HISTOPATHOLOGICAL STUDY OF ENDOMETRITIS IN SLAUGHTERED BUFFALOES

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ABSTRACT

The present study was designed to investigate histopathological changes of endometritis in 110 slaughtered buffaloes from local abattoir in Junagadh (Gujarat). Grossly, 44 genitalia exhibited thickening of uterine wall and presence of varying degree of mucopurulent / purulent exudate noted in 18 uterine samples. Histopathologically, the lesions observed were acute, subacute and chronic changes in 25.45%, 20.90% and 34.55% respectively. Acute endometritis was characterized by severe congestion along with marked stromal edema, degenerative changes and focal denudation of luminal epithelium, focal hemorrhage in sub epithelial zone, infiltration of inflammatory cells predominantly polymorphonuclear cells and mononuclear cells in lamina propria, infiltration of mononuclear cells in glandular lumina and peri-endometrial glands. Subacute endometritis consisted of denudation of luminal epithelium, congestion, stromal edema and Focal haemorrhagic spot, infiltration of mononuclear cells in lamina propria, glandular lumina and around atrophied endometrial gland, glandular dilation, hyperplasia of mucosal epithelium, atrophy of endometrial glands and thickening of blood vessels. The main features of chronic endometritis were desquamation of mucosal epithelium, infiltration of mononuclear cells and plasma cells in sub epithelial zone, dilatation of endometrial glands with degenerative changes, infiltration of mononuclear cells in glandular lumina and periglandular region with narrowing of glandular lumina, perivascular and periglandular fibrosis leading to severe thickening of blood vessels resulting in narrowing of their lumina and transformation of endometrial epithelial cells into low cuboidal against the normal columnar epithelium. Beside the above histopathological lesions three uterine samples revealed adenomyosis and twenty two genitalia showed metritis.

Keywords: *Bubalus bubalis*, buffalo, endometritis, histopathology

INTRODUCTION

Uterine diseases include endometritis,

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metritis, pyometra, mucometra, perimetritis, uterine abscessation and neoplasm of various kind. Among these endometritis is the most common ailment under field / farm conditions in buffaloes causing decreased fertility resulting in high economic losses (Agarwal and Tomer, 2003). Endometritis is defined as inflammation of endometrium (uterine mucosa) not extending to myometrium (muscular coat) without showing any systemic signs / symptoms in the host (Bondurant, 1999). Whereas, the inflammation of entire thickness of uterine wall is known as metritis and may be considered an extension of endometritis. The great majority of uterine inflammatory conditions are initiated first in the endometrium. Uterine infections delay in the recovery of ovarian functions and uterine involution, increase 'days open' and thus extend the calving interval. It not only makes the animals infertile but leads to sub-fertile state even after successful clinical resolution of the disease. Endometritis precludes fertilization, prevents successful implantation / development of viable embryo leading to unsuccessful pregnancy in large number of cases (Semambo *et al*., 1991). Endometrial biopsy is the most reliable diagnostic tool and an important key to identify the nature of infertility and to confirm clinical cases of endometritis (Bretzlaff, 1987). Diagnosis of sub clinical endometrial changes is possible only by histopathological examination of biopsy samples (Singh *et al*., 1983). A high correlation between sub fertility and demonstrable endometrial histopathology has been reported (Hartigan *et al*., 1972).

MATERIALS AND METHODS

A total 110 full thickness uterine tissues were collected at horn-body junction of uterus in 10% neutral buffered formalin for histopathological examination from local abattoir in Junagadh (Gujarat). Further, these tissues were processed by routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Tissue sections of 5 to 6 µ thicknesses were prepared and routinely stained by Hematoxylin and Eosin staining method. The sections were observed under microscope and the histopathological alterations in the tissue were recorded (Luna, 1968). Where needed the duplicate sections were stained by Masson's trichrome. Further, histopathological changes were observed in different genitalia, the findings were categorized as acute, subacute and chronic endometritis (Azawi *et al*., 2008) and additional lesions were also recorded, if any.

RESULTS AND DISCUSSION

Out of 110 female buffalo genitalia collected from local slaughter house at Junagadh showed gross anomalies in 62 genitalia (56.36%) i.e. mild to moderate thickening of uterine wall in 44 genitalia (40%) and presence of varying degree of mucopurulent / purulent exudate with mild to moderate degree of congestion and shreds of tissue debris. Over lapping of these two features occasionally existed together.

