Journal of Animal Research: v.9 n.2, p. 351-357. April 2019

DOI: 10.30954/2277-940X.02.2019.21

Prevalence, Risk Factors and Impact of Subclinical Endometritis on Reproductive Performance of Nili-Ravi Buffalo

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Received: 07 Mar., 2019 **Revised:** 06 April, 2019 **Accepted:** 15 April, 2019

ABSTRACT

Subclinical endometritis (SCE) is the inflammation of endometrium without systemic illness, hence remains mostly undiagnosed and untreated. The early diagnosis necessitates the evaluation of important risk factors. The objective of this study was to identify the risk factors of SCE and their effects on reproductive performance of Nili-Ravi buffalo. 100 buffaloes approaching parturition were selected. During calving, various risk factors viz., type of calving, peri-parturient disorders, sex and birth weight of calf, gestation period and season of calving were recorded. Buffaloes were subjected to endometrial cytology using cytobrush method on 45 day postpartum (DPP) and divided into 2 groups viz., buffaloes 'with SCE' (>5% PMN; n=38) and 'without SCE' (≤5% PMN; n=62). Buffaloes in estrus were artificially inseminated and fertility parameters were recorded. The occurrence of SCE was significantly affected by calving assistance (OR=11.74; P<0.001), peri-parturient disorders (OR=6.87; P<0.05) and gestation period (OR=1.16; P<0.05). Sex, birth weight of calf and season of calving were not associated with SCE. The service period of buffaloes did not vary between two groups. The median days open was significantly (P<0.05) higher in buffaloes with as compared to buffaloes without SCE (141 vs. 117 d). Buffaloes with SCE had significantly (P<0.05) lower first service conception (21.1 vs. 43.5%) and took more (P<0.05) mean number of services per conception (2.71 vs. 1.62) compared to buffaloes without the SCE. It may be concluded that risk factors around the time of calving control the onset of SCE which in turn has negative impact on reproductive performance of Nili-Ravi buffaloes.

Keywords: Buffalo, Prevalence, Reproductive performance, Risk factors, Subclinical endometritis

Subclinical endometritis (SCE) is the inflammation of the endometrium without systemic signs of illness or clinical infection (Barlund *et al*., 2008). Hence, the disease condition remains mostly undiagnosed and untreated. As compared to cattle, buffaloes have higher incidence of uterine infections including subclinical endometritis. Ptaszynzka (2003) attributed the reasons for higher incidence of uterine infections in the buffaloes to poor hygiene, morphometry of vulval lips, vaginal stimulation for milk let down and wallowing habit. Diagnosis of subclinical endometritis in bovine species has been hampered by lack of a universally accepted definition of disease and, simple and effective diagnostic techniques. Subclinical endometritis has also been described as 'cytological endometritis' due to an increased proportion of ploymorphonuclear (PMN) cells in endometrial cytology samples obtained by endometrial cytobrush or low-volume uterine lavage (Dubuc *et al*., 2010). In cows and buffalo, several tests have been developed to evaluate the inflammatory processes of the endometrium viz., histopathology (Pascottini *et al*., 2016), endometrial cytology (Kasimanickam *et al*. 2004; Singh *et al.,* 2018; Nehru *et al.,* 2019), cytology of small volume uterine lavage (Gilbert *et al*., 2005; Dar *et al*., 2015) and optical density of uterine lavage (Machado *et al.,* 2012).

Cytological assessment using cytobrush technique is the reference method for diagnosis of subclinical endometritis, owing to ease of collection of samples, quality of the samples, rapid results, and repeatability of the test (Wagener *et al.,* 2017). The vast variety of diagnostic cut-off values (5 to 25% PMN cells), to differentiate affected and unaffected animals taking into account different days of sampling after calving (Kasimanickam *et al.,* 2004; Barlund *et al.,* 2008; Galvao *et al.,* 2009), is the only limitation of cytobrush based evaluation. The wide range of PMN thresholds suggest that several animal-level and herd-level risk factors exist that affect the prevalence of this disease. Since the disease condition mostly remains undiagnosed. Identifying risk factors for SCE may allow for dairy management interventions to aid in controlling this costly disease. Over the last decade, consensus has been arrived vis-a-vis proportion of PMN cells in endometrial cytosmears. Accordingly, presence of >5% PMN at 40-60 days postpartum (DPP) is universally accepted as diagnostic threshold for SCE in bovine species (Gilbert *et al.,* 2005).

