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Steroid Hormones and Inflammatory Markers in the Uterine Tissue and Follicular Fluid of Cattle and Buffalo with Uterine Bacterial Infection

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Abstract

ENDOMETRITIS is the most common reproductive infertility problem in dairy animals. This study aimed to investigate the relation between the histopathological characterization of endometritis and ovarian steroid hormones in both uterine tissue and follicular fluids (FF) in addition to the proinflammatory cytokines and myeloperoxidase (MPO). Uteri collected from slaughter houses were subjected to hematoxylin and eosin (H&E) routine staining and Antisyndecan-1 immunohistochemistry aids to classify the type of endometritis. Bacteria in the apex of the examined uterine horns were isolated and identified. FF and uterine tissues homogenate were used to assay E2, progesterone (P4), tumor necrosis factor α (TNFα) and MPO. Results showed that sample-species type (cow uteri, buffalo uteri, cow FF, and Buffalo FF fluids) influenced (P<0.0001) MPO, E2, and P4. Uterine (Ut) pathology (acute suppurative, acute lymphocytic, chronic suppurative, chronic metritis, and normal); the isolated bacteria; Ut Pathology × Sample-Species; Ut Pathology × bacteria; and Bacteria ×Sample-Species influenced (P<0.0001) TNFα, MPO, E2, and P4. Ut Pathology × bacteria × Sample-Species affected TNFα, MPO, and P4. In conclusions, Immunohistochemistry is a reliable method to confirm the chronicity of endometritis. Both TNFα and MPO would be reliable markers for determining endometritis. Endometritis likely altered the FF proinflammatory markers and the steroid hormones and sequentially the future fertility. Buffaloes seem sensitive to uterine pathology caused by bacteria.

Keywords: Cattle, Buffalo, Endometritis, Immunohistochemistry, MPO, Ovarian hormones, tumor necrosis factor α.

Introduction

Endometritis in dairy animals is the main cause for culling and economic losses. The increase in the open days and number of services required for conception results in increasing the inter-calving interval while the cow or the buffalo remains dry for longer interval adversely affected the next calf crop and milk production. The increase in the open days and the number of services per conception together with the cost of medication required to treat endometritis are contributed to the high economic losses caused by endometritis. The improper treatment and misuse of medication result in flourishing multidrug-resist bacteria. In cows, the cytobrush can be used to take cellular samples from the endometrium to facilitate the diagnosis [1]. The development of clinical endometritis (acute or chronic) is associated with the presence of influx of polymorphonuclear cells (PMNs) leading to the presence of purulent or mucopurulent discharges [2, 3]. The count of three hundred polymorphonuclear cells or one-hundred cells by the high power in 10 fields was recommended to confirm the diagnosis of subclinical endometritis [4].

In the bovine uterine infections, Escherichia coli, *Trueperella pyogenes, Fusobacterium necrophorum* and *Prevotella* species are the most important pathogens [3]. Twenty-four days postpartum, *Hemolytic streptococci* and *T. pyogenes* increased the risk of endometritis but the presence of *E. coli* and coagulase-negative *Staphylococci* did not affect the prevalence [5]. In clinical endometritis, *Escherichia coli, Streptococcus spp (Strept. Uberis), Trueperella pyogenes (T. pyogenes), Actinomycetales, Lactobacillales, Bacillales, Burkholderiales, Caulobacteriales, Enterobacteriales, Pasteurellales* and *Pseudomonadales* were isolated [6, 7].

In bovine infected uteri, tumor necrosis factor-α (TNF-α) and many interleukins are produced [8] and

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their inflammatory markers gene expression were upregulated in the peripheral blood [9, 10] and the endometrium [8, 11] shortly after infection. In bovine subclinical endometritis, the non-detection of vaginal purulent materials with altered cellular composition reflected by a higher messenger RNA (mRNA) expression of lipocalin-type prostaglandin D synthase, IL1A, IL1RN, IL6, TNF, and CXCL8 confirmed the condition [1]. Concentrations of nitric oxide in blood and uterine secretions increased in animals with subclinical and clinical endometritis [12]. Cattle developed subclinical endometritis indicated lower polymorphonuclear cell MPO activity during the first eight days after parturition [13]. In bovine postpartum metritis, *Escherichia coli*–stimulated monocytes expressed fewer key proinflammatory cytokine genes (TNF, IL1B, and IL6) for two weeks after calving [14].

Therefore, this study aimed to identify the most common bacteria associated with clinical endometritis in the culled cows and buffaloes, classify the type of endometritis, confirm the endometritis with the histopathology and immunohistochemistry, and compare the ovarian hormones, TNFα and MPO concentrations and activities in the uterine tissue and FF collected from culled animals.

Material and Methods

All samples were collected shortly after animals' slaughter in Giza abattoirs according the Animal Care and Use Committee of the National Research Centre which approved the protocol without requiring any formal ethics approval in this particular case of bringing samples from already slaughtered animals.

Genitalia of buffaloes (N=52) and cows (N=36) were brought from the slaughter house as soon as possible. In the laboratory, sections from the infected parts were obtained and kept in 10% formalin for the histopathology and immunohistochemistry. Other sections were kept at -20°C for preparing uterine tissue homogenate. The apex of each uterine horn was cut and used for bacteriological examinations. The FF were aspirated in clean Eppendorf tubes for hormonal assays. Samples were labelled explaining animal species and the condition of the uterus identified by the gross anatomy of the collected samples.

Bacteriological examination

Sample were kept in transport medium Carry-Blair (Difco). Media used for isolation and studying the colonial morphology of the bacteria include Blood agar base medium (Oxoid), Mannitol salt agar medium (Oxoid), MacConkey's agar medium (Oxoid), Brain heart infusion broth, and Nutrient agar medium (Oxoid; [15]). Media used for biochemical identification of bacterial isolates are Nitrate reduction medium (Oxoid), Christensen's urea agar medium, Simmon's citrate agar medium, Sugar fermentation media, Oxidation - Fermentation medium (Difco), Gelatin agar media, Triple sugar iron agar medium, Glucose phosphate broth, and Peptone water 2 % [15]. Media used for preservation of bacterial isolates were 1. 0.4 % semisolid soft agar and Glycerol broth. Reagents and chemicals for catalase test are hydrogen peroxide H_2O_2 3%, paraffin oil, and citrated rabbit plasma. Reagents used for nitrate reduction test included 0.8% sulfanilic acid 5N, Acetic acid and 0.5% Alpha naphthylamine and acetic acid 5N. Reagents used for Voges-Proskauer test included 5 % alpha naphthol in absolute ethyl alcohol and 40% potassium hydroxide in Voges-Proskauer test. For the preparation of methyl red, 200 ml distilled water was added to 300 ml ethyl alcohol 95 % then 0.1 g methyl red was added. Kovac's reagent used for indole test, isoamyl alcohol 150 ml, pure dimethyl amine benzaldhyde 10 g, and concentrated HCl 50 ml. Gram stains was used for morphological identification of the isolates [15]:

Collected swab samples were streaked onto blood agar plates, MacConkey agar plates, and Mannitol salt agar plates. All samples were streaked in duplicate plates and incubated aerobically and anaerobically for isolation of aerobic, facultative anaerobic as well as anaerobic bacteria. Aerobic plates were incubated at 37 °C for 24 hrs. However, anaerobic plates were incubated anaerobically in an anaerobic jar using anaerobic system (BD) and were incubated at 37 °C for 48-72 hrs. After incubation, the discrete colonies were picked up and recultivated onto nutrient agar slants then re-incubated again. Gram's staining was carried out then bacteria were divided according to their microscopical appearance into Gram-positive and Gram-negative bacteria [15]. A smear from pure culture from each isolate was microscopically examined for determining of shape, size, arrangement and Gram staining reaction [15].

