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Predisposing factors, diagnostic and therapeutic aspects of persistent endometritis in postpartum cows

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Abstract. A certain level of endometrial bacterial infection and inflammation is involved in bovine uterine involution during the puerperal period. Factors that hamper normal uterine involution expose the uterine environment to pathological conditions, causing different endometritis levels. The lack of proper diagnostic tools extends the time to conception. Efforts have been made to elucidate the postpartum uterine environment, including bacterial flora, changes in transient endometrial inflammation, and the pathophysiology of endometritis, to improve bovine reproductive performance. *E. coli* and *Trueperella pyogenes* in the uterus are likely to cause persistent infection, and *Mycoplasma bovis* infection is associated with dystocia and cytological endometritis in postpartum dairy cows. Due to the widespread use of cytobrush as a diagnostic tool for bovine subclinical endometritis (SE) that enables quantification of the degree of inflammation, we found that endometritis at week 5 postpartum was associated with delayed first ovulation. Approximately 30% of open cows have SE during the postpartum period, and cows with low blood glucose during prepartum have a high risk of developing SE. Additionally, cows with purulent vaginal discharge do not always have endometritis but only vaginitis and/or cervicitis. Intrauterine infusion of polyvinylpyrrolidone-iodine (PVP-I) improves fertility and promotes endometrial epithelial cell regeneration after inducing transient uterine inflammation, suggesting that PVP-I could be a good alternative to antibiotics. In conclusion, prepartum management to prevent glucose deficiency, prompt diagnosis to identify causative agents and intrauterine inflammation levels, and appropriate treatment to minimize antimicrobial resistance is beneficial for tackling endometritis and improving reproductive performance in bovine herds.

Key words: Bovine uterus, Endometritis, Intrauterine infection, Pathogenic bacteria, Treatment

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Introduction

The decline in the conception rate of dairy and beef cows is a major reproductive management problem in Japan and other parts of the world [1]. It is important to identify the predisposing factors, establish proper diagnostic and therapeutic strategies for reproductive disorders in the early postpartum period, and breed healthy animals to tackle this decline. After calving, the pituitary gland exerts its luteinizing hormone response to gonadotropin-releasing hormone (GnRH) as early as 7 days postpartum [2], and ovarian function resumes a few weeks postpartum. Complete uterine involution takes more time than ovarian function, and the uterus is usually not ready for the embryo to implant until approximately 5 weeks postpartum in cows. When the ovaries start functioning normally, and uterine involution becomes complete, some cows can conceive by artificial insemination (AI) after estrous detection or a timed AI protocol without estrous detection [3–8]. However, some other cows cannot get pregnant because the uterine environment is not yet ready to accept the embryo for implantation because of persistent intrauterine inflammation beyond the puerperium.

Factors involved in persistent intrauterine inflammation include

improper metabolism, abnormal calving, and unhygienic barn conditions (Fig. 1). Heavy bacterial colonization after dystocia, poor hygiene, and poor uterine defense mechanisms can lead to puerperal uterine infections [9, 10]. Pathogenic microorganisms cause inflammation in the endometrium, delay uterine involution, and hinder embryo survival [11]. However, the diagnostic and therapeutic protocols that should be implemented to obtain the best subsequent reproductive performance remain unclear. This article reviews some of the predisposing factors and the diagnostic and therapeutic aspects of persistent endometritis in postpartum cows.

Predisposing Factors for Persistent Endometritis in Cows

Metabolism

Nutrition and its management are important factors that affect the uterine environment of cows. We investigated the impact of metabolic traits and body condition score (BCS) during early lactation on subclinical endometritis (SE) diagnosed at weeks 5, 6, and 7 postpartum [12]. In animals diagnosed at week 5, blood urea nitrogen (BUN) and BCS at weeks 6 and 7 were significantly lower in animals with SE (SE group) than in those without SE (Non-SE group). In animals diagnosed at week 6, blood glucose at week 4 postpartum was significantly lower in the SE group than in the non-SE group. BCS at weeks 3 to 7 was significantly lower in the SE group at week 7 than in the non-SE group. Therefore, low blood glucose, BUN, and BCS could be risk factors for developing cytologically diagnosed endometritis at weeks 5, 6, and 7 postpartum. Later, we found that low postpartum and prepartum glucose levels were

