REVIEW ARTICLE
JIVA 22 (2) August 2024
https://doi.org/10.55296/JIVA/22.2.2024.7-27

JIVA
OFFICIAL JOURNAL OF INDIAN VETERINARY ASSOCIATION, KERALA

Received on 25/06/2024
Accepted on 22/07/2024

DIAGNOSIS OF SUBCLINICAL UTERINE INFECTION AND ITS THERAPEUTIC MANAGEMENT IN CATTLE AND BUFFALOES: AN UPDATE

A. Kumaresan[1*], Anand Kumar[2] Yadav, Kishan Kumar[3] Fataniya and A. Manimaran[4]

[1] ICAR National Fellow and Principal Scientist, [2]Ph.D. Scholar, [3] M.V.Sc. Scholar,
[4] Senior Scientist, *Theriogenology* Laboratory,
Southern Regional Station of ICAR-NDRI,
Bengaluru 560030, Karnataka

*Corresponding author ogkumaresan@gmail.com

ABSTRACT

Optimum fertility is a pre-requisite for enhancing productivity as well as life time production of dairy bovines. However, increasing evidences indicate that with increasing milk yields, fertility has been declining. Analysis of the existing information indicates that dairy animals in our country calve at a later age, conceive at very later stage of post-partum period and the conception rate is low. Among the several reasons for extended service period (in turn calving interval), post-partum uterine infection alone accounts to around 30 per cent. Infectious diseases affecting reproductive organs can create losses throughout the reproductive cycle by decreasing ovulation rates, fertilization rates, embryonic survival rates and fetal survival rates. Understanding the molecular basis of uterine disease and identification of bio-molecules to detect uterine infection at an early stage besides developing effective preventive and therapeutic strategies is the need of the hour to realize the dream of obtaining a calf per cow per year. It is in this regard, the developments in the field of diagnosis and treatment of uterine infection, with special reference to subclinical uterine infection, are compiled, analysed and presented in this paper to provide the readers with the updated information on these aspects.

Keywords: Cattle, Buffalo, Uterine Infection, Diagnosis, Therapy

INTRODUCTION

One of the major reasons for reduced life-time milk production by a dairy bovine is transient loss of fertility or infertility. The causes of infertility in dairy animals are many and can be complex. They relate to follicle development and maturation, onset of estrus, successful coitus/insemination, ovulation, fertilization, implantation, the development and delivery of the normal foetus and its membranes, proper uterine involution and cleansing, resumption of

ovarian cyclicity, estrus expression and conceive again at right time (Kumaresan and Srivastava, 2022). In a life time, eight to ten such cycles are expected to occur in dairy cattle and buffaloes. Anything interfering with the routines of this cycle, such as diseases, poor nutrition, inadequate herd management, hereditary and congenital factors, hormonal disturbances or environmental changes, can make the animal infertile, if only temporary in occurrence. In general, the causes of infertility in dairy animals can be classified into congenital morphological, functional, infectious and unknown causes, among which post-partum uterine infection alone accounts for around 30 per cent (Kumaresan *et al.,* 2022).

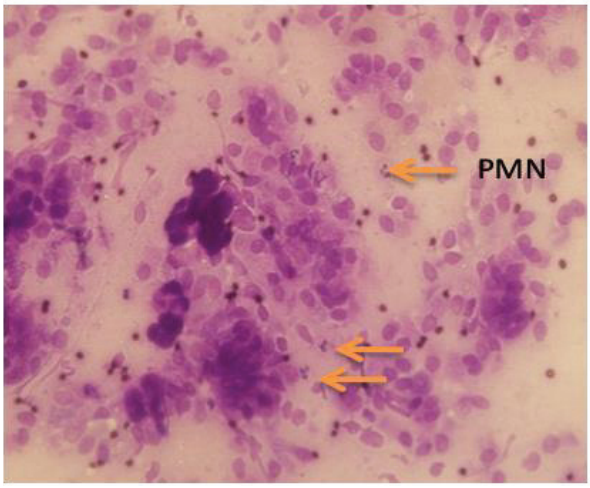
During the first two weeks after calving, a majority of the cows (>90 per cent) acquire microbial contamination from external environment that settle inside the uterus. As the days pass by during the post-partum period, the normal cow responds against the microbial infection by means of activation of the innate immune system that recruits its first line of defensive agents i.e. neutrophils. From blood, the neutrophils migrate to the uterus and by means of phagocytosis, kill the microbes especially bacteria and nullify the infection in the uterus which ultimately brings the environment inside the uterus to normal (Sheldon *et al*., 2009). While a considerable proportion of apparently healthy cows expel out the microbial contaminants, some of the cows (40 per cent) still have the microbes inside the uterus even three weeks after calving. During the postpartum period, the gravid uterus undergoes the process of involution which is facilitated by $PGF_2\alpha$ and oestrogen, where the size of the pregnant horn returns to its normal non-gravid state and is extremely important in order to decrease the further multiplication of the infectious agents. If the gravid uterus does not return to its normal non-gravid state at a desired period of time and also if the calving area is not hygienic, there is chance of entry of infection into the uterus and possible setting up of inflammation leading to the development of uterine disease.

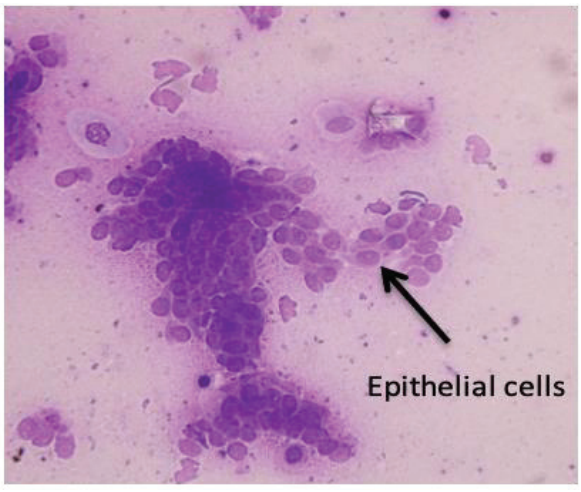
Postpartum uterine disease is one of the main reasons for bovine infertility associated with prolonged days to first service, days open and increased culling rate (Gilbert *et al*., 2005). Among the post-partum uterine diseases, subclinical endometritis (SCE) is the most prevalent one, affecting around 30 per cent of lactating dairy cows, with prevalence rate that ranges from 11 to >70 per cent in some dairy herds, resulting in huge economic losses to the dairy industry (Kasimanickam *et al*., 2004). One of the biggest problems with this disease is that it runs without any clinical signs in the animal, as a result,

majority of the cows are left undiagnosed. Diagnosis of SCE in dairy animals still remains a challenge because of lack of clinical signs and the sensitivity and specificity of the available diagnostic tests are low. In a study, it was reported that SCE is one of the main reasons for cows to become a repeat breeder with a prevalence of 52.7 per cent in repeat breeding dairy cows (Salasel *et al*., 2010). Keeping in view the serious effects of SCE, it is important to accurately diagnose the condition at the earliest possible time so that proper therapy could be taken up. In this paper, updated information on detection of SCE in dairy cattle and buffaloes, and the developments in therapy are detailed.