For Gram positive bacteria, the biochemical tests that were used for Gram positive bacteria were catalase test, coagulase test, urease test, nitrate reduction test, gelatin liquefaction, carbohydrate fermentation tests, Oxidation-Fermentation test as well as the hemolytic activity on blood agar and detection of hemolysis activity also pigment production [16, 17]. For Gram negative bacteria, the biochemical tests that were used for Gram negative bacteria were indole test, methyl red test, Voges Proskauer test, Simmon's citrate test as well as growth on triple sugar iron agar and urease test also sugar fermentation test [15].

Histopathological examination

All collected samples of uterine tissues were fixed in formalin solution (10 %), followed by dehydration in different concentrations of alcohol,

and then embedded in paraffin, sectioned at a thickness of 4-5 µm and stained with (H&E). The tissue sections were examined and photographed with the camera of Olympus light microscope (Olympus BX 51, Tokyo, Japan; [18]). According to the histopathology, endometritis was classified into acute suppurative (N=32), acute lymphocytic (N=14), chronic suppurative (N=18), chronic metritis (N= 8), and normal uteri (N=16)

Immunohistochemical assessment of Anti-Syndecan-1 antigen

Paraffin embedded uterine Anti-Syndecan-1 antigen receptor subunits were detected using the avidin–biotin–peroxidase immunohistochemical method [19]; ProTaqs 2 step detection system Quartet- Berlin- Germany) with a primary Anti-Syndecan-1 polyclonal antibody (SL 1309R). The reaction cellular site of the appeared brown in the cell membrane surface.

Collection of FF, preparation of tissue homogenate, and hormonal assay

Follicles of any size from both ovaries were aspirated and preserved at -20°C until assaying hormones and inflammatory markers. From each uterus, 1gm from each sample was sliced into small pieces and a phosphate buffer saline (1.00 ml) was added and homogenized using a homogenizer. The supernatant was obtained and kept in an Eppendorf and stored at -20 °C for further analysis. The ovarian hormones (E2 and P4), bovine MPO of double antibody sandwich enzyme linked immunosorbent assay (SunRed, www.srbooo.com, China) has a sensitivity of 0.738 ng/ml and range from 0.8 to 200 ng/ml. Bovine TNF α (TNFα, Chongqing Biospes Co., LTD, www.biospes.com, China) has a sensitivity of 2.5ng/ml and the range from 25 to 30 ng/ml. Both MPO and TNFα were assayed in the tissue homogenate of the uteri and the aspirated FF. The intra and inter assay precisions of TNF α and MPO were <10% and <12%.

Statistical analysis

Data of E2, P4, TNFα, and MPO are subjected to statistical analysis using SPSS 20.0. Simple one-way ANOVA was used to study the effect of endometritis classified according to the pathological findings, the effect of sample, and the isolated bacteria. Duncan's Multiple Range test was used to differentiate between significant means. Two-Way ANOVA was used to study the effect of four sample types (two species uterine tissues homogenate and two FF) with three types of endometritis, metritis, and control (apparently normal) specimens and isolated bacteria to study the effect of seven main isolated bacteria with three types of endometritis using general linear models. Pearson's correlation coefficient was also performed. Univariate General Linear model (GLM) was conducted to study the effect of uterine pathological condition, the effect of species and sample type, and the effect of isolated bacteria as a covariate.

Results

The prevalence of isolated bacteria from cows and buffalo genitalia of all samples were *E. coli* (100.0%), *Pseudomonas* spp. (Ps.; 67.4), *P. vulgaris* (Pv; 50.0%), *S. aureus* (Sa; 39.1%), *Citrobacter* spp. (C; 30.4%), *S. epidermidis* (Se; 28.3%), and *Micrococcus* spp. (M; 15.2%).

On gross examination of the uterus, hyperemia of the mucosa and purulent luminal exudate as well as focal hemorrhages in both cows (Fig. 1A) and buffaloes (Fig. 1B) were found.

The gross macroscopic examination showed enlarged uterus, edematous, hyperemic and the endometrial surface was irregular, might have visible yellowish mucopurulent and purulent exudate as well as focal hemorrhages and patches of necrosis and ulceration in both cows (Fig. 1A) and buffaloes (Fig. 1B).

The histopathological examination of buffaloes' uterine tissues samples with acute suppurative endometritis (36%) showed partial denudation and desquamation of the lining pseudo-stratified columnar epithelial cells (Fig. 2A) associated with severe focal as well as diffuse submucosal infiltration of mononuclear inflammatory cells, mainly neutrophils within the endometrial stroma and uterine glands (Fig. 2B). In the acute lymphocytic endometritis (16%), focal area of mononuclear inflammatory cells infiltration mainly lymphocytes, plasma cells and macrophages were observed (Fig. 3A-B) some examined sections showed diffused submucosal inflammatory cells infiltration mainly lymphocytes (Fig. 3A-B).

The chronic endometritis (20 %) was characterized by severe and extensive mucosal cells desquamation with severe mononuclear inflammatory cells infiltration in the subepithelial zone. Blood vessels within the endometrium were congested. Hemorrhage (Fig. 4A-B) and focal uterine degenerative changes, reduction of endometrial glandular lumina with degenerative changes in the glandular epithelium, glandular atrophy associated with peri-glandular fibrosis were noticed (Fig. 4B).

Chronic metritis constituted 9% of sampled uteri and showed cellular infiltration reached the tunica muscularis with infiltration of mononuclear cell in myometrium and serosa along with edematous changes, medial hypertrophy of blood vessels with stenosis of lumina and infiltration of inflammatory cells in lamina propria. Further thickening of the endometrial blood vessels, and severe peri-glandular fibrosis were seen (Fig. 5A-B).

The response of studied uteri to anti-Syndecan-1

antigen immunoreactivity showed positive immunoreaction expressed in brown color on the cell membrane surface (Fig. 6A-B).

