Indian Journal of Animal Sciences **88** (4): 415–419, April 2018/Article

Bacterial presence and fertility in subclinical endometritic buffaloes at oestrus

HARPREET SINGH[1], PARKASH SINGH BRAR[2], A K ARORA[3], S S DHINDSA[4] and M HONPARKHE[5]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 012 India

Received: 2 October 2017; Accepted: 13 December 2017

ABSTRACT

Study evaluated the diagnosis of subclinical endometritis (SCE) at the time of oestrus in relation to uterine bacterial contamination and fertility in buffaloes (*Bubalus bubalis*). Normal cyclic healthy buffaloes (115) presented for insemination at spontaneous oestrus were diagnosed (by endometrial cytology) for SCE and divided into control (<5% PMN) and SCE (≥5% PMNs) groups. The prevalence of 22.61% for SCE was recorded. Significantly higher proportion of buffaloes were infected in SCE (65.4 vs 21.3%) than in control group. The seven different bacteria (*Staphylococcus* spp., *Bacillus* spp., *Proteus* spp., *Escherichia coli, Klebsiella* spp., *Streptococcus spp*. and *Pseudomonas spp*.) in both the groups with *T. pyogenes* only in SCE group were isolated. The presence of SCE as well as bacteria recorded a detrimental effect on fertility, with significantly lower conception rate (23.08 vs 37.08%) in SCE and tendency for higher number of services per conception in buffaloes with infected uteri, compared to their healthy counterparts. A differential trend for antibiotic sensitivity to the bacterial isolates was recorded.

Key words: Antibiotics, Bacteria, Buffaloes, Fertility, Oestrus, Subclinical endometritis

Uterine infection and the associated inflammatory response causes subfertility and infertility in cows and buffaloes (Azawi 2010). Uterine infection implies the adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelia and release of bacterial toxins that lead to establishment of uterine diseases. Subclinical endometritis (SCE), also called as 'cytological endometritis' is the inflammation of endometrium without any clinical manifestation or systemic illness (Kasimanickam *et al.* 2004) and is characterized by an increased proportion of polymorphonuclear (PMN) cells in endometrial cytology by cytobrush or low-volume uterine lavage techniques (Dubuc *et al*. 2010). Subclinical endometritis is one of the most important causes of subfertility in dairy herds (Galvao *et al*. 2011). The economic impact of SCE remains mostly unknown due to diagnostic issues, delayed effects and requirement of deliberate statistical analysis. However, losses due to SCE are speculated to exceed billions of dollars annually for the global dairy industry (Ghasemi *et al.* 2012).

The incidence of SCE was reported to be 23.08% in Indian buffaloes (Gahlot *et al.* 2016). Similarly, 22.61% of normal cyclic healthy buffaloes presented for artificial insemination were recorded to be suffering from SCE (Personal data). Various bacteria from endometritic bovine uteri have been reported (Azawi 2010). However, the relationship of uterine bacterial presence with proportion of PMNs in the endometrium (Brodzki *et al*. 2014) is still ambiguous, particularly in oestral buffaloes. Hence, the present study was conducted to evaluate the relationship between occurrence of SCE and uterine bacterial contamination at oestrus and their consequences on ensuing fertility in buffaloes.

MATERIALS AND METHODS

Healthy and normal cyclic buffaloes (parity 2nd to 5th; mean age of 6.4±1.7 years; BCS = 2.5 to 4; average 305 day lactation yield was 2403±110.6 kg) in spontaneous oestrus without clinical endometritis and any other reproductive anomaly presented for artificial insemination (115) were enrolled after reproductive evaluation by trans-rectal ultrasonography and quality of cervico-vaginal discharge.

Prior to insemination, two endometrial samples were collected by cytobrush technique (Barlund *et al.* 2008) from uterine horn of the ovarian side with dominant follicle (confirmed ultrasonographically) at each occasion, one for cytology and another for bacterial isolation. The first cytobrush was aseptically put into a sterile 15 ml tube containing 2.0 ml of autoclaved Brain Heart infusion (BHI) broth media for bacteriological study. The cytological smears made from second cytobrush were fixed with methanol and stained with modified Wright Giemsa stain (Sigma-Aldrich Inc. USA) as per the manufacturer's

Present address: [1]PhD Scholar (hsinghvet@gmail.com), [2]Professor (parkashbrar@gmail.com), [4]Assistant Animal Scientist (Reproduction) (shahbazdhindsa76@yahoo.com), [5]Gynaecologist (honparkhem@gmail.com), Department of Veterinary Gynaecology and Obstetrics. [3]Professor (aroraak65 @yahoo.com), Department of Veterinary Microbiology.

guidelines. Slides were examined using light microscopy and 300 cells per slide were counted to record the % of PMN cells and buffaloes were designated into control (<5% PMN cells) and subclinical endometritic (SCE, ≥5% PMN cells) groups. All the buffaloes were inseminated with good quality frozen thawed semen at the same oestrus (twice as per AM/PM schedule) and were confirmed for pregnancy by ultrasonography at day 40 post-insemination.

