HISTOPATHOLOGICAL CHANGES IN THE UTERUS OF INFERTILE SLAUGHTERED BUFFALOES

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ABSTRACT: Agribusiness is the foundation of Pakistan's economy as it contributes 18.9 percent to the nation's GDP. Buffalo is a significant investor with a populace of 38.8 million heads. The profitable characteristics of Nili Ravi wild ox are inimitable with some other buffalo breeds on the planet, while the conceptive qualities are still nadir. The most widely recognized regenerative issues experienced are postponed time of pubescence, helpless estrus articulation, low ripeness with AI, deferred baby blues anestrous, and irregularity. These attributes have made it amazingly hard to increase quick hereditary advancement in this variety. Conceptive biotechnologies assume a key job in breed improvement by bypassing these obstacles. For this study eighty animals were selected and slaughtered due to reproductive issues and reproductive tracts were collected from the slaughterhouse and observe the tract grossly (texture and shape, any exudate, ovarian structures). Then cut the uterine tissue sample with the size of 2 × 2 × 2cm was fixed in formalin 10% for histopathology. After processing microscopy was performed to find histopathologic changes (Endometrial epithelium, uterine gland, and inflammatory cells and caruncles status). The grading of animals is labeled as Normal (-), Mild (+), Moderate (++), and Severe (+++) on basis of the degree of infection. The white side test was negative for 40% of samples while 60% of animals are positive with different degrees of affection. Endometrial epithelium erosion is absent in 31.43% of animals while 68.67% of animals have erosions. Atrophy of uterine glands was absent in 41.43% of animals while 58.67% of animals contained atrophy of glands. 11.43% of animals were negative for inflammatory cells while 88.66% of animals were positive for inflammatory cells. 20% of animals had normal caruncles while 80% of animals had swelled caruncles. Discharge was collected for the white side test from the cervix. 62.86% of animals had serious discharge while 37.14% of animals had a purulent discharge. The color and texture of the tract are divided into two categories pale and pale reddish with swelling. 54.74% had a pale color while 45.26% had a pale and reddish with swelling. In conclusion, 60% of animals were affected by endometritis including mild, moderate, and severe. Histopathological parameters and the nature of exudate also showed a significant relationship with endometritis which was indicated by the white side test. The treatment of endometritis should be done after the white side test before insemination. In this way, we can improve reproductive performance and conception rate. Further studies are warranted to perform the culture sensitivity test and treat the animals with intra-uterine and systemic antibiotic therapy.

Keywords. Buffalo, Histopathology, white side test.

(Received 23.10.2022 Accepted 27.01.2023)

INTRODUCTION

Pakistan is a developing state and the per capita income of Pakistan is 1130 US Dollars (Khan 1990). Pakistan's economy is agriculture based and the share of agriculture in the GDP of the country is 19.2%. There are two main parts of agriculture one part is that includes crops and cultivation and the other part is livestock which includes all animals and entities related to animals. The crops account for 39.9% while the livestock accounts for 60.1 in this total of 19.2% (Economic Survey of Pakistan, 2020-21). Livestock plays a pivotal role in the development of the state and provides respectful resources of earning to the citizens of the country. Eight million Pakistanis are engaged in this source of earning. The country has a total of more than 200 million animals including cows, buffaloes, sheep, and goats with a population of 51.5 million, 42.4 million, 31.6 million, and 80.3 million respectively. The major products include beef and milk while pluck and offals are also rendered useful (Economic Survey of Pakistan, 2020-21). According to the Economic Survey of Pakistan, the total milk production of the country is 63.7 million tons per year. Nilli Ravi known as the black gold of Pakistan is the largest milk-producing species in the country with a share of 61% in the total milk production. The fat

percentage of buffalo milk is about 7% (Han *et al*. 2007). Beef is another major product with a total production of 2380 metric tons per annum (Economic Survey of Pakistan, 2020-21).

Despite having many good dairy characteristics, the reproductive performance of the buffalo is not so impressive. The main reproductive problems encountered by the specie are delayed puberty, seasonal anestrous, repeat breeding, postpartum anestrous, and low fertility with artificial insemination (AI) (Drost 2007b). Due to mentioned reason the goal of calf a year is difficult to achieve in buffaloes. To overcome this problem, scientists used many assisted reproductive technologies (ART) so that a goal of calf a year can be achieved easily. Along with this goal, genetic improvement was also made by using ART. But some issues are not going to sort out including repeat breeding and some uterine diseases. The bovine uterine disease reduces milk yield, impairs fertility, and has implications for animal welfare. During involution, the uterus is usually exposed to multiple potential bacterial pathogens which are cleared by successful action of the local inflammatory response. Repeat breeding is an issue that is still present in high prevalence, especially in buffalo. A lot of treatments and protocols were applied to sort out this issue without fruitful results.