DIAGNOSTIC TESTS FOR SUBCLINICAL UTERINE INFECTION

Endometrial cytology: Subclinical cases of endometritis are best diagnosed based on the evaluation of neutrophil leukocyte infiltration by cytological examination (Barlund *et al*., 2008). The percentage of neutrophil leukocytes and the neutrophil leukocyte/lymphocyte ratio increase in postpartum uterine infections. Endometrial cytology technique involves the measuring of the proportion of neutrophils in the uterine samples collected using cytobrush or low volume uterine lavage technique. While several reports indicate its usage in diagnosing SCE, a few studies opined that, at this moment, it is doubtful whether endometrial cytology can be used as routine technique in veterinary practice due to wide variation in the threshold values of neutrophils (5-18 per cent) (Wagener *et al*., 2017). In addition, sample preparation, sampling procedure and evaluation is time consuming, therefore, it is considered to be a reference method but not an accurate diagnostic test for SCE (Walsh *et al.,* 2011).





Uterine fluid cytology: Although endometrial cytology is considered as good technique for diagnosis of SCE, restlessness temperament and comparatively thinner and coiled uterus in buffaloes (Srivastava *et al*. 2013; Srivastava and Kumaresan 2014) pose problems in using cytobrush techniques. Further, the availability of the cytobrush designed for use in the buffalo is very much limited. Personal experience of the authors indicate that collection of uterine fluid is easier in buffaloes when compared to cytobrush technique because the sheath used for collection of uterine fluid does not require physical contact with endometrial lining and thus is less traumatizing and causes less discomfort to the buffalo. Therefore, we modified the method and used uterine fluid cytology for diagnosis of SCE in buffaloes and reported that the percentage of PMN leukocyte in uterine fluid cytology can be used as a tool for diagnosis of subclinical endometritis in buffaloes (Gahlot *et al*., 2017).

Bacteriological study: Bacteriological studies conducted for determining puerperal metritis and clinical endometritis conditions found that majority of the bacteria were related to nonspecific mixed infections that invade the uterus at parturition or shortly after parturition (El-Azab *et al*., 1988; Sheldon *et al*., 2002). Recently, Madoz *et al.* (2014) conducted a bacteriological study for the diagnosis of sub-clinical endometritis in grazing dairy cows and recommended that bacteriology would not be a reliable tool because no bacteria were isolated from cows affected with sub-clinical endometritis.

Uterine lavage sample optical density test (ULSOD): Uterine lavage sample optical density test (ULSOD) is used for diagnosis of endometritis. Briefly, 20 mL of sterile saline solution is infused into the uterus and agitated gently, and a sample of the fluid (5 to 15 mL) is aspirated and processed for optical density measurement at 620 nm. This test measures the concentration of proteins or cells in the uterine lavage sample and the recommended threshold for $ULSOD_{620}$ for clinical endometritic animal is >0.058 with specificity of 78 per cent. $ULSOD_{620}$ measurement could be used as a substitute for endometrial cytology diagnostic technique as the former procedure is less laborious than acquiring endometrial cytology details (Machado *et al.,* 2012).

Reagent strips test: Reagent test strips have also been used in the last few years to diagnose endometritis that is not clinical (Santos *et al*., 2006). It has been found that animals with endometritis have elevated pH levels as well as elevated leukocyte esterase and protein levels. Leukocyte esterase test (LES) is another method recently developed for detection

of sub-clinical endometritis in uterine flushing fluids of dairy cattle, where leukocyte esterase is an enzyme produced by neutrophils and is therefore known to be indicative of inflammation. Although results obtained by LES were positively correlated with endometrial cytology results, to fully recommend the use of LES test as a cow-side diagnostic tool, this method requires further refinement in sample preparation and processing (Couto *et al.,* 2013).

Biochemical tests: The biochemical composition of uterine fluid is altered in SCE, which can also help in detection of the condition. A study by Gahlot *et al*. (2018) indicated that urea and urea N concentrations were significantly higher in normal buffaloes as compared to buffaloes affected with subclinical endometritis. On the other hand, cholesterol and total bilirubin concentrations were significantly lower in normal buffaloes when compared to those affected with subclinical endometritis. Concentrations of alkaline phosphatase were significantly higher in buffaloes affected with subclinical endometritis when compared to normal buffaloes. It was reported that the likelihood ratio was 3.63 for urea indicating that buffaloes having less than the threshold concentration (47.5 mg/dL) of urea in their uterine fluid were 3.6 times more risk to be affected with SE. The likelihood ratio for urea N, cholesterol, ALP and bilirubin was 2.33, 2.54, 2.12 and 1.65, respectively. It was concluded that ALP, urea, urea N and cholesterol concentrations in uterine fluid may serve as tools for diagnosing subclinical endometritis in the water buffalo. The levels of acute-phase proteins (creatine kinase, haptoglobin, α1-acid glycoprotein), non-esterified fatty acid (NEFA), β-hydroxybutyric acid (BHBA), and nitric oxide can also be used to diagnose uterine infections (Hirvonen *et al*., 1999; Humblet *et al*., 2006).When compared to healthy animals, animals with subclinical endometritis and puerperal metritis had considerably higher serum levels of NEFA and BHBA (Hammon *et al*., 2006).