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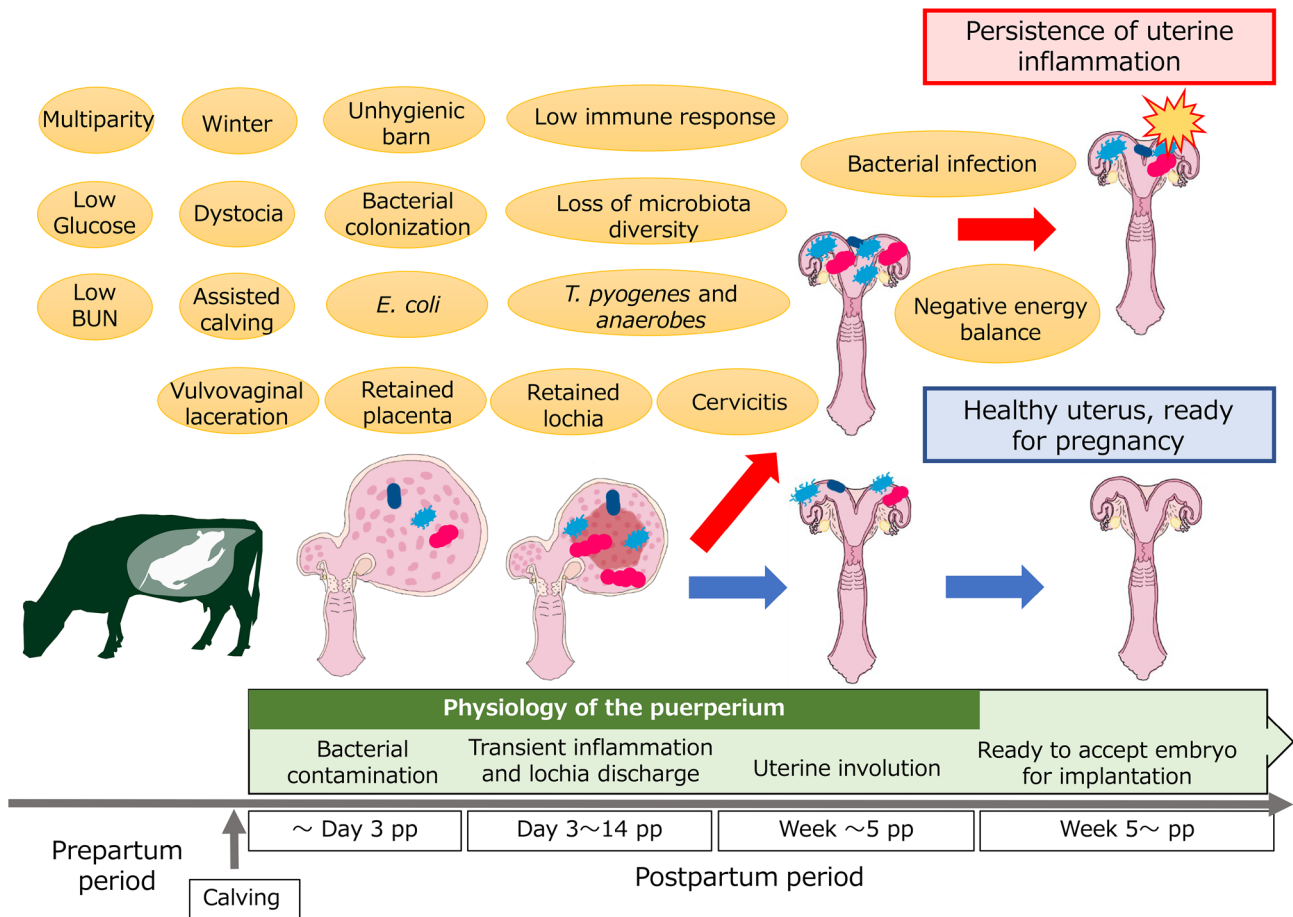


Fig. 1. A schematic picture of predisposing factors (yellow circles) for persistent endometritis along with prepartum and postpartum periods in the cow.

