Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Bacteriological Finding, Vaginal Discharges, and Endometrial Cytology for Endometritis Detection in Postpartum Buffaloes

Salah Noori Mohammed

Department Of Surgery and Obstetrics

College of Veterinary Medicine - University of Diyala, Iraq

* Corresponding author's E-mails:

salah.n@uodiyala.edu.iq

Salah.laser@yahoo.com

ORCID:0000-0002-0509-1234

Abstract

Background: Endometritis is one of the most common diseases in cattle and occurs several weeks postpartum. It causes severe economic losses, including increased open days, calving intervals, and the number of services needed to achieve conception.

Aim of study: This study aimed to demonstrate the incidence of endometritis during 20–30 days postpartum in buffaloes. Moreover, we determined common risk factors that affect the rate of endometritis in these animals.

Methods: A total of 72 buffaloes, aged 3-12 years and at 20–30 days post-calving, were enrolled in this study. All the animals were checked by transrectal palpation, ultrasound, and vaginal secretion collection. A four-grade system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge including < 50% pus, and 3 = involving > 50% pus) was used to categorize the vaginal secretions of these cows. Endometrial cytology and bacteriological samples were then collected using the cytobrush technique.

Results: Ten of 72 buffaloes (13.8%) had abnormal vaginal secretions (grades 1-3) and indicated clinical endometritis (CE), and twelve of 72 clinically healthy buffaloes (16.6%) had subclinical endometritis (SCE, ≥ 8% PMN buffaloes). Escherichia coli was the most common bacteria isolated from SCE (38.9%) buffaloes. Moreover, E. coli was the major bacteriological risk factor for SCE occurrence. A. pyogenes (28.1%) and E. coli (21.1%) were the most common risk factors for the occurrence of CE. Poor to moderate agreement was found among PMN%, bacteriological findings, and vaginal discharges.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Conclusion: The current study showed minimal effects of SCE and the most isolated bacteria on the pregnancy rate of buffaloes, while the study demonstrated CE and A. pyogenes as the main risk factors for the reproductive performance of these animals.

Keyword: Buffalo postpartum, Cytological endometritis, Uterine infections, PMN

Introduction

Uterine infections can be classified as puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis (SCE). SCE is the inflammation of the uterine endometrium without mucopurulent material accumulation in the vagina and any systemic symptom [1]. SCE is also known as cytological endometritis [2, 3]. Dubuc et al. [3] described cytological endometritis as "an elevated ratio of polymorphonuclear cells (PMN) in endometrial cytology samples obtained through cytobrush (CB) or low-volume uterine lavage (LVF)." Clinical endometritis (CE) is an endometrial inflammation with purulent or mucopurulent discharge; moreover, this disease can be detected 21 days postpartum and is associated with clinical signs of disease [1]. The term "purulent vaginal discharge" was adopted as a substitute for CE because the presence of abnormal genital discharge does not necessarily indicate endometrial inflammation [3]. Endometritis is prevalent in highly productive dairy cows and has been associated with decreased pregnancy per insemination, extended pregnancy intervals, and increased culling rate [2].

Precise diagnosis of endometrial infections in cattle is hindered by the lack of consensus on an acceptable definition of bovine endometritis [1,2]. Most cows experience some degree of endometritis during normal uterine involution after birth. Transrectal palpation of the uterus is the most common method of diagnosing postpartum uterine diseases; however, this method lacks the accuracy to identify endometritis and subsequent reduced fertility [4, 5]. Several approaches, such as the collection of endometrial and inflammatory cells using a guarded cotton swab [6], uterine biopsy [7], LVF [2], or CB [8], are used to detect cytological endometritis. Moreover, CB and LVF are less invasive techniques compared with uterine biopsy [9]. CB application is less harmful than LVF because the fluid (normal saline, 0.9%) used in LVF produces endometrial irritation. Moreover, the saline solution extends the time required to obtain samples (a 17% failure to obtain saline) and increases the alteration of cells harvested via LVF [9]. However, a previous study described CB as the most reliable method for diagnosing bovine cytological endometritis [10].

Mateus et al. [11] found that ultrasound uterine measurement is convenient and

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

allows for reliable result comparison. Ultrasonographic intrauterine fluid determination 3 weeks postpartum exhibits good sensitivity and specificity and is reliable for diagnosing endometritis [8, 10].

The occurrence of endometritis in buffaloes in Iraq remains unclear, bacterial spectra, sensitivity to antibiotics, and the relationship between bacterial contaminations has been rarely investigated. The present study aims to assess the relationship among bacterial contamination, vaginal discharges, and endometrial cytology findings in postpartum buffaloes and also to determine the risk factors that affect of CE and SCE occurrence.

Materials and methods

Animals

A total of 85 buffalo at 15 days to 30 days post-calving period were obtained from three different private buffalo farms. All samples were collected from animals while cows remained at their farm of origin between January 2019 to October 2019. The buffalo farms are located in Diayla province. The animals were aged 3–12 years, weighed 300–450 kg, and managed under free grazing. The cows were also fed according to field management after providing concentrated feed, which consisted of alfalfa, corn silage, beet pulp, cottonseed, soybean, corn, and barley. These animals were kept in an outdoor near the rivers for wallowing and milked twice daily. Individual animal data on calving history, lactation, breed, parity were recorded. The farms used many bulls with a high fertility and passed a breeding soundness examination conducted every two month. These farms used 20:1 as cow-to-bull ratio for natural mating after the postpartum period. The body condition score (BCS) of the cows was evaluated by using a 5-point scale [12]. Pregnancy diagnosis for cows was achieved by using B-mode ultrasound attached with a linear probe of 5MHz frequency (Sonosite VET 180 Plus, Bothell, WA, USA) at 150 and 200 days after calving.

