Comparison of endocervical and endometrial cytology to diagnose sub-clinical endometritis in repeat breeding cows

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ABSTRACT

Repeat breeding due to sub clinical endometritis (SCE) is often diagnosed by cytological examination and the effect of endocervical inflammation (ECI) on increased hazards of pregnancy is inadequately investigated. In this study, endocervical cytology was compared with the golden standard test 'endometrial cytology' to detect SCE as a cause of repeat breeding in cows. Influx of Neutrophils and endocervical inflammation will reflect the status of endometrium and may indirectly reflects the ongoing sub clinical form of endometrial inflammation. Hence, this study was aimed at fixing threshold for PMN in endocervical cytology and comparing the same with golden standard technique. Repeat breeding cows associated with endocervical inflammation with >8% of neutrophils during standing estrus in endocervical cytology will have increased hazards of pregnancy. Our study suggested that a moderate concordance/association between ECI and endometrial inflammation (EDI) in repeat breeding cows, these findings were based on the endocervical and endometrial cytology. Based on the endocervical cytology examination 85% of repeat breeding cow posses EDI (SCE) with a diagnostic sensitivity of 86% and showing moderate clinical acceptability for application of endocervical cytology as diagnostic aid to detect SCE in repeat breeding cows.

Key Words: endocervical cytology, subclinical endometritis, repeat breeding, cross bred cows

INTRODUCTION

Repeat breeding due to subclinical endometritis (SCE) often creates inappropriate uterine environment which seriously impedes the fertilization process and embryonic growth (Sheldon *et al*., 2009). Vagaries of studies have demonstrated various diagnostic techniques to detect SCE with varying degree of accuracy and clinical applicability (Barlund *et al.,* 2008; Sheldon *et al*., 2008 and deBoer *et al*., 2014).

Cows with SCE were diagnosed by the uterine lavage based endometrial cytology harvesting bovine endometrial cells and PMNs with uterine lavage is considered to be a consistent golden standard technique

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and reflects the endometrial status. PMN cut –off of more than 3% in endometrial cytology beyond 35 Days In Milk (DIM) was designated as SCE or cytological endometritis (Galvão *et al*., 2011; El-Amrawi and El-Karim, 2019). Ahmadi *et al*., (2016) demonstrated the collection of endocervical mucus (ECM) in cows and their easy handling in contrast to uterine lavage (UL).It was hypothesized that the changes in endometrial cytology during the diseased condition will be reflected in the cervical cytology. Hence the present study was envisaged to investigate the efficiency of ECM in comparison with UL to detect SCE in cows.

MATERIALS AND METHODS

Experimental Animals

The present study was conducted at Gynaecology and Obstetrics section, Veterinary Clinical complex, Veterinary College and Research Institute, Tirunelveli. Pluriparous cows presented with an anamnesis of infertility were subjected for gynaeco-clinical and ultrasonographic examinations. A total of 28 crossbred cows with repeat breeding syndrome were selected for the study.

Assessment of PMN cells in endocervical mucus

During the observed oestrus the ECM was collected from the cows as described by Adnane *et al*. (2017). The vulva was cleaned with 0.1% potassium permanganate solution and a sterile uterine sheath attached to a 20 ml syringe was fixed at the endocervix by recto-vaginal method and ECM was aspirated. The mucus was smeared on glass slides and stained using Leishman stain for cytological evaluation as described by Adnane *et al*. (2017) with slight modification. Briefly the aspirated and agitated mucus was smeared on the grease free glass slide and air dried before staining, the dried smear was then stained by using Lieshmann stain . A total of 100 nucleated cells were counted in 10 random high-power fields (10 cells per HPF), and PMN cells ratio were averaged by HPF100 method (Adnane *et al*., 2018).

Assessment of PMN cells in uterine lavage

After collection of ECM, UL was collected from the same animal by adopting a slight modification (i.e low volume uterine lavage technique) in the collection procedure described by Cheong *et al*. (2012). Ten millilitre of sterile 0.9% normal saline solution was infused into the uterus using guarded sterile uterine sheath attached to a 20 ml syringe. The uterus was gently massaged and the fluid was aspirated. The recovered fluid was centrifuged at 2500 xg for 10 min. The sediment was smeared on the glass slides and stained using Leishman stain. A total of 100 nucleated cells were counted in 10 random high-power fields (10 cells per HPF), and PMN cells ratio was averaged by HPF100 method (Melcher *et al*., 2014).

