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Research Article

Comparative efficacy of immunomodulators and sensitive antibiotic on endometrial histopathology in endometritis in crossbred cows

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ABSTRACT

The present study was undertaken to evaluate the efficacy of immunomodulators (*E. coli* LPS & oyster glycogen) and sensitive antibiotic on endometrial histopathology used for treatment of endometritis in crossbred cows. Forty eight cows tested positive for endometritis on the basis of appearance and white side test were randomly divided into 4 groups with 12 cows in each group. In group A, 100 µg of *E. coli* LPS once, in group B, 500 mg oyster glycogen (OG) once, in group C, most sensitive antibiotic i.e. enrofloxacin was infused intrauterine for 3 consecutive days and in group D, 20 ml PBS was infused once which served as control. Endometrial biopsies were collected at 0 hour i.e. before treatment and at 72 hours after treatment from all the groups for histopathological study.

The incidence of acute/mild type of endometritis was significantly ($P<0.01$) higher than subacute and chronic endometritis. A significant reduction in the cases of endometritis was observed after treatment with LPS ($P<0.01$) and OG ($P<0.05$). Treatment with most sensitive antibiotic also reduced the cases of endometritis but this reduction was non significant. Infusion of LPS and OG reduced the cases of denuded epithelium and completely cured pseudostratification of epithelium. Increased infiltration of PMN cells and mononuclear cells in the endometrial epithelium after PLS and OG infusion are indicative of increase in the degree UDM. Infusion of LPS caused complete recovery of the different types of pathological conditions of the endometrial glands except one case of cystic dilatation. Infusion of OG also led to significant recovery except 3 chronic cases of endometritis. All lymphoid follicles were also cleared following OG infusion probably because of its immunomodulatory effect. Infusion of antibiotic intrauterine resulted in to 25% increase in histopathological changes associated with endometrial epithelium. In untreated control group, histological studies revealed no change in endometrial epithelium as compared to before treatment. It was revealed that LPS and OG significantly cured all types of endometritis and is better therapy for endometritis than antibiotic. Repeated intervention of endometrium for intrauterine infusion of antibiotic markedly enhanced the condition of mild endometritis.

Key words: Endometritis, immunomodulator, *E.coli* LPS, oyster glycogen, antibiotics, endometrium, hostopathology, crossbred cows.

Endometritis often has been the major cause of repeat breeding (Roberts, 1971; Arthur *et al.*, 1989). The incidence of endometritis amongst the repeat breeder bovines has been reported from 8.5 to 56.6% in cows (Rahumathulla *et al.*, 1986; Maneta *et al.*, 1990; Singh and Sinha 1991; Mohanty *et al.*, 1992; Sawamukai *et al.*, 1994). Bacterial endometritis occurs more often as compared to viral, protozoal or fungal endometritis. The incidence of bacterial endometritis amongst the repeat breeding cows has been found to be 77 to 100% (Easley *et al.*, 1951; Dholakia *et al.*, 1987; Shukla, 1988; Singh *et al.*, 1989; Dohmen *et al.*, 1996).

In a study on repeat breeder cows, an incidence of 53.33% subclinical endometritis was observed. Inflammatory cell infiltration, degeneration and

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desquamation of epithelial cell lamina, glandular dilatation and periglandular fibrosis were determined as 53.33%, 33.33%, 20.0% and 13.33% of the cows having endometritis (Dogan *et al.*, 2002). Javed and Khan (1991) observed mild endometritis in 45.45% and moderate in 54.55% cows with history of repeat breeding problem.