Intrauterine oxygen reductase potential: Intrauterine oxygen reductase potential is used as an indicator for uterine infection. *Trueperella pyogenes* either alone or in combination with anaerobic bacteria like *Fusobacterium necrophorum* and Bacteroides species is frequently associated with uterine infection and thus leading to a drop in the intrauterine oxygen reductase potential creating an anaerobic environment in the uterus favouring the growth of anaerobic bacteria and aggravating uterine infection as a whole (Dohmen *et al.,* 1995).This drop in intrauterine oxygen reductase potential may be associated with either metabolism of microorganisms or increased oxygen utilization by neutrophils.

Myeloperoxidase activity and cytochrome C reduction assay: Recent studies demonstrated that cows suffering with uterine health disorders had decreased neutrophil function. The phagocytic ability of neutrophils could be evaluated by two indices, the myeloperoxidase activity and the cytochrome c reduction assay. Myeloperoxidase is released when the phagosome fuses with primary granules of the neutrophil. Whereas, Cytochrome c reduction assay measures the amount of superoxide anions produced by the neutrophils during the oxidative metabolic burst associated with phagocytosis (Hammon *et al.,* 2006).

Ultrasonography: Nowadays, ultrasonography is an essential part of routine clinical examination of reproductive tract of dairy cattle. With respect to subclinical endometritis, it is an easy and fast technique available basing on the presence of fluid accumulation in uterine lumen. The diameter of the uterine content and its echogenicity (anechoic, mixed echogenicity, or hyperechoic) can be distinguished by transrectal uterine ultrasonographic examination. However, this method is biased at times due to location of the probe on the uterine horn, endometrial thickness and fluid accumulation is also noticed in non-affected SCE cow at the time of estrus (Kasimanickam *et al.,* 2004).

White side test: Under field conditions white side test can be used as a routine test to ascertain the grades of non-specific genital tract infections. Briefly, 1ml of cervico-vaginal discharge is heated with equal volume of 5 to 10 per cent sodium hydroxide up to boiling point followed by cooling of the sample. The intensity of color depends on the severity of endometritis and concentration of leucocytes which is graded as normal (no color), mild infection (light yellow color), moderate infection (yellow color) and severe infection (dark yellow color) (Bhat *et al*., 2014).

Endometrial biopsy: Undoubtedly endometrial biopsy is considered to be the gold standard method for detection of SCE. Endometrial biopsies are graded based on the rate of recurrence, inflammation status, peri glandular fibrosis and glandular nesting. In acute inflammation, neutrophils are the prime indicators, whereas, lymphocytes are the prominent feature in chronic inflammation (Snider *et al*., 2011). Previous studies reported that this procedure itself is harmful and induce uterine pathology and affects the future fertility of the animal (Simon and McNutt, 1957; Zaayer, 1986). However, a recent study reported that this procedure is safe and reliable for the assessment of post-partum uterine health of cows, if it is carried out properly (Chapwanya *et al*., 2010).

EMERGING MOLECULAR TOOLS

Earlier reports indicate that no cow-side test has until now been established for detection of subclinical endometritis in dairy cows (Pascottini and Americo, 2016). Therefore, it is necessary to discover appropriate molecule(s) that could facilitate development of highly sensitive and specific cow side test for diagnosis of SCE. Transcriptional and protein-level research for diagnosis of subclinical endometritis have been conducted in recent years because routine diagnostic methods had failed in one way or another. At the molecular level, uterine immune responses from subclinical endometritic animals showed an increased transcriptome (mRNA) expression of certain pro-inflammatory mediators being cytokines, acute phase proteins and antimicrobial peptide and few protein contents. Due to hormonal changes, the amalgam of uterine fluid is also changed during different phases of the oestrous cycle and also in experimentally induced uterine infections (Kasimanickam *et al.,* 2013). Evidences indicate that several changes in the uterine fluid protein levels of infected animals (Gahlot *et al*., 2017). Few previous trials have evaluated one or few proteins in uterine fluid of subclinical endometritis cow (Brodzki *et al*., 2015a; Brodzki *et al*., 2015b). Very recently 6 candidate protein markers were identified in the uterine fluid of cows affected with SCE (Kurati*,* 2019). Enhanced expression of mRNA and protein levels of pro-inflammatory mediators in cow uterus during subclinical endometritis reported in several studies are given in Table 1.

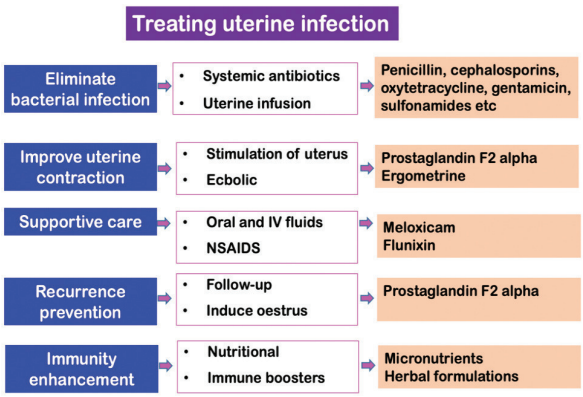
THERAPY FOR UTERINE INFECTION

Treatment of uterine infections is generally executed through intrauterine (i.u.) or systemic administration of antibiotics. The major objectives of using antimicrobials are reduction of bacterial contamination and inflammation. Intra uterine administration of antiseptic solutions and systemic administration single or combined hormonal preparation such as PGF2α and GnRH, etc., are also common practice (Manimaran *et al.,* 2022). Hormones such as $PGF_2\alpha$ are mainly used for induction of luteolysis and consequent induction of estrus with increased uterine contractility and clearance. The most important line of treatment for uterine infection is parenteral antibiotic. Several broad spectrum antibiotics have been used for systemic administration including penicillin, oxytetracycline, ceftiofur, however, ceftiofur is the antibiotic of choice for the treatment of metritis in dairy cows (Galvao, 2011). Ceftiofur is a broad-spectrum third-generation cephalosporin. Long acting ceftiofur formulations are

Table 1. Increased mRNA and protein levels of pro-inflammatory mediators in cow uterus during subclinical endometritis