Histopathology of the uterus can assist us to find out the exact problem and is also helpful in the treatment of the issue. Histopathology can be performed by taking a tissue sample from the uterus in two ways biopsy and autopsy (Rhyaf 2010). A biopsy is an invasive process that is performed on live animals and also has the risk of infection. An autopsy is a safe process if performed under controlled conditions and proper history of the animal. Histopathology of the uterus will guide us through the common problem in specific areas where tracts are collected. This study was conducted on female reproductive tracts of buffalo and compare the characteristics and histopathology parameters with normal parameters of the tract. It was helpful to determine the common disorders in that population of buffalo.

Two main factors are considered the cause of repeat breeding fertilization failure and early embryonic death. These factors lead to infertility. The major cause of infertility in buffalo is ovarian inactivity but some issues have causes still unknown such as repeat breeding. In the past, there is a lot of studies working on infertility but were unable to sort out the issue completely. Infertility covers a variety of disorders some of which are untreatable due to non-specific causes. Histopathology is done by using autopsy techniques that differentiate in parameters of normal and infertile or repeat breeder animals. This study was also able to describe the major issue or disease in a specific area where these tracts were collected. The main objective of our study is the comparison between histopathological parameters of normal and infertile or repeat-breeding buffalo. To find the relationship between the white side test and histopathological changes in the reproductive tract.

Histopathology and culture sensitivity are the major tools to evaluate reproductive issues and infertility. Histopathology better describes the status of endometrial glands, caruncles, inflammatory cell infiltration, hemorrhages, and the thickness of uterine layers (Thasmi *et al*. 2018). Currently, the problem is a limitation of knowledge about the cause of repeat breeding and infertility in buffalo. In this study, the histopathological changes in the uterus of infertile or repeat breeder-slaughtered buffalo were observed.

MATERIALS AND METHODS

Site and Animals: This study was carried out at the PAMCO (Punjab Agriculture & Meat Company) slaughterhouse and the histopathology lab, department of Histopathology, Faculty of Biosciences, University of Veterinary and Animal Sciences Lahore. A total of eighty samples from slaughtered animals were included in this study. These animals were slaughtered due to repeat breeding (Not conceiving after three consecutive services and not any apparent disorder) and infertility (Having corpus luteum on the ovary and not presenting any apparent vaginal discharge) on the base of history taken from the owners. The age of animals was between 4-6 years which was calculated by observing the dentition (presence of six permanent incisors) of animals. These animals have a 3 to 3.5 body condition score. Animals were on rest for a minimum of eight hours before slaughtering to reduce the stress factor in the holding pen of the slaughterhouse. In this holding pen, observation was performed to check apparent disorder, dentition, and body condition scores.

Whiteside test: Samples for the white side test were collected from the cervix of the reproductive tract (Kumar *et al*. 2015). Then samples were carried to the office PAMCO to perform the white side test to check the endometritis. To perform this test 1ml of 10% NaOH was added to the sample and mixed. Then boil the mixed sample on a spirit lamp and evaluate the results. This test was evaluated on the base of color after boiling dark yellow for severe endometritis, yellow for moderate endometritis, and light yellow for mild endometritis (S *et al*. 2012). Whiteside test classifies into four groups normal (-), mild (+), moderate (++), and severe (+++) (Soundara Pandian and Punniamurthy 2007).

Histopathology

Sample collection; Unless otherwise stated, all of the chemicals used in this research were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Sample

collection equipment included plastic 250ml lid containers having 10% formalin solution, and glass test tubes to collect the sample for cervical mucus. Sample collection was done in the evening during slaughtering days twice a week. Reproductive tracts were collected including the vagina, cervix, uterus, and ovaries. The tracts selected for the processing had corpus luteum and no vaginal discharge was observed.

Samples were collected for histopathology from the uterus. 1-2cm part of the uterine body was taken by using a scissor and stored these samples into 10% formalin solution. Label these samples by using animal ID which was assigned A1-A80 and also having the record of other parameters of the same animal. Then these samples were carried to the histopathology lab, department of Histopathology, Faculty of Biosciences, University of Veterinary and Animal Sciences Lahore. These samples were stored at room temperature and processed in two sessions with the steps and protocols of the lab for tissue (Ahmed 2016).

Trimming and fixation: The microscopic parameters were studied after the histopathology of these samples. Histopathology of these samples was performed by following this procedure. A small part of the organ (uterine horn) of slaughtered buffalo which showed pathological changes was cut into 2 × 2 × 2 cm size. Uterine horn tissues were immersed in 10% formalin fixative for the fixation.

Dehydration and embedding: Samples of uterine horn that were fixed in 10% formalin solution were then washed and dehydrated in an ethanol series with increasing concentrations (80% alcohol, 90% alcohol, and absolute alcohol), cleared in xylene, and embedded in paraffin wax.

