BACTERIOLOGICAL AND BIO-HISTOPATHOLOGICAL STUDIES IN REPEAT BREEDING COWS

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SUMMARY: Biopsy tissues of uterine endometrium were taken from 11 repeat breeding cows and examined histologically. Of the 11 cows 5 (45.45%) showed mild while 6 (54.45%) moderate endometritis. Mild endometritis was characterized by denudation of lining epithelium and moderate infiltration of neutrophils and lymphocytes in the lamina propria. Some of the endometrial glands showed degeneration and necrosis of their epithelial cells. In moderate endometritis, in addition to the above findings there was dense infiltration of lymphocytes and plasma cells in the stratum compactum and early fibroblastic proliferation around some endometrial glands. There was also found periglandular and perivascular leukocytic infiltration. The endometrial glands at some places were distorted and cystic. Uterine mucus was also collected from the repeat breeding cows and the organisms isolated were correlated with the type of endometritis. Mild endometritis was associated with Staphylococcus (19.05%), Bacillus (19.05%), Streptococcus (9.52%), Escherichia (4.76%) and Proteus (4.76%). In moderate endometritis there were found Staphylococcus (14.28%), Streptococcus (14.28%), Bacillus (9.52%) and Corynebacterium (4.76%).

Key Words: Endometritis, histopathology, bacteriology.

INTRODUCTION

There is no alternate of fertility, however reproductive disorders causes infertility ultimately leading to substantial economic losses through increased calving intervals, loss of milk production and culling of useful breeding animals. Specific and non-specific infectious agents during pre and postpartem periods frequently invades the uterus and produces metritis and endometritis leading to repeat breeding. The isolation of micro-organisms along with histopathological studies of uterine endometrium by biopsy are known to be of paramount importance in the diagnosis, prognosis and rational treatment of repeat breeding animals (3,2,1). Such vital studies have scarcely been conducted in Pakistan. Therefore the present project was undertaken to find out the uterine bacterial organisms associated with different types of histopathological changes in repeat breeding cows.

MATERIAL AND METHODS

Eleven repeat breeding cow were selected from among animals brought for artificial insemination (Al) at the clinic of the Department of Animal Reproduction, University of Agriculture, Faisalabad and breeding history was recorded. That included last data of service, previous number of services provided and number of previous parturitions. These cows showed uterine discharge consisting of few pus flakes and were not conceived at least by three previous inseminations (A. I or natural). For bacteriological studies uterine discharge from all the cows was collected by passing a sterilized plastic rod (normally used for A. I) through the cervix. To the outer end of the rod, a syringe with a short rubber tube was connected for suction of mucus and after suction, both ends of the rod were hermetically sealed. The rods were then brought to the laboratory for further analysis.

Each rod was broken and the mucus was cultured in nutrient broth at 37ºC for overnight. The broth was sub cultured on plates of nutrient, blood, MacConkey's agar and staph-110 medium. Bacterial growth was recultured on nutrient agar plates to obtain pure culture. The cultural, morphological and biochemical tests were applied to identify and characterize the type of organisms (4).

Biopsy tissue of uterine endometrium were obtained with a biopsy punch from all the cases under observation. The tissues were preserved in Bouin's solution for 24 to 48 hours and dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-6 micron thick were cut, stained with H and E stain (12) and examined under microscope for histopathological studies.

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Table 1: Bacterial isolates from 33 metritic cows.

Bacteria Isolated	As single isolates No. (%)	As mixed isolates No. (%)	Total No. (%)
Staphylococcus spp.	3(14.29)	4(19.05)	7(33.34)
Streptococcus	2(9.52)	3(14.29)	5(23.81)
Bacillus	-	6(28.57)	6(28.57)
Escherichia	-	1(4.76)	1(4.76)
Proteus	-	1(4.76)	1(4.76)
Corynebacterium	-	1(4.76)	1(4.76)
Total	5(23.81)	16(76.19)	21(100)

Table 2: Correlation between bacterial isolate with type of endometritis.

Bacteria Isolated	Type of endometritis		
	Mild (n=5)	Moderate (n=6)	Total (n=11)
Staphylococcus	4(19.05)	3(14.28)	7(33.33)
Streptococcus	2(9.52)	3(14.29	5(23.81)
Bacillus	4(19.05)	2(9.52)	6(28.57)
Escherichia	1(4.76)	-	1(4.76)
Proteus	1(4.76)	-	1(4.76)
Corynebacterium	-	1(4.76)	1(4.76)
Total	12(57.14)	9(42.85)	21(100)

RESULTS

Isolation and identification of organisms from mucus samples of 11 repeat breeding cows under observation showed bacterial isolates as given in Table 1.

