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Review Article

CYTOBRUSH: A NOVEL TECHNIQUE TO DIAGNOSE CYTOLOGICAL ENDOMETRITIS IN BOVINES

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Abstract: In the last decade, several new concepts in the field of diagnosis of cytological or subclinical endometritis came into light. This review summarizes the recent discussion about diagnosis and etiopathogenesis of subclinical endometritis. Subclinical endometritis is recognized by findings of endometrial cytology, which is usually done with the cytobrush-technique or by low-volume flushing of the uterus. The sampling procedure is negligibly invasive and has no adverse effect on successive conception rate. The impact of subclinical endometritis on reproductive performance is categorized by reduced conception rates, and prolonged days to first service and days open. In addition, it has been well established that subclinical endometritis has an effect on survival and quality of the embryo. Out of different methods of collection of uterine samples, cytobrush is the most reliable method for diagnosing bovine cytological endometritis.

Keywords: *Cytobrush-technique, Flushing, Uterus*

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Introduction

Cytological endometritis or subclinical endometritis (SCE) is single most vital reproductive impairments in dairy animals studied in the past decade. It can be defined as the superficial inflammation of the endometrium (no deeper than the stratum spongiosum) without noticeable clinical signs, but remarkably affecting reproductive performances [1-3]. The absence of pus in the postpartum genital tract does not mean that the tract is normal. The importance of subclinical endometritis has emerged over the last 15 years, with the realisation that cytological evidence of inflammation of the endometrium is associated with reduced fertility. Trans-rectal palpation of the uterus is the most common method of diagnosing postpartum uterine diseases; however, it is very difficult to find out SCE by this method [4,5].

Cytology is considered the best technique to diagnose SCE due to its feasibility and fair reliability. Different techniques have been described to obtain endometrial samples for cytological examination in both mares and cows, such as cotton swab, uterine biopsy, low volume uterine lavage (LVF), or Cyto brush (CB) [6,7]. Out of different methods of collection of uterine samples, CB is the most reliable method for diagnosing bovine cytological endometritis [8]. In 2004, for the first time, the CB was used to obtain endometrial samples in cows [9].

Prevalence and detrimental effects of subclinical endometritis

SCE is the inflammation of the uterine endometrium without mucopurulent material accumulation in the vagina and any systemic symptom [10]. Its prevalence varied between 20 - 53 % from 20 - 60 days postpartum.

The incidence of SCE in cattle and buffaloes was found 15 % and 26 %, respectively. Postpartum uterus is very prone to get several types of microbial contamination that can cause severe economic losses to farmers in the form of an increasing in the number of services per pregnancy, increasing the length of calving-conception interval, abortions, infertility and death.

Etiopathogenesis

SCE is the inflammation of the endometrium without clinical signs and evidence of infection [11,12]. In early postpartum period, uterine pathogens may compromise the reproduction both by causing direct endometrial damage and by producing toxins [13,14].

Pathogenic bacteria which are associated with this disease condition are *Escherichia coli, Trueperella pyogenes, Fusobacterium necrophorum, Prevotella* and *Bacteroides* [15-18]. They produce bacterial endotoxins which are known to elicit negative impact on reproduction as they may affect estradiol and progesterone secretion and alter follicular growth and the normal development of the corpus luteum; causing ovulation failure by interfering with LH production; prolong the life span of corpus luteum by increase PGE2 secretion and induce embryo mortality [18-22].

Polymorphonuclear granulocytes represent the first and principal immunologic defense mechanism in the uterus [23-25]. An elevated number of polymorphonuclear neutrophils (PMNs) in the uterine lumen indicate an inflammatory reaction of the endometrium. The suggested threshold value for polymorphonuclear cells (PMN) as diagnostic for subclinical endometritis depends on the time postpartum and varies from 5 to 18% [26]. It has also been publicized that an overall threshold of 5% PMN is entitled for all cows and buffaloes between 21 and 62 days postpartum.

Kasimanickam *et al.* (2004) found >18 percent neutrophils at 20-33 days postpartum or >10 percent neutrophils at 34-47 days postpartum in uterine samples as an indicative of subclinical endometritis whereas, Gilbert *et al.* (2005) found 5 percent neutrophils at 40 to 60 days postpartum as an indicator of sub-clinical endometritis, while Barlund *et al*. (2008) used a neutrophil threshold value of 8 percent at 28-41 days postpartum in cattle to declare endometritis. Various research workers have used different threshold values of PMN cells for the identification of sub-clinical endometritis.

Different methods of cytological sample collection

Cytology is considered the best technique to diagnose SCE due to its feasibility and fair reliability. Different techniques have been described to obtain endometrial samples for cytological examination in both mares and cows, such as cotton swab, uterine biopsy, low volume uterine lavage (LVF), or Cyto brush (CB). Out of different methods of collection of uterine samples, CB and LVF are found less invasive. Further, the CB method is less harmful than LVF because the fluid (normal saline, 0.9%) used in LVF produces endometrial irritation. Moreover, the saline solution extends the time required to obtain samples (a 17% failure to obtain saline) and increases the alteration of cells harvested via LVF [26]. So, CB as the most reliable method for diagnosing bovine cytological endometritis. Animals with subclinical endometritis do not show any clinical sign of endometritis. Endometrial cytology is the most used technique to diagnose SCE both in field and research [27]. The measurement of the proportion of PMNs in cytology slides is the hallmark for SCE diagnosis.

Cytological sample collection by cytobrush and slide staining

Cytobrush assembly consists of stainless-steel rod and cytobrush, which is guarded by a stainless-steel sheath. This assembly needs to be introduced into the uterine body through the vagina and cervix. Then, stainless-steel sheath is retracted to expose the cytobrush and rotated twice in a clockwise direction to obtain cells from the endometrium. After removing the cytobrush assembly from the vagina, the cytobrush containing cellular material is rolled onto a glass slide and air dried. The slides should be stained using Giemsa stain followed by examination under a microscope (400× magnification). The numbers of epithelial endometrial cells and PMNs are counted (up to 200 cells per slide) and the percentage of PMNs present thus calculated [28-30].

Future prospect

Cytobrush technique is simple, non-invasive, consistent and efficient diagnostic aid which provides adequate uterine cells to accomplish both cytology and gene expression analysis in single sample. Research studies have supported that endometrial cytology by cytobrush technique is most efficient and early diagnostic technique which can be used along with microbial assay for detecting existence and severity of endometritis. Also, antimicrobial sensitivity analysis of cytological sample helps in accurate selection of antibiotic thereby minimising both cost of therapy and antimicrobial resistance. Thus, cytobrush technique is advantageous in diagnosis as well as treatment of subclinical endometritis in bovines.

Application of review: Article will provide information on cytobrush technique for diagnosing subclinical endometritis.

Review Category: Veterinary and Animal Sciences

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