Sectioning and staining: The wax block was then sectioned at a thickness of 5 µm by using a microtome then stained with hematoxylin-eosin to observe histopathological changes.

Microscopic examination: Then slides were observed at 4x, 10x, 40x and 100x.Microscopicparameters endometrial epithelium, uterine gland atrophy, Presence of inflammatory cells and caruncles status were observed in microscopy. Endometrium epithelium erosion was observed at 10x and 40x. Uterine gland atrophy was observed at 40x. Inflammatory cells were observed at 40x and 100x. While caruncles swelling was observed at 10x and 40x. All these parameters were observed and classify them into two categories (-) and (+).

Macroscopic parameters: Macroscopic parameters were included color of tract, nature of exudate, white side test. These parameters were observed and noted before collect the samples for histopathology. Color of tract indicated the metritis. Exudate nature which was serous or purulent indicated the degree of infection.

Microscopic parameters: The microscopic parameters included erosion of epithelium endometrium, uterine gland atrophy, inflammatory cells, and caruncles swelling. These parameters were observed at different resolutions.

Statistical Analysis: The data obtained is expressed in percentages. "Chi-square" was used to examine the parameters. Pearson Chi-Square was used to compare the normal, mild, moderate, and severe infections in the selected animals from the slaughterhouse ($P<0.05$).

RESULTS

The total 80 samples of cervical mucus and uterine tissues were analyzed by white side test to check the endometritis and histopathological changes in uterine tissues.

The grading of animals was labeled as Normal (-), Mild (+), Moderate (++), and Severe (+++) on basis of the degree of infection. The whiteside test was negative for 40% of samples, while 60% of animals are positive with different degrees of infection. Endometrial epithelium erosion was not observed in 31.43% of animals, while 68.57% of animals had epithelium erosions. Atrophy of uterine glands was absent in 41.43% of animals and 58.57% of animals contained atrophy of glands. 11.43% of animals were negative for the presence of inflammatory cells while 88.57% of animal's positive. 20% of animals had normal caruncles and 80% of animals had swelled caruncles.

Discharge was collected for the white side test from the cervix. 62.86% of animals had serous discharge while 37.14% of animals had a purulent discharge.

Color of tract: The color and texture of the tract are divided into two categories, pale and pale reddish with swelling. 54.74% of tracts were normal and pale in color while 45.26% of tracts are swollen and pale reddish. The association of the color of the tract with the white side test for endometritis was found by using Pearson's CHI square. $P<0.05$ in Table 4.4 is shown a significant association between the color and the White side test.

Exudate: Exudate was observed using two categories serous and purulent. 62.86% of tracts having serous discharge while 37.14% of tracts have purulent discharge. The association between the exudate and white side test is shown in Table 4.5. The $P<0.05$ showed a significant relationship.

Endometrial epithelium: 31.43% of tracts have normal and intact epithelium while 68.57% have ruptured epithelium. Pearson's Chi-square shows the association between epithelium and the white side test in Table 4.6. The $P<0.05$ value show a significant association.

Table 1: Percentage of animals with the grading of infection (n=80).

Sr. no.	Parameters	Normal(-) Percentage	Mild(+) Percentage	Moderate(++) Percentage	Severe(+++) Percentage
1	Whiteside test	40.00	14.29	31.43	14.29

Table 2: Grading of animals with infection (n=80).

Sr. No.	Parameters	Normal (-) %	Positive(+) %
1	Exudate, serous(-) purulent(+)	62.86	37.14
2	Endometrial epithelium Erosion	31.43	68.57
3	Uterine gland atrophy	41.43	58.57
4	Inflammatory cells	11.43	88.57
5	Caruncle swelling	20.00	80.00

Table 3: Color and texture of tract (n=80).

Sr. No.	Parameter	Pale %	Pale reddish and swelling %
1	Color and texture	54.74	45.26

Table 4 Chi-Square tests to check the association between color and WST.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	80.000[a]	3	.000
Likelihood Ratio	110.703	3	.000
Linear-by-Linear Association	66.773	1	.000
N of Valid Cases	80		