Animals physical examination

All practical examinations and scoring for clinical findings were done by the same veterinarian (author) for postpartum buffaloes of the current study.

All animals were checked by transrectal palpation 20 to 30 days after calving to evaluate degree of uterine involution, symmetry of uterine horns, and position of the uterus relative to the pelvic brim. Genital tract discharge of all cows was checked through examination of vaginal secretions by using hands covered with clean disposable long gloves at 20 days to 30 days post-calving to identify CE cases. A four-grade system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge including < 50% pus, and 3 = involving > 50% pus) was used to categorize vaginal secretions. The vulva and perineum region were washed, cleaned, sterilized with iodine, and dried using clean, sterile paper towels. The area was then lubricated (Triad Sterile Lubricating Jelly, H&P Industries Inc., Mukwonago, WI, USA). The hand covered with a long sterile disposable glove was inserted into the vagina far enough to allow observation of the nature of the fluid; if necessary, a light

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

source was used to obtain evidence of abnormal cervical secretion [10].

Ultrasound examination

Ultrasound examination was done to determine the uterine cervix diameter and fluid accumulation in the bovine uterine lumen [8,10]. All buffaloes were scanned using the ultrasonographic technique (Sonosite VET 180 Plus, Bothell, WA, USA). The buffaloes were grouped into two categories: endometritis and healthy buffaloes. Buffaloes with uterine cervix measurement (CM) higher than 5 cm and uterine horns containing fluid in the uterus (FIU), regardless of the amount or nature (hyperechogenic or hypoechogenic), upon ultrasonography, were classified under the endometritis group, as described by Kasimanickam et al. [8]. A buffalo was categorized as healthy when its uterine cervix diameter was <5 cm, with no abnormal discharge externally or in the uterus based on ultrasonographic findings, as described by Zobel [13].

Endometrial cytological examination

Endometrial cytological samples were collected from cows by using a sterile cytobrush Plus GT, Medscand Medical, Germany (Fig. 1) was modified for utilization in cows (14). The handle was shortened to 2 cm and threaded to enable it to be inserted into a stainless steel rod (artificial insemination gun; 65 cm × 4 mm). The cytobrush and stainless steel rod combination were then inserted into a plastic sheath (Chemise Sanitaire, IMV Technologies, and France) to avoid vaginal contamination), and the device introduced into the vagina. Next, a sleeved arm was introduced into the rectum to facilitate passage of the instruments through the genital tract and os cervix. Once the device has passed through the cervix, the CB was exposed and turned (360º) to get cellular materials from the adjacent endometrium (body of uterus). Collected samples were then rolled 2-3 times on a clean glass slide.

The sample was rolled on the sterile microscopic slide (75mm×25mm) and stored in a transport medium (LABCHEM SDN.BHD, Malaysia) for bacteriological analysis. All slides were fixed with methanol for 30 min, transported to the laboratory within 3 h, stained with 5% Giemsa stain for 3 min, and dried. All of the slides were evaluated by counting 300 cells at 400× magnification (Leitz Labourlux-S, Wetzlar, Germany) to determine the percentage of neutrophils (PMN %). Endometrial threshold value ≥ 8% was used (10) to determine the SCE occurrence in the farms between 20 and 30 days postpartum.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Figure 1: Cytobrush used to obtain endometrial cytological samples.

Bacterial isolation

Bacteriological samples were transferred into sterile tubes containing thioglycolate broth as transport medium (Sterile transport media, LABCHEM SDN. BHD, Malaysia). The samples were transported to the laboratory in an ice box and immediately processed for bacteriological examination. Samples were cultured aerobically on sheep blood agar, MacConkey agar, and nutrient agar. Bacterial growth on the culture plates was scored semi-quantitatively after 18 hours of incubation at 37 °C for aerobic growth. Bacteria were identified based on the shape of the bacterial colony, Gram stain, hemolysis, morphology, and biochemical tests such as coagulase, oxidase, catalase, indole production, and methyl red.

Statistical analysis

Clinical findings and all data were recorded and encoded into excel software (Excel 2007, Microsoft Office Corporation, Redmond, WA, USA). The data included BCS, parity, date of parturition, type of calving, postpartum diseases, vaginal clinical findings (discharge score), cytological examination findings, bacteriological isolation results, and reproductive performance of cows. All the statistical methods were performed by SPSS software (version 18.0, IBM SPSS Inc., Chicago: USA) and Excel 2007. The occurrence of SCE and CE was determined based on clinical examination results, vaginal discharges, and endometrial cytological samples. The agreement among vaginal discharge grades, the percentage of PMN, and bacteriological findings were examined using Kappa analyses (Santos *et al*., 2009b). Chi-square analysis was used to compare the bacteriological results of healthy and infected cows. Moreover, the relative risk factor for the occurrence of SCE and CE was measured, considering isolated bacteria (*E. coli*, *Staphylococcus*, and *Bacillus*) by using SPSS software consequently; just isolated bacteria with P value < 0.10 were considered to have significant effects and included in regression model. A binary logistic regression model was used to analyze risk factors for the occurrence of SCE and CE. These

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

factors included infection with *E. coli* (0 = no infection, 1 = infection), infection with *Bacillus* spp. (0 = no infection, 1 = infection), infection with *Staphylococcus* spp. (0 = no infection, 1 = infection), calving (0 = normal, 1 = dystocia), and BCS (0 = ≥ 3, 1 = < 3), parity (0= primiparous, 1= multiparous). Reproductive performance parameters were reported by calculating the percentage of pregnancy rate at 150 and 200 days postpartum diagnosed by using ultrasound technique.