Keeping UL PMN ratio of > 3% as cut off value for the detection of SCE (Fischer *et al.,* 2010), animals were categorized as with or without SCE. Based on these criteria the PMN % in ECM and UL were compared and analyzed.

Statistical analysis

The results obtained from enumeration of PMN from ECM was compared with UL for its diagnostic sensitivity, specificity, Lin's concordance correlation coefficient and Bland-Altman plot to assess the agreement between two assays and their concordance will be interpreted according the criteria established by Smith (2009).

RESULTS AND DISCUSSION

The mean percentages of PMN cells in animals with and without SCE were presented in Table 1. Based on the 'gold standard' of UL PMN cut-off ratio, 24 (85.71%) out of 28 repeat breeder cows were found to be positive for SCE.

The average PMN in the SCE positive animals was 5.90 (Tab.1) which was above the cut off value of 3 per cent (Sheldon *et al*., 2009 and Madoz *et al*., 2013). Influx of PMN into the endometrial mucosa of cows above threshold values is always associated with acute active endometrial inflammation and conception failure often leads to repeat breeding in cows (Turner *et al*., 2012).

Examination of ECM cytology revealed that the entire entire animals positive for SCE (as per UL cytology) had a clear differentiation in the mean percentage of PMN cells (8.20 ± 0.82) (Fig.1) when compared with those animals without SCE (3.75 ± 1.3) (Fig.2). The finding suggested that the endometrial cytological changes during SCE can be extrapolated to the endocervical cytology and hence a positive correlation could be ascertained between the two genital environments.

It was observed that the diagnostic sensitivity of endocervical cytology was 86% and specificity of 75% and Lin's correlation coefficient of 0.61 with moderate agreement ($p<0.05$). Our findings imply that appearance of PMN above threshold values in endocervical cytology of repeat breeding cows demonstrates a moderate concordance with occurrence of SCE (Table 1). Hence, our study found that the presence of >8% PMN in endocervical cytology can detect SCE in repeat breeding cows and such cows will have increased hazards of pregnancy failure. The mean difference in PMN's of endocervical () and endometrial region () was plotted by the Bland-Altman graphical method and the plot revealed moderate concordance between inflammatory status of the endometrial and endocervical region. The Bland-Altman plot method indicated a mean difference in PMN% between endometrial and endocervical specimens of 5% and 8% respectively with 95% limits of agreement of -14 to 12 % (Fig.3). This plot suggests that the difference in the inflammatory status of endocervix and endometrial mucosa can be compared with acceptable magnitude of mean difference between assays.

It can be concluded that the cows with endocervical cytology of above threshold value of > 8% PMN could be considered positive for SCE. By virtue of easy collection and handling, more efficient method requiring less expertise when compared to UL for collection and processing of samples, endocervical cytology can be used as a diagnostic criterion to detect SCE in repeat breeding cows.

Table: 1. Mean±SE values of PMN% in repeat breeder cows with or without SCE

	(%PMN)	
	SCE Negative (n=4)	**SCE Positive (n=24)**
Uterine Lavage (UL)	1.75±.025	5.90 ± 0.80
ECM	3.75 ±1.3	8.20 ± 0.82