TNF-α concentrations (Table 1) declined (P=0.14) in the FF compared to the uterine tissues' homogenates of cows and buffalo (Table 1). In the acute suppurative, TNF-α increased (P< 0.0001) in the FF of buffaloes compared to the uterine tissues' homogenates of buffaloes. It decreased in the FF of cows compared to the cow's uterine tissues' homogenates. In the chronic suppurative endometritis (P<0.017) and chronic metritis (P<0.0001), TNF-α increased in the follicular tissues of cows compared to their uterine tissues' homogenates (Table 1). In the uterine tissues of cows, TNF-α decreased (P=0.032) in the chronic metritis. TNF-α tended (P>0.05) to increase in the uterine tissues of normal buffaloes. In the FF of buffaloes, TNF-α increased (P<.0001) in the acute suppurative endometritis (Table 1). The concentrations of TNFα (Table 6) are influenced by the uterine pathology (P<0.0001), the bacteria isolated from the uteri (P<0.0001), the uterine pathological × species-Sample type (P<0.0001), Uterine pathology × bacteria isolated (P<0.0001), Bacteria × species-Sample type (P<0.0001), and uterine Pathology × bacteria × Sample-Species (P<0.0001)

MPO increased (P=0.006) in the FF of normal buffaloes compared to their uterine homogenate (Table 2). In the acute suppurative endometritis, MPO increased (P<0.0001) in the FF of buffaloes and decreased in the FF of cows compared to their uterine tissue homogenates. In the chronic suppurative endometritis (P<0.0001) and acute lymphocytic endometritis (P<0.001), the FF of cows and buffaloes got higher MPO than the uterine tissue homogenates. In the uterine tissue of cows, MPO increased (P<0.001) in both acute suppurative endometritis and chronic metritis compared to normal uteri, chronic suppurative and acute lymphocytic endometritis (Table 2). In the follicular tissue of cows, MPO increased (P=0.003) in the chronic suppurative endometritis (Table 2). In the uterine tissue of buffalo, MPO increased (P<0.05) in chronic endometritis. The concentrations of MPO (Table 6) are influenced by the uterine pathology (P<0.0001), Sample-Species (P<0.0001), the bacteria isolated from the uteri (P<0.0001), the uterine pathological × species-Sample type (P<0.0001), Uterine pathology × bacteria isolated (P<0.0001), Bacteria × species-Sample type (P<0.0001), and uterine Pathology × bacteria × Sample-Species (P<0.0001)

E2 in the FF increased (P<0.0001) five times compared to the uterine tissue of either normal, acute suppurative, chronic suppurative, acute lymphocytic, and chronic metritis of cows or buffaloes (Table 3). In the uterine tissue of buffalo (P<0.001) and cows (P=0.09), E2 concentrations increased in the acute suppurative endometritis (Table 3). The lowest (P<0.0001) E2 concentrations are noted in the FF of normal cows. The concentrations of E2 (Table 6) are influenced by the uterine pathology (P<0.0001), Sample-Species (P<0.0001), the bacteria isolated from the uteri (P<0.0001), the uterine pathological × species-Sample type (P<0.0001), Uterine pathology × bacteria isolated (P<0.0001), and Bacteria × species-Sample type (P<0.0001).

In normal, acute suppurative, acute lymphocytic endometritis, and chronic metritis P4 concentrations increased (P<0.0001) in the FF of cows and buffaloes compared to their FF (Table 4). In the chronic suppurative endometritis, the lowest (P=0.017) P4 concentrations are noticed in the uterine tissue of cows. Low P4 are noticed in the normal (P<0.0001) uterine tissue of buffaloes, and in and the FF (P=0.054) and uterine tissue of buffaloes with acute lymphocytic endometritis (P<0.0001). In the FF of cows, P4 declined (P=0.002) in the chronic metritis and is maximum in the acute lymphocytic endometritis (Table 4). The concentrations of P4 (Table 6) are influenced by the uterine pathology (P<0.0001), Sample-Species (P<0.0001), the bacteria isolated from the uteri (P<0.0001), the uterine pathological × species-Sample type (P<0.0001), Uterine pathology × bacteria isolated (P<0.0001), Bacteria × species-Sample type (P<0.0001), and uterine Pathology × bacteria × Sample-Species (P<0.012)

In the FF, TNFα increased (P=0.012) in the acute and suppurative endometritis. MPO increased in acute suppurative and acute lymphocytic endometritis (Table 5). Only FF of normal animals has low (P=0.033) E2 and FF of acute lymphocytic endometritis has low (P=0.057) P4 concentrations (Table 5).

In the uteri of normal animals, high (P<0.0001) TNFα concentrations were recorded (Table 6). The lowest MPO were observed in the uteri with chronic suppurative endometritis but high MPO (P=0.014) were noted in acute suppurative endometritis and chronic metritis (Table 6). The acute suppurative endometritis was associated with the highest E2 (P<0.0001) concentrations but the chronic metritis is associated with high P4 (P<0.0001).

The TNFα (Fig. 7) increased (P<0.0001) to reach the maximum value in animals with acute suppurative endometritis and the isolated bacteria were *Pseudomonas Spp*. (Ps.) with *P. vulgaris* (Pv.) with isolating either *S. epidermis* (Se) or *Citrobacter Spp*. (C). In the acute lymphocytic endometritis, TNFα declined (P<0.001) when Se, or Sa, or C were isolated and increased to reach the maximum values when Ps+Pv -/+ Se or C were isolated and PS+Sa +/- C were isolated (Fig. 7). In the chronic suppurative endometritis, TNFα declined (P<0.01) when Ps.+Pv.

-/+ Se or C were isolated and increased when PS. -/+ C or M or Se. (Fig. 7). In animals with chronic metritis, TNFα declined (P<0.0011) when Ps.+Sa. +/-C were isolated and reached the maximum value when Ps.+Pv. -/+ Se or C were isolated (Fig.7). TNFα increased in normal animals (P<0.001) when PS+Sa+Pv -/+M or Se or C were isolated.

Within animals of acute suppurative endometritis, MPO started to increase (P<0.001) when the bacteria of PS-/+C or *Micrococcus Spp*. or Se were isolated and the maximum value when PS+S. aureus (Sa)+Pv -/+ M or Se or C were isolated (Fig. 8). In the chronic suppurative endometritis, MPO declined (P<0.05) when Ps.+Pv. -/+ Se or C were isolated and increased when Ps.+Sa. +/-C were isolated (Fig. 8). The uteri of animals with chronic metritis showed lower (P<0.01) MPO when Ps.+Pv. -/+ Se or C were isolated (Fig. 8). In normal animals, MPO reached the maximum concentration (P<0.001) Ps +/-C or M or Se were isolated and reached the minimum values when PS+Sa */+ C or Pv or Se+/- M were isolated (Fig. 8).

Discussion

Though the early diagnosis and treatment of endometritis (subclinical) is important to understand the progress of the disease to modify the treatment strategies. Most of samples collected for the current study were from culled dairy animals because of repeat breeder and the poor response to treatment and all were classified either acute (suppurative or lymphocytic) or chronic endometritis. Similar to cows, the huge abnormal number of polymorphonuclear cells during the acute endometritis [20] has been confirmed in the histopathological sections of the acute suppurative endometritis which are responsible for the secretion of muco-purulent discharges with no systemic illness symptoms [2, 3]. In ewes, E. *coli* with *Staphylococcus aureus* mixed with *S. epidermidis or Micrococcus* were isolated where partial degeneration and desquamation of pseudo stratified columnar epithelial cells associated with submucosal infiltration of neutrophils in the suppurative one and lymphocytes in the lymphocytic endometritis [21]. In agreement with cattle and buffalo chronic endometritis of this study, the partial degeneration and desquamation of lining epithelial cells with diffuse infiltration of inflammatory cells, the atrophy of some uterine glands associated with peri-glandular fibrosis was also reported in ewes [21].