associated with uterine infection in postpartum dairy cows [13]. In this study, the blood glucose concentrations at week 3 and week 1 prepartum were significantly lower in cows with infection at both weeks 5 and 7 postpartum (persistent bacterial infection) than those in cows without infection at both weeks 5 and 7 postpartum, those with infection at week 5 but without infection at week 7 postpartum, and those without infection at week 5 but with infection at week 7 postpartum (Fig. 2). This result indicates a negative energy balance prepartum in cows exhibiting persistent bacterial infection at weeks 5 and 7 postpartum. This decrease in glucose concentrations might be partially due to inadequate dry matter intake. However, a more important contributor is the increased glucose clearance from blood because of nutrient partitioning, which directs glucose toward the insulin-insensitive tissues, primarily the mammary gland [14]. These findings are supported by a previous report [15], which showed that cows with uterine infection suffer disturbances in energy metabolism until 4 weeks postpartum and delay in the physiological adaptations required to meet their energy needs. This delay might be confirmed by decreased blood glucose concentrations at 4 and 6 weeks postpartum in cows diagnosed with endometritis at 6 weeks postpartum [12]. Glucose availability to fuel neutrophil function may contribute to the impaired capacity observed during the transition period [16]. A positive association was detected between greater plasma insulin-like growth factor-I concentrations and clinical endometritis (CE) during 3 to 5 weeks postpartum in multiparous dairy cows [17]. These findings highlight the benefits of identifying cows at risk of developing uterine

infections around the calving period in terms of early implementation of preventive strategies. Veterinary practitioners or herd managers should monitor pre-and postpartum variations in blood metabolite concentrations and BCS in cows since such variations might render cows more susceptible to uterine infection.

Abnormal calving

Stillbirth and dystocia both have a significant impact on the subsequent reproductive performance and productivity of the cattle industry [18]. Additionally, vulvovaginal laceration has been recognized as a risk factor for postpartum uterine disease [19]. Since there is an association between abnormal calving and subsequent endometritis, understanding the risk factors for abnormal calving will help to implement measures to reduce the incidence of endometritis. We identified the risk factors for stillbirth and dystocia in Japanese black cattle by retrospectively analyzing 41,116 calving records of 15,378 animals from 905 farms in the suburban areas of Miyazaki City, located on the southeastern coast of Kyushu, Japan, and found that low temperatures during the late pregnancy period, primiparity, premature birth, and excessive fetal weight were risk factors for stillbirth and dystocia in this cohort of animals [20]. In addition, we clarified the association between selenium and liposoluble vitamins with stillbirth [21]. In this study, selenium and vitamin A serum levels in cows with stillborn calves were significantly lower than those in cows that had normal calving. Adequate selenium and vitamin A levels in the serum are important for successful calving, reducing