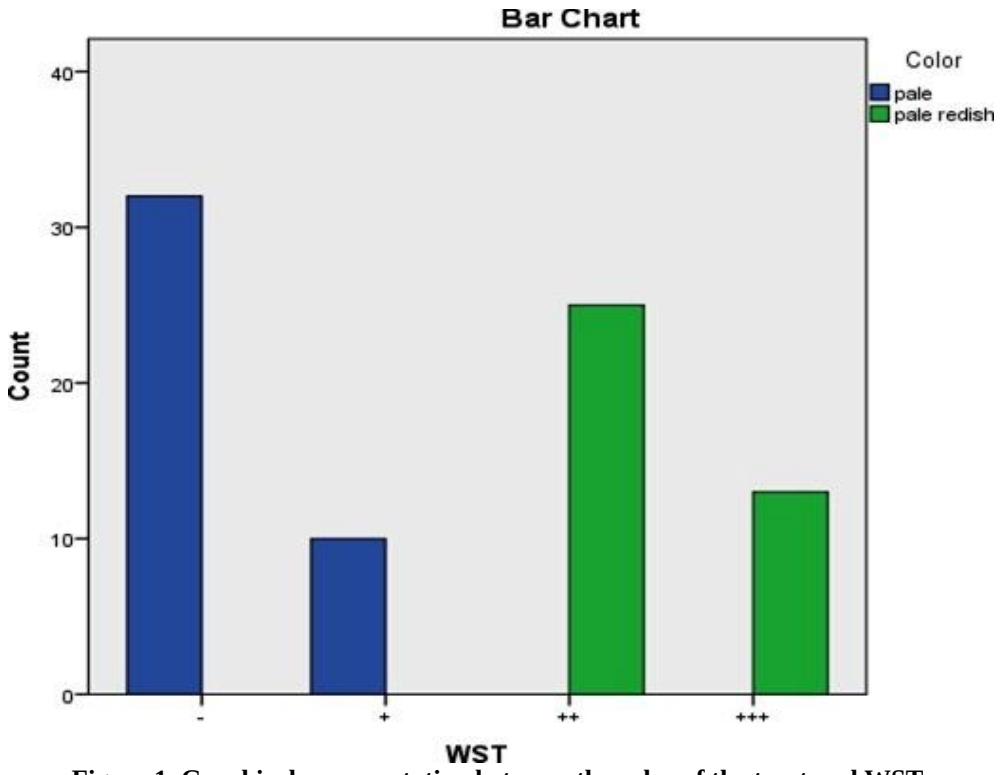

Figure 1. Graphical representation between the color of the tract and WST.

Table 5 Chi-Square tests to check the association between exudate and WST.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	55.075[a]	3	.000
Likelihood Ratio	72.104	3	.000
Linear-by-Linear Association	50.421	1	.000
N of Valid Cases	80		

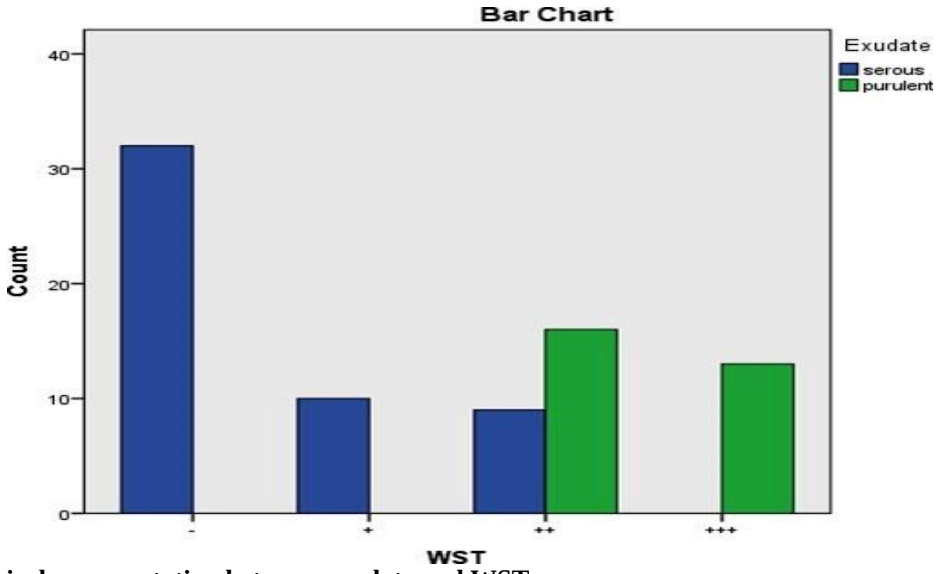

Figure 2. Graphical representation between exudate and WST.

Table 6 Chi-Square tests to check the association between epithelium and WST.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	54.545[a]	3	.000
Likelihood Ratio	65.753	3	.000
Linear-by-Linear Association	42.104	1	.000
N of Valid Cases	80		