In recent years, several researchers have reported a negative impact of SCE in dairy cattle and buffaloes on subsequent reproductive performance. Cows diagnosed with SCE had prolonged days open (Kasimanickam *et al*., 2004; Gilbert *et al*., 2005; Barlund *et al*., 2008), and a negative correlation between first service conception rate in cows and PMN count was also reported (Kaufmann *et al.,* 2009). A recent study in buffaloes found drastic reduction in first service conception rate, increase in number of services per conception and increase in time to pregnancy in buffaloes with SCE (Nehru *et al.,* 2019). The extra days without conception (Kasimanickam *et al.,* 2004; Gilbert *et al.,* 2005; Barlund *et al.,* 2008), extra artificial inseminations (Gilbert *et al.,* 2005; Barlund *et al.,* 2008), and subsequent culling result in huge economic losses.

Our objectives were to (1) determine risk factors for SCE, and (2) evaluate the effects of SCE on reproductive performance in an organized herd of Nili-Ravi buffalo.

MATERIALS AND METHODS

Experimental animals

The present study was conducted at the Central Institute for Research on Buffaloes, Nabha, in the Punjab State of India. The experimental station is located at 30°22′ N latitude and 76°12′ E longitude, having tropical climate characteristics where the weather is hot and sub-humid for most of the year, yearly temperature ranges from a minimum of 2°C to a maximum of 48°C and yearly relative humidity ranges from a minimum of 50% to a maximum of 85%.

Buffaloes in 2nd - 5th parity approaching calving were identified and kept under close observation until the day of calving. During calving, various risk factors such as type of calving (normal or dystocia), peri-parturient disorders (cervico-vaginal prolapse, uterine prolapse, retained placenta, etc), sex of calf, body weight of calf, gestation period and season of calving were recorded. Seasons were categorized as follows: summer – March to June; rainy – July to October; and winter – November to February. All the selected buffaloes were closely monitored and were subjected to endometrial cytobrush based cytology on 45 DPP.

Endometrial cytology

Pap smear brush for human use (Mayfair Surgical Corporation, Ludhiana) fixed on the tip of stylette and passed through a bovine uterine swab catheter was used as cytobrush assembly. The cytobrush assembly was passed through cervix and guided past the uterine bifurcation into previously gravid uterine horn (Barlund *et al.,* 2008). The pap smears was pushed out through the anterior end of uterine catheter and rotated across the endometrium to collect cellular material. The cytobrush was withdrawn and smears were prepared. The cytosmears were fixed with methanol and stained with Modified Wright Giemsa Stain (Sigma-Aldrich Inc., USA) as per the guidelines. Slides were examined using light microscopy under 400 × magnification to identify endometrial epithelial and PMN cells. A total of 300 cells per slide were counted (Melcher *et al.,* 2014).

Allocation into groups

The threshold value for percentage of PMN cells in endometrial cytology for diagnosis of subclinical endometritis was adopted as reported by Gilbert *et al.* (2005). Accordingly at 45 DPP, buffaloes with >5% PMN cell count in endometrial cytosmears were designated as positive (n = 38) and buffaloes with ≤5% PMN cell counts were designated as negative (n = 62) for subclinical endometritis (Fig. 1). Buffaloes of both the groups were observed for signs of first postpartum overt estrus. Buffaloes in estrus were artificially inseminated (twice as per AM/PM rule) and fertility parameters (up to 120 DPP) were recorded. Pregnancy was confirmed by ultrasonography at day 45 post-insemination.

