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Histopathological Evaluation of Important Uterine Pathological Affections in Riverine Buffalo (*Bubalus bubalis*): An Abattoir Study

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ABSTRACT

Infertility or sterility due to uterine pathology is one of the major reasons for culling of buffalo in India. Abattoirs serve as a good source of biological samples in order to study different pathological conditions. Major affections of uterus in buffalo include endometritis, metritis, pyometra, hydrometra, mucometra and certain congenital abnormalities. Mostly diagnosis of such cases is based on gross morphological examination observed in abattoir or sometime on clinical and post-mortem examination. Histological based diagnosis of major affections of uterus in buffalo is less and as such very few reports are available especially on hydrometra and mucometra conditions. The present study reports the morphological and histopathological evaluation of cytological and purulent endometritis, hydrometra and mucometra conditions in riverine buffalo.

Key words: Buffalo, uterus, histopathology, abnormalities, infertility

INTRODUCTION

Reproductive disorders are key determinants affecting fertility to a great extent and ultimately causing huge economic losses to livestock industry. It is estimated that about 18-40% cattle and buffalo are culled every year due to infertility or sterility in India (Kaikini, 2002). Majority of buffaloes in India reach slaughterhouse on account of infertility due to different pathological or functional disorders (Sharma *et al*., 1993; Agarwal *et al*., 2005). Hence, abattoirs serve as a good source of biological samples in order to study different pathological abnormalities of reproductive tract in buffalo which are severe enough to cause infertility or even complete sterility (Dobson and Kamonpatana, 1986; Azawi *et al*., 2008b). Major affections of uterus reported in cattle and buffalo include endometritis (subclinical and clinical), puerperal and septic metritis, pyometra, perimetritis, parametritis, hydrometra, mucometra and certain congenital abnormalities (Hatipoglu *et al*., 2002; Ali *et al*., 2006; Saxena *et al*., 2006; Azawi *et al*., 2008b; El-Sakkar *et al*., 2008; Rhyaf, 2010; Modi *et al*., 2011). Among different uterine affections, endometritis especially subclinical endometritis is considered as a major cause of reproductive failure in cattle as well as buffalo (Moghaddam and Mamoei, 2004; Sheldon *et al*., 2008; Senosy and Hussein, 2013). Lack of proper diagnosis of subclinical endometritis is a major concern especially for field veterinarians. Different diagnostic approaches currently in use for diagnosis of endometritis in cattle and buffalo

includes ultrasonography, vaginoscopy, endometrial cytology, white side test, bacteriology and endometrial biopsy (Barlund *et al*., 2008). Each of these techniques has its own limitation over others. Histopathology of endometrial biopsy is one of the diagnostic tools for detection of endometritis or any other pathological changes occurring at tissue level in uterus. Reports are available in cattle on histopathological details of endometritis and other affections of uterus (Ali *et al*., 2006; Singh *et al*., 2008; Rhyaf, 2010). However, most of the studies in buffalo reported only gross pathological affections of uterus observed in abattoir, diagnosed clinically or at post-mortem examination. Meagre information is available on histopathological changes of certain abnormalities of buffalo uterus particularly Iraqi buffalo (Azawi *et al*., 2008a; Sayyari *et al*., 2011; Azawi and Ali, 2015). In general, histopathological studies diagnosing endometritis in riverine buffalo have been reported (Ghora *et al*., 1996; Babu *et al*., 2013). However, detail investigations on other important uterine pathological affections including hydrometra and mucometra have not been studied.

MATERIALS AND METHODS

Sample collection: The female genital tracts of buffalo cows (n = 400) were collected randomly from local slaughter house, Bareilly, Uttar Pradesh during the period January, 2014 to December, 2014. Information regarding breed, identity and history of the animals were not available, however, apparently these buffaloes were observed to be graded Murrah, which is a predominant breed of buffalo in Rohilkhand region. The genital tracts were collected immediately after exsanguination and transported on ice to laboratory in plastic bags.