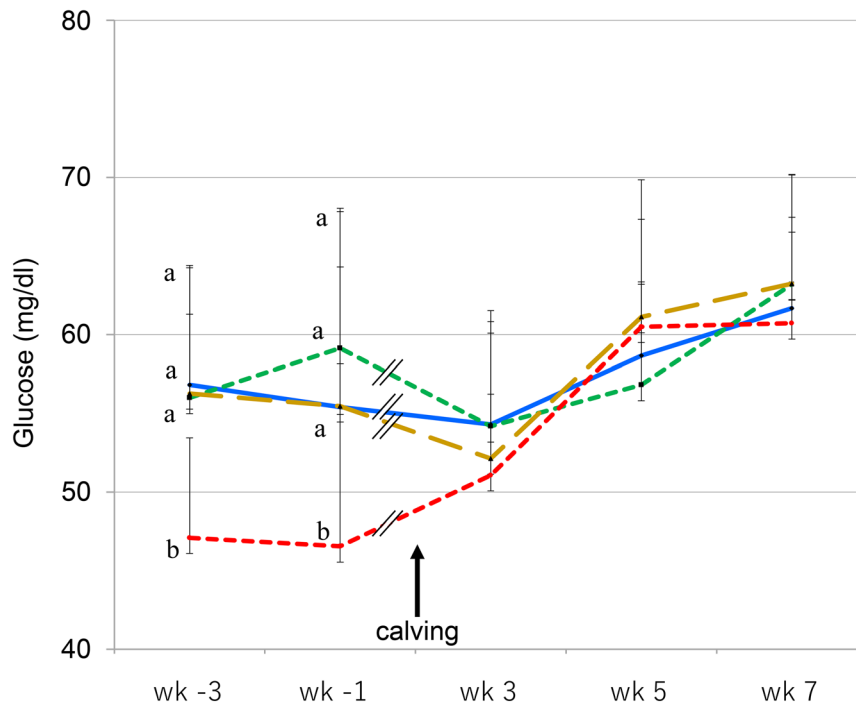


Fig. 2. Blood glucose concentrations (mean \pm SD) in cows of different test groups during the prepartum (3 weeks and 1 week before calving; wk -3 and wk -1, respectively) and postpartum period (3, 5, and 7 weeks after calving; wk 3, wk 5 and wk 7, respectively) (Reproduced with permission of the Society for Reproduction and Development from Ghanem *et al.* *J Reprod Dev* 62: 457–463, 2016 [13]). Negative for infection (Neg-Neg: Blue line) at 5 (wk 5) and 7 (wk 7) weeks after calving, positive for infection at wk 5 but not wk 7 (Inf-Neg: Green line), positive for infection at wk 7 but not wk 5 (Neg-Inf: Brown line), and persistent uterine bacterial infection at wk 5 and wk 7 (Inf-Inf: Red line).

the subsequent risk of developing endometritis.

Microbial contamination and infection

In cows, CE and SE are characterized by a purulent uterine discharge existing in the vagina and an enlarged cervix beyond 3 weeks postpartum [22], and a certain number of polymorphonuclear leukocytes (PMNs) exist in the endometrium in the absence of clinical signs, respectively [23]. Whether the disease is clinical or subclinical, endometritis is triggered by uterine microbial infection following contamination through the dilated cervix during parturition and colonization of the endometrium. Causative agents of uterine infection include microorganisms, such as bacteria, viruses, protozoa, and fungi, which cause infertility, embryonic death, and abortion in cattle [24–29]. Among them, bacteria are the most reported agents for bovine endometritis.

Over 90% of cows have microorganisms in their uterus for the first 2 weeks following calving, 78% between days 16 and 30, 50% between days 31 and 45, and 9% between days 45 and 60 postpartum [30]. Most of these bacteria are environmental contaminants and are cleared by the uterus without impairing fertility [31]. Bacteriological studies have identified *Trueperella pyogenes*, *Bacteroides* spp., *Fusobacterium necrophorum*, *Escherichia coli*, *Streptococcus* spp., *Clostridium* spp., *Pseudomonas aeruginosa*, and *Staphylococcus* spp. as the bacteria most likely to be associated with bovine endometritis [32, 33]. *T. pyogenes*, *Prevotella* spp., *F. necrophorum*, and *Escherichia coli* are the major uterine pathogens [34].

T. pyogenes cooperates with aerobic-facultative anaerobic bacteria and/or gram-negative obligate anaerobes such as *F. necrophorum* and *Prevotella* spp. in formulating uterine bacterial flora [35]. Severe endometrial lesions are mainly caused by *T. pyogenes*, which is the

most prevalent bacterial type in the late postpartum period and acts synergistically with anaerobic pathogens such as *F. necrophorum* [34, 36, 37]. However, limited data are available on the persistence of *T. pyogenes* and anaerobic bacteria in the uterus of cows and their association with cytological endometritis and purulent vaginal discharge (PVD) under field conditions. Therefore, we examined the persistence of bacterial infections caused by *T. pyogenes* and anaerobic bacteria isolated during the postpartum period (weeks 5 and 7 postpartum) using cytobrush techniques in relation to PMN percentage (PMN%) and the condition of vaginal mucus discharge in dairy cows. We observed that 15% of the animals had bacterial infections both at weeks 5 and 7 postpartum (persistence of infection) and that there was a positive correlation between the severity of cytological endometritis, PVD, and the persistence of infection [38]. Additionally, cows with persistent bacterial infection at both weeks showed a significantly higher prevalence of cytological endometritis than those without bacterial infections at weeks 5 and 7 postpartum (Table 1). In this study, *T. pyogenes* was not isolated alone, except in one cow, but was isolated together with aerobic-facultative anaerobic bacteria such as *F. necrophorum* and *Prevotella* spp. This highlights the importance of these pathogens in inducing endometritis. *F. necrophorum* produces a leukotoxin, *Prevotella melaninogenicus*, which inhibits phagocytosis, and *T. pyogenes* produces a growth factor for *F. necrophorum* [22]. Contrastingly, although *E. coli* plays a key role in the metritis–endometritis syndrome complex [29, 34, 39], the number of *E. coli*-positive samples was surprisingly low in our study [38]. These results indicate that the prevalence of *E. coli* infection decreases in the late postpartum period, while that of *T. pyogenes* and anaerobes increases, which supports previous observations that *E. coli* dominated the uterus in the first few days