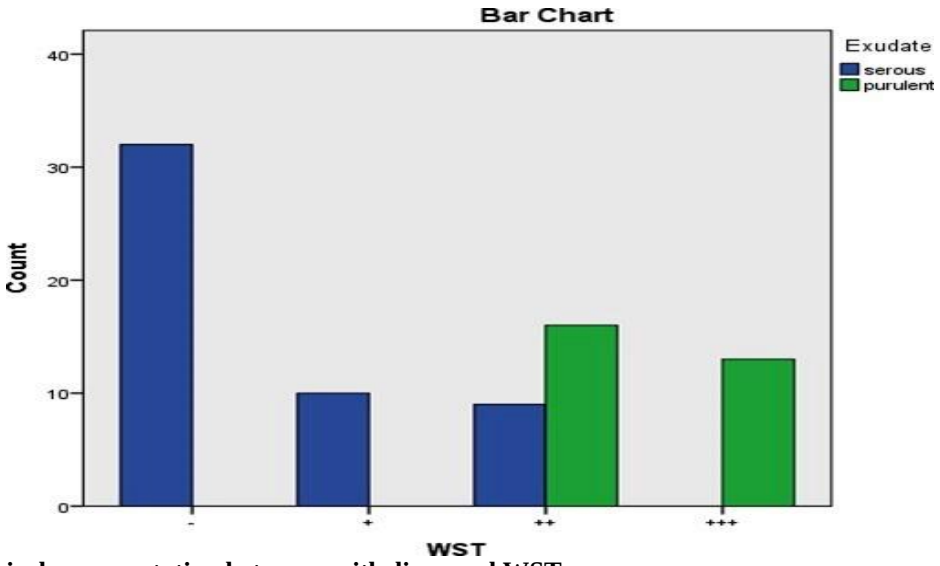

Figure 3. Graphical representation between epithelium and WST.

Uterine gland: 41.43% of uterine glands were normal while atrophy of glands was observed in 58.57%. Pearson's Chi-square showed the association between uterine gland atrophy and the white side test in Table 4.7. The P<0.05 value show the significant association between them.

Table 7 Chi-Square tests to check the association between the uterine gland and WST.

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	45.517[a]	3	.000
Likelihood Ratio	54.357	3	.000
Linear-by-Linear Association	35.135	1	.000
N of Valid Cases	80		

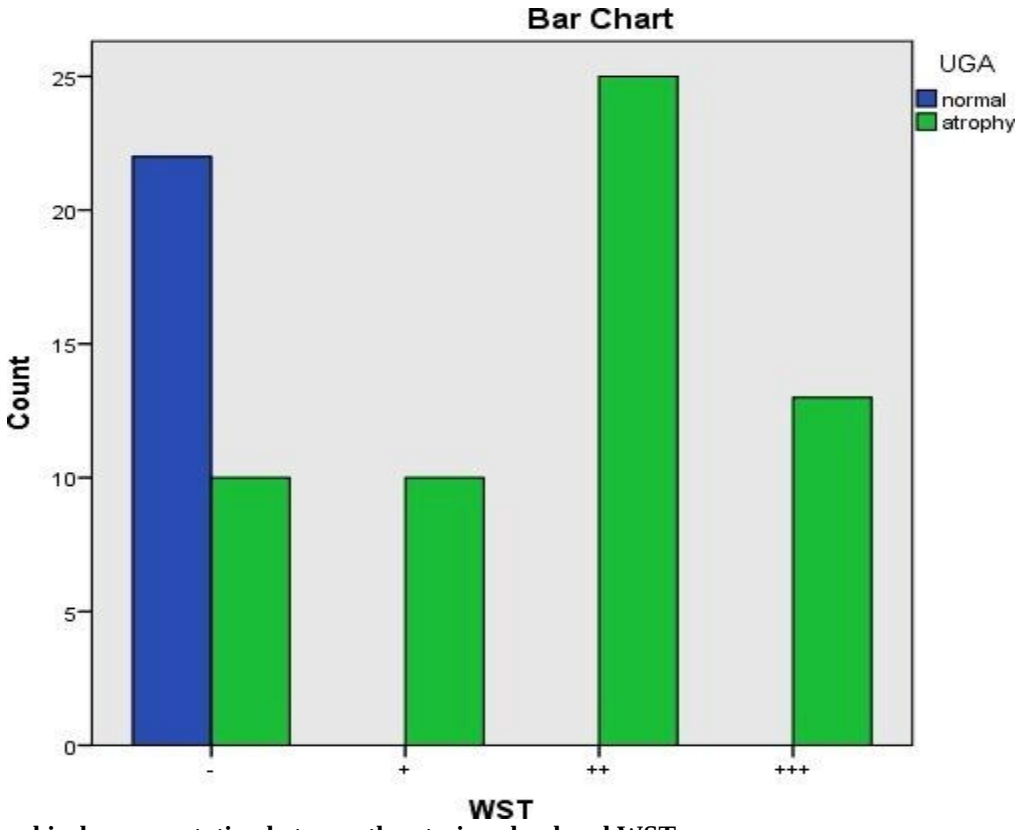

Figure 4. Graphical representation between the uterine gland and WST.