Isolation, identification of bacteria and antibiotic sensitivity: After incubation at 37°C for 6–8 h, the sample broths were gently streaked onto sterile culture media (Brain Heart Infusion agar, Mannitol Salt agar, Eosin Methylene Blue agar, MacConkey Lactose agar, Blood agar) plates. Isolation and identification of aerobic bacteria was further carried out based on the morphology, cultural characteristics, Gram stain and biochemical tests in the positive samples as described by Quinn *et al.* (1999). Plates with 1 or more colony-forming units were designated as positive for bacterial growth and with more than three species or with mixed culture were considered contaminated.

Bacterial isolates were aseptically processed by the standard disk diffusion method for *in-vitro* antibiotic sensitivity as per the standard protocol (CLSI 2004). The isolates were tested against 14 antibiotics for their sensitivity using antibiotic sensitivity discs, viz. ceftriaxone (30 mcg), gentamicin (30 mcg), ciprofloxacin (5 mcg), enrofloxacin (10 mcg), penicillin-G (2 units), oxytetracycline (30 mcg), chloramphenicol (25 mcg), cephalexin (30 mcg), cloxacillin (200 mcg), cefotaxime (30 mcg), cefoperazone (75 mcg), ampicillin (25 mcg), furazolidone (50 mcg) and norfloxacin (5 mcg). The results were interpreted as per the zone size interpretative chart supplied by the manufacturer (Hi-Media Laboratories Limited, Mumbai).

Statistical analyses: Data were analyzed by Student's t-test using SPSS for Windows (SPSS version 24.0, Chicago, USA). $P<0.05$ was considered to be statistically significant. The effect of SCE and bacterial presence on fertility parameters was analyzed by Kaplan-Meier survival analysis using MedCalc statistical software (MedCalc version 15.8, Belgium).

RESULTS AND DISCUSSION

Twenty six buffaloes out of 115 recorded ≥5% PMN in endometrial cytology and accordingly a prevalence of 22.61% (26/115) for SCE at oestrus was recorded. The remaining parameters were analyzed accordingly for the two groups, i.e. buffaloes diagnosed with (SCE group, n=26) or without (Control group, n= 89) subclinical endometritis at oestrus.

Uterine bacterial isolates: Out of total 115 buffaloes enrolled, 31.3% (36/115) were bacteriologically positive and 70.4% (79/115) showed no bacterial growth. However, bacterial growth was observed in 65.4% (17/26) of buffaloes of SCE group compared to only 21.3% (19/89) of control group. Reports regarding uterine bacterial presence in oestral cows or buffaloes suffering from SCE are lacking in the available literature.

In the present study, no bacterial growth was observed in 78.7% of non-endometritic as compared to 34.6% of endometritic buffaloes. Earlier studies have also failed to isolate bacteria from subclinical endometritic postpartum cows (Madoz *et al.* 2014, Ricci *et al.* 2015). In the present study, a low but significant correlation ($r = 0.295$, $P<0.01$) between uterine bacterial presence and endometrial PMN content was observed. Earlier in cows also, low level of association between presence of intrauterine pathogens and endometrial PMN percentage was reported (McDougall *et al.* 2011). These observations support the no correlation reported between the endometrial PMN content and bacteriological growth density on agar plates, suggesting that not all cases of SCE are associated with bacterial infection or that inflammation is long lasting than the infection (Baranski *et al.* 2012). Moreover, recently Wagener *et al.* (2017) reviewed studies on subclinical endometritis and summarized that uterine infections with known pathogens play a minor role in SCE than in clinical endometritis and metritis. They speculated that SCE could be a response to a more or less nonspecific uterine infection. Hence, exploiting culture independent DNA based methods to identify nonspecific bacteria in large number buffaloes with SCE is warranted. It cannot be excluded that cytobrush sampling technique may miss contaminated or inflammatory regions of endometrium. However, Werner *et al.* (2012) concluded that a single sample by cytobrush is valuable to evaluate infectious and inflammatory properties of endometrium in cows. Ellenberger *et al.* (2006) also revealed that the whole endometrium was affected in 82% of endometritic cows. Therefore, it was assumed that cytobrush technique is suitable to identify most cases of endometritis.