Table 1. Prevalence of cytological endometritis in cows with and without bacterial infection at weeks 5 and 7 postpartum

Cows	Week 5			Week 7		
	PMN †		Endometritis (%)	PMN †		Endometritis (%)
	≤ 6%	≥ 6%		≤ 4%	≥ 4%	
Cows negative for bacterial infection at both weeks	29	3	9.4 ^a	24	8	25 ^a
Cows positive for bacterial infection at both weeks	1	9	90 ^b	1	9	90 ^b
Total	30	12		25	17	

(Reproduced with permission of the Society for Reproduction and Development from Ghanem, *et al.* *J Reprod Dev* 61: 54–60, 2015 [38]). Bacterial infection was defined here as infection by *T. pyogenes* and anaerobes. †Diagnostic criteria for cytological endometritis were ≥ 6% and ≥ 4% PMN at weeks 5 and 7, respectively. a–b: different letters reflect differences between groups ($P < 0.001$).

after calving. *T. pyogenes* was later found in animals with severe CE [31, 40], and *E. coli* was a pioneer pathogen that predisposed cows to infection with *T. pyogenes*, which was strongly associated with CE [41]. In metagenomic analyses of 16S rRNA gene sequences from endometrial samples of postpartum dairy cows, *T. pyogenes* was the predominant bacteria in animals with CE, and the microbiome in cows with CE had a loss of bacterial diversity [42]. Vaginal *E. coli* from cows with metritis or endometritis possessed the virulence factor gene as well as elevated motility and biofilm formation capacity that could confer the ability to colonize the bovine genitalia, and *E. coli* strains from phylogroup B1 were associated with postpartum uterine disease [43]. The shift in the uterine microbiome in cows that develop metritis is characterized by heterogeneity loss and a decrease in bacterial richness [41].

Effective prevention and treatment of *T. pyogenes*, certain *E. coli* strains, and anaerobic bacterial infections should be implemented to mitigate the persistence of uterine infections and inflammation, thereby shortening the days open in postpartum dairy cows. Other bacteria, such as *Staphylococcus* spp., *Streptococcus* spp. or non-*E. coli* aerobic gram-negative rods have also been isolated as additional flora along with major uterine pathogens [44]. In a recent study, common uterine pathogens were rarely detected, and the composition and diversity of the microbiota consisted of *Staphylococcus*, *Bacillus*, and *Streptococcus* spp. in the uterus of cows with mild endometritis [45].

Mycoplasma species can also be isolated from the bovine reproductive tract. The first isolation of *Mycoplasma bovis* from the genital tract of cows was reported in 1947 [46]. While *M. bovis* has been found in cows with low fertility, in whom no other cause of infertility has been found [47], it has also been found in the vaginal mucus of normal cows, which has led to speculation concerning its role as a pathogen [48, 49]. However, the association between mycoplasma infection and cytological endometritis based on PMN% remains unclear. We investigated the incidence of mycoplasma infection in the uterus of 209 postpartum Holstein cows using a novel and rapid PCR to detect 7 mycoplasma species and detected only *M. bovis* in 7.4% of the samples [50]. We also found that the incidence of dystocia was significantly higher in mycoplasma-positive cows than in mycoplasma-negative cows. This might be due to the microbial entrance into the uterus at calving or reduced immune function in the genital tracts of cows that have recently experienced dystocia. In addition, the incidence of cytological endometritis was significantly higher in mycoplasma-positive than in mycoplasma-negative cows at week 7 postpartum. This might indicate that *M. bovis* aggravated the severity of endometritis when it was associated with dystocia. Moreover, *M. bovis* seems to be pathogenic to the endometrium and not opportunistic. These results indicate that *M. bovis* infection

in the uterus might be associated with recent dystocia and cytological endometritis in postpartum dairy cows. Additional investigations in a large population of postpartum cows are required to confirm this. Future studies should focus on the follow-up of infected cows to evaluate the pathogenicity of mycoplasma, the ability of cows to self-cure the infection, and the subsequent reproductive performance of infected animals.