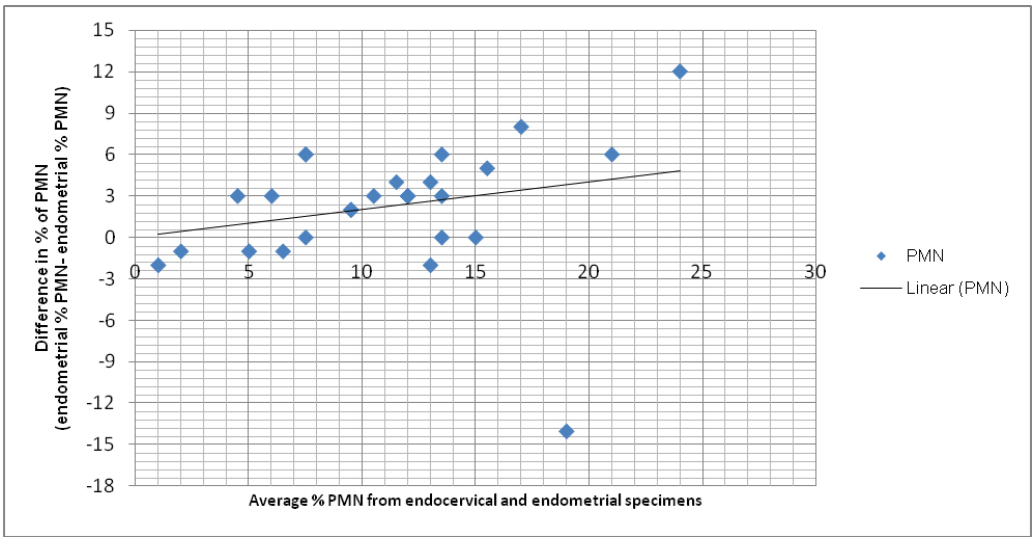

Fig.3. Bland-Altman plot of the data of the percentage of neutrophils (%N) in endometrial and endocervical specimens for each cow (n=24)

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REFERENCES

Adnane, M., Kieran, G., Meade and O' Farrelly, C. (2018). Cervico-vaginal mucus (CVM) – an accessible source of immunologically informative biomolecules. *Veterinary Research Communications*, **42:** 255–263.

Adnane, M., Rachid, K., Christian, H and England, G. (2017). Risk factors of clinical and subclinical endometritis in cattle: A review. *Turkish Journal of Veterinary and Animal sciences,* **41**: 1-11.

Ahmadi, M., Kadivar, R and Vatankhah, A.M. (2016). Evaluation of polymorphonuclear (PMN) cells in cervical sample as a diagnostic technique for detection of subclinical endometritis in dairy cattle. Asian *Pacific Journal of Reproduction*, **1:** 1-5.

Barlund, C.S., Carruthers, T.D., Waldner, C.L and Palmer, C.W. (2008). A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology*, **69:** 714 –723.

Cheong, S.H., Nydam, D.V., Galvao, K.N., Crosier, B.M., Ricci, A and Caixeta, L.S. (2012) Use of reagent test strips for diagnosis of endometritis in dairy cows. *Theriogenology*, **77**(5): 858-864

deBoer, M., LeBlanc, S., Dubuc, J., Meier, S., Heuwieser, W., Sebastian, A., Gilbert, R and Mcdougall, S. (2014). Invited review: Systematic review of diagnostic tests for reproductive-tract infection and inflammation in dairy cows. *Journal of Dairy Science,* **97:** 3983-3999

El-Amrawi and El-Karim(2019). Effect of subclinical endometritis on pro-inflammatory cytokines, prostaglandin-e2, muc-1 and cortisol levels in uterine lavage of repeat breeder dairy cows. *Advances in Animal Veterinary Sciences*, **7(s2):** 1-5.

Fischer, C., Drillich, M., Odau, S., Heuwieser, W., Einspanier, R and Gabler, C. (2010). Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the oestrous cycle and elevated in case of sub-clinical or clinical endometritis. *Reproduction Fertility and Development,* **22:** 818-829.

Galvão, K.N., Santos, N.R., Galvão, J.S and Gilbert, R.O. (2011). Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. *Theriogenology*,**76:**290-299.

Madoz, L., Giuliodori, M., Migliorisi, L., Jaureguiberry, M and de la Sota, R. (2013). Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of dairy science,* **97:** 2013

Melcher., Yvonne., Prunner., Isabella and Drillich, Marc.(2014). Degree of variation and reproducibility of different methods for the diagnosis of subclinical endometritis. *Theriogenology*, **82:** 1016

Sheldon, I. M., Williams, E. J., Aleisha, N. A., Deborah, M. N. and Herath, S. I. (2008). Uterine disease in cattle after parturition. *The Veterinary Journal.* **176:** 115-121.

Sheldon, I.M., Cronin, J., Goetze, L., Donofrio., G. and Schuberth, H. J. (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biology of Reproduction*, **81:** 1025-1032.

Smith, D.R., (2009). Veterinary Clinical Epidemiology: A Problem-Oriented Approach. Second Edition. London CRC-Press Ltd, P-59.

Turner, M., Gareth, H and Sheldon, I.M. (2012). Immunity and Inflammation in the Uterus. *Reproduction in Domestic Animals*, **47:** 402-409.