Inflammatory cells: 11.43% of tracts did not contain inflammatory cells while 88.57% of tracts had inflammatory cells. Table 4.8 shows the association between the presence of inflammatory cells and with white side test. The P<0.05 value shows the significant association between them.

Table 8 Chi-Square tests to check the association between inflammatory cells and WST.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.143[a]	3	.001
Likelihood Ratio	20.534	3	.000
Linear-by-Linear Association	13.233	1	.000
N of Valid Cases	80		

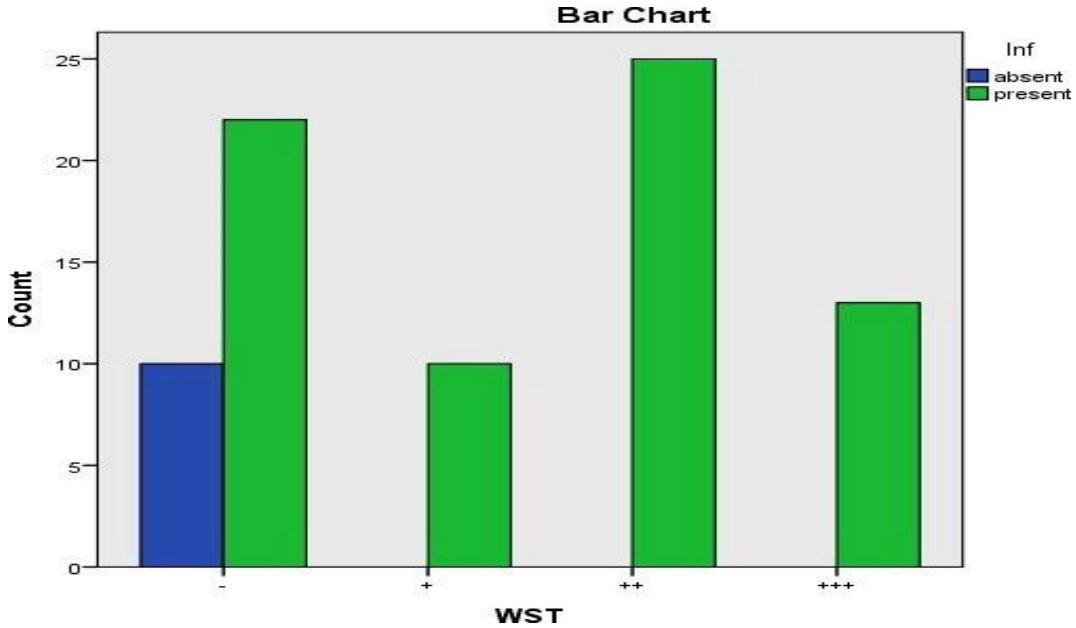

Figure 5. Graphical representation between inflammatory cells and WST

Caruncles: 20% of tracts had normal size and shape of caruncles while 80% of tracts were swelled and misshaped. Pearson's Chi-square showed the association between caruncle status and the white side test in Table 4.9. The P<0.05 value show the significant association between them.

Table 9 Chi-Square tests to check the association between caruncles status and WST.

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	30.000[a]	3	.000
Likelihood Ratio	35.703	3	.000
Linear-by-LinearAssociation	23.157	1	.000
N of Valid Cases	80		