Diagnoses for Bovine Endometritis

Establishing regular CE and SE diagnoses during the early postpartum period is important because it could minimize negative effects on reproductive performance, and hence, maximize the benefits gained from improved reproductive performance. The Metricheck device for evaluating vaginal mucus turbidity during weeks 3 and 4 postpartum and fluid detection in the uterus by ultrasonography and vaginoscopy during week 6 postpartum can be used as good diagnostic tools for CE and as a good predictive tool to predict the subsequent reproductive performance of dairy cattle [51]. However, the diagnosis of SE requires the detection of intrauterine (endometrial) inflammation in the absence of PVD. Although the risk factors and pathophysiology of PVD and cytological endometritis are at least partly shared, uterine and cervical tissue trauma and bacterial infection appear to play a greater role in PVD, whereas regulation of the immune response appears to play a greater role in cytological endometritis [52].

Several diagnostic techniques for detecting the occurrence of inflammatory processes in the uterus include ultrasonography [53], bacterial culture from the uterus [54], uterine biopsy [55], and uterine cytology have been described [56]. Ultrasonography and bacterial culture have low sensitivity, and uterine biopsy followed by histological examination is expensive, time-consuming, and clinically inaccessible under practical field conditions.

In human and veterinary medicine, PMNs collected using a cytobrush are commonly studied as biomarkers to monitor the severity of inflammation and the relationship between a high PMN% and reproductive disturbances [57, 58]. PMNs exhibit rapid chemotaxis toward pathogenic bacteria along with macrophages and uterine inflammation can be detected rapidly as PMNs migrate into the endometrium [23]. Thus, endometrial cytology using cytobrush techniques has been developed as a useful method for diagnosing CE and SE in cows. Additionally, PMN% determination is an objective method that can evaluate the extent of local inflammation within the uterus since the occurrence of cytological endometritis has been reported to have a detrimental effect on subsequent postpartum fertility [59, 60]. While endometrial cytology using a cytobrush is relatively uncomplicated under field conditions, it still requires

staining smear samples on slides and microscopic examination to make a diagnosis. It is important to be able to rapidly diagnose postpartum endometritis using validated cow-side techniques to improve reproductive performance at the herd level. Several research groups, including ours, have proposed the determination of leukocyte esterase (LE) activity, an enzyme produced by neutrophils, as a cow-side test for diagnosing postpartum SE [61–63]. Test strips can analyze LE activity in urine samples, uterine lavage fluid, or uterine cytobrush samples. Our results showed that LE activity correlated significantly with PMN% at 3, 5, and 7 weeks postpartum (Fig. 3). The finding that LE activity increased significantly in cows with SE agreed with that of previous studies [61, 62] and showed that the association was the strongest and many cows had high PMN% at week 3 because neutrophils were present at greater numbers than at weeks 5 and 7 postpartum. Cows in the early postpartum period tend to have high neutrophil proportions in endometrial cytology [56], which may have helped to improve the sensitivity of the reagent strip test. Our results suggest that evaluation of LE activity by test strips at week 3 postpartum may be an alternative to endometrial cytology for diagnosing SE.

Effect of Persistent Endometritis on Ovarian Status

We observed that PMN% was higher in animals with anovulatory follicles (> 23 mm) with low progesterone (P_4) than in those with follicles of 10–23 mm, and animals showed corpus luteum (CL) at 6 weeks postpartum [64]. These results support other reports, concluding that cows with bacterial infections in the genital tract had slower growth of dominant follicles and were less likely to ovulate [35, 65].