Results

85 buffaloes enrolled in this study, 13 cows were excluded because of the presence of postpartum metrits (5 buffaloes) clinical mastitis (2), sold (1), lameness (2) and inadequate endometrial cytological sampling (3 cows). The remaining 72 buffalo cows were identified as primiparous 18 % (13/72), and multiparous 82 % (59/72). Calving assistance (dystocia) 12.5% (9/72), retained placenta 13.8% (10/72), and twins 1.3% (1/72) was recorded in buffaloes of the current study.

Vaginal examination

Vaginal examination on postpartum days of 15-24 showed ten buffaloes (13.8%) with abnormal vaginal secretions. Four buffaloes had a vaginal secretion score of 1, four buffaloes were scored 2, and two buffaloes were scored 3.

Bacterial isolation

Regarding bacterial isolates, 20 different microorganisms were identified among the total 114 bacterial isolates obtained from 55 of the 72 (76.3%) postpartum cows; no bacterial isolates were obtained from the remaining 17 buffalo cows (15 healthy and 2 SCE buffalo). Multiple bacterial species were isolated from 41 of the 55 cows (76.4%), whereas a single bacterial species was isolated from the remaining 14 cows. Bacterial isolates are shown in Table 1. *E. coli*, *Bacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp. accounted for 22.8 % (26/114), 13.1% (15/114), 9.6% (11/114), and 13.1 % (15/114) of the bacteria species isolated, respectively (Table 1). *Escherichia coli* was the most commonly isolated bacteria from SCE (38.9%; 7/18), which was significantly higher ($P < 0.05$) than healthy buffalo cows 18.7% (12/64). The commonly isolated bacteria from CE cows were *A. pyogenes* (28.1%), *Escherichia coli* (21.8%), and *Staphylococcus aureus* (18.7 %). were higher ($P < 0.05$) than in healthy cows. Moreover, no bacteria were isolated from two cows (2/12) with SCE.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Table 1: List of bacteria isolated from healthy, CE, and SCE buffaloes.

Species of bacteria isolated	Isolation rate		
	Healthy Cows (50 buffalo)	CE* (10 buffalo)	SCE** (12 buffalo)
Escherichia coli	12 (18.7 %) [a]	7 (21.8%) [a]	7 (38.9 %) [b]
Bacillus spp.	10 (15.6 %)	2 (6.2 %)	3 (16.6 %)
Staphylococcus schleiferi	2 (3.1 %)	-	1 (5.5 %)
Staphylococcus aureus	2 (3.1 %)	6 (18.7 %) [d]	-
Streptococcus bovis	3 (4.8 %)	1 (3.1 %)	1(5.5 %)
Streptococcus pneumonia	2 (3.1 %)	1 (3.1 %)	1(5.5 %)
Streptococcus agalactia	3 (4.8 %)	-	1(5.5 %)
Streptococcus	3 (4.8 %)		-
A. pyogenes	-	9 (28.1 %)	1(5.5 %)
Proteus spp.	-	3 (9.3 %)	-
Enterobact *faecium*	3 (4.8 %)	1 (3.1 %)	-
Alcaligenes faecalis	3 (4.8 %)	-	-
Enterobacter cloacae	3 (4.8 %)	-	1(5.5 %)
Klebsiella pneumoniae	-	2 (6.2 %)	1(5.5 %)
Acinetobacter baumannii	3 (4.8 %)	-	1(5.5 %)
Acinetobacter lwoffii	3 (4.8 %)	-	-
Acinetobacte rcalcoaceticus	3 (4.8 %)	-	-
Chromobacterium	3 (4.8 %)	-	-
Pantoea agglomerans	2 (3.1 %)	-	-
Lactobacillus fermentus	2 (3.1 %)	-	-
Pasteurella spp.	2 (3.1 %)	-	-
Total bacterial isolation	64 (100 %)	32 (100 %)	18 (100 %)
Clean (no bacteria)	15	-	2

*CE: Clinical endometritis, **SCE: Subclinical endometritis.
[a b c d]Different lowercase letters in the same row indicate significant differences ($P < 0.05$).

Endometrial cytology

All cows were sampled by cytobrush, of which, 12 cows manifested SCE (16.6%, determined by using ≥ 8% PMN as threshold value and vaginal discharge score = 0). The average PMN % on the slides ranged from 0 to 29%. The agreement among endometrial examination, PMN %, and vaginal discharge score (0–3) was good ($k = 0.80$, $P < 0.05$; Table 2). There is a poor agreement between vaginal discharge score (0–3) and bacteriological finding ($k = 0.24$, $P < 0.05$) also between bacterial finding and PMN ($k = 0.30$, $P < 0.05$) (Table 2).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Table 2: Agreement among endometrial cytology, bacterial isolation and vaginal discharges for postpartum buffaloes.

