



## Infertility in Female Buffaloes Due to Some Uterine Disorders

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**Abstract** | The purpose of this study is to establish that certain uterine disorders may play a significant role in female buffalo infertility. A total of 340 reproductive tracts of female buffaloes with a previous history of infertility was collected from different farms in Egypt through the period of two years. Uteri have been visually and manually inspected as well as examined bacteriologically and histologically. Several uterine lesions either external or internal were noted in 78/340 animals (22.94%) as follows: internal lesions as endometritis in 33 animals (9.71%), metritis in 11 animals (3.24%), endometrial hyperplasia in 7 animals (2.06%), adenomyosis in 5 animals (1.47%) and fibroma in 3 animals (0.88%). External uteri lesions as perimetrial nodules in 10 animals (2.94 %). Perimetrial cysts in 9 animals (2.65%). Furthermore, many bacteria were isolated and the most common was *Klebsiella oxytoca*, *Proteus vulgaris*, *Proteus vulgaris* and *Enterobacter aerogenes*. The conclusion is that uterine disorders play a crucial role in female buffaloes' infertility. It is highly recommended to use specific clinical parameters and biopsy techniques for early diagnosis in order to proceed with the appropriate treatment.

**Keywords** | Buffaloes, Uterus, Infertility

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## INTRODUCTION

The current world buffalo population is about 200 million (Hegde, 2019). More than 97 % of the world's population of water buffalo is kept in Asia. The uterine infertility in dairy animals is various and may be attributed to a number of conditions like hormonal disturbances, infectious diseases, nutritional causes, congenital and other pathological conditions. Among the most possible causes, uterine abnormalities were considered as one of the major causes like perimetritis, adenomyosis, hydrometra, mucometra, endometritis, pyometra, perimetritis and serositis. Out of the total affected buffaloes, the incidence of perimetritis was the highest (10.7%) followed

by endometritis (7.14%), perimetrial cyst (5.35%), adenomyosis (3.57%) and pyometra (3.57%), hydrometra (1.78%), mucometra (1.78%) and serositis (1.78%) (Mittal *et al.*, 2009; Ponraj *et al.*, 2017; Hamouda *et al.*, 2020; Kumar *et al.*, 2022). The fertility of buffaloes still low due to bad nutrition, management and other environmental factors (Pasha and Hayat, 2012). The rate of uterine infection in buffaloes is more than in cows (Azawi, 2010). Endometritis have been reported previously in buffaloes (Dawar *et al.*, 2017; Babji *et al.*, 2020; Elmetwally *et al.*, 2020, Hamouda *et al.*, 2020). Postpartum metritis is a major disorders in buffaloes leading to a fetid vulvar discharge and uterine infections can be life-threatening, leading to prolonged inter calving intervals and involuntary culling (Azawi

*et al.*, 2013). Bacterial species are involved in most cases of endometritis and in some cases fungal infection was recorded due to the irrational use of antibiotics (Shokri and Yadollahi, 2017; Rashid *et al.*, 2020). Subclinical endometritis in buffaloes is a main cause of infertility and represents a major role of repeat breeding syndrome in dairy animals (Elsayed *et al.*, 2020; Singh *et al.*, 2020). A repeat breeder is animal fails to conceive after three services associated with true oestrus. There are a number of factors implicated in this condition. In general, uterine infections together with anoestrus and environmental factors are the main causes (Saraswat and Purohit, 2016). In buffloes, failure of fertilization and early embryonic death has also been considered (Mohyuddin *et al.*, 2019). There are different diagnostic techniques for the diagnosis of subclinical endometritis including biopsy, endometrial cytology, ultrasonography and vaginoscopy (Singh *et al.*, 2020; Parikh *et al.*, 2022). Uterine tumours are very few and rare among dairy animals. However, leiomyomas are the most common tumour among cattle and buffaloes (Sachan *et al.*, 2018; Azawi and Al-Sadi, 2010). In a conclusion, the present study clarified that uterine disorders play a major role in female buffaloes infertility.

## MATERIALS AND METHODS

### STUDY PERIOD AND LOCATION

Uteri of 340 of buffaloes (3-6 years) with a history of infertility have been collected from different farms in Egypt for a period of two years (2021-2022).

### PATHOLOGICAL EXAMINATION

A gross inspection of the body of the uterus and two horns was performed for any external abnormalities. Endometrium was examined for colour, the nature of the fluid, and any internal lesions. Specimens from affected parts were collected and fixed in neutral buffered formalin (10%). The specimens were processed, embedded in paraffin, sectioned at 3-5 um, and stained with Hematoxylin and Eosin, as well as Masson's trichome stains (Suvarna *et al.*,

2019).