PMN% was significantly higher in the non-CL group than in the CL group at week 5 postpartum [66]. The PMN% and vaginal mucus scores during week 5 postpartum in cows ovulating by day 28 were significantly lower than the PMN% and vaginal mucus scores in those ovulating by day 49. These results suggest that a higher PMN% at week 5 postpartum is associated with delayed resumption of ovarian cyclicity in high-producing dairy cows. Delayed onset of ovarian activity in cows with endometritis compared to that in healthy cows has been reported [67, 68]. However, no relationship was found between bacteriological findings and ovarian activity [39]. Another group found no consistent relationship between the stage of the estrous cycle and uterine neutrophil function [69].

Besides the first ovulation, uterine infection affects the length of the luteal phase after the first postpartum CL formation. Cows with persistent *T. pyogenes* and anaerobic bacterial infections had a significantly prolonged luteal phase (PLP) than cows without infection, indicating that persistent *T. pyogenes* and anaerobic co-infection could diminish ovarian activity [38]. Postpartum cows with uterine infections are less likely to ovulate because the growth of the dominant follicle is slower, and there are lower plasma estradiol concentrations [22]. If cows ovulate, endometrial cytokines might alter steroidogenesis by luteal cells, which could contribute to a lower P_4 secretion or an increase in the prostaglandin (PG) E_2 /PGF $_2$ ratio, which might extend the luteal phase.

It is difficult to determine whether the presence of *T. pyogenes* and anaerobes retards the resumption of ovarian cyclicity or whether delayed postpartum ovarian activity enhances infection and persistence of *T. pyogenes* and anaerobes. When ovulation occurs before the uterus expels all the exudates and debris, a heavy bacterial growth such as that of *T. pyogenes* occurs, and CL is retained for a prolonged interval [70]. Furthermore, the first CL to develop in cows with uterine

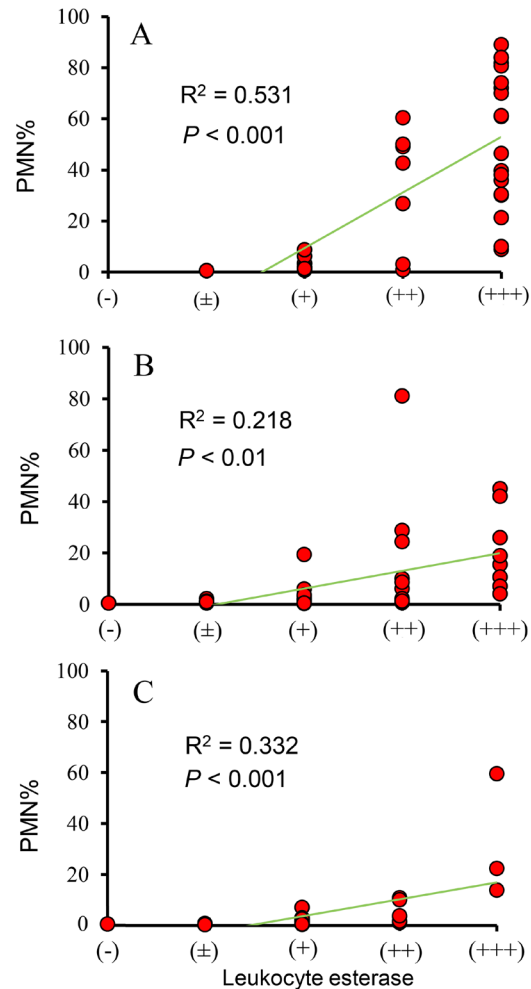


Fig. 3. Associations of levels of leukocyte esterase (LE) ($n = 43$) with PMN% in endometrial samples from dairy cows (Reproduced with permission of the Japanese Society of Veterinary Science from Nazhat *et al.* *J Vet Med Sci* 80: 302–310, 2018 [63]). Graphs A to C show the associations between LE levels and PMN% at W3 (A), W5 (B), and W7 (C).

disease secretes less P_4 than normal animals [65]. *T. pyogenes* infusion into the uterus caused luteal regression; consequently, first-wave dominant follicles, which normally become atretic and ovulated in half of the infused cows [71]. However, CL did not regress in the remaining cows, and the mechanism determining the fate of CL remains unclear [72]. Serum P_4 concentrations in cows with PLP were higher than those in cows with normal ovarian activity, indicating that the occurrence of PLP in clinically healthy, high-producing dairy cows was not associated with uterine infection [73].