Treatments of endometritis with parenteral or intrauterine infusions of various drugs have met with varying degree of success (Oxender and Seguin, 1976; Shukla and Pandit, 1989). The inconsistent results, repeated treatment, chances of development of drug resistance, high cost of treatment and compulsory milk disposal after antibiotic treatment makes it uneconomical (Hussain, 1989). More recently, besides traditional procedures of treatment of endometritis in cows some new designated as biologically active immunomodulators have been used. The intrauterine infusion of *E. coli* lipopolysaccharide (*E.coli* LPS), Oyster glycogen (OG), Leukoterine B4, Hyper immune serum etc. makes a part of them (Singh *et al.*, 2003; Saini *et al.*, 1999; Zerbe *et al.*, 1996). Some authors observed effective influx of neutrophils and proteins in to the uterine lumen by the intrauterine infusion of these immunumodulators. However, no work regarding the effect of immunomodulators on endometrial histopathology has been conducted to prove its efficacy at cellular level. Hence the present study was conducted to investigate the endometrial histopathological changes in relation to treatment with immunomodulators and sensitive antibiotics.

MATERIALS AND METHODS

Forty eight crossbred cows with endometritis were selected on the basis of history of repeat breeding, thorough per rectal examination and physico-chemical characteristic of cervical mucus. Cows with purulent or muco-purulent estrual discharge or containing white flakes and positive reaction to white side test were considered positive for endometritis. Moreover, animals with history of repeat breeding and clear discharge but positive reaction to white side test were also included in the study. All the cows were examined per-rectally to rule out any anatomical defects of -genitalia and ovari abnormalities. Cows were divided randomly into 4 groups. In group A cows, *E. coli* LPS (100 µg) was infused intrauterine once, in group B cows, oyste glycogen (500 mg) was infused intrauterine once, in group C cows, sensitive antibiotic i.e. enrofloxacin (1000 mg in 30 ml distilled water) on the basis of *in-vit* antibiogram was infused intrauterine for 3 days and group D cows were served as untreated control in which PBS (20 ml) was infused intrauterine once.

Endometrial biopsies were collected from all the cows of each group before the start of treatment (at 0 hour) and at 72 hour after the beginning of treatmen The biopsy technique consists of collecting a representative piece of endometrium aseptically using Nielson's Uterine biopsy punch from either of the horns, 2 to 3 inches deep from the uterine body. The biopsy punch was introduced into one of the horns of the uterus as in the artificial insemination. The piece of endometrium which lodged in to groove of biopsy instrument was released and preserved in vials containing 10% buffered neutral formalin for further histopathological examination.

The tissues were dehydrated in graded alcohol ranging from 70% to absolute, cleared through xylol and embedded in melted paraffin. The tissues were micro-sectioned (4-6 micron thickness) and stained with Haematoxylin and Eosin stain for histopathologica examination (Lillie, 1965).

The slides were studied in relation to -

1. Nature of the endometrial epithelium (denudation, pseudostratification, infiltration of cells within the epithelial cytoplasm and sub epithelial regions).
2. Nature of endometrial glands (hypertroph hyperplasia, atrophy or necrosis and glandular cysts).
3. Cellular infiltration in the stratum compactum or in the stratum spongiosum.
4. Lymphoid nodule/follicle formation.
5. Hyaline degeneration

RESULTS AND DISCUSSION

A total of 48 endometrial biopsy samples were collected from crossbred cows suffering from endometritis. Present study revealed significantly ($P<0.01$) higher incidence of acute/mild type (58.33%) of endometritis than sub acute (14.58%) and chronic (27.08%) endometritis indicating that pathogens causing acute inflammatory conditions of the uterus are the important factor responsible for endometritis in cows. When this acute/ mild inflammatory condition overcomes the natural defense mechanism of the uterus may lead to subacute or chronic endometritis. However, contrary to it Javed and Khan (1991) observed mild endometritis in 45.45% and moderate in 54.55% cows with history of repeat breeding problem.

A significant recovery ($P<0.01$) from the endometritis (58.34%) was observed following LPS infusion. Infusion of OG also led significant recovery ($P<0.05$) in 33.34% cases. Infusion of enrofloxacin cured only 25.00% cases while in untreated control 8.34% cows were recovered (Table 2).

The various histopathological changes observed in the endometrium of cows suffering with endometritis compared before and after treatment.