Molecules	Reference
mRNA	
IL1A, IL1B, IL6, NOS2 and TLR4	Herath et al. (2009)
CXCL5, ILIB, IL8, TNF, PTGS2 and HP	Gabler et al. (2010)
CXCL5, IL1B, IL8 and TNF	Fischer et al. (2010)
IL-1B, IL-6 and IL-8	Galvao et al. (2011)
IL6, IL8 and TNFα	Ghasemi et al. (2012)
SAA3 and TAP	Chapwanya et al. (2013)
CXCL1/2, CXCR2, IL1A, IL1B, IL6, IL8, PTGDS, PTGS1	Peter et al. (2015)
S100A9 and TAP	Ibrahim et al. (2016)
Proteins	
Cathelicidins and S100	Wheeler et al. (2012)
IL6, IL10, Hp and SAA (Early postpartum)	Brodzki et al. (2015a)
TNFα, IL6, IL10, SAA and Hp (Late postpartum)	Brodzki et al. (2015b)
Alkaline phosphatase	Gahlot *et al*. (2018)

showing better results since it maintains the minimum inhibitory concentration for five days or above against pathogenic bacteria including *A. pyogenes, E. coli, F. necrophorum* in blood and endometrial tissue. The recommended dose for treatment of metritis in postpartum dairy cows is 2.2 mg/Kg body weight. Although oxytetracycline is widely used, it is not the optimum treatment because there is evidence for bacterial resistance to this antimicrobial and high concentrations of the antibiotic are required to inhibit bacterial growth (Sheldon *et al*., 2009). Although several trials have been conducted to see the clinical efficacy of these drugs, the results are not consistent and therefore treatment protocol often varies among



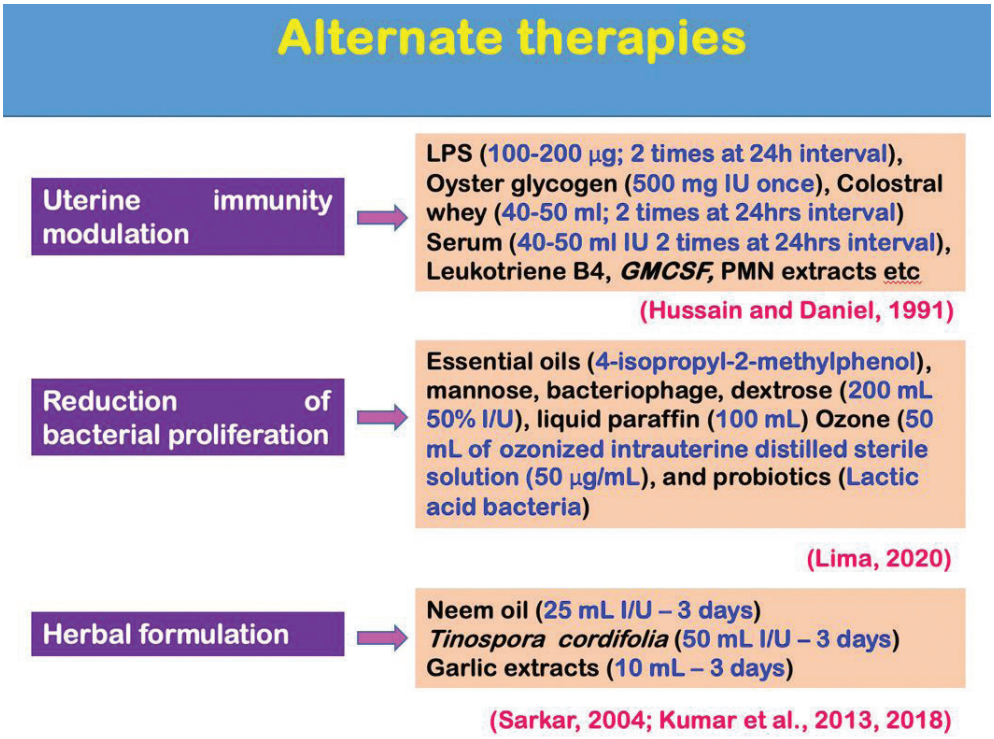
veterinarians (Lefebvre and Stock, 2012). The five basic aims of treatment of uterine infection are shown in figure.

ALTERNATE THERAPIES FOR UTERINE INFECTION

Animals treated with antibiotics experience variable rates of recovery, development of microbial resistance, a

reduction in the innate uterine defence system due to low PMN cell phagocytic activity, and withdrawal of milk, hence alternative therapies may be considered as a means of activating natural immune responses.

Immunomodulators: It is believed that the chemoattractant properties of *E. coli* lipopolysaccharides (LPS) draw PMNs to the uterine lumen. This rise in PMNs in the endometrium may aid in the recovery of endometritis in cows and mares (Asbury and Hansen, 1987). Rashid Dar *et al*. (2019) used primary bubaline endometrial stem cells to assess the immunomodulatory effects of curcumin, LPS, and/or flagellin on prostaglandin E_2 (PGE_2) and proinflammatory cytokines production. They found that while flagellin had less effect on PGE_2 synthesis, LPS had a more stimulatory effect. Compared to IL1 and TNF, LPS dramatically increased the transcripts of IL8 and IL6 in bubaline endometrial stromal cells. Oyster glycogen is a polymer that has immunomodulatory properties; it has the ability to draw PMN cells into the lumen of the uterus. In a study, Oyster glycogen was administered intrauterine at a dose of 10 mg/mL to treat subclinical endometritis in repeat breeding crossbred cows. There was no discernible difference in the conception rate between the treatment and control groups, despite the recovery rate being greater in treatment



group compared to control group (Krishnan *et al*., 2015). Leukotriene B4 is a strong chemoattractant that can cause PMN cells to migrate into the lumen of the uterus in cows. Zerbe *et al*., (1996) showed that Leukotriene B4 at a concentration of 30 nmol/L had the ability to increase intrauterine PMN cell counts by up to 5–10 times in a 24-hour period. Recombinant bovine granulocyte colony-stimulating factor, an endogenous hematopoietic growth factor, encourages progenitor cells in the bone marrow to produce and differentiate neutrophils. A study by Canning *et al*., (2017) assessed the impacts of granulocyte colony-stimulating factor and showed that while there was a rise in polymorphonuclear leukocyte cells in circulation, the incidence of uterine illnesses did not decrease. IL8 is a proinflammatory cytokine and the primary neutrophil chemoattractant. It was shown that when compared to control cows the incidence of puerperal metritis in cows treated with L-rbIL-8 and H-rbIL-8 was lower (Zinicola *et al*., 2019).