The bacteria of seven distinct genera were isolated in both the groups, whereas *T. pyogenes* was isolated only from endometritic buffaloes (Table 1). *E. coli* was most prevalent among bacteria isolated in non-endometritic buffaloes; whereas, *E coli* and *Staphylococcus spp.* were equally prevalent in endometritic buffaloes (Table 1). Numerous bacteria in diverse combinations have been isolated from the postpartum bovine uteri. Our results corroborated with previous reports in which *E. coli* was found to be associated with 18.15% (Shukla and Sharma 2005) to 25.25% (Arora *et al.* 2000) cases of clinical endometritis. However, Samatha and Babu (2013) reported *Salmonella* (34.37%), *Staphylococcus* (28.87%) and *Pseudomonas* (15.62%) as major organism in uterine fluid of repeat breeding buffaloes at oestrus. However, no attempt has been made to decipher the uterine microbiota at oestrus and its effect on the subsequent fertility.

The number of buffaloes with mixed infection was double in SCE than in control group (10 versus 5 buffaloes). This could be attributed to synergistic action of one bacteria to promote growth of other bacteria, which could be associated with persistent inflammation (Singh *et al.* 2008). In the present study, *T. pyogenes* was isolated from four

Table 1. Distribution of bacterial isolates in bacteriologically positive buffaloes

Bacterial isolate (Total 52)	Control (Positive buffaloes = 19) (Total bacterial isolates = 24)			SCE (Positive buffaloes = 17) (Total bacterial isolates = 28)		
	Present as single isolate	Present as mixed infection	Percent of total isolates (n=24)	Present as single isolate	Present as mixed infection	Percent of total isolates (n=28)
Staphylococcus spp.	1	3	16.7	1	4	17.9
Streptococcus spp.	0	2	8.3	1	3	14.3
Bacillus spp.	4	0	16.7	0	4	14.3
E. coli	6	2	33.3	1	4	17.9
Pseudomonas spp.	2	1	12.5	1	1	7.1
Klebsiella spp.	0	1	4.2	0	2	7.1
Proteus spp.	1	1	8.3	0	2	7.1
Truperella pyogenes	0	0	0.0	3	1	14.3

buffaloes with SCE and none in control. Azawi *et al*. (2007) suggested that a earlier uterine infection with *E. coli* affected the phenotype and function of PMN cells and might support a coinfection with *T. pyogenes* at a later stage. *T. pyogenes* was demonstrated to be associated with severity of endometritis and caused uterine disease when infused into uterus (Dar *et al*. 2015, Wagener *et al.* 2017).

Reproductive performance of buffaloes: In the present study, presence of SCE or bacteria and their interaction resulted in a significant effect on fertility parameters in buffaloes (Table 2). In dairy cows also, a negative impact of SCE on subsequent reproductive performance (prolonged days open, reduced conception rate at first AI) was reported (Kasimanickam *et al*. 2004, Barlund *et al*. 2008). Studies have demonstrated the presence of SCE to be a key factor in pregnancy rate, with an increase in days open ranging from 25 days (Dubuc *et al.* 2011) to 30 days (Madoz *et al*. 2013) in cows with SCE. Likewise, significantly lower pregnancy rate (60 versus 80%) in cows with SCE compared to healthy cows upto 200 days postpartum was reported (Barrio *et al*. 2015). However, the single study (Ata *et al*. 2010) analyzing uterine bacterial presence at oestrus in cattle and reported no adverse effect on conception rate.

Table 2. Reproductive parameters in buffaloes classified as per presence or absence of subclinical endometritis and bacteria at spontaneous oestrus

Group	No. of AI per conception[#]	Days to pregnancy from first AI[#]	Conception rate at first AI[$] (in percentage)
SCE/bact+ (n=17)	2.77±0.30[b]	81.38±16.34[b]	17.65
SCE/bact- (n=9)	2.29±0.18[ab]	59.43±5.74[ab]	33.34
Control/bact+ (n=19)	2.05±0.15[ab]	44.46±6.59[ab]	31.58
Control/bact- (n=70)	1.67±0.24[a]	31.11±12.08[a]	38.14

SCE/bact+, buffaloes with SCE and positive for bacterial isolation; SCE/bact-, buffaloes with SCE but negative for bacterial isolation; Control/bact+, healthy buffaloes positive for bacterial isolation; Control/bact-, healthy buffaloes positive for bacterial isolation. [a,b]Values with different superscripts within a column differ significantly (P<0.05). [#]Groups compared by ANOVA followed by DMRT post hoc test. [$]Groups compared by Chi-square test.

