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Pathological Study of Uterine Abnormalities in River Buffaloes (*Bubalus bubalis*) in Basrah

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Abstract | A study was conducted to assess the type and prevalence of abnormalities occurring in the female reproductive tracts. This research aimed to determine the frequency and abnormalities in the female reproductive systems of 50 buffalo cows slaughtered at the Basrah slaughterhouse. Fifty genitalia of female cows were confiscated from a nearby slaughterhouse. The results showed gross inflammatory changes of endometritis in 50 animals and classified according to histological examination as acute endometritis (24 %; 12/50), subacute endometritis (18 %; 9/50) chronic endometritis (30 %; 15/50), and while metritis cases (16 %; 8/50) were of the chronic type of inflammation. In all six cases of perimetritis (1.5% of the total) (1.5%; 6/50), the uterine serosa was found to be thicker than usual, and pus of a yellowish hue was observed to accumulate. The pathological changes of acute endometritis includes stromal edema and severe engorgement of a few endometrial blood vessels. In most cases, the endometrial epithelium showed signs of degeneration and localized denudation. Mild to moderate infiltration of inflammatory cells, mostly polymorphonuclear and mononuclear cells. Subacute endometritis, which was characterized by a modest to moderate loss of the luminal epithelium, congestion, and swelling of the stroma. Focal hemorrhagic foci, as well as glandular dilation. The lesions exhibited a transformation of uterine endometrial epithelial cells from their normal columnar look to a low cuboidal shape. Additionally, there was infiltration of inflammatory cells in the lamina propria in chronic cases.

Keywords | Uterine abnormality, Endometritis, Buffalo, Basrah**Received** | August 15, 2024; **Accepted** | November 10, 2024; **Published** | December 09, 2024***Correspondence** | Batool S. Hamza, Department of Pathology and Poultry Diseases, Veterinary Medicine College, Basrah University, Basrah, Iraq; **Email:** hamzabatool5@gmail.com**Citation** | Hamza BS, Al-Amery TS (2024). Pathological study of uterine abnormalities in river buffaloes (*Bubalus bubalis*) in Basrah. J. Anim. Health Prod. 12(s1): 300-305.**DOI** | <https://dx.doi.org/10.17582/journal.jahp/2024/12.s1.300.305>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Iraqi river buffaloes (*Bubalus bubalis*) belong to the river-type Mediterranean buffalo family (Al-Jamas, 1997; Bhadaniya *et al.*, 2019). In comparison to all other domesticated animals, buffaloes yield milk of superior quality, featuring over 3.5-4% protein and 7-10% fat (Mohamed, 2017).

Diseases uterus include endometritis, pyometra, perimetritis, metritis, mucometra, uterine abscessation, and neoplasm of various kinds. Endometritis is the prevailing affliction in buffaloes in field/farm circumstances, leading to reduced fertility and significant economic losses (Agarwal and Tomer, 2003; Ganaie *et al.*, 2018). The presence of pathogenic microbes in the uterus can lead to several complications, including inflammation, endometrial

histological lesions, a slowed rate of uterine involution, and even embryo endangerment (Sheldon *et al.*, 2006). A decreased conception rate, longer time intervals between calving and first service or conception, and an increase in cattle culled due to infertility are all connected with uterine disease (Huszenicza *et al.*, 1999; Rahawy, 2021).

Endometrial invasion of the myometrium is a hallmark of adenomyosis. There is also a widespread enlargement and thickening of the surrounding myometrial muscles (Botsis *et al.*, 1998; Bazot *et al.*, 2002; Minervino *et al.*, 2020; Rahawy, 2021). There are two different types of adenomyosis: Nodular and diffuse circumscribed nodular-type tumors can be mistaken for leiomyomas; however, nodular tumors do not have clear borders and instead contain anywhere from one to many cystic spaces (Pelage and Jacobe, 2005). A total of 24 uterine biopsies were successfully obtained from 40 repeat-breeding buffaloes. Neutrophil infiltration into the uterine glands, stroma, and endometrium was evident in fifteen of the buffaloes. Some instances of chronic endometritis were marked by infiltration of lymphocytes and plasma cells, cystic dilatation of the glands, and peri-glandular fibrosis (Singh *et al.*, 1983).