Of the 11 cows 5 (45.45%) yielded single and 6 (54.55%) mixed type of bacteria.

Based on degree of reaction, the histopathological findings of uterine tissues obtained by biopsy revealed mild endometritis in 5 (45.45%), and moderate in 6 (54.55%) cows. In mild endometritis, the changes included denudation of lining epithelial cells and moderate infiltration of neutrophils and lymphocytes in the lamina propria. Some of the endometrial glands showed degeneration and necrosis of their epithelial cells. In moderate endometritis, in addition to the above findings, there was dense infiltration of lymphocytes and plasma cells in the stratum compactum and early fibroblastic proliferation around some endometrial glands. There was also found periglandular and perivascular leukocytic infiltration.The endometrial glands at some places were distorted and cystic. The correlation of bacterial isolated with histopathological findings has been presented in Table 2.

The predominent organisms involved in mild endometritis were Staphylococcus, Bacillus and Streptococcus, in moderate endometritis these were Staphylococcus and Streptococcus, while Corynebacterium which is known to be associated with severe type of endometrial reaction was isolated in one case of moderate endometritis.

DISCUSSION

Repeat breeding in animals has great economic importance as it causes increased calving interval, less number of offsprings, decreased milk production and wastage of time and money on treatment. Among various causes of repeat breeding, bacterial infections has prime importance.

The incidence of bacterial isolate in the present study was in confirmity with Osman *et al.* (16), who reported 42 per cent single and 48 per cent mixed type of bacterial population. No sterile case was detected in the present study which was not in congruence with the above author, he reported 9 per cent incidence of sterile cases. This could be due to less number of case records and malpractices adopted by milk made for let down of milk as insertion of switch of tail in to vagine, hence introducing infection.

The type of bacterial isolates in the present study coincided with the findings of Osman *et al.* (16) and Zafrucas (22). They also found Staphylococcus, Streptococcus, *E. coli*, Corynebacterium, Proteus, in addition Pseudomonas, which was not recovered in our study. Rahman *et al.* (17), in addition reported Klebsiella and Pseudomonas. Shouman *et al.* (20), in addition reported yeasts and moulds. The organisms like Klebsiella, Pseudomonas were not recovered in our study, may be because of less number of case recorded or the animals included in the study were repeat breeder with minor pus flakes in their uterine discharge. Further, the uterine wall appeared relatively normal when examined through the rectum.The efforts were not made to isolate yeasts and moulds.

Seitaridis and Tsangaris (19), among 50 repeat breeder cows have detected light, moderate and severe degree of endometritis in 20, 50 and 26 per cent respectively. In an other study, Hartigan *et al.* (11), has reported mild in 21, moderate in 4 and severe endometritis in 9 cows. The present limited study conducted, favors the findings of Seitaridis and Tsangaris (19) as high incidence of moderate endometritis. Severe type of endometritis

was not detected, this may be because of less number of animals examined in the study and the other factors as cited above.

The histological changes recorded in mild endometritis were the same as has been reported by Sagartz and Hardenbrook (18) and Brus (3). In moderate endometritis, the histological changes were in line with the studies of Brus (2), DeBois (5), Kampelmacher (13) and Deeb *et al.* (6).

In mild and moderate type of endometritis Staphylococcus and Streptococcus were the organisms recovered with high incidence, while Corynebacterium was isolated only from single case. Aziz-ud-Din (1) isolated Corynebacterium and Staphylococcus. Nunn (15) reported saprophytic bacilli, Staphylococcus and Streptococcus. In an other study Glatgel and Chadli (8) reported Staphylococcus and Streptococcus from endometritic cows.

The present literature does not give any clear answer as to whether the uterus of cows ready for successive insemination (repeat breeder) is free or not from micro-organisms. Some worker believes that the uterus of cows at the time of first or successive inseminations is nearly always sterile (5, 7) while several other described that repeat breeding is mainly due to the presence of sub clinical infections in the uterus (9,10,14).

Keeping in view the present findings and cited statement by later authors, it is suggested that, of the repeat breeding animals, bio-histopathological study along with isolation of micro-organisms and sensitivity be routinely performed to ascertain the cause and prognosis of the case.

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