		Bacterial Isolation		Kappa
		Positive	**Negative**	
Cytobrush	≥ 8 % PMN	24	3	K=0.30
	< 8 %PMN	24	21	P<0.05
		Vaginal Discharges		
		Positive	**Negative**	
Bacterial isolation	Positive	17	31	K=0.24
	Negative	1	23	P<0.05
		Vaginal Discharges		
		Positive	**Negative**	
Cytobrush	≥ 8 % PMN	18	6	K=0.80
	< 8 %PMN	0	48	P<0.05

Buffalo cows with <8% and ≥ 8% of polymorphonuclear leukocytes (PMN) in endometrial cytology samples, negative (score 0) or positive (scores 1, 2, and 3) gross vaginal inflammation score, and the presence or absence of bacterial contamination at days 15–24 post calving. The Kappa statistic (K) is a measure of the level of agreement between the tests, where 1 = complete agreement and 0 = no agreement.

Ultrasound examination

Ultrasound evaluation 4 weeks postpartum showed that 28 buffalo cows had FIU (Table-3); this result presented a 0.64 kappa agreement with the cytological method, as well as 83.3% sensitivity and 83.3% specificity. According to the CM 4 weeks postpartum, 23 cows showed more than 5 cm and yielded a 0.64 kappa agreement, 75% sensitivity, and 89.5% specificity. When the parameters were combined (FIU + CM) to increase the accuracy of diagnosing endometritis, an improved kappa agreement was observed between ultrasound and CB methods. Consequently, a 0.70 kappa value, 91.6% sensitivity, and 89.5% specificity were obtained (Table 3).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Table 3: Agreement among diagnostic methods for endometritis 4 weeks postpartum.

Week 4 post calving		Cytobrush		Kappa (p= value)
		PMN ≥ 8 %	PMN < 8 %	
Ultrasound (FIU)	Positive	20	8	K=0.64(P<0.05)
	Negative	4	40	
		Cytobrush		
		PMN ≥ 8 %	PMN < 8 %	
Ultrasound (CM)	≥ 5	18	5	K=0.65 (P<0.05)
	< 5	6	43	
		Cytobrush		
		PMN ≥ 8 %	PMN < 8 %	
Ultrasound (FIU+ CM)	Positive	22	5	K=0.70 (P<0.05)
	Negative	2	43	

Endometrial cytology results showed that buffalo cows with %PMN <8 are healthy, and those with ≥8 are suffering from endometritis 4 weeks postpartum. FIU is the fluid in uterine, which is negative (no fluid) or positive (present fluid) upon ultrasonographic evaluation. CM is the uterine cervix diameter: <5 cm, healthy; ≥5 cm, endometritis, upon ultrasonographic evaluation. Kappa statistic measures the level of agreement between tests, where 1 = complete agreement and 0 = no agreement.

Risk factors affecting the occurrence of CE and SCE

Table 4 shows the significant relative risk factor of *Trueperella pyogenes*, *E. coli* and *Staphylococcus aureus* for the occurrence of CE. *E. coli* was the major bacteriological relative risk factor for the occurrence of SCE. (odds ratio (OR) = 6.1; 95% CI = 1.70–21.97; P < 0.05). *Trueperella pyogenes* was the major bacteriological risk factor for the occurrence of CE (OR = 5.1; 95% CI = 0.95–20.53; P < 0.05). Calving assistance was considered a risk factor for CE occurrence in buffaloes (OR = 10.6; 95% CI = 1.48–76.09; P < 0.05), whereas BSC was a risk factor only for SCE occurrence (OR = 11.7; 95% CI = 1.24–113.32; P < 0.05). Other isolated bacterial species and parity did not have obvious risk effects on the occurrence of CE and SCE (Tables 4 and 5).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Table 4: Bacteriologic relative risk factors for buffaloes diagnoses with clinical endometritis and subclinical endometritis between 20 and 25 days postpartum.

Factor	Relative Risk	95 % Cl	P Value
Diagnosis CE*			
Bacillus	1.1	0.314-5.73	P > 0.05
E coli	3.7	0.93-26.66	P < 0.05
Streptococcus sp.	2.1	0.35-13.08	P > 0.05
*Staph*ylococcus aureus	3.1	0.25-24.6	P < 0.05
Trueperella pyogenes	5.1	0.95-20.53	P < 0.05
Diagnosis SCE**			
E coli	6.1	1.70-21.97	P < 0.05
Bacillus	1.4	0.34-6.15	P > 0.05
Streptococcus sp.	1.2	0.28- 5.08	P > 0.05

*CE: Clinical endometritis, ** SCE: Subclinical endometritis.

Table 5 : Results of binary logistic regression analysis for the risk of SCE and CE in buffaloes examined at 20 days to 25 days postpartum.

Variables*	Odds Ratio	95 % Cl	P Value
Diagnosis CE			
E coli	6.4	1.80-44.2	P < 0.05
Trueperella pyogenes	8.4	1.88-65.91	P < 0.05
Parity	0.57	0.154-3.08	P > 0.05
Calving assistance	9.7	1.92-71.09	P < 0.05
retained placenta	10.2	2.22-75.4	P < 0.05
Diagnosis SCE			
E coli	7.3	1.89-39.68	P < 0.05
BCS	8.7	1.92-113.32	P < 0.05
Calving assistance	2.8	0.45-12.6	P > 0.05
Parity	0.83	0.22-3.18	P > 0.05

Variables*: CE: clinical endometritis, SCE: Subclinical endometritis, OR:0dds ratio, BCS: body condition score, infection with E coli (0= infection:1= no infection), infection with bacillus (0= infection:1= no infection), infection with Staphylococcus (0= infection:1= no infection), calving assistance(0=assisted calving: 1=normal calving): BCS :(0= < 3: 1= ≥3), parity : (0=primparous: 1=multiparous).