Varying degree of inflammatory changes with infiltration of different inflammatory cells in the endometrium of 89 (81%) out of 110 uterine samples were noted whereas remaining 21 (19%) uterine samples did not show any significant infiltration / inflammatory changes on histopathology. Based on infiltration and inflammatory changes, the uterine samples (89/110) were classified into acute, subacute and chronic endometritis (Table 1).

Acute endometritis

Twenty eight (25.45%) uterine samples out of one hundred and ten samples revealed acute endometritic changes. Severe engorgement of a few endometrial blood vessels along with marked stromal edema (Figure 1) was noted in almost all these cases. Degenerative changes and focal denudation of endometrial epithelium was noted in majority of the cases (Figure 2). Twelve samples revealed focal areas of hemorrhage in sub epithelial zone in addition to congestion and edema (Figure 3). In twenty four cases, mild to moderate infiltration of inflammatory cells predominantly polymorphonuclear cells and mononuclear cells in lamina propria was noticed. Four cases revealed moderate to severe infiltration of polymorphonuclear and mononuclear cells in subepithelial zone (Figure 4). Mild to moderate infiltration of mononuclear cells in glandular lumina and peri-endometrial glands in nineteen and six cases, respectively were discernible (Figure 5). In addition enlargement of endometrial glands having degenerative changes was also noticed in nine cases (Figure 6).

Subacute endometritis

Twenty three (20.90%) uterine samples revealed subacute endometritis and changes were characterized by mild to moderate denudation of luminal epithelium, congestion and stromal edema. Focal mild haemorrhagic foci were seen in eight cases. Moderate to severe infiltration of mononuclear cells in eleven cases and moderate to severe infiltration of mononuclear cell in twelve cases in lamina propria were also discernible. Mild to moderate infiltration of mononuclear cells in glandular lumina in ten cases and in six cases mild to moderate infiltration of mononuclear cells around atrophied endometrial gland occurred. In five cases mild to moderate glandular dilation were noticed. Seven uterine samples revealed mild to moderate hyperplasia of mucosal epithelium leading to stratification (Figure 7) along with denudation of endometrial epithelium and atrophy of endometrial glands in three cases were observed. Nineteen cases revealed mild to moderate hypertrophy of vascular medial coat leading to its thickening (Figure 8).

Chronic endometritis

Chronic endometritis was noticed in thirty eight (34.55%) uterine samples. The lesions were characterized by extensive desquamation of mucosal epithelium and severe infiltration of mononuclear cells and plasma cells in subepithelial zone in all the cases (Figure 9). Dilatation of endometrial glands with degenerative changes was noticed in six cases. Mild to moderate infiltration of mononuclear cells occurred in glandular lumina and periglandular region in few cases. In 34 cases severe degenerative/necrotic and other irreversible changes were seen resulting in atrophy of endometrial glands (Figure 10), reduction in endometrial glandular lumina and degenerative changes in glandular epithelium. Thirty five cases revealed moderate to marked thickening of blood vessels leading to narrowing of lumen along with infiltration of lymphocytes and plasma cells in sub epithelial zone (Figure 11 and 12). The number, size and shape of uterine glands varied depending on the severity of stromal and periglandular fibroplasia. In a few cases mild to moderate perivascular and periglandular fibrosis occured (Figure 13). In sixteen cases the uterine endometrial epithelial cells transformed to low cuboidal in nature instead of normal columnar appearance (Figure 14).

Besides the above histopathological lesions of acute, subacute and chronic endometritis, three

uterine samples showed lesions of adenomyosis and metritis was seen in twenty two samples. Adenomyosis was characterized by the presence of basilar uterine glands along with adjacent stromal tissue amidst myometrium (Figure 15). Metritis was characterized by infiltration of mononuclear cell in myometrium and serosa with or without edematous changes (Figure 16).

In present study we could observe acute (25.45%), subacute (20.90%) and chronic (34.55%) cases of endometritis out of 110 cases studied and supported the earlier reports made on such survey by a number of workers ranging from 0.30 to 39%, 1.51 to 33.92% and 4.16 to 58%, respectively, for acute, subacute and chronic endometritis (Tafti and Darahshiri, 2000; Prajapati *et al*., 2004; Prasad *et al*., 2006; Saxena *et al*., 2006; Azawi *et al*., 2008; Raju and Madhuri, 2009; Babu *et al*., 2013; Samatha *et al*., 2013; Ali and Ameen, 2014).