MATERIALS AND METHODS

The sample was acquired from a local slaughterhouse in Basrah in October, November, and December 2023. The buffalo cows female genital tracts (n=50) were chosen at random. Data were scarce regarding the animals' breeds, identities, and histories. Immediately following exsanguination, the genital tracts were removed placed in plastic bags, and shipped to the laboratory on ice.

Histopathology: For histopathological analysis, pieces of uterine tissues from all conditions mentioned were kept in 10% neutral buffered formalin. The tissue process was conducted at the Department of Pathology and Poultry Diseases, Veterinary Medicine College, Basrah University. The samples were placed in an increasing concentration of alcohol solution (75%, 85%, 95%) for two hours in each step as a dehydration process. Cleared with xylene to remove the remaining alcohol for 1 hour twice. After that, the specimens were infiltrated in paraffin wax and embedded in blocks. Following tissue processing, sections with a thickness of 5 µm were produced and fixed in paraffin. The samples were stained by Hematoxylin and eosin (H and E) stain (Bancroft *et al.*, 2018), which was applied to these sections, and they were then viewed under a microscope for a histological assessment.

RESULTS AND DISCUSSION

Out of 75 female cows genitalia were examined at a local

slaughterhouse in Basrah Province south of Iraq. The results showed gross inflammatory changes of endometritis and were found in 50 cases, which were classified according to histological examination as; acute endometritis (24 %; 12/50), subacute endometritis (18%; 9/50) chronic endometritis (30 %; 15/50), and while metritis cases (16 %; 8/50) were of the chronic type of inflammation. In all six cases of perimetritis (1.5% of the total) (1.5%; 6/50), the uterine serosa was found to be thicker than usual, and pus of a yellowish hue was observed to accumulate.

Out of 50 samples, uterine tissues with acute endometritis showed acute endometriotic alterations. In nearly every one of these patients, there was significant stromal edema and severe engorgement of a few endometrial blood vessels (Figure 1A). In most cases, the endometrial epithelium showed signs of degeneration and localized denudation (Figure 1B). Figure 1C shows congestion and edema as well as focal foci of bleeding in the subepithelial zone. Figure 4 shows mild to moderate infiltration of inflammatory cells, mostly polymorphonuclear and mononuclear cells, into the lamina propria in other cases. Subepithelial zone infiltration of polymorphonuclear and mononuclear cells ranged from moderate to severe in certain cases (Figure 1D). It was possible to observe mild to moderate infiltration of mononuclear cells in the glandular lamina and, in certain instances, in the peri-endometrial glands (Figure 2A). Furthermore, there were enlargements of endometrial glands accompanied by degenerative alterations (Figure 2B).

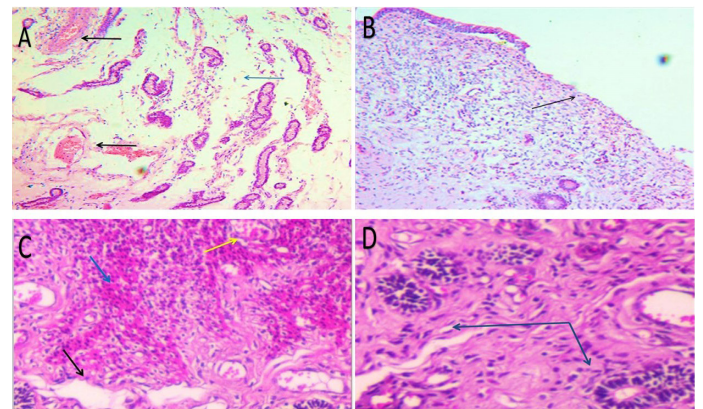


Figure 1: A: Acute endometritis: stromal edema (blue arrow) and severe engorgement of a small number of endometrial blood vessels (black arrows) H&E stain 100x. B: Acute endometritis: The endometrial epithelium showed localized denudation, as indicated by the black arrow. H&E stain 100x. C: Localized regions of subepithelial hemorrhage (blue arrow), together with congestion (yellow arrow) and edema (black arrow). H&E stain 100x. D: Acute endometritis: There is a moderate to mild infiltration of inflammatory cells, particularly polymorphonuclear and mononuclear cells, into the lamina propria (blue arrow). H&E stain 400x.