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Reproductive performance

Cox regression model analysis showed that CE, and *Trueperella pyogenes* have significantly affect the risk for non-pregnancy at 200 days after calving P < 0.05) (Table 6). The pregnancy rates at 150 and 200 days postpartum were 27.7% (20/72) and **4**0. 2% (29/72), respectively. The percentages of pregnant cows at 150 days postpartum were 32% (16/50), 25% (3/12), and 10% (1/10) in healthy, SCE, and CE groups, respectively. The percentages of pregnant buffalo cows at 200 days postpartum were 46% (23/50), 33.3% (4/12), and 20% (2/10) in healthy, SCE, and CE groups, respectively (Table 7).

Table 6: Results of Cox regression for the hazard of non-pregnancy within 200 days in buffaloes examined 20 days to 30 days after calving.

Factors*	**Non- pregnancy**		
	HR	**CL 95%**	**P value**
CE	3.49	0.66-3.34	P < 0.05
SCE	1.2	0.5-2.94	P > 0.05
E coli	1.1	0.63-2.75	P > 0.05
Trueperella	3.7	0.69-2.75	P < 0.05
Staphylococcus	0.9	0.28-2.91	P > 0.05
Calving assistance	0.51	0.26-1.22	P > 0.05
BCS	0.76	0.42-1.37	P > 0.05

*Factors: CE: clinincal CE: clinincal endometritis, SCE, Subclinical endometritis, HR, hazard ratio, BCS: body condition score, CE(0= CE: 1= no CE diagnosed), SCE(0= SCE: 1= no SCE diagnosed), infection with E coli (0= infection:1= no infection), infection with bacillus (0= infection:1= no infection), infection with Staphylococcus (0= infection:1= no infection), calving assistance(0=assisted calving: 1=normal calving), BCS: (0= < 3: 1= ≥3).

Table 7: Reproductive performance in buffalo cows.

Variable		Total (%)	healthy	SCE*	CE**
Number of cows		72	50	12	10
Pregnancy rate	At 150 days post calving	20/72 (27.7 %)	16/50 (32%)	3/12 (25%)	1/10 (10%)
	At 200 days post calving	29/72 (40.2 %)	23/50 (46%)	4/12 (33.3%)	2/10 (20%)

* SCE: Subclinical endometritis, **CE: Clinical endometritis.

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

Discussion

Cattle are the primary source of production animals for the meat industry and many problems like dystocia and postpartum problems are affecting the productivity of these cows (15). The effects of these factors on CE and SCE rates and reproductive performance were also investigated. The overall occurrence of CE was 13.8% and this in agreement with previous study found by Azawi et al.,(16) who reported 13.3% in cycling Iraqi buffaloes. Also, this incidence is lower than higher incidence rate of endometritis 22.4%, 24.7%, and 25% obtained by (17, 18) in Iranian, and Egyptian buffaloes, respectively

The SCE occurrence in the present study was 16.6%, which is lower than that in a previous study (20%) in fourth week postpartum buffaloes in Egypt (19) and other study in dairy cows (14). The discrepancy in the results may be due to decreased post-calving problems, such as dystocia, retained placenta, and metabolic disorders. Furthermore, most cows in this study depended on grazing and had low milk production, thereby developing few stress factors and minimal exposure to uterine infection after calving.

Thus far, no consensus has been established with regard to the effect of threshold value and time of uterine sampling on SCE diagnosis. SCE can be diagnosed using different cut-off values, such as PMN % range of 5%–18, and various techniques using cytobrush and low-volume lavage (10). Other studies depended on the thresholds of PMN % according to the effects on the reproductive performance (20,21). Kasmanickam *et al*. (8) depended on >18% PMNs as threshold value between 20 -33 days postpartum and >10% PMNs between 34 and 47 days postpartum using the cytobrush to diagnosis endometritis while Gilbert *et al*. (9) used >5% PMNs as a significant cut-off point for diagnosis endometritis in cows using lavage between 40 and 60 days postpartum. The low SCE prevalence may be attributed to differences in geographic area, environment, and endometrial cells counted among the studies. A total of 300 cells were counted per slide in the present study, whereas 100 cells were counted in previous studies (10).

E. coli was the common bacteria isolated from healthy (18.7%) and endometritic cows (21.8% in CE cows and 38.9% in SCE cows) in the period between 20 days to 30 days postpartum. These results are either similar (22,23) or higher than those reported in previous studies (21). Another previous study reported E. coli (23%), Archanobacterium pyogenes (13%) and Staphylococcus aureus (10%) were mostly isolated bacteria from uterus buffaloes with repeat breeding (16)

Most studies confirmed that *E. coli* was isolated in the early period of 0 day to 15 days after calving, and the percentage gradually decreased with the advancement of the postpartum period (24). The present results agree with previous study demonstrating that *E. coli* and *A. pyogenes* were the common uterine pathogens in the postpartum period (23). Werner *et al*. (24) reported the lack of association between the abnormal vaginal discharge and *E. coli* infection in endometritic cows. Although *E. coli* is a common bacteria in the environment, specific strains of this species have been isolated from cows

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

with uterine diseases (1). Endometrial pathogenic *E. coli* is more adherent and invasive in the endometrium compared with *E. coli* isolated from the uterus of clinically unaffected cows. These strains pathogenic *E. coli* develop diseases of endometrial surfaces such as postpartum metritis or endometritis in the bovine genital tract (1). In agreement to most studies, the present study showed a increased number of *A. pyogenes* (nine isolate) in CE cases, leading to many cases of purulent vaginal discharge (scores 2 and 3) (13). Also, the current study registered 7 isiolated of *E. coli* (28.1%) in CE fuffaloes. However, Sens and Heuwieser (23) reported in the previous study about bacterial isolation during postpartum period that *E coli and A. pyogenes* were the dominant bacteria that isolated from uterus between 7-24 days postpartum.