Shortly after reaching the bacterial pathogens to the uterine cavity, the Toll-like receptors in the endometrial lining epithelium detects the peptidoglycans of the bacterial cell wall and the lipopolysaccharides toxins secreted by the invaded bacteria are activated [22, 23]. The activation of these receptors initiated the secretion of TNFα to the circulation which promoted the migration of special types of immune cells to the uterine cavity [24]. In cows with subclinical endometritis, blood mononuclear cells of B-cells, NK-cells, and CD172a-positive monocytes in addition to neutrophils were significantly elevated [25].

In the present study, though the increase of TNFα in the chronic endometritis and the increase of MPO in the acute endometritis of this study were insignificant in acute, chronic and pregnant cattle and buffaloes, but on the molecular level, TNF mRNA expression increased 20-fold higher in the cytobrush samples collected from subclinical endometritis-positive cows [1]. Regression analysis of gene expression levels (cycle threshold) versus polymorphonuclear (PMFs) frequency in the cytosmear decreased by 2.4% for each additional cycle threshold required to detect TNFα gene expression [1]. The *tnf* gene expression correlated positively with both *il8* and *il6* [1]. In cows with subclinical endometritis, a higher copy numbers of TNFα were expressed from blood leukocytes [10]. The uterine tissue homogenate of buffaloes with acute suppurative and acute lymphocytic endometritis showed the highest levels of TNFα which increased in their FF when the endometritis became chronic. The negative correlation found between TNF and steroid hormones, reversed the effect of TNFα in stimulating Prostaglandin F2α from the endometrium and altered the corpus luteum function and the next ovulation [25]. Plasma collected from cows with subclinical endometritis did not alter the gene expression of whole leukocytes and polymorphonuclear cells but elevated expressed messenger RNA copy numbers of CXCL8, CXCL1, and IL1B in intermediate monocytes of healthy cows [10]. The secretion of proinflammatory cytokines (TNFα and MPO) not only recorded here in the uteri of infected animals as previously reported [26] but also higher concentrations were determined in the FF of affected cows and buffaloes with endometritis and was also detected at the fetal maternal interface of pregnant cows [27]. Similar to the increased TNFα in the early pregnant cows and buffaloes included in this work, higher physiological mRNA transcripts of TNFα genes were recorded eighteen days after conception associated increased neutrophils and peripheral blood mononuclear cell [28]. The inverse correlation between the ovarian hormones and TNF and their direct one with MPO confirm their pro- and anti-inflammatory functions in ovarian follicles and the uterus during pregnancy and after infection [29, 30]. The presence of TNFα in the uteri and FF of cattle and buffaloes not only stimulated prostaglandin F2α (PGF2α) secretion in endometrium but also interfered the production and actions of hormones in the endometrium and ovarian follicles cells [25].

MPO is a member of the mammalian peroxidase family and is known to be effective in killing

microorganisms and its increased activities indicated the presence of endometritis [31]. The increased MPO during acute endometritis and its decrease during the chronic one is mainly referred to its action after secretion from neutrophils following bacterial infection to trap and kill the bacteria outside the cells [32]. The present study recorded lower MPO in the chronic endometritis than the acute suppurative one. This decrease could be attributed to the decreased neutrophils in both pyometra and chronic endometritis compared to the acute metritis in buffaloes [33]. Conversely, lymphocytes decreased in the acute metritis compared to either pyometra or chronic endometritis [33].

The early pregnant animals in this study were still having some pathogenic bacteria isolated from acute and chronic endometritis. Similarly, the pathogenic bacteria isolated from healthy cows and those with subclinical or clinical endometritis increased until five weeks postpartum [34]. Similar to the bacteria isolated from endometritis of this study, pregnant and nonpregnant heifers showed similar bacterial profiles with the abundance of both unclassified *Pasteurellaceae* and *Fusobacterium* in nonpregnant heifers and the abundance of *Pasteurella multocida* in pregnant heifers [35]. In normal cows subjected to the timed insemination technique, *Corynebacterium* was abundant in the uterus two days before artificial insemination and also in pregnant cows *Staphylococcus*, *Prevotella*, *Microbacterium*, *Butyrivibrio*, *Ralstonia*, and unassigned genera from family *Alcaligenaceae*, and family *Comamonadaceae* showed higher abundance in nonpregnant cows than pregnant one [36].

The decrease of P4 in the chronic endometritis with the isolation of *E. coli* and *Citrobacter species* with either *S. aureus* and *Pseudomonas species* or *Micrococcus species* and *S. epidermis* agree with its decreased concentration in association with the relative abundance of *Firmicutes* at both day -9 and day -2 before timed insemination. The increased P4 in associated with the isolation of *E. coli* and *Citrobacter species* with either *S. aureus* and *P. vulgaris* in the acute suppurative or with *Micrococcus species* and *S. epidermis* the acute lymphocytic endometritis agrees with its significant correlation to an increase in relative abundance of *Proteobacteria* in the vagina of nonpregnant cows [36]. Moreover, the isolation of *Arcanobacterium pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum*, *Prevotella melaninogenica* and *Proteus* species from intrauterine infection in cows did not relate to the severity of the disease [37] because six to eight weeks postpartum, most of (70–80%) of isolated pathogenic bacteria from mild or subclinical bovine endometritis whatever the organisms, resolved before starting the breeding [38]. This early postpartum subclinical endometritis increased FF P4 concentrations and decreased E2, concentrations for long-term which influences on the steroid concentrations of ovarian follicles long after infections had self-resolved resulting in reduced oocyte quality, conception rates and elongated interval between calving [38]. These adverse effects of the bacterial endometritis on the oocyte quality were also noticed when experimental bacterial infusion in cattle altered the oocyte transcriptome differently at Day 4 and Day 60, suggesting the susceptibility of different follicle stages to damage due to the uterine infection [39]. The presence of the proinflammatory cytokines in the FF of the acute and chronic endometritis of cattle and buffaloes of this study and the pregnant animals are in agreement with the ability of granulosa cells to offer more predicted and persisted upstream regulators of differentially expressed genes than the endometrium and the oviduct for three months after the intrauterine infusion of *E. coli* and *Trueperella pyogenes* in cows [40]. In the current study, the altered TNFα and MPO in association with the isolated bacteria in the acute, chronic, or pregnant animals were also reported to occur for 146 days after intrauterine bacteria infusion and 16 days after artificial insemination and conception which upregulated 140 genes and downregulated 31 genes in 16 days pregnant cows after synchronization and insemination 130 days after uterine infusion of *E. coli* and *Trueperella pyogenes* [41]. Uterine infections increased the early embryonic loss from Day 7 after transfer of embryos from healthy cows to recipients resolved from endometritis or inseminated and conceived cows recovered from uterine infection [42, 41].