Nature of endometrial epithelium

In 18 cases (37.50%), changes associated with endometrial epithelium were observed prior to start of treatment. The endometrial epithelium in 10 cases (20.83%) was denuded (fig.1). This might be due to the presence of mild irritant(s) in the form of bacterial or viral infection of low virulence which can provoke catarrhal inflammation of the surface epithelium and may produce necrosis and desquamation of the epithelium and may also be responsible for infiltration of mononuclear cells and plasma cells with little hyperemia. Along with catarrhal inflammation in one case (2.08%), infiltration of mononuclear cells was observed in the surface epithelium. Pseudostratification of surface epithelium was observed in 7 cases (14.58%) showing sub-acute/chronic reaction (Table 1, fig 2).

Infusion of LPS and OG reduced the cases of denuded epithelium due to mild irritation to the endometrium. All the cases of pseudostratification of epithelium were also cured. This may be because of infusion of LPS and OG causes chemotaxis of polymorphonuclear neutrophil granulocytes (PMNs) into uterine lumen resulting into phagocytosis of pathogens present in the uterine lumen and hence enhancing the natural healing processes in cases of denuded epithelium

Table 1: Overall histopathology of endometrium of cows with endometritis before and after treatment.

Sl. No.	Histopathological changes	Before Treatment	After Treatment
1	Nature of endometrial epithelium	18(37.50%)	20(41.66%)
	(a) Denuded epithelium	10(20.83%)	8(16.66%)
	(b) Pseudostraitification	7(14.58%)	2(4.16%)
	(c) Infiltration of cells	1(2.08%) [a]	10(20.83%) [b]
2	Nature of endometrial glands	15(31.25%)	9(18.75%)
	(a) Hyperplasia	2(4.16%)	1(2.08%)
	(b) High columnar cells	1(2.08%)	-
	(c) Denuded & detached epithelial cells	2(4.16%)	2(4.16%)
	(d) Cystic dilatation	4(8.33%)	4(8.33%)
	(e) Infiltration of cells	4(8.33%)	1(2.08%)
	(f) Atrophy of gland	2(4.16%)	1(2.08%)
3	Cellular infiltration in the stratum compactum and stratum spongiosum	10(20.83%) [a]	2(4.16%) [b]
4	Lymphoid nodule formation	3(6.25%)	1(2.08%)
5	Hyaline degeneration	2(4.16%)	1(2.08%)
	Total	48 (100%)[A]	33 (68.75%)[B]

Means with different superscripts (a, b) between columns vary significantly ($P<0.05$).
Means with different superscripts (A, B) between columns vary significantly ($P<0.01$).

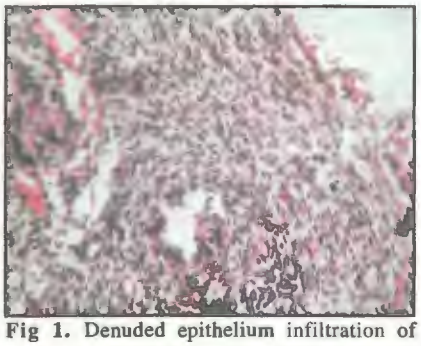

Fig 1. Denuded epithelium infiltration of mononuclear cells, hemorrhage in stromal tissue & disruption of endometrial gland, H & E stain,400 x.

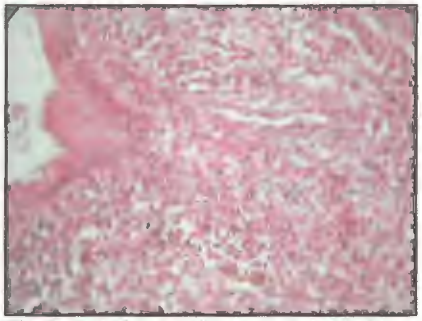

Fig 2. Psuedostraitification of epithelium along with distorted stroma, H & E stain, 400 x.

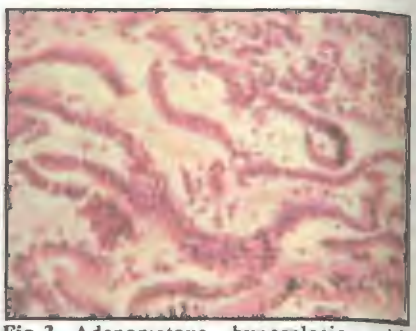

Fig 3. Adenomatous hyperplasia with complete loss of stroma, H & E stain, 400 x.