Moreover, *Streptococcus* isolation from cows in the present study was less frequent and did not severely affect CE and SCE; similarly, previous studies reported *Streptococcus* as an opportunistic bacteria in the postpartum uterus (25). In contrast to the present findings, Werner *et al*. (24) reported that infection with *Streptococcus* during the early postpartum period increased the risk for the occurrence of abnormal vaginal discharge and elevated uterine PMN %. In the present study, *S. aureus* was isolated from CE buffaloes and affected the risk factor for its occurrence. These results are consistent with previous studies, thereby confirming the serious effect of *Staphylococcus* sp. on uterine infection (26). However, the results were in contrast to other studies reporting the absence of significant effect of *Staphylococcus* on CE cows (25).

Most pathogenic bacteria isolated from postpartum buffaloes with uterine diseases are *Escherichia coli*, *Prevotella* spp. (1). Also, many studies isolated *Streptococcus* sp. *Staphylococcus* sp., or non-coliform aerobic gram-negative rods (8). To choose a suitable and effective antimicrobial drug to treat postpartum uterine diseases, it is very important to know the sensitivity of the pathogen bacteria to antibiotics

This study focused on comparison between ultrasound technique and cytological method in the diagnosis of endometritis in buffaloes. After 4 and 5 weeks postpartum, the involution of the genital tract of cows was almost complete in the healthy cows, while it was delayed in the infected cows. The cows with uterine cervix diameter of more than 5cm after four weeks developed uterine diseases and more likely to have reduced fertility in the future [4].

A delay the involution of the uterus and uterine contamination with bacterial species after calving was associated with accumulation of uterine fluid that was detected by ultrasonic examination[8]. This study also showed not good agreement between ultrasound evaluation and cytological technique at week 4 postpartum. These results agree with previous studies that reported poor agreements between ultrasonic measurements of uterine fluids and cytobrush methods in diagnosis of endometritis in dairy cows [10, 20]. The studies explained that both forms of endometritis; one associated with the cellular influx of PMN and the second by the accumulation of fluids inside the lumen of the uterus with a low percentage of PMN with decreased uterine clearness have been diagnosed

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

with these methods[9, 10]. In this study, both uterine fluid and cervical diameter were useful tools in the detection of infected cows. Based on our results, a better sensitivity, specificity, and kappa agreement was observed with cytological method, which is reported as the gold method in diagnsosis of endometritis. This was also trueonce two parameters were combined to diagnose endometritis giving a high Kappa agreement = 0.70. This result is in agreement with Barlund et al. [10], and Meira et al. [27] who found that the ultrasound technique was a good non-invasive, useful and practical method used to estimate uterine fluid or cervical diameter in order to diagnose endometritis. The efficiency of ultrasonic technique in the diagnosis of endometritis can be increased once it is combined with uterine fluids, thus giving a 70% kappa.

This study showed the effect of calving assistance (dystocia) and retained placenta as a risk factor on CE (Table 5); the result is in agreement with previous study reporting the effect of calving assistance on the rate of possible uterine contamination after calving [14]. In cattle, dystocia is often accompanied with many post-partum disorders, such as retained placenta and impaired uterine involution, and that develop endometritis [1]. Moreover, abnormal calving increases indirectly the chance for the development of both clinical and subclinical endometritis by increasing the probability of uterine infection like metritis [1]. dystocia can induce severe trauma of the pelvic canal and also allow to the introduction of huge bacteria into the uterus and develop endometritis [28]. Sheldon *et al*. [1] mentioned in one study that cows which had dystocia has more chances to suffer from uterine infections and increased culling rate in the future than those that calved without assistance. Buffaloes with retained placenta, dystocia or other parturient problem had significantly lower subsequent fertility [16], increased days open and increased number of services per conception. However, this result is in contrast with another study that did not find any effect of calving assistance on SCE occurrence (26).

In the present study, no association was found between BCS and CE occurrence; similarly, previous studies that failed to demonstrate any effect of BCS on CE *(29*). However, Cheong *et al*. (20) reported that BSC significantly affected the rate of CE in cows. The present study also demonstrated the effect of BCS on SCE occurrence, which may be attributed to the effect of negative energy balance (NEB) after calving on the immunity of cows, thereby increasing the probability of uterine infection (9). Hammon *et al*. (30) mentioned that uterine infection was accompanied with NEB, which begins before birth and continues through the early lactation; this study also reported that cows with acute NEB exhibit reduced neutrophil function and developed SCE. body energy reserves at calving consider an essential factor influencing reproductive performance in cattle, and it is a most important factor determining when heifers and cows will resume cycling after calving and response to postpartum nutrient intake (31).