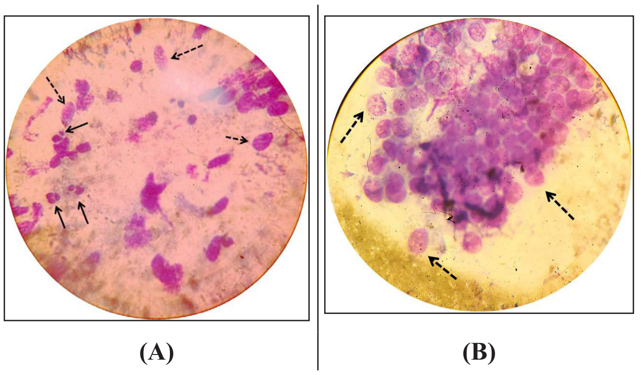

Fig. 1: Endometrial cytology of Nili-Ravi buffalo with **(A)** and without **(B)** subclinical endometritis at 45 days postpartum. Modified Wright Giemsa Stain [Magnification = 400 ×]. Neutrophils (solid arrow) and endometrial epithelial cells (dashed arrow)

STATISTICAL ANALYSIS

The statistical analysis was carried out using SPSS Statistics 16.0 (SPSS, Chicago, USA). For logistic regression model, subclinical endometritis (without = no, with = yes), peri-parturient disorders (0 = no, 1 = yes), type of calving (0 = normal, 1 = dystocia), sex of calf (0 = female, 1 = male), season of calving (0 = summer, 1 = summer, 2 = rainy), in addition to variables like body weight of calf (kg) and gestation period (days) were included as factors. Fertility parameters, viz. calving to first estrus and calving to conception, were analyzed using Kaplan– Meier survival curve analysis, and median values were compared using log-rank test. Buffaloes were censored if not diagnosed as being pregnant before culling, death, or the end of the data collection period, which was 200 DPP. For logistic regression and survival analyses, CI was set at 95%. For all statistical analyses level of significance was set at α = 0.05.

RESULTS AND DISCUSSION

The overall prevalence of SCE was 38% (38 affected and 62 unaffected). Buffaloes with SCE (>5% PMN) had significantly (P<0.001) higher polymorphonuclear cell count (PMN %) at 45 DPP as compared to buffaloes without SCE (≤5% PMN) (Fig. 2). Based on endometrial cytology (>5% PMN), the incidence of subclinical endometritis in Indian buffaloes has been reported to be between 23 (Gahlot *et al*., 2017) and 33.7% (Nehru *et al.,* 2019); however, a slightly higher incidence based on uterine lavage cytology has also been recorded (41.7%) (Dar *et al*., 2015).

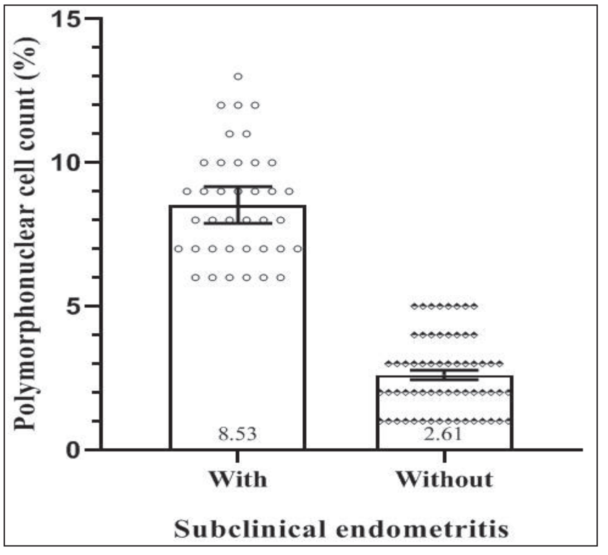

Fig. 2: Mean percentage of polymorphonuclear cells in endometrial cytosmears of buffaloes with and without subclinical endometritis at 45 DPP. Buffaloes with SCE by cytology had mean PMN % of 8.53 (95% CI: 7.89-9.16) and buffaloes without SCE had mean PMN % of 2.61 (95% CI: 2.27-2.95). Error bars represent the 95% CI for the means (P<0.001).