Antibiotic sensitivity pattern of bacterial isolates: The results of *in-vitro* antibiotic sensitivity test of different isolates to the antibiotics varied as depicted in Table 3. Overall, the highest number of bacterial (>70%) isolates was sensitive to cefotaxime, cefoperazone, ciprofloxacin, norfloxacin, gentimicin, enrofloxacin, pencillin-G followed by (>60%) chloramphenicol, oxytetracycline, ceftrixone and less sensitive (>50%) to ampicillin, furazolidone and cloxacillin. In another study, uterine *E. coli* isolates from endometritic buffaloes displayed good sensitivity (³90%) to tetracycline, cotrimoxazole, gentamicin and chloramphenicol, with moderate resistance to nitrofurantoin and amoxicillin (Ingale *et al*. 2016). Present findings were in partial agreement to the highest number of isolates from endometritic cows reported to be sensitive to ceftriaxone (64%), followed by gentamicin, enrofloxacin and chlortetracycline (32%) and minimum with chloramphenicol (Udhayavel *et al*. 2013). A higher sensitivity of various uterine bacterial isolates to ciprofloxacin and gentamicin had been reported in previous studies (Arora *et al*. 2000, Seh *et al*. 2000). In agreement with our results, Arora *et al.* (2000) also reported resistance to penicillin-G, nitrofurantoin and furazolidone and attributed it to large scale use of these antibiotics in veterinary practice.

In the present study, *Pseudomonas spp.* isolates were having the highest resistance levels among all type of bacteria. The least sensitivity of *Pseudomanas* isolates from clinical endometritic cows had been reported previously (Udhayavel *et al*. 2013).

The variability reported in the pattern of antibiotic sensitivity could be associated with the variation in the drug resistance developed by micro-organisms by indiscriminate antibiotic usage. Another key factor of anaerobic uterine environment resulting in loss of antibiotic potency should

Table 3. Antibiotic sensitivity pattern of bacterial isolates from healthy and subclinical endometritic buffaloes at oestrus

Antibiotic	Bacterial isolates (number)								
	Staphylococcus spp. (9)	*Streprococcus* spp. (6)	*Bacillus* spp. (8)	*E. coli* (13)	*Pseudomonas* spp. (5)	*Klebsiella* spp. (3)	*Proteus* spp. (4)	*T. pyogenes* (4)	Overall (total isolates=52)
	Sensitivity in% (No. of isolates)								
Oxytetracycline	66.7 (6)	83.4 (5)	87.5 (7)	53.8 (7)	40 (2)	66.7 (2)	75 (3)	66.7 (3)	67.3 (35)
Pencillin-G	77.8 (7)	83.4 (5)	87.5 (7)	76.9 (10)	0 (0)	66.7 (2)	75 (3)	66.7 (3)	71.2 (37)
Ceftrixone	55.6 (5)	83.4 (5)	87.5 (7)	46.2 (6)	60 (3)	66.7 (2)	75 (3)	100 (4)	67.3 (35)
Chloramphenicol	44.5 (4)	66.7 (4)	62.5 (5)	76.9 (10)	40 (2)	100 (3)	75 (3)	100 (4)	69.3 (36)
Cephalexin	77.8 (7)	50 (3)	62.5 (5)	69.2 (9)	40 (2)	100 (3)	50 (2)	100 (4)	65.4 (34)
Cloxacillin	55.6 (5)	50 (3)	62.5 (5)	76.9 (10)	20 (1)	66.7 (2)	25 (1)	33.3 (1)	53.8 (28)
Cefotaxime	77.8 (7)	66.7 (4)	87.5 (7)	100 (13)	40 (2)	100 (3)	75 (3)	100 (4)	82.7 (43)
Cefoperazone	66.7 (6)	83.4 (5)	87.5 (7)	94.6 (12)	60 (3)	100 (3)	75 (3)	0 (0)	75.0 (39)
Ciprofloxacin	66.7 (6)	66.7 (4)	75 (6)	61.5 (8)	80 (4)	100 (3)	100 (4)	66.7 (3)	73.1 (38)
Gentamycin	88.9 (8)	66.7 (4)	62.5 (5)	61.5 (8)	40 (2)	66.7 (2)	100 (4)	100 (4)	71.2 (37)
Ampicillin	55.6 (5)	66.7 (4)	50 (4)	53.8 (7)	40 (2)	66.7 (2)	75 (3)	67.7 (3)	57.7 (30)
Furazolidone	77.8 (7)	66.7 (4)	62.5 (5)	53.8 (7)	20 (1)	66.7 (2)	0 (0)	67.7 (3)	55.8 (29)
Norfloxacin	66.7 (6)	66.7 (4)	50 (4)	84.6 (11)	80 (4)	66.7 (2)	100 (4)	67.7 (3)	73.1 (38)
Enrofloxacin	77.8 (7)	66.7 (4)	100 (8)	61.5 (8)	20 (1)	33.7 (1)	100 (4)	100 (4)	71.2 (37)