Edema seen in connective tissue in lamina propria might be due to failure of ionic pump mechanism as a result of injury, accumulation of sodium, calcium and water into cells result in swelling and rupture of cell (Coster, 1977; Chatterjee *et al*., 1979). Cystic endometrial glands develop due to accumulation of secretions and cellular debris and might be due to lack of tone and peristalic massage action by myometrium (Azawi, 2008). Singh *et al*. (1983) observed cystic endometrial glands in repeat breeders. Endometrial glands when occluded results in cysts in severe endometritis. When inflammation subsides, the endometrial glandular epithelium flatten following fibroplasia. The cysts may remain indefinitely indicating evidence of previous infection. Cystic endometrial hyperplasia may also occur in the uterus of cow, ewes and bitch in association with hyper estrogenic activity (McEntee, 1990).

A consistent feature observed in most of the uterine samples in the present study was cellular infiltration in lamina propria and lumen of endometrial glands. These inflammatory changes could be due to the presence of pathogenic microorganisms or their toxins/endotoxins (Javed and Khan, 1991). During inflammatory process, the endometrial glands are filled with leucocytes and later the glandular epithelium is totally destroyed and replaced by fibrous tissue, leaving only the masses of infiltrating cells to mark the glandular sites (Dawson, 1963). The variation in the intensity of the uterine inflammatory changes was attributed to host resistance, environment, virulence of microorganisms and hormonal influence (Amer, 1998; Abd El-Wahab, 1991).

Subacute and chronic infection seen in endometrium might have caused arteriosclerotic

Table 1. Types of endometritis observed in slaughtered buffaloes.

Sr. No.	Various conditions diagnosed	No. of uterine sample	Percentage (%)
1	Normal / without any significant lesion	21	19.10
2	Acute endometritis	28	25.45
3	Subacute endometritis	23	20.90
4	Chronic endometritis	38	34.55

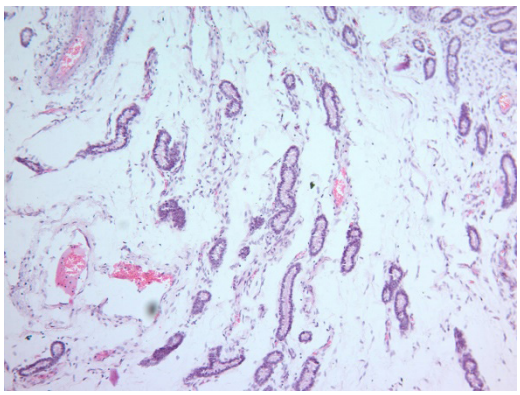

Figure 1. Acute endometritis: Marked stromal edema with mild infiltration of inflammatory cell in lamina propria and engorgement of a few blood vessels (100x H & E).

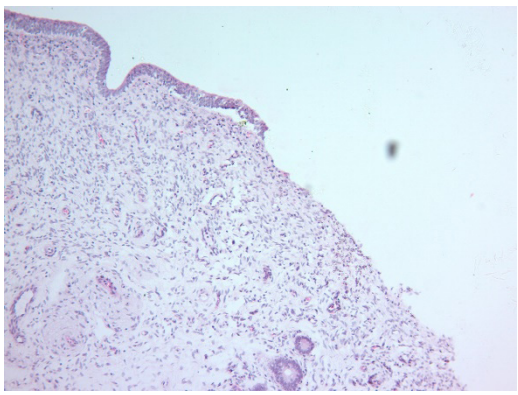

Figure 2. Acute endometritis: Partial denudation of endometrial lining with infiltration of inflammatory cell in lamina propria (100x H & E).

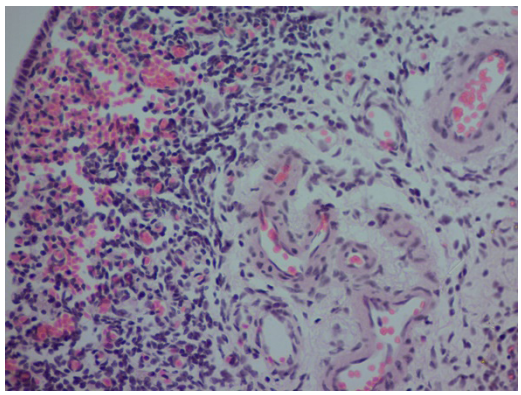

Figure 3. Acute endometritis: Marked congestion / hemorrhages in sub epithelial zone with infiltration of mononuclear cells and edema (100x H & E).