Gross examination and classification of different affections of uterus: Genitalia were examined grossly in the laboratory for the presence of different morphopathological abnormalities. After gross examination, all the genitalia were opened by incising caudo-cranially, starting from cervix to uterotubal junction. Endometrium was examined for colour change, nature of the fluid (pus/mucus) and presence of any pathological lesions. Endometrial cytological smears were prepared using modified cytobrush by spreading cells on the glass slide and allowed to dry at room temperature for 10-15 min. Smears were fixed with absolute methanol for 1 min and stained with modified Giemsa stain for 60 min, washed under tap water and air dried. Cytological assessment was performed by counting 200 cells (both endometrial and PMNs) per slide at 40X magnification and classified into epithelial and PMN cells based on cellular and nuclear morphology. Endometrial cytology smear of the apparently normal uterus with >5% PMN cells (Fig. 1) and no gross inflammatory lesions were classified as Cytological Endometritis (CE) (Gilbert *et al*., 2005; Loyi *et al*., 2013). Uterus filled with purulent or mucopurulent material and presence of inflammatory lesions like haemorrhage, necrotic lesions and exudate debris in the carancular and intercarancular areas in the endometrium was categorized as Purulent Endometritis (PE) (Fig. 2). However, genitalia with symmetrical enlargement of both the uterine horns due to fluid accumulation were categorised separately. Fluid present in the uterus was collected to evaluate volume and based on its consistency (watery/mucus), condition has been classified as "hydrometra" and "mucometra" accordingly. Hydrometra reveals characteristic thinning of uterine wall along with atrophy of carancular tissue due to accumulation of clear watery fluid (about 100-350 mL) in both uterine horns and body of uterus (Fig. 3), normal cervix and

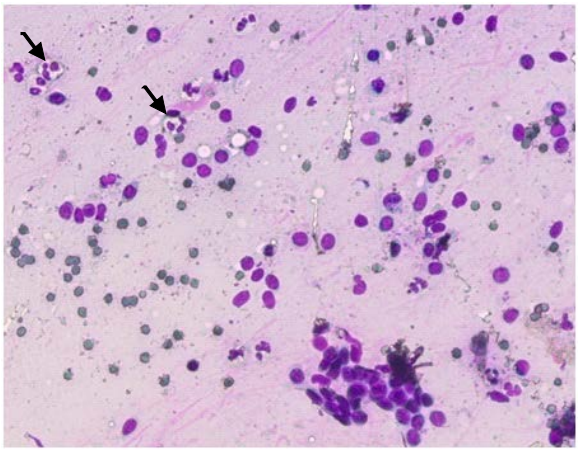

Fig. 1: Endometrial cytology smear showing >5% PMN cells (black arrows), 40X

Fig. 2: Purulent endometritis with mucopurulent material inside lumen of uterus

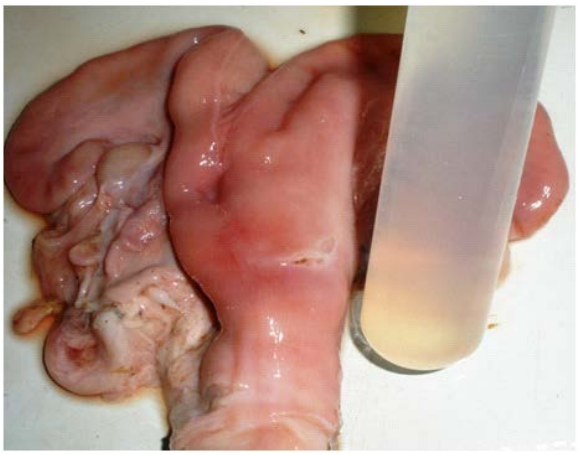

Fig. 3: Hydrometra: Accumulation of watery fluid in lumen of uterus

Fig. 4: Mucometra: accumulation of mucus in lumen of uterus with thick cervical seal

presence of >8 mm diameter follicle on both the ovaries. Similarly, mucometra condition was taken into consideration based on the presence of about 400 mL of non-odorous opaque thick mucus in both uterine horns and body of uterus along with the presence of corpus luteum in right ovary (Fig. 4) and thick tenacious gummy cervical material almost plugging the cervical canal. Therefore, cervical tissue was also subjected for histopathology. In the present study, 8 observations each of cytological and purulent endometritis and hydrometra were subjected to histopathology. However, during the study period only one case of mucometra was recorded and included in the study.