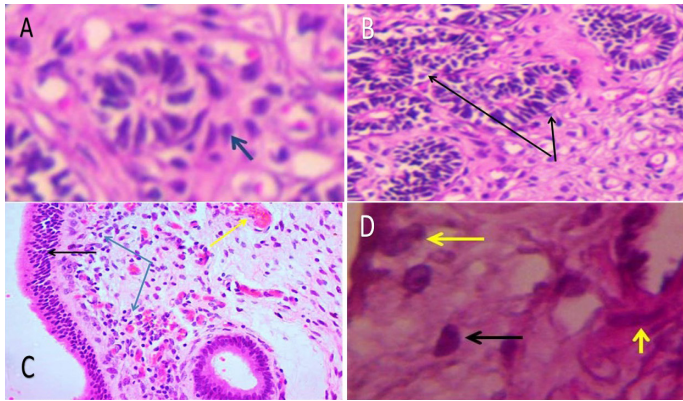


Figure 2: A: Acute endometritis: The glandular lamina and peri-endometrial glands show mild to moderate infiltration of mononuclear cells (blue arrow). 400X H&E stain. B: Acute endometritis: enlargement of endometrial glands with degenerative changes (blue arrow) 100X H&E stain. C: Subacute endometritis: Marked hyperplasia of luminal epithelium (black arrow) with congestion (yellow arrow), edema and infiltration of inflammatory cells in lamina propria with glandular epithelial hyperplasia (blue arrow) (100x H & E). D: Subacute endometritis There was moderate to severe infiltration of mononuclear cells especially plasma cells (black arrow) as well as epithelioid cells in lamina propria around endometrial gland (yellow arrow) 400x H&E stain.

Subacute endometritis occurs in 18% of cases. The uterine samples showed subacute endometritis, which was characterized by a modest to moderate loss of the luminal epithelium, congestion, and swelling of the stroma. Focal hemorrhagic foci, as well as glandular dilation, were noticed. Figure 2C shows that certain uterine samples exhibited a slight to moderate increase in the growth of the mucosal epithelium, resulting in the formation of layers, as well as the loss of the endometrial epithelium and the shrinkage of the endometrial glands in other cases. There was moderate to severe infiltration of mononuclear cells, especially plasma cells as well as the presence of epithelioid cells in lamina propria around the endometrial gland (Figure 2D). Figure 3A shows the presence of mild to moderate hypertrophy of the vascular medial coat, resulting in its thickness.

Thirty percent of the uterine samples showed signs of chronic endometritis. The lesions exhibited a transformation of uterine endometrial epithelial cells from their normal columnar look to a low cuboidal shape. Additionally, there was infiltration of inflammatory cells in the lamina propria, as depicted in Figure 3B. The presence of mononuclear cells and plasma cells in the subepithelial zone was observed to be severe in all cases (Figure 3C). Peri-glandular and perivascular fibrosis, accompanied by the infiltration of inflammatory cells, might be observed in certain instances (Figure 3D). Figure 4A shows the presence of severe degenerative changes, including necrosis and other

irreversible alterations, which have led to the atrophy of the endometrial glands. Figure 4B shows a significant increase in the thickness of blood vessels, resulting in the narrowing of the inner space, as well as the presence of lymphocytes and plasma cells infiltrating the area.