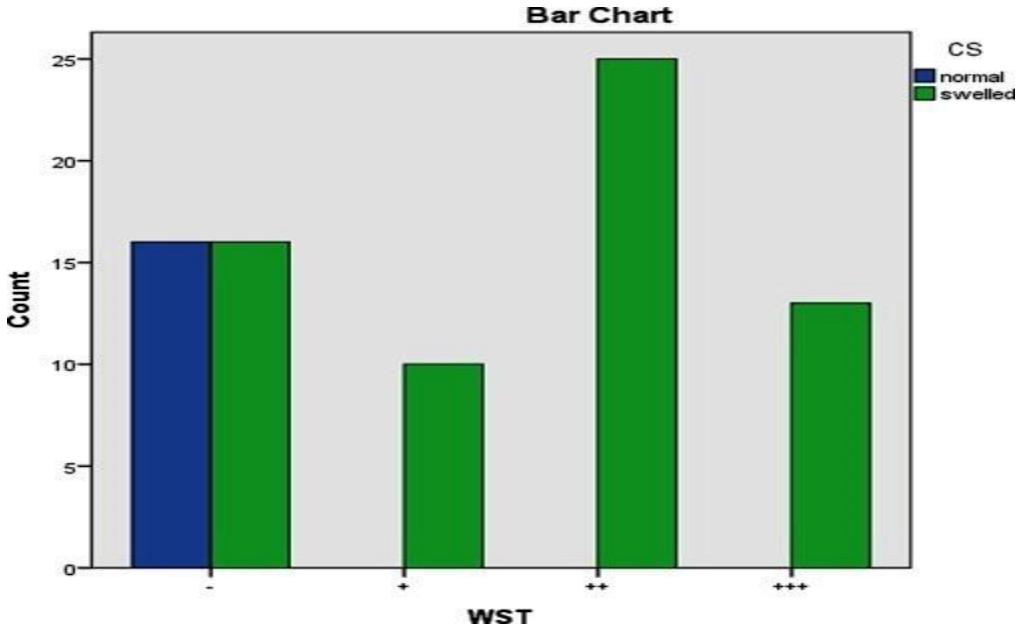

Figure 6. Graphical representation between caruncles and WST

DISCUSSION

The macroscopic parameters (texture and shape, exudate, ovarian structures) and microscopic parameters (endometrial epithelium, uterine gland, inflammatory cell, and caruncles status) of infertile slaughtered buffalos were observed and compared with normal parameters. About 70% of female buffalos were slaughtered in slaughterhouses within slaughtering days due to complaints of conception failure after multiple services. This study was conducted to find out the common problems of these slaughtered buffalos due to which they failed to conceive. A white side test was performed to check the incidence of sub-clinical endometritis. The dark yellow color of samples indicates severe (+++) endometritis, the yellow color indicates moderate (++) infection and the light yellow color indicates mild infection having an incidence of 14%, 31.4%, and 14.3% respectively. About 60 % overall slaughtered buffalos had sub-clinical endometritis. The treatment of endometritis should be done after the white side test before insemination. In this way, we can improve reproductive performance and conception rate. Another study concluded that treatment of sub-clinical endometritis leads to improvement in the general health condition of the animals which might be helpful to increase the conception rate of repeat breeder cows (Pandey *et al.*). The grading of endometritis was labeled as Normal (-), Mild (+), Moderate (++), and Severe (+++) on basis of the degree of infection.

The whiteside test was negative for 40% of samples while 60% of animals are positive with different degrees of infection. Endometrial epithelium erosion was absent in 31.43% of animals while 68.67% of animals have erosions. Atrophy of uterine glands was absent in 41.43% of animals while 58.67% of animals contained atrophy of glands. 11.43% of animals were negative for the presence of inflammatory cells while 88.66% of animals were positive for inflammatory cells. 20% of animals had normal caruncles while 80% of animals had swelled caruncles. The status of epithelium is directly related to the white side test in the condition of endometritis. The number of inflammatory cells also increased as the severity of endometritis increased. Under field conditions, subclinical endometritis must be diagnosed with the help of a white side test and treated with intrauterine therapy or systemic antibiotic therapy. In this way, the reproductive performance can be improved and reduce the culling rate.

The whiteside test for endometritis is a valuable tool for confirmation after building an association with inflammatory cells in the present study. The whiteside test was utilized to determine changes in cervical mucus color from normal to a light yellowish color. There was a significant correlation in the detected subclinical endometritis by endometrial cytology that indicated an increasing percentage of PMNs up to 12%≥ 18% with the increased age of the affected cows (5.43± 0.841) in the selected population (Bedewy and Rahaway 2019). The whiteside test also showed high associations with endometrial epithelium. The animals which were positive for the white side test have a discontinuity in the endometrial epithelium while the animals which are negative for the white side test mostly have intact epithelium. A ratio of polymorph nuclear cells (PMNs) to epithelial should be calculated. If the ratio is more than 12% PMNs to epithelial cells, subclinical endometritis is significant (Bedewy and Rahaway 2019).

In this study white-side tests also have an association with the uterine glands. The animals which were positive for the white side test have degenerated and atrophy of glands. The epithelium of glands is also disturbed. The most perceptible changes observed in the uterine endometrial glands include a reduction in number, density, extent of branching, and lumen width in both superficial and deep endometrial zones. Glandular epithelium in some endometrial glands revealed degenerative changes and initial vacuolization. Histologically, the presence of moderately dilated endometrial glands with muco-protenacious material inside the glandular lumen was observed. It was also associated with adenomyosis characterized by the presence of endometrial glands and stroma embedded within the myometrium. However, endometrial glands within the myometrium were lined by a single layer of cuboidal epithelium and atrophied without containing any material in the lumen.

Periglandular infiltration of lymphocytes and plasma cells along with atrophy and degeneration of endometrial glands were observed (Chethan et al. 2015).

In conclusion, 60% of animals were affected by endometritis including mild, moderate, and severe. Histopathological parameters and the nature of exudate also showed a significant relationship with endometritis which was indicated by the white side test. The treatment of endometritis should be done after the white side test before insemination. In this way, we can improve reproductive performance and conception rate. Further studies are warranted to perform the culture sensitivity test and treat the animals with intra uterine and systemic antibiotic therapy.

