Red arrow- PMN cells

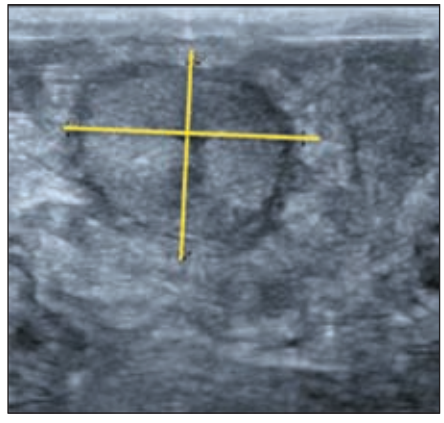
Fig. 2 Transrectal ultrasonography



2a. Cervical diameter



2b. Endometrial thickness



2c. Uterine horn diameter

In the second phase of study (V2), EC was performed on SCE positive cows (n=24) on the first day of exhibited oestrus after 45th day PP. The ratio of PMN cells to the endometrial epithelial cells was evaluated and animals with more than four per cent PMN on days 45 to 60 PP were considered positive for SCE (Madoz *et al.,* 2013). On these days, TRUS was also performed on 24 cows and the diameter of the cervix and uterine horn, as well as endometrial thickness, were recorded.

The data obtained were statistically analysed by independent t-test and repeated ANOVA to determine the efficiency of EC and uterine changes by TRUS on the diagnosis of SCE.

Results and discussion

Endometrial cytology

The samples for endometrial cytology were collected and evaluated for the per cent of PMN cells for the diagnosis of SCE. In the first phase (V1), a total of 74 EC samples were collected on 30th DPP. Out of 74 samples collected, 32 samples were positive for SCE on 30th day, accounting to 43.24 per cent. The mean (±SE) PMN cells per cent in the normal and SCE group were 5.55 ± 0.41 and 20.54 ± 0.48, respectively (Table 1). There was a significant difference between the normal and SCE group ($p< 0.05$) on 30th DPP. In the second phase (V2), 24 SCE positive animals were considered and the per cent of PMN (mean ± SE) cells was 7.56 ± 0.32. There was significant reduction in the mean PMN cells per cent (20.54 ±0.48 to 7.56 ±0.32) over time (30 to 54.63± 1.13 d) in cows with SCE.

Endometrial cytology at 30th DPP (V1), showed more than 18 per cent PMN cells in SCE positive animals (20.54 ± 0.48). The diagnostic criterion for SCE was replicated by Kasimanickam *et al.* (2004) in which they defined the presence of ≥18 per cent of PMN cells on 20 to 33 DPP period as confirmatory of SCE. Further, these authors reported that modified cytobrush had the sensitivity and specificity of 36 and 94 per cent, respectively for diagnosis of SCE. In the second phase (V2), the PMN cell per cent was 7.56 ± 0.32 which indicated SCE, as the diagnostic criterion used in this study was from Madoz *et al*. (2013) who defined a cut-off value of four per cent PMN cells for SCE after 45th DPP. The decline in PMN cells from phase I to II of the study (20.54 ± 0.48 *vs* 7.56 ± 0.32) corroborates to the reports of Bonnett *et al*. (1991), Gilbert and Schwark (1992) and Kasimanickam *et al*. (2005) who reported that there was a decrease in the number of PMN cells with the histological involution occurring after 40th DPP.

Transrectal ultrasonography

Transrectal ultrasonography was done on 30th DPP along with EC to determine its value in the diagnosis of SCE. All 74 animals in the first phase (V1), were considered in the study and evaluated for cervical diameter (CD), endometrial thickness (ET) and uterine horn diameter (UD). Mean (±SE) of CD, ET and UD on 30th day was 30.21 ± 3.73, 7.09 ± 2.81, 21.06 ± 3.28 mm for SCE negative and 31.58 ± 3.29, 7.1 ± 1.2, 21.2 ± 3.12 mm in SCE positive animals (Table 1). Statistical analysis of CD, ET, and UD of cows positive and negative for SCE revealed that there was no significant difference ($p > 0.05$) between them. In the second phase

Table 1 Endometrial cytology and TRUS findings in normal and SCE positive animals on different days of assessment

Group	Mean (±SE)			
	EC	TRUS		
	PMN (%)	CD (mm)	ET (mm)	UD (mm)
Normal (n=42) (30th DPP)	5.55±0.41[a]	30.21±0.73	7.09±0.41	21.06± 0.64
SCE (n=32) V1 (30th DPP)	20.54±0.48[b]	31.58±0.7	7.1±0.23	21.2±0.71
SCE (n=24) V2 (> 45 DPP)	7.56 ± 0.32[c]	31.24±0.71	8.22±0.24	21.82 ± 0.49

Mean with different superscripts differ significantly (a-b-c in columns) (p< 0.05).