According to our findings, abnormal parturition, peri-parturient disorders (cervico-vaginal prolapse, uterine prolapse, retained placenta, etc) and increased gestation period were associated with an increase in prevalence of SCE at 45 DPP (Table 1). The occurrence of SCE was affected by calving assistance (OR=11.74; CI=3.08-44.83; P<0.001), peri-parturient disorders like cervico-vaginal prolapse, uterine prolapse, retained placenta, etc (OR=6.87; CI=1.47-32.16; P<0.05). Higher gestation period was associated with increase in the prevalence of subclinical endometritis (OR=1.16; CI=1.03-1.30; P<0.05). Other factors, for, examples, sex of calf, body weight of calf, and season of calving, were not associated

Table 1: The logistic regression for the odds of subclinical endometritis in herd of postpartum Nili-Ravi buffaloes

Variable	Class	Estimate	S.E.	Sig.	Odds Ratio (O.R.)	95% C.I. for O.R.
Type of calving	Normal				1	
	Dystocia	2.46	0.68	0.001	11.74	3.08-44.83
Peri-parturient disease	No				1	
	Yes	1.93	0.78	0.014	6.87	1.47-32.16
Gestation period	—	0.14	0.05	0.012	1.16	1.03-1.30
Sex of calf	Female				1	
	Male	-0.95	0.37	0.055	0.32	0.10-0.97
Birth weight of calf	—	0.13	0.06	0.058	1.14	1.00-1.29
Season of calving	Summer				1	
	Rainy	-0.66	0.85	0.435	0.52	0.10-2.72
	Winter	0.78	0.69	0.260	2.18	0.56-8.46
Constant		-54.08	8.58			

with the diagnosis of subclinical endometritis. Overall, the model was significant (Chi square=45.54; df=6, P<0.001) and correctly classifying the outcome for 77.0% of cases as compared to 62% in the null model. The Negelkerke R square value of 0.498 indicated that model explained 49.8% variability in the outcome.

The main predisposing factors related to the incidence of uterine infections were calving assistance, twin births, malpresented calves and retained placenta (Bell and Roberts, 2007). Similarly, Kasimanickam *et al.* (2004) reported that cows with peripartum reproductive events (retained placenta, twins, assisted calving) were 3.15 times more likely to have SCE at 20–33 DPP and 3.18 times more likely to have SCE at 34–47 DPP. Peri-partum obstetrical conditions were associated with delay in uterine involution, expulsion of lochia, closure of cervix and regeneration of endometrium (Sheldon *et al.,* 2008). It has been observed that dystocia and retained placenta lead to decrease in phagocytic activities of uterine and peripheral blood neutrophils (Paisley *et al*., 1986; Lewis, 1997). This may result in increasing the decreased antimicrobial activity and persistence of uterine infection.

Although, sex of calf (male calf) was significantly associated with occurrence of clinical endometritis (Potter *et al.,* 2010), we found no significant effect. The association of sex of calf with endometrial inflammation in earlier study might be due to increased gestation and higher birth weight of male offspring that increases the predisposition to postpartum obstetrical conditions. Interestingly, we found that increased gestation period was associated with occurrence of SCE, which may be due to overgrown calves that result in dytocia and thus indirectly affecting subclinical endometritis.

Although, season of calving was previously reported to be significantly associated with occurrence of uterine infection (Hossein-Zadeh and Ardalan, 2011), we found no association between season of calving and subclinical endometritis. It was reported that calving occurring between November and April dramatically increased the incidence of clinical infection of the uterus during the first month postpartum. This may be due to the fact that during the rainy and winter seasons, the general health of cows decreases, animal are kept indoors in unhygienic conditions making them more vulnerable to uterine infections (Markusfeld, 1984; Bruun *et al.,* 2002). Study on a large herd (57,301 dairy cows) confirmed the direct relation between calving during winter months and clinical metritis (OR=2.4) (Hossein-Zadeh and Ardalan, 2011). However, our study is in agreement with others which reported no significant influence of calving season on prevalence of clinical endometritis (Kim and Kang, 2003) and subclinical endometritis (Carneiro *et al.,* 2014).