Chitosan microparticles: Chitosan is a chemical that is derived from chitin and is a linear polysaccharide generated by deacetylation of chitin, is nontoxic, bioadhesive, biocompatible, and biodegradable (Baldrick, 2010). Chitosan microparticles have broad-spectrum antibacterial action at both acidic and neutral pH levels, making them a promising alternative to traditional antibiotics (Jeon *et al*., 2014). It was reported that intrauterine administration of Chitosan microparticles on the day following calving reduced uterine infection (Daetz *et al*. 2016). However, further studies are needed before recommending its use in treatment of uterine infection.

Sugars: The exact mechanism by which dextrose or other sugars reduces uterine infection is unknown. Nevertheless, the usage of sugars is justified by the fact that one type of sugar (hypertonic sucrose) prevented *E. Coli* from growing in human wounds, while another type of sugar (mannose) prevented *E. Coli* from adhering to horse endometrial cells (Lima, 2020). Following its application to cows, researchers have hypothesized that the benefits of dextrose would be mediated by enhancing uterine tone, inhibiting bacterial development, and nourishing endometrial cells. In a study by Maquivar *et al*. (2015) it was reported that dextrose-treated cows had improved pregnancy per AI at the first treatment and a decreased incidence of endometritis 14 days later.

Ozone: The logic behind the use of ozone is because of its ability to inhibit or inactivate infectious microorganisms like fungi, bacteria, viruses, and spores. It also enhances the anti-inflammatory effect by inhibiting proinflammatory

cytokines and phospholipase A2, as well as by stimulating the activity of immune-suppressive cytokines like TNF β1 and IL10 (Sagai and Bocci, 2011). In a study, after the initial endometrial cytology, cows were either left untreated or treated with 50 mL of ozonized intrauterine distilled sterile solution (50 µg/mL) on day 35 after calving. Comparing treated cows to control cows, the second endometrial cytology test showed that ozone therapy decreased the incidence of subclinical endometritis (5.0 vs. 50.0 per cent) (Escandón *et al*., 2020).

Probiotic bacteria: Probiotic usage is a novel and exciting field of study for the prevention or treatment of uterine illness. Probiotics are live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host. Probiotics have a variety of mechanisms of action such as improving tight junction signalling-related gene expression, biofilm formation on mucosal layers, immune modulation, competition for adherence and prevention of pathogen bacterial adhesion, competition for nutrients, synthesis of antimicrobial compounds (bacteriocins, organic acids, antimicrobial proteins, enzymes, H_2O_2 and CO_2), and preservation of optimal vaginal pH (Rosales and Ametaj, 2021). Ametaj *et al*. (2014) studied the impact of a combination of lactic acid bacteria on the prevalence of purulent vaginal discharge/clinical endometritis, plasma haptoglobin concentrations, and milk production in dairy cows. Treatments were conducted once a week beginning two weeks prior to calving and continuing until week four postpartum. The cocktail administered intravaginally lowered acute phase protein haptoglobin in weeks two and three after delivery and the incidence of clinical endometritis at three weeks postpartum. Another study investigated the impact of an alternative lactic acid bacteria cocktail given intravaginally and intrauterine on metritis incidence, non-esterified fatty acid concentrations, and proinflammatory cytokine gene expression in blood neutrophils and the endometrium (Genís *et al*., 2018). Metritis incidence decreased from 31.1 per cent in the control group to 13.3 per cent after intravaginal lactic acid treatment.

Vaccines against metritis: In recent times, different studies evaluated the effect of immunization against metritis, a herd-specific vaccination made from uterine swabs of primiparous cows with a diagnosis of metritis that contains whole inactivated bacterial cells of the *Streptococcus uberis, Bacteroides, Escherichia coli, Trueperella pyogenes,* and *Peptostreptococcus species*. Subcutaneous vaccinations were administered to late-pregnant heifers six weeks prior to calving and again three

weeks later. There was no difference in the incidence of clinical endometritis or reproductive outcomes (such as the time between calving and conception, pregnancy per AI at first service) between the vaccine and control groups of cows (Freick *et al*., 2017). In another experiment, heifers were randomized to receive one of four treatments: control, vaccine 1 (containing bacterin and subunit proteins), vaccine 2 (containing bacterin), and vaccine 3 (containing recombinant subunit proteins) and found that puerperal metritis was less common after receiving metritis vaccination (9.1 per cent vs. 14.9 per cent, respectively) with respect to control (Meira *et al*., 2020).

Phytotherapy: Garlic *(Allium sativum)* has historically been utilized for ages to treat infectious diseases by a variety of societies throughout. Gopikrishnan *et al*. (2022) showed that using an ethanolic extract of garlic to treat endometritis resulted in higher conception rates and a substantial drop in bacterial count compared to the control and other treatment groups. For almost three millennia, ashwagandha has been a significant herb in the indigenous and ayurvedic medical systems. It is an anti-inflammatory, sedative, and diuretic that is typically used to boost stamina and energy (Mandhwani *et al*., 2017). Rahi *et al*. (2014) stated that the most successful treatment among all treated groups was determined to be garlic + ashwagandha extract; as a result, it may eventually take the place of conventional antibiotics for bacterial endometritis that causes repeat breeding conditions in crossbred cows. Neem is the most often used traditional medicinal plant in India, the neem plant is medicinal in almost every component, and it has been shown to have antioxidant, immunomodulatory, anti-inflammatory, antifungal, antibacterial, and antiviral qualities (Mandhwani *et al*., 2017). In a few studies, it was shown that administration of neem oil (25 mL) intrauterine for three days cured the subclinical uterine infection in cattle and buffaloes. Similarly, the intrauterine administration of *Tinospora cordifolia* (50 mL) or Garlic extracts (10 mL) for three days was shown to help curing the uterine infection in cattle and buffaloes (Kumar *et al*., 2013; 2018). Recently, Japheth *et al.* (2021) developed a polyherbal mixture comprising of a combination of *Trachyspermum ammi* L., *Curcuma longa* L., *Cuminum cyminum* L., *Trigonella foenum-graecum* L., *Foeniculum vulgare* Mill., *Anethum graveolens* L. and *Zingiber officinale* Roscoe and showed that supplementation of this mixture improved the immunity of the buffaloes, facilitated early involution of cervix and uterus, efficient cleansing of lochia, downsized the uterine infection and improved subsequent fertility.