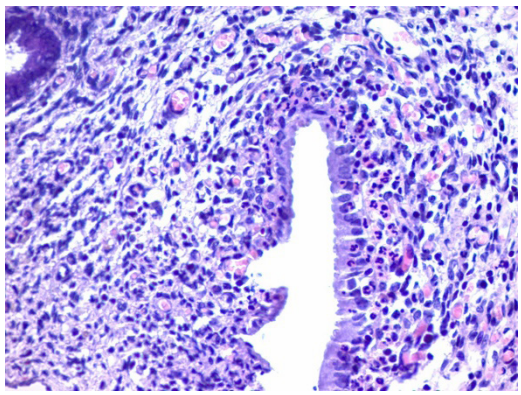

Figure 4. Acute endometritis: Sever infiltration of polymorphonuclear cell and mononuclear cell in lamina propria (400x H & E).

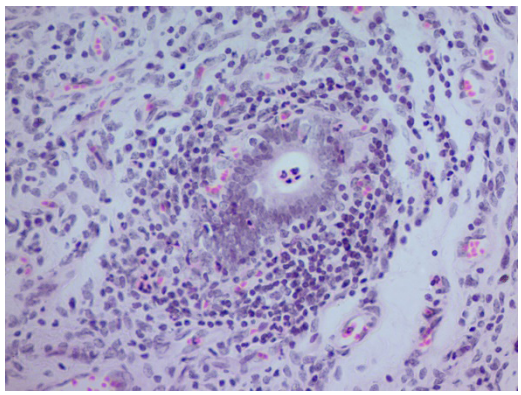

Figure 5. Acute endometritis: Marked infiltration of mononuclear cells in periglandular area with a few mononuclear cells in glandular lumen (400x H & E).

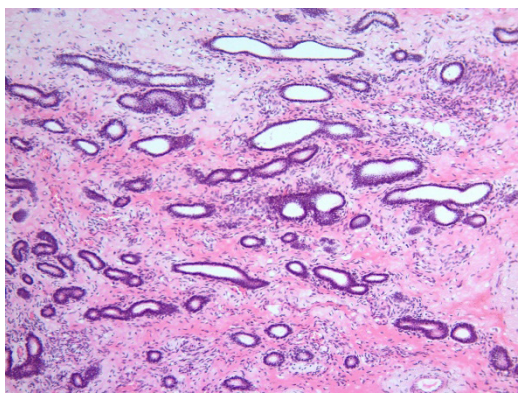

Figure 6. Acute endometritis: Dilated endometrial glands with degenerative changes in glandular epithelium, edema and infiltration of inflammatory cell in surrounding lamina propria (100x H & E).

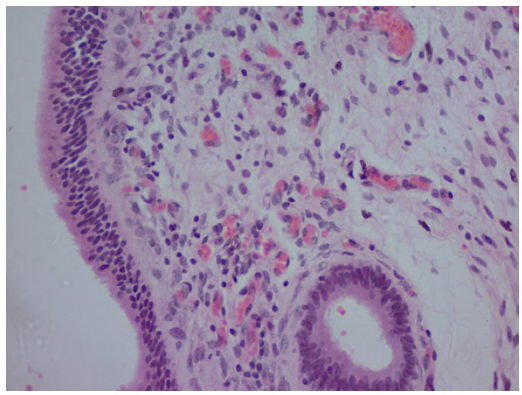

Figure 7. Subacute endometritis: Marked hyperplasia of luminal epithelium with congestion, edema and infiltration of inflammatory cells in lamina propria with glandular epithelial hyperplasia (400x H & E).

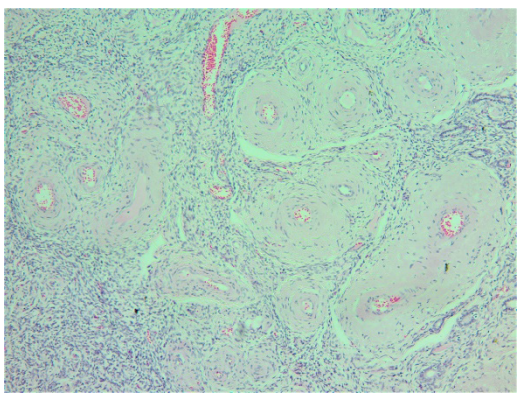

Figure 8. Subacute endometritis: Moderate medial hypertrophy leading to luminal stenosis with infiltration of inflammatory cells in lamina propria (100x H & E).