Syndecan-1 or CD138 is a cell surface heparan sulfate proteoglycan (HSPG) which is usually involved in the modulation of inflammatory diseases and infection [43]. The expression of anti-syndecan-1 in the uteri with endometritis had been also indicated the presence of chronic endometritis [44]. Syndecan-1 immunohistochemistry (IHC) was previously recommended to diagnose chronic endometritis because it stains only plasma cells (Plasmacytes; [45]). Plasmacytes exist only in the chronic endometritis [46]. Syndecan-1 binds to and regulates many mediators of any disease pathogenesis and infections.

Conclusion

It could be concluded that buffaloes are more susceptible to endometritis than cows. The response of buffaloes to uterine infection is stronger on the buffalo uterine tissue and FF which interfered their future fertility. Syndecan-1 immunohistochemistry can be used as a rapid confirmatory test for the development of chronic endometritis. MPO increased in the acute endometritis but TNFα increased in the chronic one.

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Declaration of Conflict of Interest

The authors declare that they do not have any competing interest.

Ethical of approval

Ethical approval and consent to participate are not applicable.

TABLE 1. Mean ±Std. Deviation of bovine TNF pg/ml in different types of endometritis, cattle and buffalos, and different samples

Endometrial status	Uterine homogenate		FF		P-value
	Buffalo	Cow	Buffalo	Cow	
Normal	105.47±13.82^{by}	102.99±9.48^{bxy}	97.05±1.38^{ax}	99.01±0.77^{x}	0.014
Acute suppurative	97.29±1.22^{ax}	102.12±8.85^{byz}	104.11±10.21^{bz}	98.62±0.65^{xy}	0.0001
Chronic suppurative	97.26±0.9^{ax}	97.91±0.67^{abx}	97.03±0.69^{ax}	103.09±10.98^{y}	0.017
Acute lymphocytic	96.85±1.20^{a}	100.54±7.51^{b}	97.77±1.98^{a}	98.28±0.91	0.053
Chronic metritis	97.53±0.78^{ay}	94.91±0.07^{ax}	98.13±0.09^{ay}	97.70±1.63^{y}	0.0001
P-value	0.085	0.032	0.0001	0.126	

Means with different superscripts (a, b, c) within column or (x, y, z) within row are significantly different at P<0.05

TABLE 2. Mean ±Std. Deviation of bovine MPO pg/ml in different types of endometritis, cattle and buffalos, and different samples

Endometrial status	Uterine homogenate		FF		P-value
	Buffalo	Cow	Buffalo	Cow	
Normal	3.49±0.37^{ax}	5.57±1.70^{abxy}	8.29±0.89^{y}	4.28±0.39^{ax}	0.006
Acute suppurative	4.05±2.27^{ax}	8.09±1.05^{by}	8.62±2.06^{y}	4.99±0.51^{ax}	0.0001
Chronic suppurative	2.83±0.86^{ax}	3.29±0.66^{ax}	4.93±0.98^{y}	7.04±0.63^{bz}	0.0001
Acute lymphocytic	4.04±1.87^{ax}	4.54±1.73^{abx}	7.29±1.55y	6.24±0.41^{aby}	0.001
Chronic metritis	5.96±1.16^{b}	7.32±0.67^{b}	6.59±0.76	6.01±0.83^{ab}	0.899
P-value	0.015	0.0001	0.221	0.003	

Means with different superscripts (a, b, c) within column or (x, y, z) within row are significantly different at P<0.05

TABLE 3. Mean ±Std. Deviation of bovine E2 pg/ml in different types of endometritis, cattle and buffalos, and different samples

Endometrial status	Uterine homogenate		FF		P-value
	Buffalo	Cow	Buffalo	Cow	
Normal	41.99±6.09^{ax}	51.61±10.01^{ax}	1247±14.20^{z}	1014±75.45^{ay}	0.0001
Acute suppurative	318.97±79.64^{bx}	217.84±15.85^{bx}	1198±173.84^{y}	1255±18.11^{by}	0.0001
Chronic suppurative	48.31±6.68^{ax}	91.31±20.71^{abx}	1271±26.49^{y}	1257±12.48^{by}	0.0001
Acute lymphocytic	32.22±3.63^{ax}	119.31±21.51^{abx}	1241±81.32^{z}	1079±22.12^{aby}	0.0001
Chronic metritis	182.69±32.55^{aby}	41.89±4.92^{ax}	1276±13.59^{z}	1234±12.19^{bz}	0.0001
P-value	0.001	0.009	0.242	0.0001	

Means with different superscripts (a, b, c) within column or (x, y, z) within row are significantly different at P<0.05

TABLE 4. Mean ±Std. Deviation of bovine P4 ng/ml in different types of endometritis, cattle and buffalos, and different samples

Endometrial status	Uterine homogenate		FF		P-value
	Buffalo	Cow	Buffalo	Cow	
Normal	20.23 ±2.69^{ax}	18.32 ±1.71^{x}	40.64 ±3.22^{aby}	42.71 ±1.48^{bcy}	0.0001
Acute suppurative	25.11 ±1.87^{abx}	22.22 ±2.31^{x}	41.66 ±2.38^{aby}	39.51 ±3.63^{aby}	0.0001
Chronic suppurative	27.87 ±1.86^{by}	18.33 ±2.58^{x}	43.30 ±0.59^{bz}	41.81 ±3.71^{bcz}	0.017
Acute lymphocytic	20.30 ±1.46^{ax}	19.92 ±1.24^{x}	36.35 ±2.85^{ay}	44.04 ±3.25^{cy}	0.0001
Chronic metritis	30.32 ±2.91^{bx}	23.71 ±2.25^{x}	42.37 ±0.54^{aby}	37.84 ±4.71^{ay}	0.0001
P-value	0.0001	0.449	0.054	0.002	

Means with different superscripts (a, b, c) within column or (x, y, z) within row are significantly different at P<0.05

TABLE 5. Mean ±Std. Deviation FF, TNF*α*, MPO, E2, and P4

Endometrial status	TNF	MPO	E2	P4
Normal	98.01±1.42^{a}	6.79±0.89^{ab}	1159±194^{a}	41.42±2.86^{b}
Acute suppurative	101.99±8.42^{b}	7.22±0.63^{ab}	1220±139^{ab}	40.83±3.08^{ab}
Chronic suppurative	101.07±9.35^{ab}	6.34±0.41^{a}	1262±19^{b}	42.31±2.74^{b}
Acute lymphocytic	97.84±1.843^{a}	7.14±0.28^{ab}	1219±95^{ab}	37.45±2.49^{a}
Chronic metritis	97.84±1.32^{a}	6.30±0.76^{a}	1255±25^{b}	40.10±4.00^{ab}
P-value	0.012	0.84	0.033	0.057

Superscript letters (a, b, c) within column indicate significant difference at P<0.05.