Proteolytic enzyme therapy: Chymotrypsin, trypsin, and papain are the most often utilized proteolytic enzymes for intrauterine therapy. Trypsin and chymotrypsin are endopeptidases, but papain is an exopeptidase. Proteases such as trypsin, chymotrypsin, and papain serve as biological scalpels in inflamed tissue, causing fibrinolysis and proteolytic activity. Infection in the uterus causes an alkaline pH, leading to increased enzyme activity, these enzymes perform effectively in inflammatory or alkaline conditions, this leads to the breakdown of infection products, damaged cells, and tissues found in lochia material (Gulia *et al*., 2023). Singh *et al*., (2019) examined the impact of intrauterine proteolytic enzyme therapy on treating subclinical endometritis and improving reproductive performance in postpartum water buffalo cows. The pregnancy rate in subclinical endometritic cattle treated with proteolytic enzymes was better compared to controls.

CONCLUSION

The incidence of uterine infection in cattle and buffaloes is high leading to colossal loss to farmers and also affects the animal welfare. Early and accurate diagnosis of the SCE is essential to avoid such losses and also to prevent the animal developing into clinical uterine infection. Although several tests are available, their efficacy and easy to use varies with the production system and there is no single 100 per cent effective tool to diagnose SCE in cattle and buffaloes. With the developments in OMICs, now it is possible to profile the complete set of a molecules (transcripts, proteins, metabolites, small RNAs etc) in normal and diseased conditions for identification of biomarkers for the condition. Among these molecules, proteomic profiling of uterine fluid may be extremely useful to identify the precise changes in dairy cattle affected with subclinical endometritis, thereby, a cow-side diagnostic test can be developed, which is the need of the hour. Regarding therapy, several systematic studies confirm the beneficial effects of cephapirin and ceftiofur drugs in treating cows with clinical endometritis and clinical metritis, respectively. Lack of side effects or residue violation due to cephapirin is also an advantage for its usage. Understanding the relationship of uterine pathogens, its extent of inflammatory response (quantifiable through standard methods like uterine cytology, etc.) and its clinical manifestation (diagnosed by vaginal discharge) along with antibiotic sensitivity tests is very basis for developing suitable treatment protocol for uterine infections. Selection of the most appropriate drug at its optimal dosage and duration is also important to cure an infection and reduce the resistant strains development. Combined use of hormones

and antibiotics could also be explored to increase the elimination of uterine infections. With increasing public health concern about antimicrobial resistance, systematic studies with alternative herbal therapies would be long term solution to manage uterine infections in dairy animals.

REFERENCES

Ametaj, B. N., Iqbal, S., Selami, F., Odhiambo, J. F., Wang, Y., Gänzle, M. G., Dunn, S. T. and Zebeli, Q. 2014. Intravaginal administration of lactic acid bacteria modulated the incidence of purulent vaginal discharges, plasma haptoglobin concentrations, and milk production in dairy cows. *Res. Vet. Sci.* **96:** 365–370.

Asbury, A. C. and Hansen, P. J. 1987. Effects of susceptibility of mares to endometritis and stage of cycle on phagocytic activity of uterine-derived neutrophils. *Pub. Med.* **35**: 311–316.

Baldrick, P. 2010. The safety of chitosan as a pharmaceutical excipient. *Reg. Toxicol. Pharmacol*.**56:** 290–299.

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.

Bhat, F. A., Bhattacharyya, H. K., and Hussain, S. A. 2014. White side test: A simple and rapid test for evaluation of nonspecific bacterial genital infections of repeat breeding cattle. *Vet. Res. Forum*: **5**: 177.

Brodzki, P., Kostro, K., Brodzki, A., Wawron, W. and Marczuk, J. 2015a. Inflammatory cytokines and acute-phase proteins concentrations in the peripheral blood and uterus of cows that developed endometritis during early postpartum. *Theriogenology* **84**: 11-18.

Brodzki, P., Kostro, K., Krakowski, L. and Marczuk, J. 2015b. Inflammatory cytokine and acute phase protein concentrations in the peripheral blood and uterine washings of cows with subclinical endometritis in the late postpartum period. *Vet. Res.Commun.* **39**: 143-149.

Canning, P., Hassfurther, R., TerHune, T., Rogers, K., Abbott, S., and Kolb, D. 2017. Efficacy and clinical safety of pegbovigrastim for preventing naturally occurring clinical mastitis in periparturient primiparous and multiparous cows on US commercial dairies. *J. Dairy Sci*. **100**: 6504–6515.

Chapwanya, A., Meade, K. G., Doherty,

M. L., Callanan, J. J., and O'Farrelly, C. 2013. Endometrial epithelial cells are potent producers of tracheal antimicrobial peptide and serum amyloid A3 gene expression in response to E. coli stimulation. *Veterinary Immunol*. *Immunopath*. **151**: 157-162.

Chapwanya, A., Meade, K. G., Narciandi, F., Stanley, P., Mee, J. F., Doherty, M. L., and O'farrelly, C. (2010). Endometrial biopsy: a valuable clinical and research tool in bovine reproduction. *Theriogenology* **73**: 988-994.

Couto, G. B., Vaillancourt, D. H. and Lefebvre, R. C. 2013. Comparison of a leukocyte esterase test with endometrial cytology for diagnosis of subclinical endometritis in postpartum dairy cows. *Theriogenology* **79**:103-107.

Daetz, R., Cunha, F., Bittar, J. H., Risco, C. A., Magalhaes, F., Maeda, Y., Santos, J. E. P., Jeong, K. C., Cooke, R. F. and Galvão, K. N. 2016. Clinical response after chitosan microparticle administration and preliminary assessment of efficacy in preventing metritis in lactating dairy cows. *J. Dairy Sci.*:**99**: 8946–8955.

Dohmen, M.J.W., Lohuis, J.C.A.M., Huszenicza, C.R., Nagy, P. and Gacs, M. (1995). The relationship between bacteriological and clinical findings in cattle with subacute/chronic endometritis. *Theriogenology* **3** : 1379-1388.