be considered during the selection of suitable antibiotics (Sheldon and Dobson 2004). The broad usage of different antibiotics by clinicians especially in the field conditions, without a specific and targeted approach, leads to use of inappropriate antibiotics contributes to the development of microbial resistance. Hence, antibiogram study helps to identify and advocate precise antibiotics for treatment of different grades of uterine infections.

The study indicated that subclinical endometritis at oestrus was not always associated with presence of aerobic bacteria in uterus of buffaloes, although it hampered fertility significantly. Moreover, possibly a synergistic bacterial action could lead to persistence of endometrial inflammation. Although, antibiotic sensitivity varied with isolates, highest sensitivity was recorded for cefotaxime, cefoperazone, ciprofloxacin, norfloxacin, gentamicin, enrofloxacin, and penicillin-G antibiotics.

REFERENCES

Arora A K, Singh J, Pangaonkar G R and Nanda A S. 2000. Bacteriological studies on the genital tract in repeat breeder bovines. *International Journal of Animal Sciences* **15**: 205–07.

Ata A, Türütoðlu H, Kale M, Gülay M S and Pehlivanoðlu F. 2010. Microbial flora of normal and abnormal cervical mucous discharge associated with reproductive performance of cows and heifers in estrus. *Asian Australasian Journal of Animal Sciences* **23**(8): 1007–12.

Azawi O I, Omran S N and Hadad J J. 2007. Clinical, bacteriological, and histopathological study of toxic puerperal metritis in Iraqi buffalo. *Journal of Dairy Science* **90**: 4654–60.

Azawi O I. 2010. Uterine infection in buffalo cows; A review. *Buffalo Bulletin* **29**(3): 154–71.

Baranski W, Podhalicz-Dzie gielewska M, Zdunczyk S and Janowski T. 2012. The diagnosis and prevalence of subclinical endometritis in cows evaluated by different cytological thresholds. *Theriogenology* **78**: 1939–47.

Barlund C S, Carruthers T D, Waldner C L and Palmer C W. 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* **69**: 714–23.

Barrio M, Marcos V, Luis A, Quintela J, Becerra J, Pedro J, García H, Daniel M B, Francisco I, Fernandez S, Alberto P, Juan C and Ana I P. 2015. Influence of subclinical endometritis on the reproductive performance of dairy cows. *Spanish Journal of Agricultural Research* **13**(4): e05SC02.

Bondurant R H. 1999. Inflammation in the bovine female reproductive tract**.** *Journal of Animal Science* **77**: 101–10.

Brodzki P, Krzysztof N, Krzysztof K, Adam B, £ukasz K and Mrczuk J. 2014. Cytological evaluation of inflammation of the uterus and influence of endometritis on selected reproductive parameters in dairy cows. *Bulletin of Veterinary Institute of Pulawy* **58**: 235–42.

Clinical and Laboratory Standard Institute (CLSI). 2004. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Informational Supplement. NCCLS Document, M31-S1,Wayne, Pennsylvania, USA.

Dar S H, Chaudhary G R, Mehrotra S, Qureshi S D, Sankar M, Jan M H, Kumar H, Chaudhary R K, Palanivelu M and Narayanan K. 2015. Association of *Escherichia coli* and *Fusobacterium necrophorum* with subclinical endometritis in postpartum Murrah buffalo. *Indian Journal of Veterinary Pathology* **39**(4): 311–15.

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. *Journal of Dairy Science* **93**: 5764–71.

Dubuc J, Duffield T F, Leslie K E, Walton J S and Leblanc S J. 2011. Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows. *Journal of Dairy Science* **94**: 1325–38.