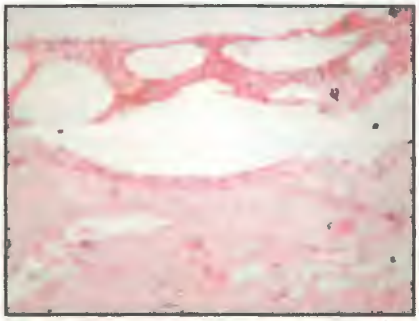

Fig 4. Extreme glandular-cystic dilatation with appreciable loss of stroma, H & E stain, 400 x.

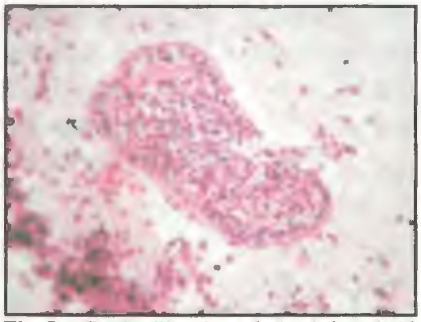

Fig 5. Cystic dilatation of gland, lymphoid nodule formation in the endometrial gland, loss of stroma with infiltration of PMN cells & mononuclear cells, H & E stain, 400 x.

Fig 6. Glandular-cystic hyperplasia with appreciable loss of stroma, flattened epithelial cells & periglandular hyalinization in few glands, H & E stain, 400 x.

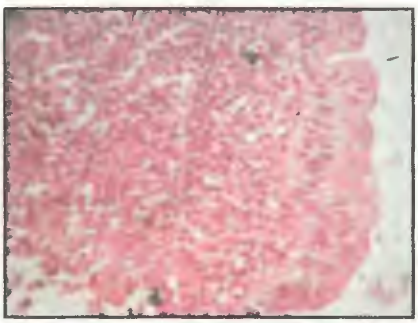

Fig 7. Normal epithelium with few PMN cells in zona compacta, 72 hr after LPS infusion, H & E stain, 400 x.

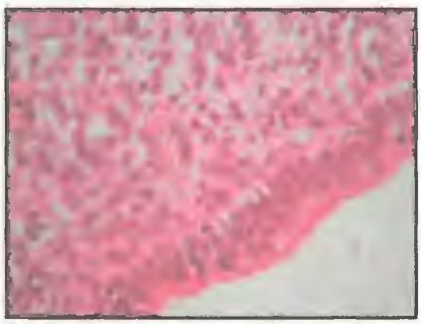

Fig 8. Normal epithelium lining with zona compacta infiltration of few PMN cells. 72 hr OG treatment H & E stain, 1000 x.

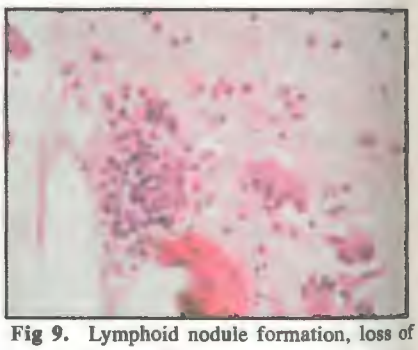

Fig 9. Lymphoid nodule formation, loss of stroma & endometrial glands along with infiltration of PMN & mononuclear cells, H & E stain, 400 x.

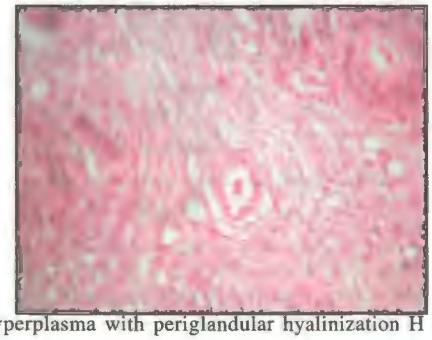

Fig 10. Glandular hyperplasma with periglandular hyalinization H & E Stain, 1000 x.