Our second hypothesis was that SCE will have a negative effect on reproductive performance in buffaloes. Although several authors have successfully quantified the effects of SCE on reproductive performance in postpartum dairy cows, this study is one of few attempts to evaluate the impact of SCE on reproductive performance in postpartum buffaloes. Survival analysis showed that the service period (calving-to-estrus interval) of buffaloes with SCE was not

Table 2: Reproductive parameters in buffaloes with and without subclinical endometritis

End point	Without subclinical endometritis (n=62)	With subclinical endometritis (n=38)	Hazard Ratio
Median days to first estrus (95% CI)[¥]	89 (77-104)	98 (93-107)	HR = 0.92 P>0.05
First service conception rate (%)[#]	43.5[b] (27/62)	21.1[a] (8/38)	
Median days open (95% CI)[¥]	117[a] (110-123)	141[b] (136-145)	HR = 0.31 P<0.05
Mean services per conception (95% CI)[$]	1.62[a] (1.41-2.34)	2.71[b] (2.24-3.14)	

Different superscript (a,b) indicate significant difference (P<0.05); [¥]Logrank test; [$]Mann-Whitney U test; [#]Chi square test; HR = Hazard ratio based on Cox's proportional hazards model.

significantly different (P>0.05) from buffaloes without SCE (Table 2). However, buffaloes positive for SCE had median days open 24 d longer (141 d, 95% CI=136–145) than that of normal cows (117 d, 95% CI=110–123; P=0.001). The hazard ratios for time to first estrus and time to pregnancy in buffaloes with SCE were calculated in reference to buffaloes without SCE. Buffaloes positive for SCE were equally likely to exhibit estrus as compared to healthy buffaloes (P>0.05). However, subclinical endometritic buffaloes had lower likelihood of conception (HR=0.31; P<0.05) as compared to healthy buffaloes.

In contrast to present study, Dubuc *et al.* (2012) showed that cows with SCE had delayed resumption of ovarian activity after calving. Similarly, cows with SCE at 42 DPP were less likely to ovulate between 63 to 70 DPP than healthy cows (Burke *et al.,* 2010). Plontzke *et al.* (2010) also reported no effect of SCE on days to first service. No significant effect of subclinical endometritis on the calving to first service was observed in Murrah buffaloes (Dar *et al.,* 2015; Nehru *et al.,* 2019). We found that buffaloes positive for SCE had significantly (P<0.05) reduced first service conception rate as compared to their negative counterparts (21.1 vs. 43.5%). Furthermore, buffaloes with SCE took significantly more (P<0.05) mean number of services per conception compared to buffaloes without the presence of SCE (2.71 vs. 1.62) (Table 2). Consequently, buffaloes positive for SCE took 24 more days to get pregnant as compared to normal buffaloes. Our results concurred with reports, which found increased days open by 25 to 40 days in cows diagnosed for SCE at 35 or 49 DPP (Galvao *et al.,* 2009; Dubuc, 2011; Madoz *et al.,* 2013; Vieira-Neto *et al.,* 2014). Our observations were also supported by various authors who concluded that animals diagnosed with SCE had prolonged days open and reduced probability of conception at first AI compared to SCE negative cows (Kasimanickam *et al.,* 2004; Gilbert *et al.,* 2005; Barlund *et al.,* 2008). Subclinical endometritis resulted in reduced proportion of cows pregnant within 200-300 DPP compared to healthy cows (60.0 versus 80.0%, respectively) (Gilbert *et al.,* 2005; Barrio *et al.,* 2015). In contrast, Carneiro *et al.* (2014) failed to arrive at an association between presence of SCE (32-70 dpp) and first service conception rate and pregnancy rate at 150 days postpartum in cows in Brazil.

The exact mechanism by which SCE decreased conception per AI and increased pregnancy loss was not clear. There was mounting evidence that cows with SCE had altered embryo quality and endometrial function (Drillich *et al*., 2012; Hailemariam *et al*., 2014). Inflammation in the endometrium had been shown to reduce fertilization in single ovulating postpartum dairy cows (Cerri *et al.,* 2009). Soto *et al.* (2003) suggested that mediators of the inflammatory cascade, including cytokines impaired early embryonic development and might be part of the mechanism by which fertility is depressed in cows suffering from inflammatory diseases. At molecular level, cows diagnosed with SCE have altered endometrial and embryonic gene expression (Hoelker *et al.,* 2012). Whether SCE per se was the causative agent of changes in endometrial and embryonic gene expression or the buffaloes that developed SCE had underlying factors that also cause changes in the transcriptome remains to be elucidated.