REFERENCES

Ahmed M. 2016. Steps of tissue processing in histopathology laboratory,Review Report. HEALTH DIGEST. 1: 26-27.

Al-Fahad T, Alwan A, Ibraheem N. 2004. Histological and morphological study of abnormal cases of female reproductive system in Iraqi buffaloes. Iraqi J. Vet. Sci. 18: 109-115.

Amiridis G, Tsiligianni T, Dovolou E, Rekkas C,

Vouzaras D, Menegatos I. 2009. Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. Theriogenology. 72(4): 542-548.

Balogh O, Túry E, Abonyi-Tóth Z, Kastelic J, Gábor G. 2014. Macroscopic and histological characteristics of fluid-filled ovarian structures in dairy cows. Acta Veterinaria Hungarica. 62(2): 215-232.

Drost M. 2007a. Advanced reproductive technology in the water buffalo. Theriogenology. 68(3): 450-453.

Drost M. 2007b. Bubaline versus bovine reproduction. Theriogenology. 68(3): 447-449. Graden AP, Olds D, Mochow CR, Mutter LR. 1968. Causes of Fertilization Failure in Repeat

BreedingCattle1. J. of Dairy Sci. 51(5): 778-781.

Gustafsson H, Emanuelson U. 2002. Characterisation of the repeat breeding syndrome in Swedish dairycattle. Acta veterinaria Scandinavica. 43(2): 115-125.

Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 64(9): 1879-1888.

H Lazim E, J Ali A, I Azawi O. 2008. Pathological and anatomical abnormalities affecting buffalo cowsreproductive tracts in Mosul. Iraqi J. of Vet. Sci. 22(2): 59-67.

Han B-Z, Meng Y, Li M, Yang Y-X, Ren F-Z, Zeng Q-K, Nout MR. 2007. A survey on the microbiological and chemical composition of buffalo milk in China. Food Control. 18(6): 742-746.

Hatipoglu F, Kiran M, Ortatatli M, Erer H, Çiftçi M. 2002. An abattoir study of genital pathology in cows: I. Ovary and oviduct. Revue de médecine vétérinaire. 153(1): 29-34.

Jainudeen M, Hafez E. 2000. Cattle and buffalo. Reproduction in farm animals. 157-171.

Khan AH. 1990. Wagner's" law" and the developing economy: a time series evidence from Pakistan.

Indian Economic J. 38(1): 115.

Kumar S, Bhardwaz A, Srivastava AK, Rao M, Kumar N. 2015. White side test--a field test on the cervical mucus of cows for diagnosis of endometritis. Intas Polivet. 16(2): 207+.

Madoz LV, Giuliodori MJ, Migliorisi AL, Jaureguiberry M, de la Sota RL. 2014. Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. Journal of Dairy Science. 97(1): 195-201.

Meira EBS, Henriques LCS, Sá LRM, Gregory L. 2012. Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. J. of Dairy Sci. 95(12): 6969-6973.

Pascottini OB, Hostens M, Dini P, Vandepitte J, Ducatelle R, Opsomer G. 2016. Comparison between cytology and histopathology to evaluate subclinical endometritis in dairy cows. Theriogenology. 86(6): 1550-1556.

Rhyaf A. 2010. Histopathological Study of Endometritis of the cows. AL-Qadisiya Journal of Vet. Med.Sci. 9(1).

Salzano A, Pesce A, D'Andrea L, Paciello O, Della Ragione F, Ciaramella P, Salzano C, Costagliola A, Licitra F, Neglia G. 2020. Inflammatory response in repeat breeder buffaloes Theriogenology. 145: 31-38.

Saraswat CS, Purohit G. 2016. Repeat breeding: Incidence, risk factors and diagnosis in buffaloes. Asian Pacific J. of Repro. 5(2): 87-95.

S R, Devanathan T, K.Kulasekar, N.Palzhanivel, C.Balachandran. 2012. White-side test and endometrial biopsy for diagnosis of endometritis in Repeat Breeding cows. Indian Journal of Animal Reproduction. 33: 56-58.

Soundara Pandian S, Punniamurthy N. 2007. Sub-clinical uterine infections in repeat breeder cows. 84: 654-655.

Tandle M, Purohit G. 2013. Genital tract affections in the female buffalo. Bubaline Theriogenology. IVIS, Ithaca NY A. 5724: 0713.

Thasmi CN, Siregar TN, Wahyuni S, Aliza D, Panjaitan B, Nazaruddin N, Sabila FN, Fallatanza M.2018. Anatomical and histological changes of uterine horn of Aceh cattle with repeat breeding. J. of advanced vet. and ani. research. 5(4): 445-453.

Wajid SJ. 2015. A pathological abattoir survey of the reproductive tracts of non-pregnant camels (Camelus dromedaries) in Iraq. J Phar and Bio Sci. 10: 84-90.