In the present study, poor to moderate agreement was found among PMN %, bacteriological findings, and vaginal

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

discharges. According to previous studies, results indicated that abnormal discharges do not necessarily indicate uterine infection [3,31]. The present study also showed poor agreement between bacterial findings and PMN, similar to the findings of other studies [32]. Not all of the bacterial species cause inflammation and infusion of PMN to the uterine endothelium because numerous bacteria may be normal inhabitants of the uterus; uterine infection after calving can also be due to other causes, such as yeast and viruses. The present study showed good agreement between PMN % and vaginal discharges. False positive diagnoses should be considered into the evaluation of studies on the diagnosis and treatment of clinical endometritis. The proportion of samples positive for A. pyogenes or samples exceeding a threshold for PMN of 5 and 18% increased with vaginal discharge score. There was a significant positive correlation between findings of A. pyogenes and PMN, but not between other bacteria and PMN [31]

Reproductive performance is one of the most common economically important traits in cattle production [33].The current study showed minimal effects of SCE, and most isolated bacteria on the reproductive performance of cows because the ability of these cows to self-cured; this finding is similar to those reported in a previous study [26].While the current study showed CE and *A. pyogenes* as risk factor for reproductive performance and this agree with most studies that showed increased days open and days to conception in cows suffering from uterine diseases, such as CE [14]. Increased polymorphonuclear cells were clear in buffaloes infected with A. pyogenes and anaerobic bacteria which suggest that these bacteria play a role in causing gramnegative anaerobes and other facultative pathogens including A. pyogenesin severe uteri inflammations. Many of previous studies confirmed that most of the clinical and reproductive consequences might be attributed to the presence of certain non-specific pathogens, mainly Actinomyces pyogenes, either alone or in company with other bacteria like E. coli and Gramnegative obligate anaerobes [16, 24].

A. pyogenes could make acute destruction of the endometrium layer and impairment of future reproductive performance of animals [16]. T. pyogenes was the most essential bacteriological risk factor for the incidence of CE and had a detrimental effect on the hazard of nonpregnancy by 200 days postpartum [26].

The current study also showed a decline in the pregnancy rate of buffaloes 40.2 % (29/ 72) at 200 days post calving because several possible factors affect the reproductive performance of these buffalo cows, such as seasonal breeding, Prolonged postpartum acyclicity in suckled cows, nutrition, breed and uterine infection. Pregnancy rates in the day 200 after birth were more than at 150 days, the reason for that may be these buffaloes have a longer period to overcome their reproductive problems and more chance to be pregnant. Extended post calving anestrus in suckled beef cows is one of the most common limitations to gaining a calf every year [34]. Climatic stress, parity, extended suckling, nutritional deficiencies, and management practices were the most common reasons of prolonged calving intervals [15]. Further studies are needed to shed light on the uterine infections in beef cows and its

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

relationship to other factors that cause weakness of the reproductive performance of these cows.

Conclusion

A moderate agreement exists among PMN %, bacteriological findings, and vaginal discharges, whereas a poor agreement exists between bacterial findings and PMN %. Results indicated that abnormal discharges do not necessarily indicate uterine infection Also, not all of the bacterial species cause inflammation and infusion of PMN to the uterine endothelium because numerous bacteria may be normal inhabitants of the uterus; endometritis after calving can also be due to other causes, such as yeast and viruses. *E. coli*, *S. aureus*, and calving assistance (dystocia) were the major factors affecting uterine infection in beef cows. CE and SCE insignificantly affected the reproductive performance of buffaloes.

References

1. Sheldon I.M., Lewis G.S., LeBlanc S. and Gilbert R.O. (2006) Defining postpartum uterine disease in cattle. Theriogenology, 65:1516–1530.
2. Gilbert R.O., Shin S.T., Guard C.L., Erb H.N. and Frajblat M. (2005) Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology, 64:1879–1888.
3. Dubuc J., Duffield T.F., Leslie K.E., Walton J.S and LeBlanc S.J. (2010) Risk factors for postpartum uterine diseases in dairy cows. J Dairy Sci., 93:5764–5771.
4. LeBlanc S.J., Duffield T.F., Leslie K.E., Bateman K.G., Keefe G.P. and Walton J.S. (2002) Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci., 85:2223–2236.
5. Runciman D.J., Anderson G.A., Malmo J. and Davis G.M. (2008) Use of postpartum vaginoscopic (visual vaginal) examination of dairy cows for the diagnosis of endometritis and the association of endrometritis with reduced reproductive performance. Aust Vet J. Wiley Online Library,86 :205–13.
6. Studer E. and Morrow D.A. (1978) Postpartum evaluation of bovine reproductive potential: comparison of findings from genital tract examination per rectum, uterine culture, and endometrial biopsy. J Am Vet Med Assoc.,172: 489–494.
7. Bourke M., Mills J.N. and Barnes A.L. (1997) Collection of endometrial cells in the mare. Aust. Vet. J. Wiley Online Library,75: 755–758.
8. Kasimanickam R., Duffield T.F, Foster R.A, Gartley C.J, Leslie K.E and Walton J.S. (2004) Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology, 62: 9–23.
9. 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. doi: 10.1016/j.theriogenology.2005.04.0

