

## CONCEPTION RATE BASED ACCURACY OF DIFFERENT METHODS TO DIAGNOSE SUB-CLINICAL ENDOMETRITIS IN COWS

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### ABSTRACT

The status of genitalia (through rectal palpation) and cervico-vaginal discharge at spontaneous estrus was examined in 140 clinically normal cows (Jersey and Jersey crossbred). Endometrial cytology (n=15), bacteriological culture analysis (n=140) and Whiteside test (n=114) used for the diagnosis of sub-clinical endometritis (SCE) in cows recorded the prevalence as 40.0, 58.8 and 55.0%, respectively. With respective methods, the conception rate in SCE positive cows was 40.0, 32.4 and 16.7% and in SCE negative cows was 48.6, 55.6 and 77.8%. Thus, based on conception rate, the endometrial cytology was the most effective test for the diagnosis of SCE.

**Keywords:** Bacteriological culture, Conception rate, Endometrial cytology, Endometritis, Whiteside test

### INTRODUCTION

In repeat breeder cows, the high prevalence of sub-clinical endometritis (SCE) is hypothesized as the main etiological factor as most of the cases go undiagnosed (Parkinson, 2009). Due to the presence of polymorphonuclear (PMN) cells in the endometrial lumen, the SCE was first described as cytological endometritis (Gilbert *et al.*, 1998), and was subsequently standardized based on the negative effects on reproductive performance (Madoz *et al.*, 2014). The most reliable method for diagnosing SCE is cytobrush cytology (Barlund *et al.*, 2008). Despite the recognized negative effects of SCE on reproduction, the standardization of SCE diagnosis is not fully established (Pascottini *et al.*, 2015). The lack of on-farm method to diagnose SCE leads to inability of farmers to routinely monitor SCE in commercial herds. In this study, attempts were made to record prevalence of SCE by using different diagnostic methods and the effect of SCE on bovine reproductive performance.

### MATERIALS AND METHODS

For this study, 140 clinically normal cows were selected based on the absence of abnormal discharge on external inspection. Apparently clear cervico-vaginal discharge samples were collected in a sterile screw capped vial containing sterile swabs. Subsequently, the cows were inseminated with frozen thawed semen (0.25 ml) and their reproductive performance was recorded.

For bacteriological culture analysis, the collected discharge samples (n=140) were inoculated in nutrient broth and incubated for 24-48 h and turbidity was observed. Whiteside test was conducted in 114 cows. One ml uterine discharge was mixed with one ml 5% sodium hydroxide solution in a test tube, heated upto boiling point and subsequently cooled in running tap water. The appearance of yellow color was positive indication of infection. Depending upon the intensity of color development, the degree of infection was classified; no color (absence of infection), mild yellow color change (mild infection), and intense yellow color (severe infection; Anilkumar and Devanathan, 1996).

Endometrial samples were collected by cytobrush in 15 cows. Sample must contain epithelial cells to

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**Table 1: Reproductive performance of non-infected and sub-clinical endometritic (SCE) cows based on different diagnostic methods.**

Methods	SCE	Inseminated	Conception rate, %
Bacteriological culture, n=77	+	40	40.0
	-	37	48.6
Whiteside test, n=55	+	37	32.4
	-	18	55.6
Endometrial cytology, n=15	+	6	16.7
	-	9	77.8

confirm the correct site of collection. If no epithelial cells are seen, there is no assurance that the sample is taken from uterus. Slides for cytological examination were prepared by rolling the cytobrush on to a clean glass microscope slide, air dried and fixed in methanol for 15-20 min and stained with Giemsa stain for 45 min. The stained smears were examined at 100x by counting 100 inflammatory and other cell types. Cows with >5% PMN in the smear were regarded positive for SCE (Gilbert *et al.*, 2005).

## RESULTS AND DISCUSSION

In the present study, the inoculation of clear cervical mucus in nutrient broth indicated SCE in 55% cows, whereas, it was 58.8% on the basis of Whiteside test and 40% according to endometrial cytology. In a previous study, bacteriological culture of cervical mucus showed growth of aerobic organisms in 57.14% and SCE was diagnosed in 72% cows based on Whiteside test (Raja *et al.*, 2012). Using the threshold of  $\geq 5\%$  PMN in endometrial cytology, the prevalence of SCE was higher in present study than a previous study with 13.8% prevalence (Pothmann *et al.*, 2013).

In this study, following the collection of uterine discharge for the analysis of SCE, the cows were inseminated. Based on microbial growth, SCE positive cows exhibited 40% conception rate, whereas based on Whiteside test and endometrial cytology findings, 32.4% and 16.7% infected cows conceived (Table 1). The conception rate in non-SCE cows based on bacteriological, Whiteside and cytology test was

48.6, 55.6 and 77.8%, respectively (Table 1). Others recorded 5% and 47% CR after sampling cows with or without SCE (Salasel *et al.*, 2010).

Thus, the comparison of diagnostic techniques indicated endometrial cytology as the best method to diagnose SCE because the CR was lowest in infected animals. In fact, neutrophils constitute the first defensive barrier against invading pathogenic organisms, resulting in an increase in the PMN cell population within the uterine lumen (Herath *et al.*, 2009). Once a pathogen comes into contact with endometrium, the endometrium is stimulated to produce cytokines and chemokines that attract immune cells, in particular PMN cells, into the uterus (Galvao *et al.*, 2011).

In conclusion, uterine cytology was the most accurate method as compared to Whiteside test and bacteriological culture to diagnose sub-clinical endometritis in cows.

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