TABLE 6. Mean ±Std. Deviation uterine tissue TNF*α*, MPO, E2 and P4

Endometrial status	TNF	MPO	E2	P4
Normal	103.93±10.29^{b}	4.79±0.54^{bc}	48.003±6.73^{a}	19.03±1.45^{a}
Acute suppurative	99.10±5.92^{a}	5.57±0.45^{bc}	281..05±41.85^{b}	24.03±1.38^{ab}
Chronic suppurative	97.69±0.76^{a}	3.13±0.24^{a}	76.97±14.14^{a}	21.51±1.93^{a}
Acute lymphocytic	96.85±1.13^{a}	4.04±0.35^{ab}	32.22±1.71^{a}	20.30±1.46^{a}
Chronic metritis	96.81±1.39^{a}	6.30±0.9^{c}	147.49±44.77^{a}	28.67±2.28^{b}
P-value	0.0001	0.014	0.0001	0.0001
Ut. pathology	0.0001	0.0001	0.0001	0.0001
Sample-Species	.470	0.0001	0.0001	0.0001
Bacteria isolated	0.0001	0.0001	0.0001	0.0001
Ut. Pathology × Sample-Species	0.0001	0.0001	0.0001	0.0001
Ut. Pathology × bacteria	0.000	0.0001	0.0001	0.0001
Bacteria ×Sample-Species	0.0001	0.0001	0.0001	0.0001
Ut. Pathology × bacteria × Sample-Species	0.0001	0.0001	.989	0.012

Superscript letters (a, b, c) within column indicate significant difference at P<0.05.

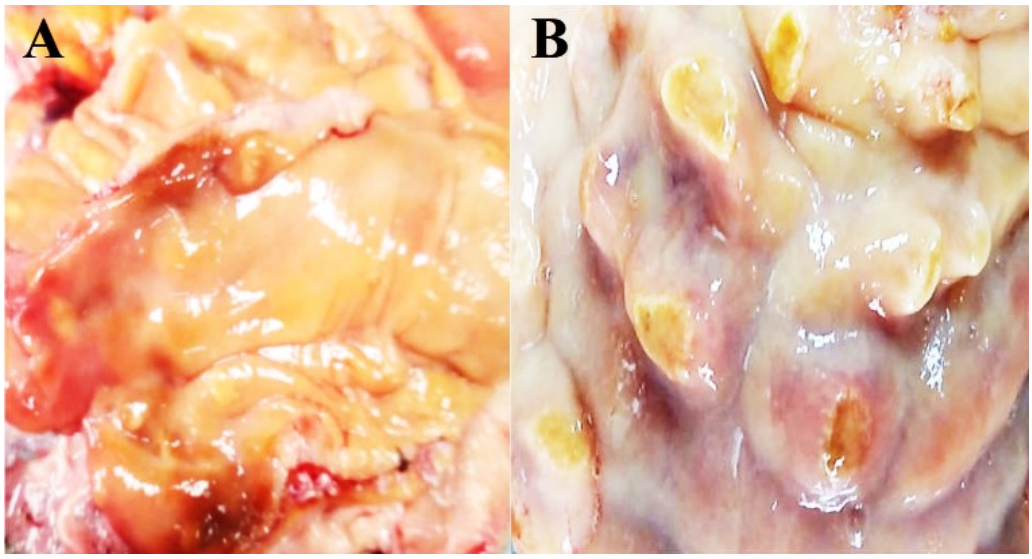

Fig. 1. The gross anatomy of endometritis in cattle (A) and buffalo (B) with yellowish mucopurulent and purulent exudate as well as focal hemorrhages hemorrhage.

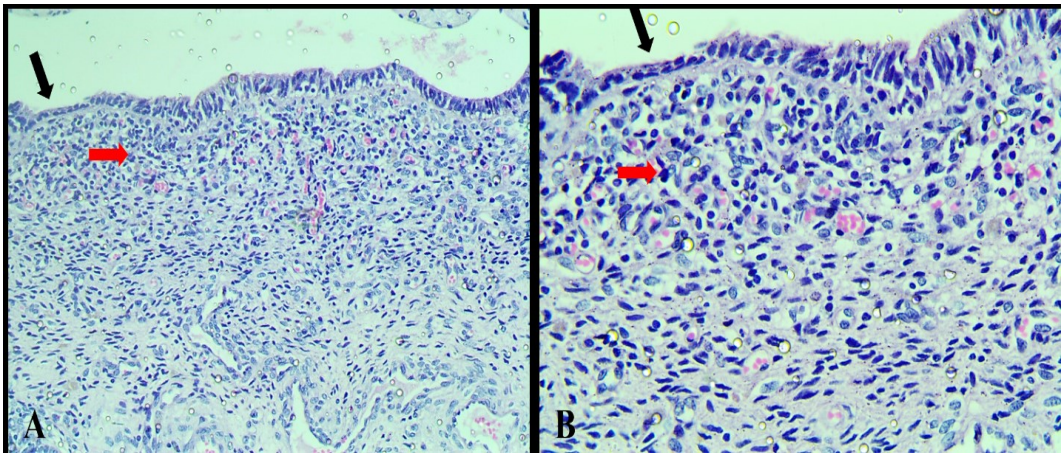

Fig. 2. Uterus of buffalo with cute suppurative endometritis shows partial degeneration and desquamation of pseudo stratified columnar epithelial cells (black arrows) associated with submucosal infiltration of mononuclear inflammatory cells mainly neutrophils (A, H&E, X200), focal area of mononuclear inflammatory cells infiltration (Red arrow) mainly neutrophils (B, H&E, X200).

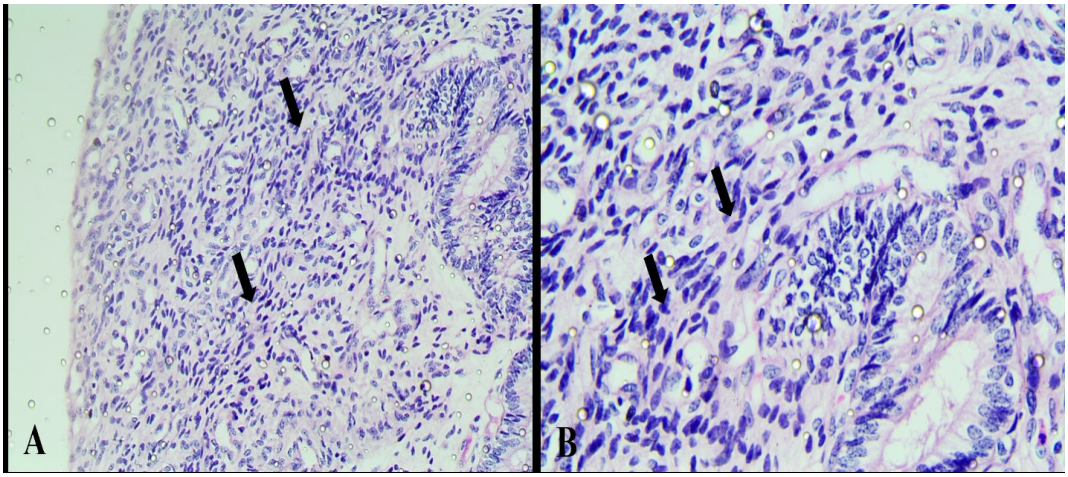

Fig. 3. Uterus of buffalo with acute lymphocytic endometritis shows diffused submucosal inflammatory cells infiltration (black arrows) mainly lymphocytes (H&E, X100).