Table 2: Histopathology of the endometrium in different groups of cows before and after treatment.

Sl. No.	Histopathological changes	Group-A (LPS)		Group-B (OG)		Group-C (Ab)		Group-D (Control)	
		0 hr	72 hr	0 hr	72 hr	0 hr	72 hr	0 hr	72 hr
1	Nature of endometrial epithelium	5(41.66%)	4(33.33%)	5(41.66%)	5(41.66%)	5(41.66%)	8(66.66%)	3(25.00%)	3(25.00%)
	(a) Denuded epithelium	3(25.00%)	**1(8.33%)**	2(16.66%)	1(8.33%)	2(16.66%)	5(41.66%)	3(25.00%)	**1(8.33%)**
	(b) Pseudostraitification	2(16.66%)	-	2(16.66%)		3(25.00%)	**2(16.66%)**	-	-
	(c) Infiltration of cells	-	3(25.00%)	1(8.33%)	4(33.33%)	-	1(8.33%)	-	2(16.66%)*
2	Nature of endometrial glands	3(25.00%)	1(8.33%)	5(41.66%)	3(25.00%)	2(16.66%)	1(8.33%)	5(41.66%)	4(33.33%)
	(a) Hyperplasia	-	-	-	-	1(8.33%)	-	1(8.33%)	**1(8.33%)**
	(b) High columnar cells	1(8.33%)	-	-	-	-	-	-	-
	(c) Denuded & detached epithelial cells	-	-	1(8.33%)	1(8.33%)	-	-	1(8.33%)	**1(8.33%)**
	(d) Cystic dilatation	1(8.33%)	**1(8.33%)**	3(25.00%)	**2(16.66%)**	-	**1(8.33%)**	-	-
	(e) Infiltration of cells	1(8.33%)	-	1(8.33%)	-	-	-	2(16.66%)	**1(8.33%)**
	(f) Atrophy of glands	-	-	-	-	1(8.33%)	-	1(8.33%)	**1(8.33%)**
3	Cellular infiltration in the stratum compactum and stratum spongiosum	3(25.00%)	-	1(8.33%)	-	3(25.00%)	-	3(25.00%)	**2(16.66%)**
4	Lymphoid nodule formation	-	-	1(8.33%)	-	2(16.66%)	-	-	**1(8.33%)**
5	Hyaline degeneration	1(8.33%)	-	-	-	-	-	1(8.33%)	**1(8.33%)**
	Total	12(100%)^{a}	5(41.66%)^{bC}	12(100%)^{A}	8(66.66%)^{B}	12(100%)	9(75.00%)	12(100%)	11(91.66%)^{D}

Means with different superscripts within groups (a, b) and between groups (C, D) vary significantly (P<0.01). Means with different superscripts within groups (A,B) vary significantly (P<0.05).
Depictions in bold letters indicate non-pregnant cows.
* One animal under this condition remained non-pregnant.

and pseudostratification. Increased infiltration of PMN cells and mononuclear cells in the endometrial epithelium after PLS and OG infusion is also one of the evidence proving the chemotaxis of PMN cells in the lumen as well as in the endometrium (Table 2).

Intrauterine infusion of antibiotic for 3 consecutive days in the cases of endometritis resulted in to 25% increase in histopathological changes associated with endometrial epithelium. 25% more cases of denuded epithelium was recorded after enrofloxacin treatment although this drug is non irritant in nature. This might because of repeated intervention of endometrium for intrauterine infusion and moreover, antibacterial drugs markedly inhibit/ destroy the phagocytic activity of uterine derived polymorphonuclear granulocytes and lower the uterine defense (Paisley *et al.*, 1986). These drugs usually harm the blood leukocytic activity (Jayappa and Loken, 1983). Reduced percentage of recovery in the cases of pseudostratification along with reduced percentage of cellular infiltration after antibiotic treatment also indicates the adverse effect of antibiotic infusion on uterine defense mechanism.