Histopathology: The sections of uterine tissues collected from right uterine horn from all the reported conditions and cervix tissue from mucometra condition were preserved in 10% neutral buffered formalin for histopathological examination. Tissues were processed, embedded in paraffin and sections of 5 μm thickness were prepared. These sections were stained with hematoxylin and eosin (H and E) stain (Luna, 1968) and examined under microscope for histopathological evaluation.

RESULTS

The histopathological findings of recorded pathological abnormalities affecting buffalo uterus during the period are detailed below.

Cytological endometritis: On histological examination, CE was characterised by hyperplasia, pseudo-stratification and discontinuity of the endometrial lining epithelium at some places. Presence of inflammatory cells including a few neutrophils, macrophages and lymphocytes in the subepithelial layer and stratum compactum (Fig. 5a). There was loosening of endometrial connective tissue stroma due to presence of oedematous fluid. Vascular changes like dilation of blood vessels, congestion and haemorrhage were also present in the lamina propria (Fig. 5b). Periglandular infiltration of lymphocytes and plasma cells along with atrophy and degeneration of endometrial glands were observed (Fig. 5c).

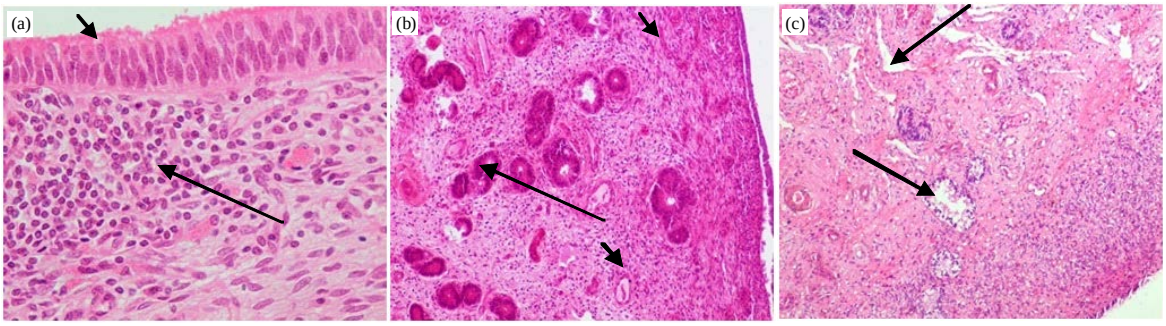

Fig. 5(a-c): (a) Histopathology of cytological endometritis showing hyperplasia (small arrow) and infiltration of inflammatory cells in sub-epithelial layer (large arrow), H and E 40X (b) Vascular changes (dilation of blood vessels, congestion, haemorrhage (small arrow)) and glandular atrophy (large arrow), H and E 10X and (c) Oedema of endometrial stroma, periglandular infiltration and glandular degeneration (large arrow), H and E 10X