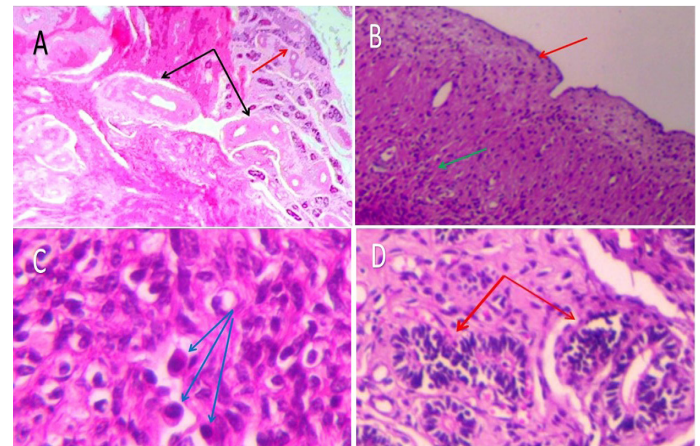


Figure 3: A: Subacute endometritis: The vascular medial coat thickened due to mild to moderate hypertrophy (black arrows), while the uterine glands atrophy (red arrows). 100x H & E stain. B: Chronic endometritis: Inflammatory cells infiltrating the lamina propria (green arrow) and low cuboidal endometrial epithelium (red arrow) (400x H & E). C: Chronic endometritis: the subepithelial zone has a heavy population of plasma cells and mononuclear cells (blue arrow). 400x, H&E stain. D: Chronic endometritis: Inflammatory cells infiltrate the periglandular fibrosis (red arrows). (400x H & E).

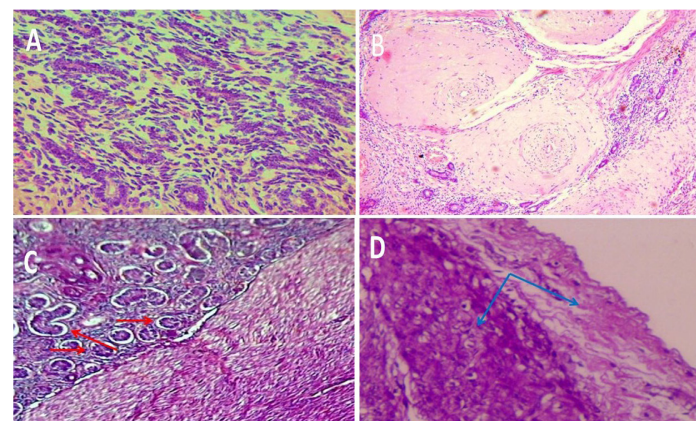


Figure 4: A: Chronic endometritis: uterine gland showing Atrophy of endometrial glands with infiltration of inflammatory cell in lamina propria (400x H & E). B: Chronic endometritis: diffuse of Medial hypertrophy of blood vessels with stenosis of lumina and infiltration of inflammatory cells in lamina propria (100x H & E). C: Adenomyosis: uterine glands along with adjacent stromal tissue within myometrium (red arrow). 100x H & E. D: Metritis: the presence of inflammatory cells and edematous fluid within the myometrium and serosa (blue arrows). 100x H & E.

In addition to the aforementioned histological lesions of acute, subacute, and chronic endometritis, three uterine samples had lesions of adenomyosis, whereas metritis was observed in certain samples. Adenomyosis is indicated by the presence of uterine glands located at the base of the uterus, together with surrounding stromal tissue within the muscular wall of the uterus (Figure 4C). Additionally, The presence of inflammatory cells and edematous fluid within the myometrium and serosa (Figure 4D) with Mononuclear infiltration in the myometrium (Figure 5). Edematous abnormalities, in addition to mononuclear cell infiltration into the myometrium and serosa, were diagnostic of metritis (Figure 1A).

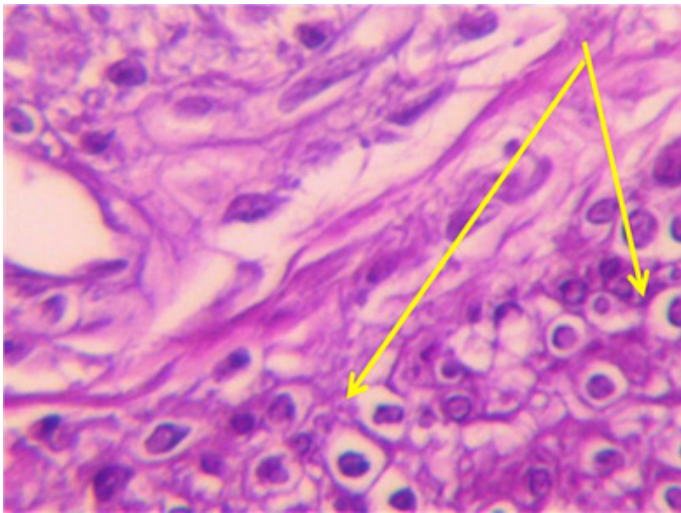


Figure 5: Metritis mononuclear infiltration in myometrium. (400x H & E stain).