No appreciable histopathological changes were recorded in endometrial epithelium in control group of cows following intrauterine infusion of PBS. Cases of denuded epithelium were reduced with appreciable increase in infiltration of mononuclear cells and PMN cells. As the changes associated with endometrial epithelium occurred mostly in mild endometritis and in untreated cases the natural defense of uterus remained least affected and hence the response in these cows were quite encouraging. In contrast infusion of antibiotic has discouraging results indicates that in mild endometritis a large proportion of animal does not require any therapy at all, especially antibiotic therapy as it might cause more harm than benefit (Hoedemaker 1998).

Nature of endometrial glands

In mild endometritis, there were no specific pathological changes in the endometrial glands. There was mild infiltration of PMN leukocytes and mononuclear cells in the stroma. A few sidrocytes (macrophages laden with haemosiderin pigments) were found in stromal tissue. This all is in agreement as reported by Cupps, 1973; Singh, 1979; Shukla, 1988. Histopathological changes in the sub-acute and chronic endometritis revealed various changes associated with endometrial gland.

In the present study overall 31.25% cases of endometritis revealed various types of changes associated with endometrial glands. Small percentage (14.58%) of cows revealed hyperplasia of endometrial glands and high columnar cells with infiltration of mononuclear cells in the glandular epithelium leading to sub-acute endometritis (Table 2).

Cystic dilatation of endometrial glands was observed in 4 cases (8.33%). Variations in these conditions included adenomatous hyperplasia, adenomatous hypertrophy and extreme glandular-cystic dilatation with necrosis of stroma (fig 3, 4, 5 & 6). This might be due to endocrine factors or due to non specific microbial irritation resulting into blockage of the duct and producing ballooning effect. However, significance of imbalance of the endocrine system and/ or deficiencies playing a primary role in the causation of these conditions is diminished because of the prevalence of inflammatory processes accompanied by the presence of reacting cells i.e. mononuclear cells, PMN cells and macrophages. In the present study 3 out of 4 case of cystic dilatations of endometrial gland were not recovered after treatment with LPS and OG and remained non pregnant. This clearly indicates the severity and chronic inflammatory condition of the uterus. Moss *et al,* (1956) also observed two main types of glandular abnormalities, the periglandular fibrosis and changes in the gland cells proper from cases of repeat breeder cows.

Denuded and detached glandular epithelium and glandular atrophy in 4.16% cases revealed a chronic inflammatory condition for prolonged period which caused denudation of epithelium in the beginning followed by complete atrophy of gland.

Infusion of LPS in the cow of group A caused complete recovery of the different types of pathological conditions of the endometrial glands except a case of cystic dilatation which did not respond to treatment.

fusion of OG in the animals of group B also led to significant recovery except in 3 chronic cases of dometritis i.e. one case of denuded glandular thelium and 2 cases of cystic dilatation of glands. The sults suggest well that these immunomodulators have little therapeutic response in chronic endometritis caused rticularly due to cystic dilatation of endometrial glands (Table 2).

Infusion of antibiotic in the cows of group C also vealed recovery from pathological conditions of ndometrial gland. However one cow from this group veloped cystic dilatation of endometrial glands (chronic ndometritis) which might be due to repeated intervention of endometrium for intrauterine infusion of antibiotic leading to chronic inflammatory condition(Table 2).

In untreated control group of cows, 4 out of 5 cases were not recovered and all remained non-pregnant. This clearly indicates that acute/mild endometritis could be recovered automatically without treatment via natural defense mechanism of uterus. However, this couldn't happen in the cases of sub-acute and chronic endometritis.

Cellular infiltration in the stratum compactum and stratum spongiosum

20.83% cases of endometritis revealed heavy infiltration of mononuclear and PMN cells in the zona compacta and zona spongiosa however, sporadic infiltration of these inflammatory cells was observed in most of the cases. Infiltration in the zona compacta was comparatively more than zona spongiosa. Presence of lymphocytes in most of the cases admixed with monocytes, macrophages and plasma cells in some cases are thought to be due to mild/low grade of chronic infections. These infiltrations cause building up the defense mechanism by way of phagocytosis, secretion of various enzymes and neutralizing substances, in the immune processes by altering antigens or transferring antigen to immunologically competent cells and farming a physical barrier between the infected and healthy areas. Infiltration of plasma cells causes in the production of immunoglobulins (humoral immune response) and these cells remain in the tissues after undergoing a change in morphology as memory cells for immunologic mechanisms (Smith *et al.*, 1972).