We conclude that the key drivers that determine outcome of subclinical endometritis are as follows: type of calving, peri-parturient obstetrical conditions and gestation period. It is clear that these risk factors around the time of calving control the onset of subclinical endometritis which in turn has drastic impact on reproductive performance of Nili-Ravi buffaloes.

ACKNOWLEDGMENTS

The authors thank the staff of Buffalo Production and management section (ICAR-CIRB Subcampus Nabha) for identifying, isolating from herd and restraining of animals prior to sampling.

REFERENCES

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:** 714–723.

Barrio, M., Vigo, M., Quintela, L.A., Becerra, J.J., García-Herradón, P.J., Martínez-Bello, D., Fernandez-Sanchez, F.I., Prieto, A., Cainzos, J. and Peña, A.I. 2015. Influence of subclinical endometritis on the reproductive performance of dairy cows. *Spanish J. Agric. Res.*, **13**(4): e05SC02.

Bell, M.J. and Roberts, D.J. 2007. The impact of uterine infection on a dairy cows performance. *Theriogenology,* **68:** 1074–1079.

Bruun, J., Ersbøll, A.K. and Alban, L. 2002. Risk factors for metritis in Danish dairy cows. *Prev. Vet. Med.*, **54**(2): 179–190.

Burke, C.R., Meier, S., McDougall, S., Compton, C., Mitchell, M. and Roche, J.R. 2010. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. *J. Dairy Sci.,* **93**(11): 5363–5373.

Carneiro, L.C., Ferreira, A.F., Padua, M., Saut, J.P., Ferraudo, A.S. and Dos Santos, R.M. 2014. Incidence of subclinical endometritis and its effects on reproductive performance of crossbred dairy cows. *Trop. Anim. Health Prod.*, **46**(8): 1435–1439.

Cerri, R.L.A., Rutigliano, H.M., Lima, F.S., Araújo, D.B. and Santos, J.E.P. 2009. Effect of source of supplemental selenium on uterine health and embryo quality in high-producing dairy cows. *Theriogenology,* **71**: 1127–1137.

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 J. Vet. Pathol.,* **39**(4): 311–315.

Drillich, M., Tesfaye, D., Rings, F., Schellander, K., Heuwieser, W. and Hoelker, M. 2012. Effects of polymorphonuclear neutrophil infiltration into the endometrial environment on embryonic development in superovulated cows. *Theriogenology*, **77**(3): 570-578.

Dubuc, J., Duffield, T., Leslie, K., Walton, J. and LeBlanc, S. 2010. Definitions and diagnosis of postpartum endometritis in dairy cows. *J. Dairy Sci.,* **93:** 5225–5233.

Dubuc, J. 2011. Postpartum uterine diseases: prevalence, impacts, and treatments. *WCDS Adv. Dairy Technol.*, **23**: 255–267.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S. and LeBlanc, S.J. 2012. Risk factors and effects of postpartum anovulation in dairy cows. *J. Dairy Sci.,* **95**(4): 1845–1854.

Gahlot, S.C., Kumar, S., Kumaresan, A., Chand, S., Baithalu, R.K., Lathika, S., Patbandha, T.K., Lathwal, S.S. and Mohanty, T.K. 2017. Efficiency of uterine fluid cytology in the diagnosis of subclinical endometritis in the water buffalo (*Bubalus bubalis*). *Reprod. Domest. Anim.*, **11:** 1–4.

Galvao, K.N., Frajblat, M., Brittin, S.B., Butler, W.R., Guard, C.L. and Gilbert, R.O. 2009. Effect of prostaglandin F2α on subclinical endometritis and fertility in dairy cows. *J. Dairy Sci.,* **92:** 4906–4913.

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.

Hailemariam, D., Ibrahim, S., Hoelker, M., Drillich, M., Heuwieser, W., Looft, C., Cinar, M.U., Tholen, E., Schellander, K. and Tesfaye, D. 2014. MicroRNA-regulated molecular mechanism underlying bovine subclinical endometritis. *Reprod. Fertil. Dev., 26*(6): 898-913.