El-Azab, M., H.L. Whitmore, I. Kakoma, B.O. Brodie, D.J. McKenna and B.K. Gustafsson. 1988. Evaluation of the uterine environment in experimental and spontaneous bovine metritis. *Theriogenology* 29: 1327-1334.

Escandón, B. M., Espinoza, J. S., Perea, F. P., Quito, F., Ochoa, R., López, G. E., Galarza, D. A. and Garzón, J. P. 2020. Intrauterine therapy with ozone reduces subclinical endometritis and improves reproductive performance in postpartum dairy cows managed in pasture-based systems. *Trop.Anim. Hlth.Prod*.**52** 2523–2528.

Fischer, C., Drillich, M., Odau, S., Heuwieser, W., Einspanier, R. and Gabler, C. 2010. Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the oestrous cycle and elevated in case of subclinical or clinical endometritis. *Reprod. Fert. Dev*. **22**:818-829.

Freick, M., Kunze, A., Passarge, O., Weber, J. and Geidel, S. 2017. Metritis

vaccination in Holstein dairy heifers using a herd-specific multivalent vaccine – Effects on uterine health and fertility in first lactation. *Anim. Reprod. Sci*.: **184** :160–171.

Gabler, C., Fischer, C., Drillich, M., Einspanier, R., and Heuwieser, W. 2010. Time-dependent mRNA expression of selected pro-inflammatory factors in the endometrium of primiparous cows postpartum. *Reprod.Biol.Endocrinol.* **8** : 152.

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. Dom. Ani.* **52** : 513–516.

Gahlot, S. C., Kumar, S., Kumaresan, A., Vairamuthu, S., Saraf, K. K., Sreela, L. and Mohanty, T. K. 2018. Biochemical analysis of uterine fluid for identification of indicators for subclinical endometritis in the water buffalo (*Bubalus bubalis*). *Reprod. Dom. Anim.* **53**: 48-53.

Galvao, K. N., Santos, N. R., Galvao, J. S., and Gilbert, R. O. 2011. Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. *Theriogenology* **76**: 290-299.

Genís, S., Cerri, R. L. A., Bach, À., Silper, B. F., Baylão, M., Denis-Robichaud, J. and Arís, A. 2018. Pre-calving Intravaginal Administration of Lactic Acid Bacteria Reduces Metritis Prevalence and Regulates Blood Neutrophil Gene Expression After Calving in Dairy Cattle. *Front. Vet. Sci*.**5** : 135.

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** :1538-1547.

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.

Gopikrishnan, D., Selvaraju, M., Palanisamy, M., Periyannan, M., Ravikumar, K and Senthilkumar, K. 2022. Therapeutic Efficacy of Garlic (Allium sativum), Turmeric (Curcuma longa) and Eucalyptus (Eucalyptus globules) Extracts in the

Treatment of Bovine Endometritis. *Ind. J. Anim. Res*. **56**: 904 – 908.

Gulia, N., Bisla, A. and Honparkhe, M. 2023. A review of applications and scope of intrauterine proteolytic enzymes therapy for treatment of uterine infections in dairy animals. *Int. J. Vet. Sci. Anim. Husb*. **8** :66–71.

Hammon, D. S., Evjen, I. M., Dhiman, T. R., Goff, J. P. and Walters, J. L. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunopath.* **113** : 21-29.

Herath, S., Lilly, S. T., Santos, N. R., Gilbert, R. O., Goetze, L., Bryant, C. E., & Sheldon, I. M. 2009. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. *Reprod. Biol. Endocrinol*. **7**: 55.

Hirvonen, J., Huszenicza, G., Kulczár, M., and Pyörälä, S. 1999. Acute-phase response in dairy cows with acute postpartum metritis. *Theriogenology* **51** : 1071-1083.

Humblet, M. F., Guyot, H., Boudry, B., Mbayahi, F., Hanzen, C., Rollin, F. and Godeau, J. M. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet, Clin. Path. **35**: 188-193.

Ibrahim, M., Peter, S., Gartner, M. A., Michel, G., Jung, M., Einspanier, R. and Gabler, C. 201). Increased mRNA expression of selected antimicrobial peptides around ovulation and during inflammatory processes in the bovine endometrium postpartum. *Theriogenology*, **86** : 2040-2053.

Japheth, K. P., Kumaresan, A., Patbandha, T. K., Baithalu, R. K., Selvan, A. S., Nag, P., Manimaran, A. and Oberoi, P. S. 2021. Supplementation of a combination of herbs improves immunity, uterine cleansing and facilitate early resumption of ovarian cyclicity: A study on post-partum dairy buffaloes. *J. Ethnopharmacol.* **272** : 113931.

Jeon, S. J., Oh, M., Yeo, W.-S., Galvão, K. N. and Jeong, K. C. 2014. Underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. *PLoS ONE* **9** : e92723.

Kasimanickam, R. K., Kasimanickam, V. R., Olsen, J. R., Jeffress, E. J., Moore, D. A. and Kastelic, J. P.

2013. Associations among serum pro-and anti-inflammatory cytokines, metabolic mediators, body condition, and uterine disease in postpartum dairy cows. *Reprod. Biol. Endocrinol*. **11**:103.

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.

Krishnan, B. B., Kumar, H., Mehrotra, S., Singh, S. K., Goswami, T. K., Khan, F. A., Patra, M. K. and Islam, R. 201). Effect of leukotriene B4and oyster glycogen in resolving subclinical endometritis in repeat breeding crossbred cows. *Ind. Anim. Res.* **49** :218.

Kumar, A., Katiyar, R., Ahmad, S. F., Balamurugan, B., Deepak, D. and Prasad, J. K. 2018. Current treatment aspects of bovine reproductive disorders. *Theriogenology* **8** : 101-109.

Kumar, H., Bhooshan, N., Dass, R. S., and Garg, A. K. 2013). Effect of treatment of immunomodulators on recovery and conception rate in endometritic buffaloes. Indian J. Anim. Sci, 83(2), 129-132.

Kumaresan, A. and Srivastava, A.K. 2022. Reproduction Efficiency in Dairy Bovine: Trends and Targets. In: Kumaresan, A. and Srivastava, A.K. (eds.). *Current Concepts in Bovine Reproduction*. Springer, Singapore, pp 7-24.