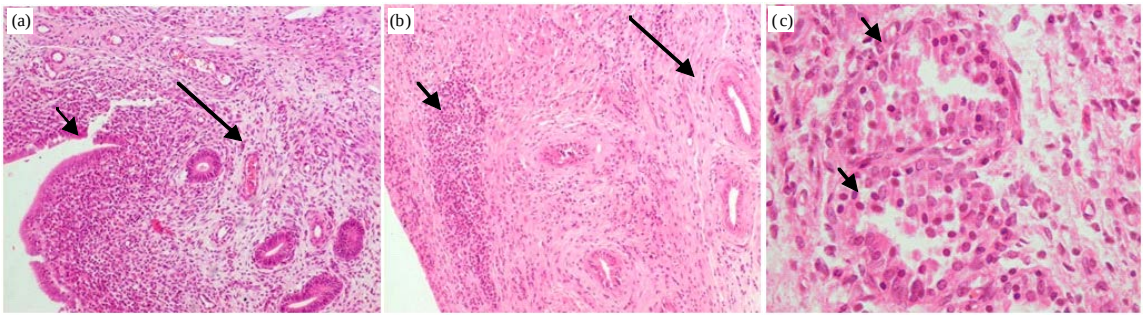

Fig. 6(a-c): (a) Histopathology of purulent endometritis hyperplasia and discontinuity of lining epithelium (small arrow), congestion of blood vessels (large aarrow), H and E 10X, (b) Infiltration of lymphocytes and plasma cells (small arrow), severe periglandular fibrosis (large aarrow), H and E 10X and (c) Complete degeneration of endometrial glands (small arrow), H and E 40X

Purulent endometritis: Histological findings revealed severe hyperplasia and discontinuity of the endometrial lining epithelium. There was severe focal as well as diffused infiltration of lymphocytes, plasma cells and macrophages in the subepithelial as well as deep layers of the stratum spongiosum (Fig. 6a). Further, thickening of the endometrial blood vessels, severe periglandular fibrosis characterized by 2-3 concentric layers of spindle shaped fibroblasts around uterine glands and proliferation of fibroblast cells were observed in the endometrial stroma (Fig. 6b). The endometrial glands were completely degenerated and infiltrated with mononuclear cells in and around the glands (Fig. 6c).

Hydrometra: Histologically, uterine tissue sections revealed compression and thinning of endometrium, submucosa and myometrium due to pressure atrophy caused by fluid accumulation in the lumen of uterus (Fig. 7). The endometrium showed compact stroma lined by low cuboidal

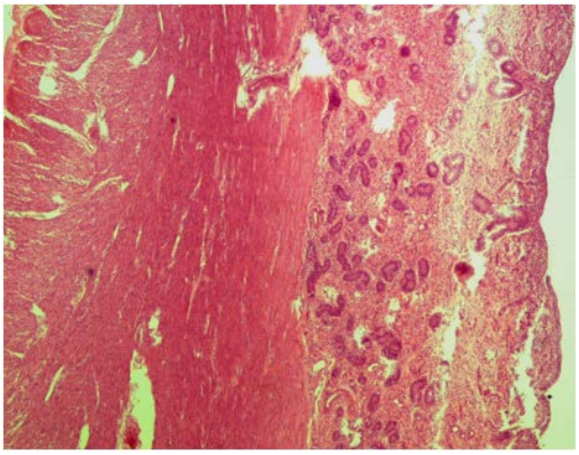

Fig. 7: Hydrometra showing atrophy of all endometrial layers, H and E 4X

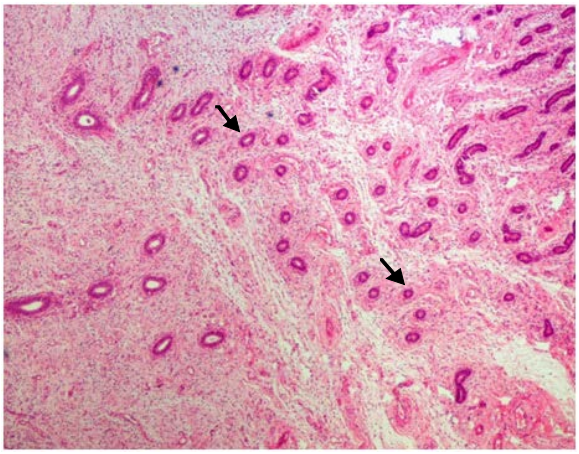

Fig. 8: Hydrometra-endometrial glands showing atrophy, reduced number, lumen width and branching (small arrow), H and E 10X

epithelium with slight hyperplasia. The most perceptible changes observed in the uterine endometrial glands include reduction in number, density, extent of branching, lumen width in both superficial and deep endometrial zones (Fig. 8). Glandular epithelium in some endometrial glands revealed degenerative changes and initial vacuolization.