Hoelker, M., Salilew-Wondim, D., Drillich, M., Christine, G., Ghanem, N., Goetze L., Tesfaye, D., Schellander, D. and Heuwieser, W. 2012. Transcriptional response of the bovine endometrium and embryo to endometrial polymorphonuclear neutrophil infiltration as an indicator of subclinical inflammation of the uterine environment. *Reprod. Fert. Dev.,* **24**: 778–793.

Hossein-Zadeh, N.G. and Ardalan, M. 2011. Cow-specific risk factors for retained placenta, metritis and clinical mastitis in Holstein cows. *Vet. Res. Commun.*, ***35***(6): 345–354.

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.

Kaufmann, T.B., Drillich, M., Tenhagen, B.A., Forderung, D. and Heuwieser, W. 2009. Prevalence of bovine subclinical endometritis 4 h after insemination and its effects on first service conception rate. *Theriogenology*, **71:** 385–391.

Kim, I.H. and Kang, H.G. 2003.Risk factors for postpartum endometritis and the effect of endometritis on reproductive performance in dairy cows in Korea. *J. Reprod. Dev.*, **49**(6): 485–491.

Lewis, G.S. 1997. Uterine health and disorders. *J. Dairy Sci.,* **80:** 984–994.

Machado, V.S., Knauer, W.A., Bicalho, M.L.S., Oikonomou, G., Gilbert, R.O. and Bicalho, R.C. 2012. A novel diagnostic technique to determine uterine health of Holstein cows at 35 days postpartum. *J. Dairy Sci.,* **95**(3): 1349–1357.

Madoz, L.V., Giuliodori, M.J., Jaureguiberry, M., Plöntzke, J., Drillich, M. and De 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**(7): 4333–4339.

Markusfeld, O. 1984. Factors responsible for post parturient metritis in dairy cattle. *Vet. Rec.*, **114**(22): 539–542.

Melcher, Y., Prunner, I. and Drillich, M. 2014. Degree of variation and reproducibility of different methods for the diagnosis of subclinical endometritis. *Theriogenology*, **82**(1): 57–63.

Nehru, D.A., Dhaliwal, G.S., Jan, M.H., Cheema, R.S. and Kumar, S. 2019. Clinical efficacy of intrauterine cephapirinbenzathine administration on clearance of uterine bacteria and subclinical endometritis in postpartum buffaloes. *Reprod. Domest. Anim.,* **54**(2): 317–324.

Paisley, L.G., Mickelsen, W.D., Anderson, P.B. 1986. Mechanisms and therapy for retained fetal membranes and uterine infections of cows: a review. *Theriogenology,* **25:** 353–381.

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

Plöntzke, J., Madoz, L.V., De la Sota, R.L., Drillich, M. and Heuwieser, W. 2010. Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina. *Anim. Reprod. Sci.,* **122**(1-2): 52–57.

Potter, T.J., Guitian, J., Fishwick, J., Gordon, P.J. and Sheldon, I.M. 2010. Risk factors for clinical endometritis in postpartum dairy cattle. *Theriogenology*, **74**(1): 127–134.

Ptaszynska, M. 2003. Compendium of Animal Reproduction, 8th Ed. *Intervet International Bv.,* pp. 230–234.

Sheldon, I.M., Williams, E.J., Miller, A.N.A., Nash, D.M. and Herath, S. 2008. Uterine diseases in cattle after parturition. *Vet. J.,* **176**(1): 115–121.

Singh, H., Brar, P.S., Arora, A.K., Dhindsa, S.S. and Honparkhe, M. 2018. Bacterial presence and fertility in subclinical endometritic buffaloes at oestrus. *Indian J. Anim. Sci.,* **88**(4): 415–419.

Soto, P., Natzke, P.R. and Hansen, P.J. 2003. Actions of tumor necrosis factor-α on oocyte maturation and embryonic development in cattle. *Am. J. Reprod. Immunol.,* **50**: 380–388.

Vieira-Neto, A., Gilbert, R.O., Butler, W.R., Santos, J.E.P., Ribeiro, E.S., Vercouteren, M.M., Bruno, R.G., Bittar, J.H. and Galvão, K.N. 2014. Individual and combined effects of anovulation and cytological endometritis on the reproductive performance of dairy cows. *J. Dairy Sci.,* **97**(9): 5415–5425.

Wagener, K., Gabler, C. 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.