Kumaresan, A., Praveen, K.S., Manimaran, A., Srivastava, A.K. 2022. Uterine Infection in Bovines: An Update. In: Kumaresan, A. and Srivastava, A.K. (eds.). *Current Concepts in Bovine Reproduction*. Springer, Singapore. pp 169-196.

Kurati S.P. 2019. Proteomic profiling of uterine fluid for identification of candidate biomarkers for sub-clinical endometritis in dairy cattle. *Ph.D. thesis*, ICAR, NDRI- Karnal, India.

Lefebvre, R. C. and Stock, A. E. 2012. Therapeutic efficiency of antibiotics and prostaglandin F2α in postpartum dairy cows with clinical endometritis: an evidence-based evaluation. *Vet. Clin. Food Anim.Pract*.**28** : 79-96.

Lima, F. S. de. (2020). Recent advances and future directions for uterine diseases diagnosis, pathogenesis, and management in dairy cows. *Anim.*

Reprod. :17 e20200063.

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** : 1349-1357.

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

Mandhwani, R., Bhardwaz, A., Kumar, S., Shivhare, M. and Aich, R. 2017. Insights into bovine endometritis with special reference to phytotherapy. *Vet. Wld*. **10 :** 1529–1532.

Manimaran, A., Kumaresan, A., Jeyakumar, S. and Rajendran, D. 2022. Therapeutic Management of Postpartum Uterine Infections in Bovines. In: Kumaresan, A. and Srivastava, A.K. (eds.). *Current Concepts in Bovine Reproduction*. Springer, Singapore. pp 197-222.

Maquivar, M. G., Barragan, A. A., Velez, J. S., Bothe, H. and Schuenemann, G. M. 2015. Effect of intrauterine dextrose on reproductive performance of lactating dairy cows diagnosed with purulent vaginal discharge under certified organic management. *J. Dairy Sci*. **98** : 3876 - 3886.

Meira, E. B. S., Ellington-Lawrence, R. D., Silva, J. C. C., Higgins, C. H., Linwood, R., Rodrigues, M. X., Bringhenti, L., Korzec, H., Yang, Y., Zinicola, M. and Bicalho, R. C. 2020. Recombinant protein subunit vaccine reduces puerperal metritis incidence and modulates the genital tract microbiome. *J. Dairy Sci*.**103** : 7364–7376.

Rahi, S., Gupta, H. P., Prasad, S. and Baithalu, R. K. 2014. Phytotherapy for endometritis and subsequent conception rate in repeat breeding crossbred cows. *Ind. J. Anim. Reprod.* **34** : 9 -12.

Rashid Dar, R., Ali, A., Firdous Ahmad, S., Singh, S. K., Patra, M. K., Panigrahi, M., Kumar, H. and Krishnaswamy, N. 2019. Immunomodulatory effect of curcumin on lipopolysaccharide and/or flagellin induced production of prostaglandin E2 and relative expression of proinflammatory cytokines in the primary bubaline endometrial stromal cells. *Reprod. Dom. Anim*.**54** : 917–923.

Rosales, E. B. and Ametaj, B. N. 2021. Reproductive Tract Infections in Dairy Cows: Can Probiotics Curb Down the Incidence Rate? *Dairy* **2** : 40–64.

Sagai, M. and Bocci, V. 2011. Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress? *Med. Gas Res*.**1** : 29.

Salasel, B., Mokhtari, A. and Taktaz, T. 2010. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. *Theriogenology* **74**: 1271-1278.

Santos, N. R., Roman, H. B. and Gilbert, R. O. 2006. The use of leukocyte esterase reagent strips for diagnosis of subclinical endometritis in dairy cows. *Theriogenology* **66** : 666-667.

Sheldon, I. M., Noakes, D. E., Rycroft, A. N., Pfeiffer, D. U. and Dobson, H. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reprod*. **123** : 837-845.

Sheldon, I. M., Price, S. B., Cronin, J., Gilbert, R. O., and Gadsby, J. E. (2009). Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reproduction in domestic animals, 44(s3), 1-9.

Simon, J. and McNutt, S. H. 1957. Histopathological alterations of the bovine uterus. II. Uterine tissue from cow of low fertility. *Am.J.Vet.Res*.**18** : 241.

Singh, H., Brar, P. S., Honparkhe, M., Arora, A. K. and Dhindsa, S. S. 2019. Subclinical endometritis in estrual buffaloes: diagnosis, prevalence and impact on reproductive performance. *Trop. Anim. Hlth. Prod.* **52** :357–363.

Snider, T. A., Sepoy, C. and Holyoak, G. R. 2011. Equine endometrial biopsy reviewed: observation, interpretation, and application of histopathologic data. *Theriogenology* **75** : 1567-1581.

Srivastava, A. K. and Kumaresan, A. 2014. Scope of buffaloes in Indian dairy industry. *Asian Buffalo Mag.* **1** : 16-27.

Srivastava, A. K., Kumaresan, A., Mohanty, T. K. and Prasad, S. 2013. Status paper on buffalo estrus biology, India. National Dairy Research Institute, Karnal, 12.

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.

Walsh, S. W., Williams, E. J. and Evans, A. C. O. 2011. A review of the causes of poor fertility in high milk producing dairy cows. *Anim*. *Reprod*. *Sci*. **123** : 127-138.

Wheeler, T. T., Ledgard, A., Smolenski, G. A., Backmann, E., McDonald, R. and Lee, R. S. F. 2012. Innate immune proteins as biomarkers for mastitis and endometritis. In *Proceedings of the 5th Australasian Dairy Science Symposium*; pp. 294-297.

Zaayer, D. 1986. Non-fertility in cows: treatment with PGF and investigation of uterine biopsies. *Cytobios*, 45(180), 55-70.

Zerbe, H., Schuberth, H.-J., Hoedemaker, M., Grunert, E. and Leibold, W. 1996. A new model system for endometritis: Basic concepts and characterization of phenotypic and functional properties of bovine uterine neutrophils. *Theriogenology* **46** : 1339–1356.

Zinicola, M., Bicalho, M. L. S., Santin, T., Marques, E. C., Bisinotto, R. S. and Bicalho, R. C. 2019. Effects of recombinant bovine interleukin-8 (rbIL-8) treatment on health, metabolism, and lactation performance in Holstein cattle II: Postpartum uterine health, ketosis, and milk production *J*. *Dairy Sci*. **102** : 10316–10328.