Mucometra: Histologically, presence of moderately dilated endometrial glands with muco-protenacious material inside the glandular lumen was observed. It was also associated with adenomyosis characterised by presence of endometrial glands and stroma embedded within the myometrium. However, endometrial glands within the myometrium were lined by single layer of cuboidal epithelium and atrophied without containing any material in the lumen (Fig. 9). Further, presence of light basophilic mucus in the lumen of cervix was also noticed (Fig. 10).

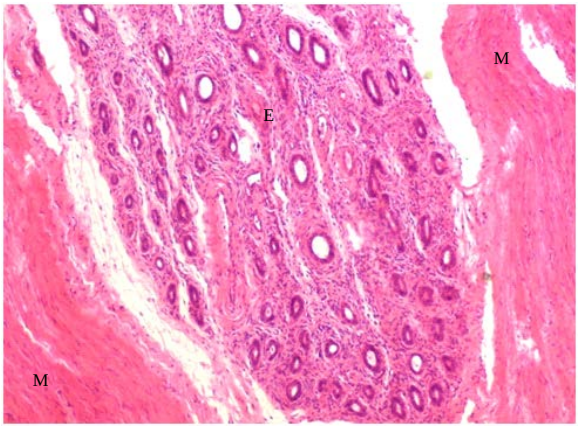

Fig. 9: Mucometra associated with adenomyosis- endometrial stroma (E) within myometrium (M), H and E 10X

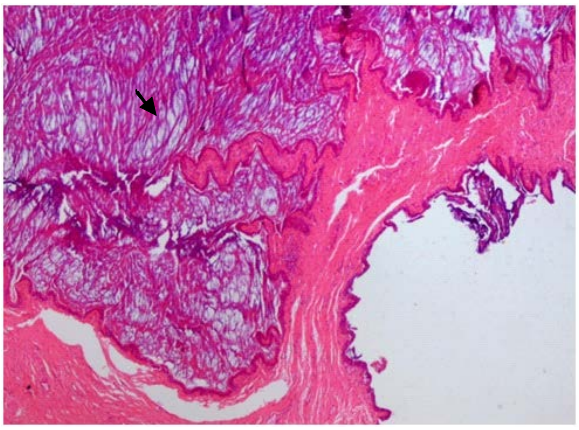

Fig. 10: Mucometra-presence of basophilic mucus inside cervix (small arrow), H and E 10X

DISCUSSION

In the present study, diagnosis of cytological endometritis was based on endometrial cytology, with the presence of >5% PMN cells. Various threshold ranging from 5-18% of PMNs in endometrial cytology have been reported in cattle, however, most of the studies in cattle and buffalo used >5% PMN cut-off to diagnose cytological endometritis (Gilbert *et al*., 2005; Loyi *et al*., 2013). Histopathological findings of buffalo uterus with cytological endometritis as observed in the present study are almost similar to observations in biopsy sample of repeat breeding cows with subclinical endometritis. However, condition has been classified further based on degree of inflammation in to mild, mild to mild chronic and chronic endometritis (Singh *et al.,* 2008). The present findings were further supported by the similar observations during endometritis recorded in repeat breeding Iraqi buffalo (Azawi *et al*., 2008a). Babu *et al*. (2013) reported mild to moderate cellular infiltration along with periglandular fibrosis in the Murrah buffalo diagnosed with endometritis based on endometrial cytology. In an abattoir study, Vala *et al*. (2011) also reported similar histopathological changes during acute and subacute endometritis in the buffalo. Hence, buffalo with apparently normal genitalia and clear uterine discharge may suffer from subclinical endometritis, therefore,