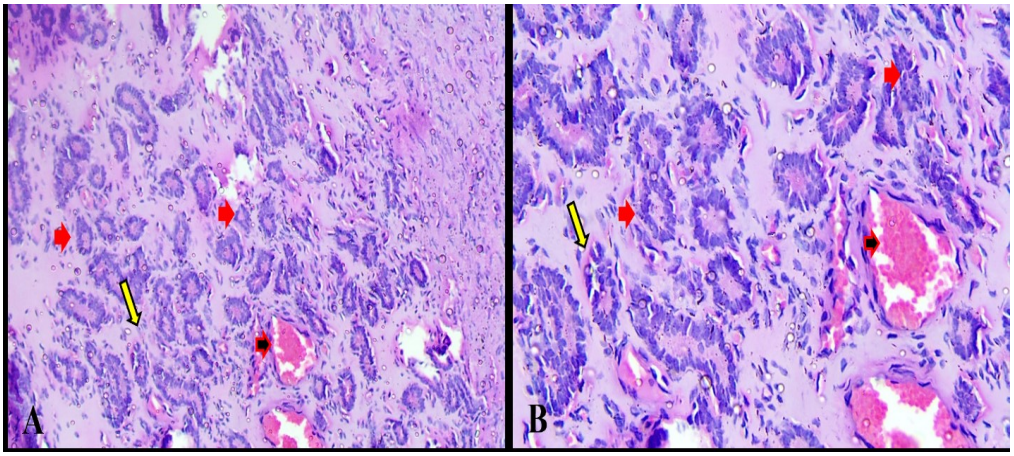

Fig. 4. Uterus of buffalo with chronic suppurative endometritis shows diffuse infiltration of inflammatory cells mainly neutrophils in the lamina propria (A), atrophy of endometrial glands (red arrow head) associated with peri-glandular fibrosis (yellow arrow) and congested blood vessels (black arrow heads; H&E 200 and 400 X magnification).

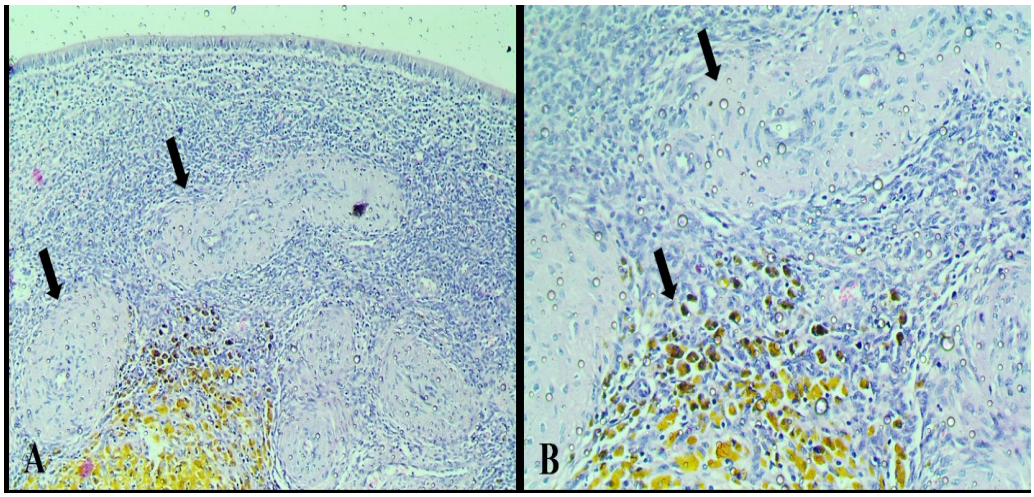

Fig. 5. Chronic metritis with severe diffuse infiltration of mononuclear cell in myometrium and serosa along with edematous changes (black arrows) (H&E 100 and 200 X magnification).

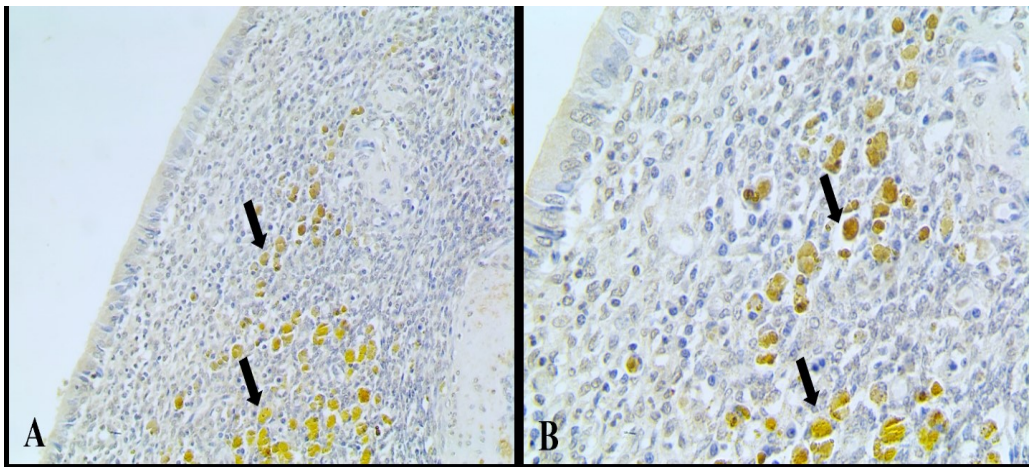

Fig.6. Photomicrograph of Anti-Syndecan-1 antigen immunoreactivity in the uterine tissue sections (X 200 and X 400 respectively) showing positive immunoreaction expressed in brown color in the cell membrane surface.