### MICROBIOLOGICAL STUDY

The uteri were thoroughly inspected immediately after exsanguination and Swabs were collected from the lesions and put in buffered peptone water transport media. Samples were kept on ice bags and sent to the Microbiology laboratory of a College of Veterinary Medicine, King Faisal University. Identification of the bacteria was carried out as described previously by (Quinn *et al.*, 1994).

### STATISTICAL ANALYSIS

The collected data from female buffaloes was recorded and analysed via SPSS 16.0 program (SPSS 16.0 for Windows Evaluation Version Release 16.0; 06 September 2007). The chi-square test was in the evaluation of different variables. P < 0.001 was considered statistically for seasonal variations.

## RESULTS AND DISCUSSION

Uterine lesions were observed in 78 animals out of 340 (22.94%). Buffaloes were more susceptible to infection in summer comparable with spring, winter, autumn (Chi-square 70.44, P <0.001) (Table 1). In summer time, there are the suppression of ovarian activity and increasing embryonic deaths (Campanile *et al.*, 2007; Ponraj *et al.*, 2017).

The acute endometritis was noticed in 6/33 animals (18.18%) and five of them were positive for bacteriological examination. Grossly, the uterine mucosa was severely congested and oedematous. Histologically, the lining epithelium of the endometrium was eroded and the endometrial stroma was highly infiltrated by neutrophils (Figure 1A). The stromal blood vessels were greatly congested and few of them showed fibrin thrombi (Figure 1B). Ultrastructural examination revealed several vacuoles inside neutrophils containing electron-dense rod-like structures (Figure 1C). The presence of bacteria indicates recent infection and early stage of inflammation (Table 2).

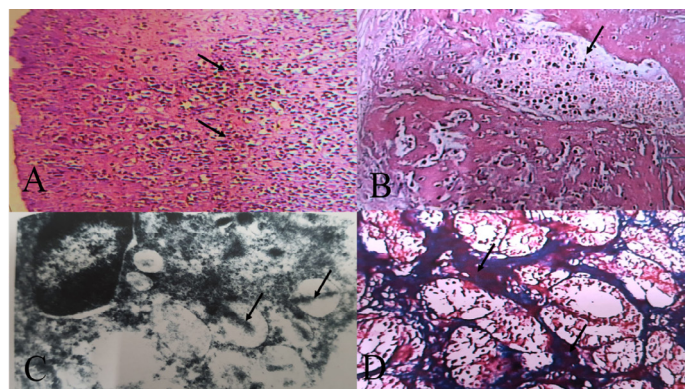
**Table 1:** Pathological lesions in the uteri of buffaloes during different seasons.

Lesions	Total animals (340)		Seasons				$\chi^2$ test	P
			Spring	Summer	Autumn	Winter		
Endometritis	33	(9.71%)	2	28	3	0	70.44	<0.001
Metritis	11	(3.24%)	1	6	3	1	6.29	0.09
Endometrial hyperplasia	7	(2.06%)	0	6	0	1	14.44	<0.01
Adenomyosis	5	(1.47%)	2	3	0	7	8.98	<0.05
Fibroma	3	(0.88%)	1	2	0	0	3.69	0.296
Perimetrial cysts	9	(2.65%)	6	1	1	1	8.56	<0.05
Perimetrial nodules	10	(2.94%)	7	1	1	1	11.13	<0.05
Total	78	(22.94%)	19/78	47/78	8/78	11/78		

$\chi^2$ , Chi-square at (P<0.05) considered significant.

**Table 2:** Isolates of bacteria and its frequency in various types of inflammation of uteri.

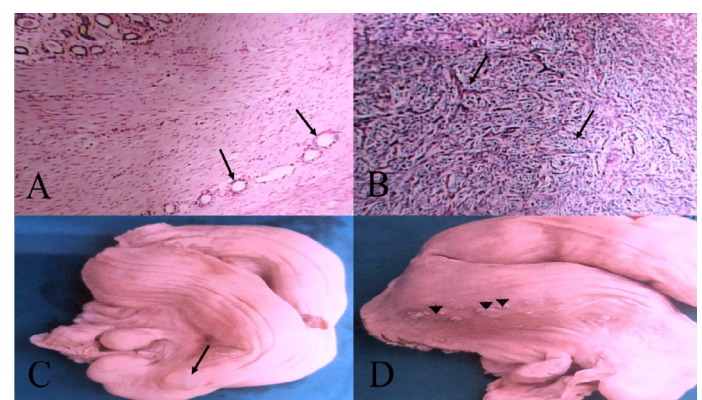
Bacterial isolates	Endometritis		Metritis	
	Acute	Chronic	Acute	Chronic
Klebsiella oxytoca	2	4	-	1
Proteus vulgaris	-	2	-	-
Esherichia coli	1	-	-	-
Enterobacter aerogenes	1	1	-	-
Pseudomonas aeruginosa	1	-	-	-
Staphylococcus aureus	-	1	-	-



**Figure 1:** (A) Acute endometritis. Heavy infiltration with neutrophils in endometrium (arrows) HE X100. (B) Acute endometritis. Fibrin thrombi in endometrial blood vessels (arrow) HEX100. (C) Acute endometritis. Rod shape bacilli in phagosome of macrophage (arrow) (Uranyl acetate and lead citrate) X28000. (D) Chronic endometritis. Periglandular fibrosis of endometrial glands (3-5 layers of fibroblasts) (arrow) Masson's trichrome stain X100.