(V2), TRUS was performed for SCE positive animals (n=24). There was no significant change from V1 to V2 in SCE positive animals with respect to CD, ET and UD (Table 1).

The diagnostic cut off value for cervical diameter was applied from Kasimanickam *et al*. (2004) in which they classified cervical diameter for diagnosing SCE as small (< 3.5cm), medium (3.5-5cm) and large (>5cm) in pure bred animals. They reported that there was a significant reduction in the size of cervical diameter over the period from 20 to 33 DPP and 33 to 47 DPP. This is in contrast to our results as maximum involution was reported before 30 DPP as all the SCE negative and positive animals had diameter less than 3.5 cm and no significant change from V1 (31.58 ± 0.7) to V2 (31.24 ± 0.71) was noticed after 30th DPP and the work was done in crossbred dairy cows which had smaller reproductive tract. The lack of any difference in CD between normal and SCE positive animals on 30th DPP as well as on days more than 45 DPP validates the findings of Kasimanickam *et al*. (2004) that SCE had less influence on CD.

The endometrial thickness in SCE negative and positive animals on 30th DPP was 7.09 ± 0.41 and 7.1 ± 0.23 mm, respectively. There was no significant difference between the groups in V1 (30th DPP). On V2 (54.63 ± 1.13 d), ET in SCE positive animals was 8.22 ± 0.24 mm. Despite the fact that there was no significant association between V1 and V2 concerning ET, a slight increase in endometrial thickness was noticed. The increase in thickness of endometrium could be attributed to oestrus in animal, caused by hyperaemia and oedematous changes in endometrium which was influenced by the modification of oestrogen and progesterone hormone ratio (Sugiura *et al.,* 2018). But in contrast to our results Purohit *et al*. (2013) appreciated a significant increase in endometrial thickness of SCE positive animals compared to negative animals. However, Polat *et al.* (2015) opined that use of TRUS for the diagnosis of SCE was leading to inconsistent results and inability to distinguish the changes in the endometrium. This is consistent with our findings that no association could be drawn between ET and SCE as no difference in ET was noticed between SCE positive and normal cows on 30th DPP. Moreover, the increase in endometrial thickness noticed in SCE positive cows on day 54.63 ± 1.13 PP could be endorsed to the response of endometrium to ovarian steroids and not to SCE because there was a reduction in PMN cells on EC during this period of study.

Sonographic assessment of uterine horn diameter (UD) in SCE positive and negative animals was 21.2 ± 0.71 and 21.06 ± 0.64 mm, respectively. There was no significant difference between SCE positive and negative animals on 30th DPP (V1). On V2, UD was 21.82 ± 0.49 mm, replicating that there was no significant variation from V1. Kasimanickam *et al.* (2004) classified UD into three categories (< 3.5, 3.5-5, >5 cm). This classification was done in purebred cattle to diagnose the SCE and to find the correlation between SCE and UD. They observed correlation between the UD and SCE due to a lack of significant association between both the variables. The size of UD slightly increased from first (21.2 ± 0.71 mm) to second phase (21.82 ± 0.49 mm) attributable to the effect of ovarian steroids on the uterus. No association could be drawn between UD and SCE as no alteration in UD was noticed between SCE positive and normal cows on 30th DPP and also from lack of a change after day 45 PP in SCE positive cows. Moreover, the UD findings did not correlate with the endometrial cytology findings to diagnose the SCE.

A lack of association between EC and TRUS for the diagnosis of SCE in PP cows was obvious in this study. This validates the findings by (Kasimanickam *et al*. 2004; Gayathri *et al*. 2020), who reported a low association between EC and TRUS in the diagnosis of SCE in cows. The nonexistence of association between these could be due to the fact that the two diagnostic modalities measured two distinctive contributory factors, EC quantified cellular response whereas, TRUS estimated clearance mechanism and physical changes in the size of uterus.

Conclusion

Endometrial cytology could discriminate between SCE positive and negative cows significantly at 30th DPP. It was

effective in diagnosing SCE after 45th DPP also, as a significant reduction in the per cent of PMN cells was obvious. However, this trend was not followed by the uterine parameters assessed by TRUS such as CD, ET and UD, as no significant difference between SCE negative and positive animals at 30th DPP and between SCE positive cows after 45th DPP was noticeable. The results of the study signify that EC endorses the merit of diagnosis of SCE in cows, while TRUS was impractical as a sole diagnostic modality for SCE in the present situation.

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Conflict of interest

The authors declare they have no conflict of interest.

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