prompt diagnosis is important to reduce the incidence of repeat breeding syndrome. Buffalo uterus with purulent endometritis having purulent/mucopurelent debris in uterine lumen as observed in the present study was histologically similar to the findings reported in cases of purulent endometritis in cattle (Hatipoglu *et al*., 2002) and Iraqi buffalo with chronic endometritis (Azawi *et al*., 2008a). The endometrial glandular degeneration and fibrotic changes were similar as reported by Babu *et al*. (2013) in the endometritic buffalo. Cytological endometritis cases in the present study were diagnosed by endometrial cytology with a cut off of >5% PMN cells, which was later confirmed by histopathology. Therefore, findings further supports the earlier observations that endometrial cytology is of diagnostic relevance to detect subclinical endometritis at field level being simple and more rapid than histopathology. Present study gives an insight about the histopathological changes occuring in riverine buffalo of Indian origin suffering from cytological and purulent endometritis as diagnosis of endometritis in one of the major concern in buffalo especially during the repeat breeding affecting fertility to a great extent. Therefore, histopathology of the endometrial biopsy might be applied in the cases where all conventional diagnosis failed to arrive any definite conclusion.

Hydrometra in buffalo was characterised by accumulation of clear watery fluid in the uterine lumen causing atrophy of the uterine wall. These findings were similar to the reports in cattle and other species including buffalo (Ghora *et al*., 1996; Yotov *et al*., 2009). In ruminants, it is reported to be associated with the prolonged administration of clenbuterol (Biolatti *et al*., 1994; Re *et al*., 1995) or exogenous administration of estrogens and clover pasture containing estrogen compounds which stimulates estrogen receptors. In canine, hydrometra cases were found to be associated with exogenous administration of estrogens (Dhaliwal *et al*., 1999). Follicular cysts and granulosa cell tumors which act as endogenous source of estrogens also reported to upregulate estrogen receptors (McEntee, 1990; Payan-Carreira *et al*., 2006). Further, excessive/chronic estrogenic stimulation induces endometrial hyperplasia leading to continuous and insidious production of watery fluid in the uterus leading to accumulation of fluids (Payan-Carreira *et al*., 2006). Histopathological findings revealed atrophy of all the layers of uterus which might be caused by pressure atrophy due to fluid accumulation for a long duration. Histological changes in the endometrial glands in the present study were similar as reported in sheep (Yotov *et al*., 2009) and dogs (Payan-Carreira *et al*., 2006). However, only gross morphology of the hydrometra cases has been reported so far in cattle and buffalo. Mucometra cases were associated with presence of thick viscid mucus in uterus and atrophy of uterine wall. The accumulation of mucus was due to obstruction of cervix caused by thick gummy cervical seal on external os and presence of corpus luteum. Histopathology revealed moderately dilated endometrial glands with muco-protenacious material inside the glandular lumen. Histology of the cervix also revealed basophilc mucus inside the lumen. The condition was also associated with adenomyosis, characterised by presence of endometrial glands and stroma embedded within the myometrium. It has been reported in cattle (Korzekwa *et al*., 2013) and buffalo (Ghora *et al*., 1996; El-Sakkar *et al*., 2008), however, in the present case adenomyosis was associated with mucometra. The condition is reported to be associated with persistant corpus luteum and the degree of hydration of mucin present in the uterus may vary from watery fluid to semi solid mass. Mucometra is the most common sequelae of chronic cases of cystic ovarian diseases (Roberts, 1986). Animals with hydrometra and mucometra conditions need to be differentiated from pregnancy because in both the condition, animal suffer from anestrus. Often animals with hydrometra and mucometra conditions are reported to be infertile for a longer period and should not be used for breeding as the hereditary nature of the condition.

The present study reported the morphological and histopathological evaluation of cytological and purulent endometritis, hydrometra and mucometra conditions affecting uterus in riverine buffaloes.

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