This finding was coincided with Bhadaniya *et al.* (2019). Chronic endometritis was noticed in 27/33 animals (81.82%) and one case was positive for bacteriological examination. Grossly, the uterine mucosa was corrugated and appeared dirty yellow in colour. Histologically, the endometrial surface was completely necrosed and the endometrial stroma was highly infiltrated by lymphoplasmacytic infiltration. Periglandular fibrosis (2-5 layers) was noticed in most cases (Figure 1D). The major chronic inflammatory cells were Lymphocytic type and/or plasma cells. Gonzalez *et al.* (1985) explained that the presence of these cells indicates the local production of sperm antibodies which interfere with conception. The presence of these types of cells may cause infertility and failure of implantation (Mohyuddin *et al.*, 2019). Periglandular fibrosis was also seen in most cases of chronic endometritis and this finding was agreed with Salemi *et al.* (2020) in water buffaloes in Iran. Messier *et al.* (1984) clarified that periglandular fibrosis plays an important role in the reduction of uterine milk, early embryonic death, and consequently repeat breeder. Metritis was observed in 5 animals (1.47%) and all cases seemed to be chronic.

Similar findings were observed in buffaloes and could be attributed to retained placenta or dystocia (Kaneene and Miller, 1994; Parmar, 2018). Endometrial hyperplasia was recorded in 5 animals (1.47%). Similar finding was recorded by several authors (Saxena *et al.*, 2006; Pereira *et al.*, 2015). Cystic graafian follicles, ovarian neoplasms (granulosa cell tumors especially), and estrogens from plants are causes of endometrial hyperplasia in the cow (Zachary, 2017). Adenomyosis was observed in 2 animals (0.58%). There were clusters of endometrial glands growing throughout the muscular layer of the uterus (Figure 2A). Similar percentage was recorded in buffaloes (Saxena *et al.*, 2006). However, Alfaris and Fahid (2009) recorded a higher percentage (20%). Adenomyosis may arise as a malformation or due to hyperplastic overgrowth of the endometrium (Jubb *et al.*, 1993). Adenomyosis could be the cause of anoestrus and repeated breeding in buffaloes (Alfaris and Fahid, 2009). Fibroma was observed in 3/340 animals (0.71%). There were bundles of collagen fibers running in different directions and mostly seen around blood vessels (Figure 2B). Perimetrial cysts were observed in 11 animals (3.24%). The cysts have thin walls containing a clear fluid and were firmly adhered to the outer uterine wall (Figure 2C). Similar cysts were recorded in buffaloes (Mittal *et al.*, 2009). McEntee (1990) explained that cysts developed in postpartum uterine involution in which the myometrium contracted leading to the infolding of perimetrium. Perimetrial nodules were observed in 10 animals (2.38%). The nodules appeared as single or multiple have red or greyish colour, and are of different sizes and shapes (0.4 in diameter) (Figure 2D). These nodules could be indicative of chronic reaction due to continuous irritation during an examination of animals with reproductive problems.



**Figure 2:** (A) Uterus. Adenomyosis. Endometrial glands migrating throughout myometrium (arrows) HEX40. (B) Uterus, Fibroma. Fibroblastic proliferation and collagen fibers around blood vessels and in different directions (arrows) HEX40. (C) Uterus (right horn). The outer surface (perimetrium) has transparent cyst (1.7X1.4 cm) (arrow). (D) Uterus. The outer surface (perimetrium) has greyish nodules of different sizes (arrowheads).

The conclusion is that uterine disorders play a crucial role in female buffaloes infertility. It is highly recommended to use specific clinical parameters and biopsy techniques for early diagnosis in order to proceed with the appropriate treatment.

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## NOVELTY STATEMENT

The present study tries to clarify that certain uterine disorders may play a major role in female buffalo infertility.

## AUTHOR'S CONTRIBUTION

MH designed the study. AJ examined histopathology. FH interpreted the data. AA prepared the manuscript. MH critically revised the manuscript. All the authors approved the final version of manuscript.

## ETHICAL APPROVAL

The methods of the study were approved by the Research Ethics Committee at King Faisal University, Saudi Arabia with Approval number KFU-REC-2022-NOV-ETHICS285.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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