Ellenberger C, Rodenbusch S, Hauffe C, Lenz M, Kießling A and Sobiraj A. 2006. Subclinical bovine fertility disorders – What contribution can pathology contribute? Subclinical Fertility Disorders in Cattle. Leipzig, pp. 28–36.

Foldi J, Kulcs A R, P´ecsi A, Huyghe B, de S, Lohuisb C M, Coxb P and Huszenicza G. 2006. Bacterial complications of postpartum uterine involution in cattle. *Animal Reproduction*

Science **96**: 265–81.

Gahlot S C, Kumar S, Kumaresan A, Chand S, Baithalu R K, Lathika S, Patbandha T K, Lathwal S S and Mohanty T K. 2016. Efficiency of uterine fluid cytology in the diagnosis of subclinical endometritis in the water buffalo (*Bubalus bubalis*). *Reproduction in Domestic Animals* **52**(3): 512–16.

Galvão K N, Santos N R, Galvãoa J S and Gilbert R O. 2011. Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. *Theriogenology* **76**(2): 290–99.

Ghasemi F, Gonzalez-Cano P, Griebel P J and Palmer C. 2012. Proinflammatory cytokine gene expression in endometrial cytobrush samples harvested from cows with and without subclinical endometritis. *Theriogenology* **78**: 1538–47.

Ingale A M, Rai R B, Saminathan M, Vadhana P, Hingade S S, Dhama K, Milton A A P and Singh R. 2016. Isolation, PCR detection, pathotyping and antibiogram profiling of *Escherichia coli* associated with endometritis in buffaloes. *Journal of Animal and Plant Sciences* **26**(5): 1247–54.

Kasimanickam R, Duffield T F, Foster R A, Gartley C J, Leslie K E, Walton J S and Johnson W H. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* **62**: 9–23.

Madoz L, Giuliodori M, Jaureguiberry M, Plöntzke J, Drillich M and De la Sota R. 2013. The relationship between endometrial cytology during estrous cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science* **96**: 4333–39.

Madoz L V, Giuliodori M J, Migliorisi A L, Jaureguiberry M and De la Sota R. 2014. Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science* **97**: 195–201.

McDougall S, Hussein H, Aberdein D, Buckle K, Roche J, Burke C, Mitchell M and Meier S. 2011. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. *Theriogenology* **76**: 229–40.

Quinn P J, Carter M E, Markey B and Carter G R. 1999. Bacterial pathogen, microscopy culture and identification. *Text Book of Clinical Veterinary Microbiology*. Mosby Publication, New York.

Ricci A, Gallo S, Molinaro F, Dondo A, Zoppi S and Vincenti L. 2015. Evaluation of subclinical endometritis and consequences on fertility in piedmontese beef cows. *Reproduction in Domestic Animals* **50**: 142–48.

Samatha V and Ramesh B K. 2013. Bacteriological and cytological studies of endometritis in buffaloes. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(2): 6–9.

Sayyari M, Farahangnia M, Ghaemmaghami S H and Sharma R H. 2012. A comparative study on bacteriology and pathology in uteri of cattle and buffalo in Ahwaz region, Iran. *Iranian Journal of Veterinary Medicine* **6**(1): 32–39.

Seh B A, Wani S A, Khan M Z and Wani G M. 2000. *In vitro* sensitivity pattern of bacteria isolated from uterine secretions of repeat breeder cows. *Indian Journal of Animal Reproduction* **21**: 61–62.

Sheldon I M and Dobson H. 2004. Postpartum uterine health in cattle. *Animal Reproduction Science* **82–83**: 295–306.

Shukla S P and Sharma R D. 2005. Bacteriological studies on the uterine biopsy and conception rate following treatment in repeat breeder crossbred cows. *Indian Journal of Animal Reproduction* **26**: 17–19.

Singh J, Murray R D, Mshelia G and Woldehiwet Z. 2008. The immune status of the bovine uterus during the peripartum period. *Veterinary Journal* **175**: 301–09.

Udhayavel S, Malmarugan S, Palanisamy K and Rajeswar J. 2013. Antibiogram pattern of bacteria causing endometritis in cows. *Veterinary World* **6**(2): 100–102.

Wagener K, Christoph G and Drillich M. 2017. A review of the ongoing discussion about definition, diagnosis and pathomechanism of subclinical endometritis in dairy cows. *Theriogenology* **94**: 21–30.

Werner A, Suthar V, Plöntzke J and 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**: 7105–14.