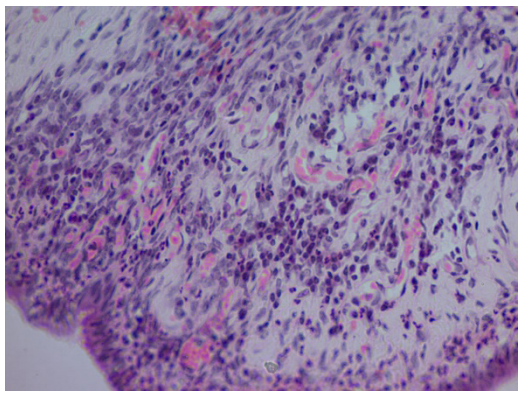

Figure 9. Chronic endometritis: Moderate congestion, severe infiltration of mononuclear and plasma cells in lamina propria (100x H & E).

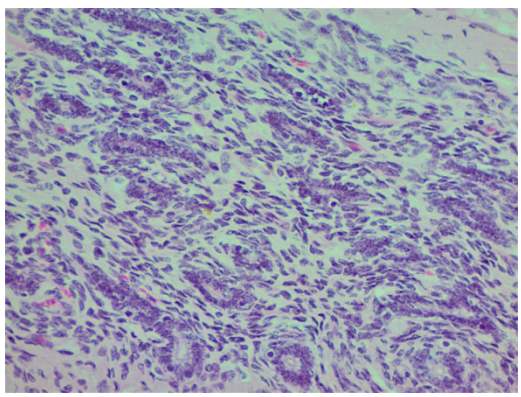

Figure 10. Chronic endometritis: Atrophy (both in number and size) of endometrial glands with infiltration of inflammatory cell in lamina propria (400x H & E).

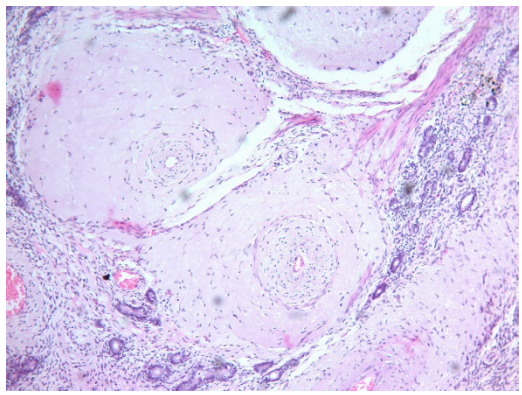

Figure 11. Chronic endometritis: Medial hypertrophy of blood vessels with stenosis of lumina and infiltration of inflammatory cells in lamina propria (100x H & E).

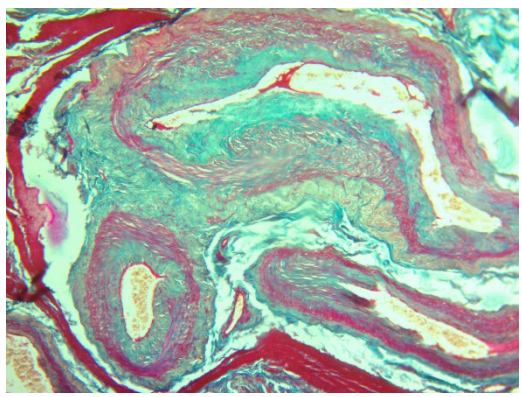

Figure 12. Chronic endometritis: Medial hypertrophy leading to thickening with narrowing of lumen (collagen fiber in green color and muscle fiber in red color, 100x Masson's trichrome).

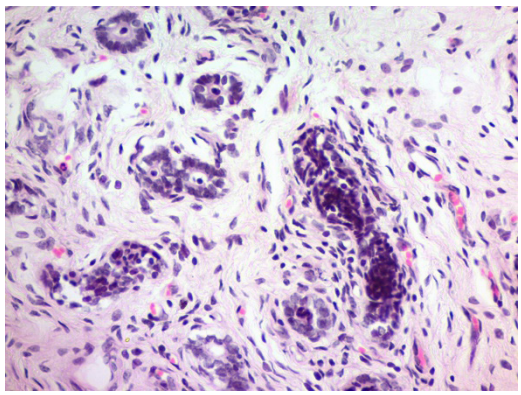

Figure 13. Chronic endometritis: Periglandular fibrosis / infiltration of inflammatory cells (400x H & E).