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One of the main causes of RB cows is endometritis, which prevents fertilization and the mortality of embryos. For RB etiology determination and treatment protocol development, it is crucial to compare clinical and postmortem examination results with histological and microbiological results (Azawi *et al.*, 2008; Ferreira *et al.*, 2008; Kaya, 2008; Polat, 2008; Ergün *et al.*, 2009; Cannazik and Polat, 2015). Various causes, including parturition, mating, infection, and others, can trigger an inflammatory reaction in the endometrium of the uterus, leading to endometritis.

From a histopathological perspective, it is marked by vascular, glandular, and stromal alterations in the lamina propria, as well as lamina epithelial degeneration, desquamation, and inflammatory cell infiltration to varying degrees (Alaçam, 2007; Kaya, 2008). Endometrial evaluations should take into account the fact that healthy cows may have a limited number of neutrophils during the

pro oestrus, oestrus, and metoestrus stages (Alaçam, 2007; Kaya, 2008; Espejel and Medrano, 2017). Endometritis was found in 39% of the cows that were used for breeding again in this investigation. Another study found rates between twelve percent to one hundred percent (Dogan *et al.*, 2002; Doğru and Güler, 2010; Garoussi *et al.*, 2010). Other studies have shown a wide range of endometritis cases, which is likely due to factors such as the number of animals used, variations in business management, and the passage of time.

The macroscopic examination revealed hemorrhage in the lumen of one uterus sample. The histopathologic examination suggested a chronic endometritis based on neutrophil infiltration, mononuclear cell infiltration, and increased in connective tissue. The determined hemorrhage may be linked to met estrus. In 25 of the uterine samples examined, degeneration and desquamation of varying grades were found in the lamina epithelialize. Additional research found the same thing when looking at endometritis instances (Dogan *et al.*, 2002; Hatipoglu *et al.*, 2002; Pothmann *et al.*, 2015). Moreover, two uterine samples from the control group showed lamina epithelial degradation and desquamation, but the investigation did not find any inflammatory cells. In both cases, mistakes made during preparation are believed to have caused the alterations seen in the lamina epithelials.

The research found that 12 uterine samples showed enlarged connective tissues around the glands in the lamina propria, with varying degrees of severity. The study by Dogan and colleagues found a periglandular fibrosis rate of 13.33%. Embryonic mortality may occur in cases of severe periglandular fibrosis due to the impairment of embryonic glandular functioning (Polat, 2008). The study hypothesized that variations in the cycle phase were responsible for histological abnormalities such as bleeding and edema in the endometrium of 33 uterine samples (52.34%), which did not show endometritis. In their investigation, Dogan *et al.* (2002) noted that the typical histological changes that did not impact fertility were present in both fertile and RB cows. Hormonal changes during the follicular period may potentially cause endometrial hemorrhage and stromal edema, according to certain reports (Espejel and Medrano, 2017; Razak *et al.*, 2023).

CONCLUSIONS AND RECOMMENDATION

Overall, our study revealed the inflammation (endometritis) of buffalo cows was in high percentage in southern Iraq (Basrah). Chronic endometritis had a higher incidence than the acute type, and the other types were in a low percentage.

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NOVELTY STATEMENT

This study considered as the first in the abnormality of reproductive organ in our college.

AUTHOR'S CONTRIBUTION

BSH: Contributed to the pathological results and writing the manuscript. TSCA: The collecting of the samples from slaughtered at the Basrah slaughterhouse and preparation of pathological sectioning.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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