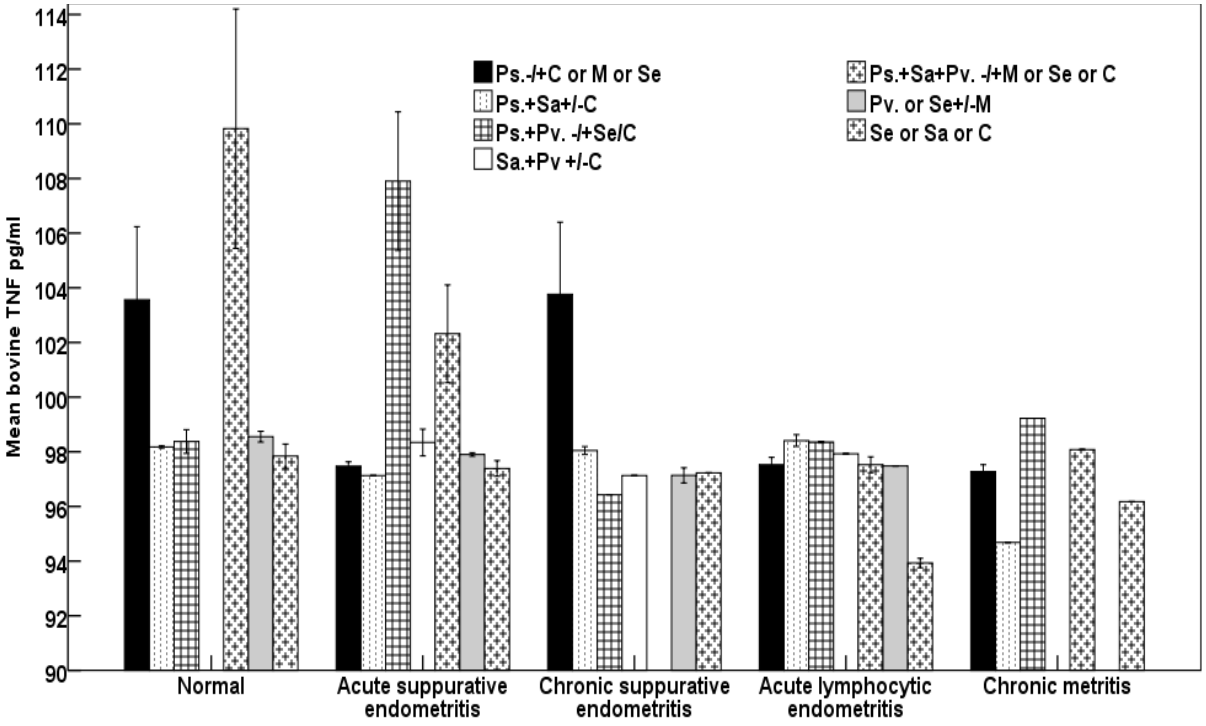

Fig. 7. Mean TNFα concentrations in relation to main bacteria isolated from the uteri of normal, acute suppurative, chronic suppurative, acute lymphocytic endometritis, and chronic metritis

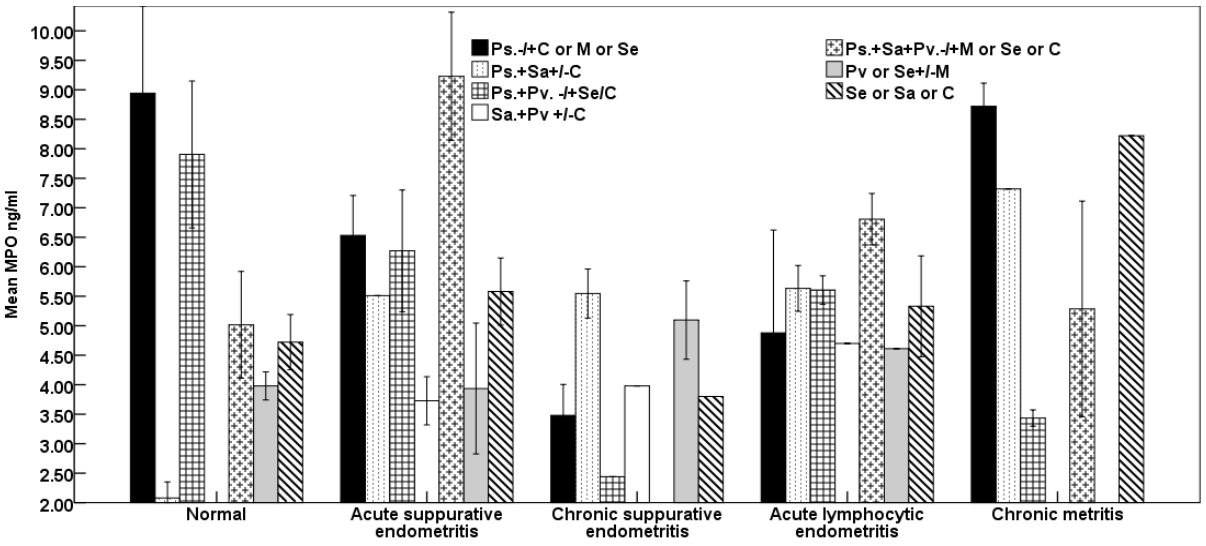

Fig. 8. Mean MPO concentrations in uteri of normal, acute suppurative, chronic suppurative, acute lymphocytic endometritis, and metritis in relation to the main bacteria isolated

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الهرمونات ومؤشرات الالتهاب في نسيج الرحم و سائل جريبيات المبيض في الماشية والجاموس المصاب بعدوى بكتيرية

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الملخص

التهاب بطانة الرحم هو مشكلة العقم التناسلي الأكثر شيوعًا في الحيوانات المنتجة للألبان. هدفت هذه الدراسة إلى التحقيق في العلاقة بين التوصيف النسيجي لالتهاب بطانة الرحم مع هرمونات الستيرويد المبيضية والسيتوكينات المؤيدة للالتهابات وميلوبيروكسيديز (MPO) في الرحم والسوائل الجريبية (Follicular fluids) بالإضافة إلى السيتوكينات المصاحبة للالتهابات وميلوبيروكسيديز. تم إخضاع الأرحام التي تم جمعها من المجازر لصبغة روتينية للهيماتوكسيلين والإيوزين (H&E) ومساعدات المناعة النسيجية Antisyndecan-1 لتصنيف نوع التهاب بطانة الرحم. تم عزل البكتيريا الموجودة في قمة قرون الارحام المفحوصة وتحديدها. تم استخدام السوائل الجريبية (Follicular fluids) ومتجانسات أنسجة الرحم (Tissue homogenates) لتحليل هرمون الاستروجين E2 والبروجسترون (P4) وعامل نخر الورم (TNFα) وMPO. أظهرت النتائج أن نوع العينة (أرحام الأبقار أوأرحام الجاموس و كذلك السوائل الجريبية للأبقار أو الجاموس) أثرت على مستوى الميلوبيروكسيديز وهورموني الاستروجين والبروجيستيرون MPO وE2 وP4. كما أن نوع التهاب بطانة الرحم طبقا للفحص النسيجي المرضي للرحم (الصديدي الحاد واللمفاوي الحاد والصديدي المزمن والتهاب الرحم المزمن والارحام الطبيعية) أثر بشكل ملحوظ على مستوى هرموني الاستروجين والبروجيستيرون وكل من الميلوبيروكسيديز والمعامل نخر الورم TNFα.

يمكننا أن نستنتج أن الجاموس أكثر عرضة للإصابة بالتهاب بطانة الرحم من الأبقار.

- تكون استجابة الجاموس للعدوى الرحمية أقوى على أنسجة الرحم والسوائل الجريبية في الجاموس والتي تتداخل مع خصوبتها المستقبلية.
- يمكن استخدام المناعة النسيجية لـ Syndecan-1 كاختبار تأكيدي سريع لتطور التهاب بطانة الرحم المزمن.
- زاد المايلوبيركسيديز في التهاب بطانة الرحم الحاد ولكن زاد TNF في التهاب بطانة الرحم المزمن.

الكلمات الدالة: الماشية ، الجاموس ، التهاب بطانة الرحم ، المناعة النسيجيةالكيمائية ، المايلوبيراكسيديز ، هرمونات المبيض ، معامل نخر الورم الفا.