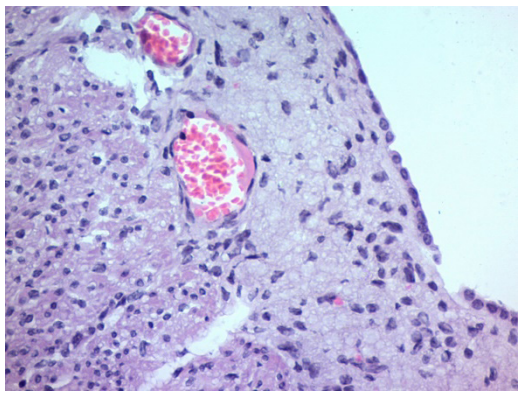

Figure 14. Chronic endometritis: Low cuboidal endometrial epithelium with infiltration of inflammatory cell in lamina propria (400x H & E).

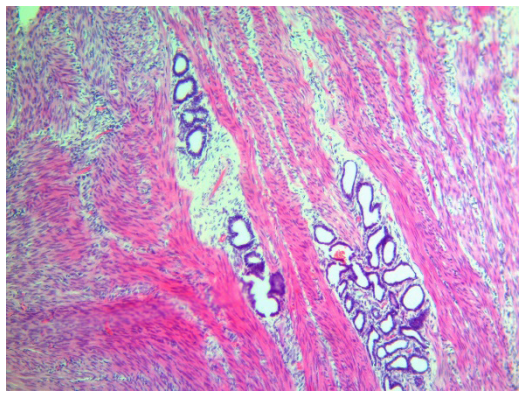

Figure 15. Adenomyosis: endometrial stroma within myometrium (100x H & E).

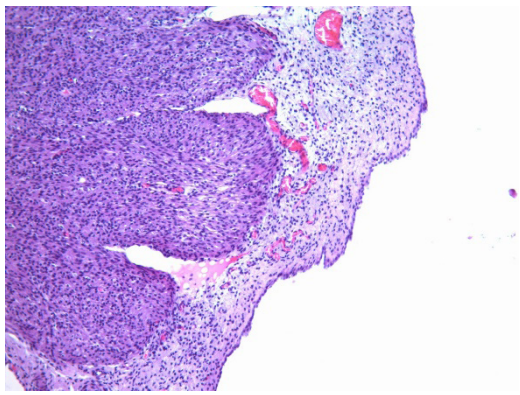

Figure 16. Metritis: Infiltration of mononuclear cell in myometrium and serosa along with edematous changes (400x H & E).

changes due to constant irritation in uterine wall and supported the views expressed by Deeb *et al.* (1976); Chatterjee *et al.* (1979); Samad and Ali (1989).

Singh *et al.* (1983) opined that periglandular fibrosis is irreversible changes leading to atrophy of endometrial glands due to pressure and reduced endometrial glandular secretory activity. Extensiveness and severity of fibrosis appears to be directly related to the ability of uterus to carry a foetus. Widespread fibrosis reduce the chance of production of foal and appears to be the most permanent cause of embryonic and early fetal death in mare (Dawson, 1963; Kenney, 1978).

Adenomyosis is triggered by the excessive estrogen production by the cystic follicles (Jones *et al.*, 1997). The precise etiology and its development are unknown. Currently, the most widely held opinion is that adenomyosis develop as down growth and invagination of basalis endometrium into myometrium (Mai, *et al.*, 1997; Ferenezy, 1998). Alfaris and Fahid (2009) opined that genesis of adenomyosis is initiated frequently during caesarean, hysterectomy and other obstetric or surgical complication, possibly due to implantation of a few endometrial glandular cells/tissues in myometrial layer accidently during surgical process. Subsequently these glandular cells/tissues develop as adenomyosis.

The above mentioned lesions (acute, subacute and chronic endometritis) are not life-threatening but may adversely influence embryo survival. Affected cows may conceive after natural service or artificial insemination but may not be able to keep the conceptus (Gilbert *et al.*, 1995; Hansen *et al.*, 2004).

Chronic endometritis was the major problems seen in slaughtered buffaloes and as these animals cannot reproduce, cause economic loss to livestock owners / farmers and this is why such animals are sold for slaughtering purposes.

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