

WHITESIDE TEST AND ENDOMETRIAL BIOPSY FOR DIAGNOSIS OF ENDOMETRITIS IN REPEAT BREEDING COWS

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ABSTRACT

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Subclinical endometritis is considered to be one of the contributing factor for the repeat breeding problems in cows and hence diagnosis of subclinical endometritis becomes imperative. The present study was aimed to assess the efficacy of endometrial biopsy and white side test for diagnosis of subclinical endometritis. Twenty nine repeat breeding and ten cyclic cows with apparently normal fertility were utilized to compare the white side test, bacteriological culture using the cervical mucus and uterine biopsy. Twenty one cows were positive for white side test and twelve showed growth in bacteriological culture with different degrees of changes in the endometrium. It was concluded that histopathology of the endometrium may clearly indicate the future fertility status when compared to white side test and bacteriological culture analysis.

Key words: Endometritis, Endometrial Biopsy, White side test.

Repeat breeder cow is one that has normal or nearly normal estrous cycle with apparently no palpable abnormalities of the reproductive tract, but fails to conceive to three or more AI with good quality semen (Roberts, 1971). Even though there are many causes for repeat breeder condition, subclinical endometritis is found to be the pathological feature (Singh *et al.*, 1983). Subclinical endometritis is characterized by inflammation of the uterus which results in significant reduction in reproductive performance in the absence of clinical signs of Endometritis (Sheldon *et al.*, 2009). Hence the present study was carried out to investigate the efficacy and usefulness of white side test and endometrial biopsy to establish status of the endometrium.

A total of 1793 cows that were treated for infertility at Madras Veterinary College teaching hospital for the past five years (2005-2009) were analyzed to record the incidence of repeat breeding. Twenty nine crossbred repeat breeding cows with the history of not conceived to three consecutive AI, calved more than once and less than ten years old were selected (group I). The vulval lips of all repeat breeding cows were observed and the deviation from vertical conformation was recorded. Gynaecological examination of these cows revealed no palpable abnormalities of the reproductive organ and had apparently normal estrual mucus. Ten normal fertile and regular cyclic animals were kept as control (group II) and the study was conducted during one year period from October 2009 to October 2010.

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With aseptic precautions using the sterile AI sheath connected with 20ml syringe, cervical mucus which represented the endometrial secretion was collected. One ml of the cervical mucus was mixed with one ml of 5 per cent sodium hydroxide solution in a test tube and

heated up to the boiling point and subsequently cooled in running tap water. The appearance of yellow colour was taken as positive indication of infection. Depending on the intensity of colour development the degree of endometritis was classified as 1 – No colour (absence of infection), 2 – mild yellow colour change (mild infection), 3 – intense yellow colour (severe infection) (Anilkumar and Devanathan, 1996).

In animals which were positive for white side test, the cervical mucus was sent for bacteriological culture and a piece of endometrial tissue was collected using the Albuchins uterine biopsy catheter, and transferred into the container containing 10 per cent formalin for histopathological examination.

The incidence of repeat breeding among infertility cases was observed to be 16 per cent. Subclinical endometritis was diagnosed in 72 per cent (21/29) of cows based on white side test of which 38 per cent (8/21) of cows had severe form of infection and 62 per cent (13/21) of cows had mild form of infection. The bacteriological culture analysis of cervical mucus showed growth of aerobic organisms in 57.14 per cent (12/21) of repeat breeding cows. The histopathology of endometrium showed marked cellular changes of different degree, such as glandular hyperplasia (13/21), moderate glandular hyperplasia (6/21), degenerative changes of the endometrium (13/21), cystic dilatation (2/21), mononuclear cell infiltration (3/21) and peri vascular fibrosis (1/21). Among the repeat breeding cows which had periglandular fibrosis 52.3% (11/21) showed involvement of 5-10 layers, 23.8% (5/21) 10-15 layers, and 9.5% (2/21) >15 layers. Twenty per cent metarrhagia and 40 per cent polymorphonuclear cell infiltration were observed in control animals.

Thirty eight per cent (8/21) of cows that had severe form of infection based on white side test showed characteristic changes in endometrium, like glandular hyperplasia with periglandular fibrosis of 15-20 layers, necrotic changes with cystic dilatation which indicated poor fertility (Mouli Krishna *et al.*, 2010), whereas 62% (13/29) of cows that had mild form of infection showed glandular hyperplasia with mononuclear cell infiltration

which is indicative of mild chronic endometritis (Morrow, 1986). The vulval conformation in 70 per cent of the repeat breeding cows was found to be in the form of inverted L shaped. During puerperium the cows should have an uneventful recovery by achieving uterine involution, eliminating bacterial content, reepithelization of endometrium and resumption of hypothalamo pituitary ovarian axis activity. This event is totally based on the nutritional status and a normal peri and postpartum period. But most of the cows suffer a nutritional imbalance and the body resources are mobilized to meet the production demand. To meet over these demand non-esterified fatty acids released from lipid stores are taken up by the liver, where they may be oxidised to carbon dioxide to provide energy, or partially oxidized to produce ketone bodies or acetate (Konigsson *et al.*, 2008). Glucose availability may be supplemented by increased catabolism of amino acids stored in skeletal muscle and other tissue proteins, thus increasing urea production. Collard *et al.* (2000) and Katoh (2002) stated that accumulation of liver lipids reduced the uterine defense mechanism and make the animal prone for uterine diseases.

Based on the study it can be postulated that this mobilization of tissue stores may cause alteration of vertical contour of the vulva to oblique or inverted L shape, which result in pooling of urine in vagina and further predispose to ascending infection of the uterus. This is supported by the results of this study in which cows with poor vulval contour are having permanent damage like periglandular fibrosis, cystic dilatation with glandular hyperplasia. Twenty per cent (2/10) cows in control showed mild form of infection based on white side test and ten per cent (1/10) showed growth of aerobic organism. The colour change observed in white side test in control cows might be due to neutrophil infiltration and metrorrhagia which are normally seen during estrogen dominance (Ohtani *et al.*, 1993). This clearly indicated the efficacy of white side test in diagnosis of subclinical endometritis. It can be inferred that animals treated for subclinical endometritis based on whiteside test at field level, when they fail to conceive repeatedly, it is suggested to obtain uterine biopsy to ascertain the endometrial status and predict the fertility status.

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