The presence of polomorphonuclear leukocytes (PMN cells) in few cases is characteristics of acute inflammatory processes. The of function these cells is principally phagocytosis besides secretion of lytic enzymes to lyse dead bacteria and dead body cells, secretion of substances which augment the inflammatory reaction and secretion of chemotactic chemicals (Smith *et al.*, 1972).

Infusion of LPS, OG and antibiotic resulted in significant recovery from infiltration of chronic inflammatory cells in their respective groups. In few cases of LPS group infiltration of few PMN cells and mononuclear cells along with deposition of golden colour hemosidrin pigments in the zona compacta was also observed (fig 7). These pigments indicate hemorrhage in that region. In OG group infiltration PMN cells with proliferation of angioblasts in few cases indicates the repair process.

Infusion of antibiotic in cows of group C revealed distortion and necrotic changes in the stromal tissue in some of the cases indicating its adverse effect after repeated infusion in the uterine lumen.

In untreated group of cows, majority of the cases remained of sub-acute nature along with infiltration of large number of mononuclear cells making the condition severe. In few cases loss of stromal tissues were also observed. In this group 16.66% cases out of total 25% cases remained non-pregnant.

Lymphoid nodule formation

Lymphoid follicles in the endometrium are not usual. Most probably they represent merely an exaggerated but physiological reaction of a local lymphatic tissue. These follicles act as a mechanism of defense against noxious agents, not only exogenous but endogenous as well (Dallenbach-Hellweg, 1981). It is considered that low grade of infections may elicit lymphoproliferative reactions with formation of lymphoid nodules and follicles to meet the demand of differentiation into other cell types i.e. plasma cells and multipotential

stem cells. These cells also supply nutrients to other cells and to participate in the immune response.

Lymphoid nodule formation was observed only in 3 cases (6.25%) in the present study. These lymphoid nodules were in periglandular areas and in stroma of stratum compactum (fig 9). In few cases focal aggregation of PMN cells and lymphocytes were also observed in the stroma. In one severe case the endometrial glands were found to be completely occluded with lymphocytic infiltration making flattened epithelial cells of the gland. McEntee, 1990 also observed such conditions in bovine endometrium suggesting its association with variety of infectious agents. The underlined gland become dilated, lined by flattened epithelial cells and were surrounded by zone of fibrous tissue. Such glands remained cystic even when the inflammation had subsided.

Intrauterine infusion of OG and antibiotic respectively in group B and C cows cleared all the lymphoid follicles probably because of its immunomodulatory and antibacterial effects.

Endometrium of one cow in untreated control group revealed lymphoid nodule formation at 72 hours of study which was not found at 0 hour. This indicates that low grade of infections for prolonged period may elicit lymphoproliferative reactions causing lymphoid nodule formation.

Hyaline degeneration

The condition of hyaline degeneration generally appears in sub-acute and chronic endometritis (fig 10). Hyaline degeneration was observed in 2 cases before treatment, one of which was cured after LPS therapy. Another such case of untreated group cows with heavy infiltration of mononuclear cells in the stroma did not subside.

The overall conception rate was high in LPS and OG group (75%) followed by antibiotic group (58.33%). Conception rate in control group cows was lowest (16.66%) with none of the cow conceived at first insemination.

In conclusion infusion of LPS and OG significantly cured the all types of endometritis in bovines and is better therapy of endometritis than antibiotic. However in chronic endometritis particularly with cystic dilatation of endometrial glands, immunomodulators have little effect. Repeated intervention of endometrium during intrauterine infusion of antibiotic markedly enhanced the histopathological changes associated with endometrial epithelium i.e. it has little or no effect on mild endometritis. Good therapeutic response could be achieved by using antibiotics in sub acute and chronic endometritis.

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