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

10. Barlund C.S., Carruthers T.D., Waldner C.L. and Palmer C.W. (2008) comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology, 69: 714–723.
11. Mateus L., da Costa L., Bernardo F. and Robalo Silva J. (2002) Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod. Domest. Anim., 37: 31–35.
12. Bercovich A, Edan Y, Alchanatis V, Moallem U, Parmet Y, Honig H, Maltz E, Antler A, Halachmi I (2013). Development of an automatic cow body condition scoring using body shape signature and Fourier descriptors. *Journal of Dairy Science*, *96*(12): 8047–8059. doi: 10.3168/jds.2013-6568
13. Zobel R. (2013) Endometritis in Simmental cows: incidence, causes, and therapy options. Turkish J. Vet. Anim. Sci., 37:134–140.
14. Madoz L.V., Giuliodori M.J., Jaureguiberry M., Plöntzke J., Drillich M. and la Sota R.L. (2013) The relationship between endometrial cytology during estrous cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows. J Dairy Sci. ,96: 4333–4339.
15. Diskin MG,Kenny DA (2016). Managing the reproductive performance of beef cows. *Theriogenology*, *86*(1): 379–387.
16. Azawi OI, Omran SN, Hadad JJ (2008). A study of endometritis causing repeat breeding of cycling Iraqi buffalo cows. *Reproduction in Domestic Animals*, *43*(6): 735–743.
17. Moghaddam AAI, Mamoei M (2004). A survey on some of the reproductive and productive traits of the buffalo in Iran. *23rd World Buiatrics*, 1910.
18. Ghanem M, Shalaby AH, Sharawy S, Saleh N(2002). Factors leading to endometritis in dairy cows in Egypt with special reference to reproductive performance. *Journal of Reproduction and Development*, *48*(4): 371–375. doi: 10.1262/jrd.48.371
19. Senosy W. and Hussein H.A. (2013) Association among energy status , subclinical endometritis postpartum and subsequent reproductive performance in Egyptian buffaloes. Anim. Reprod. Sci., 140: 40–46
20. Cheong SH, Nydam DV, Galvão KN, Crosier BM,Gilbert RO(2011). Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. *Journal of Dairy Science*, *94*(2): 762–770. doi: 10.3168/jds.2010-3439
21. McDougall S, Hussein H, Aberdein D, Buckle K, Roche J, Burke C, Mitchell M, Meier S (2011). Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. *Theriogenology*, *76*(2): 229–240. doi: 10.1016/j.theriogenology.2010.12.024

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, NO. 3, September 2024

22. Santos NR, Lamb GC, Brown DR, Gilbert RO (2009). Postpartum endometrial cytology in beef cows. *Theriogenology*, *71*(5): 739–745. doi: 10.1016/j.theriogenology.2008.09.043

23. Sens A, Heuwieser W (2013). Presence of Escherichia coli, Trueperella pyogenes, α-hemolytic streptococci, and coagulase-negative staphylococci and prevalence of subclinical endometritis. *Journal of Dairy Science*, *96*(10): 6347–6354. doi: 10.3168/jds.2013-6646

24. Werner A, Suthar V, Plöntzke J, Heuwieser W (2012). Relationship between bacteriological findings in the second and fourth weeks postpartum and uterine infection in dairy cows considering bacteriological results. *Journal of Dairy Science*, *95*(12): 7105–7114. doi: 10.3168/jds.2012-5627

25. Williams EJ, Fischer DP, Pfeiffer DU, England GCW, Noakes DE, Dobson H, Sheldon IM (2005). Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology*, *63*(1): 102–117. doi: 10.1016/j.theriogenology.2004.03.017

26. Prunner I, Pothmann H, Wagener K, Giuliodori M, Huber J, Ehling-Schulz M, Drillich M (2014). Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows. *Theriogenology*, *82*(9): 1316–1322. doi: 10.1016/j.theriogenology.2014.08.018

27. Meira E.B.S., Henriques L.C.S., Sa L.R.M. and Gregory L. (2012) Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. J. Dairy Sci., 95: 6969–6973.

28. Bruun J, Ersbøll AK,Alban L(2002). Risk factors for metritis in Danish dairy cows. *Preventive Veterinary Medicine*, *54*(2): 179–190.

29. Potter TJ, Guitian J, Fishwick J, Gordon PJ, Sheldon IM (2010). Risk factors for clinical endometritis in postpartum dairy cattle. *Theriogenology*, *74*(1): 127–134. doi: 10.1016/j.theriogenology.2010.01.023

30. Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL (2006). Neutrophil function and energy status in Holstein cows with uterine health disorders. *Veterinary Immunology and Immunopathology*, *113*(1–2): 21–29. doi: 10.1016/j.vetimm.2006.03.022

31. Wettemann RP, Lents CA, Ciccioli NH, White FJ, Rubio Ijj (2003). Nutritional-and suckling-mediated anovulation in beef cows. *Journal of Animal Science*, *81*(14_suppl_2): E48–E59.

32. Barański W, Podhalicz-Dziegielewska M, Zduńczyk S,Janowski T (2012). The diagnosis and prevalence of subclinical endometritis in cows evaluated by different cytologic thresholds. *Theriogenology*, *78*(9): 1939–1947. doi:

10.1016/j.theriogenology.2012.07.018

33. Bormann JM, Totir LR, Kachman SD, Fernando RL,Wilson DE(2006). Pregnancy rate and first-service conception rate in Angus heifers. *Journal of Animal Science*, *84*(8): 2022–2025. doi: 10.2527/jas.2005-615

34. Miller V, Ungerfeld R (2008). Weekly bull exchange shortens postpartum anestrus in suckled beef cows. *Theriogenology*, *69*(8): 913–917.