Treatments for Bovine Endometritis

Clinical endometritis

If loads of various stressors and pathogens exceed the immunocompetence of the host, the cow will fail to undergo a physiological uterine repair process, and intrauterine inflammation can occur. Subsequently, intrauterine inflammation can persist and lead to subfertility or infertility if left untreated [11, 12]. In such cases, robust treatment interventions are required. Many therapeutic methods have been employed, including hormonal administration, such as

PGF_{2α} or estradiol, and intrauterine antibiotic infusions [74]. For problems with residual hormones or antibiotics, antibiotic-resistant bacteria, withdrawal periods, and safe and proper treatments to deal with these uterine diseases are needed [75, 76].

Conversely, povidone-iodine (polyvinylpyrrolidone-iodine or PVP-I) has the advantage that it does not require a withdrawal period and does not pass into the milk [77]. While 2.0% PVP-I is commonly used to treat endometritis and pyometra, intrauterine 0.5% PVP-I treatment has positive effects on the bovine reproductive performance of repeat-breeders [78]. Therefore, an adequate PVP-I concentration and optimal treatment protocol should be developed. We determined whether 2.0% or 0.5% PVP-I is better for treating severe CE diagnosed using endometrial cytology in dairy cattle [79]. Cows with severe CE (PMN% > 30%), which had recuperated from pyometra a week before, were treated with either 2.0% or 0.5% PVP-I. The PMN% in the endometrial epithelium 2 weeks after intrauterine infusion in the 2.0% group was significantly lower than that in the 0.5% group. Moreover, the first service conception rate was higher, the number of services per conception was lower, and the time to conception was shorter in the 2.0% group than in the 0.5% group. Thus, a 2.0% intrauterine PVP-I infusion seems to be a better option than 0.5% PVP-I in treating severe CE in dairy cows and in those who recover immediately from pyometra. Presumably, the free iodine efficacy might have been reduced by the presence of even small amounts of organic materials, as ultrasonography cannot detect them in the uterus after the removal of accumulated pus. Therefore, the final iodine concentration in the 2.0% group might have been closer to 0.1%, which is the concentration known to release maximum free iodine and show antiseptic effects *in vitro* [80–82]; thus, 2.0% PVP-I was more effective in treating cows with severe endometritis. However, the threshold PMN% at the time of AI that could affect subsequent

reproductive performance and indicate the appropriate timing for treating endometritis should be clarified in future studies.

Subclinical endometritis

Intrauterine infusion of 2% PVP-I is not only effective in treating cows with severe endometritis but is also likely to be effective in promoting endometrial epithelial cell regeneration and improving fertility in cows with SE as well as CE. We randomly divided 120 lactating clinically healthy Holstein-Friesian cows at 5 weeks postpartum into 3 groups: intrauterine PVP-I infusion (PVP), saline (SAL), and no treatment (NTX); endometrial cytology was performed daily from day 0 (at week 5 postpartum) to day 7 in a subset of the cows to describe the duration of inflammation after PVP-I infusion and to determine the effect of the infusion on subsequent fertility [83] (Fig. 4). All cows received timed AI on day 17. Although PMN% significantly increased in PVP compared to SAL and NTX on day 1, it decreased to a level similar to that of the other groups by day 2 (Fig. 5), and the conception rate was significantly higher in PVP cows than in SAL and NTX cows. In addition, we conducted another experiment to evaluate the histopathology of the endometrium in dairy cows. Twenty-five cows were randomly classified into sacrifice at 24 h or 48 h after 2% PVP-I infusion, and 24, 48, 72, or 96 h after SAL infusion, and histopathology was performed on the uterus of each cow. Stratified columnar epithelium in the uterus disappeared 24 h after PVP-I or saline infusion. Subsequently, the epithelium was regenerated 48 h after PVP-I, but not after saline infusion, and regenerated 72 h after saline infusion, indicating that neutrophil infiltration in the subcutaneous tissue may have promoted endometrial epithelial cell regeneration after PVP-I administration. Future studies are needed to elucidate the relationship between epithelial regeneration and neutrophil infiltration in cows with endometritis and healthy cows

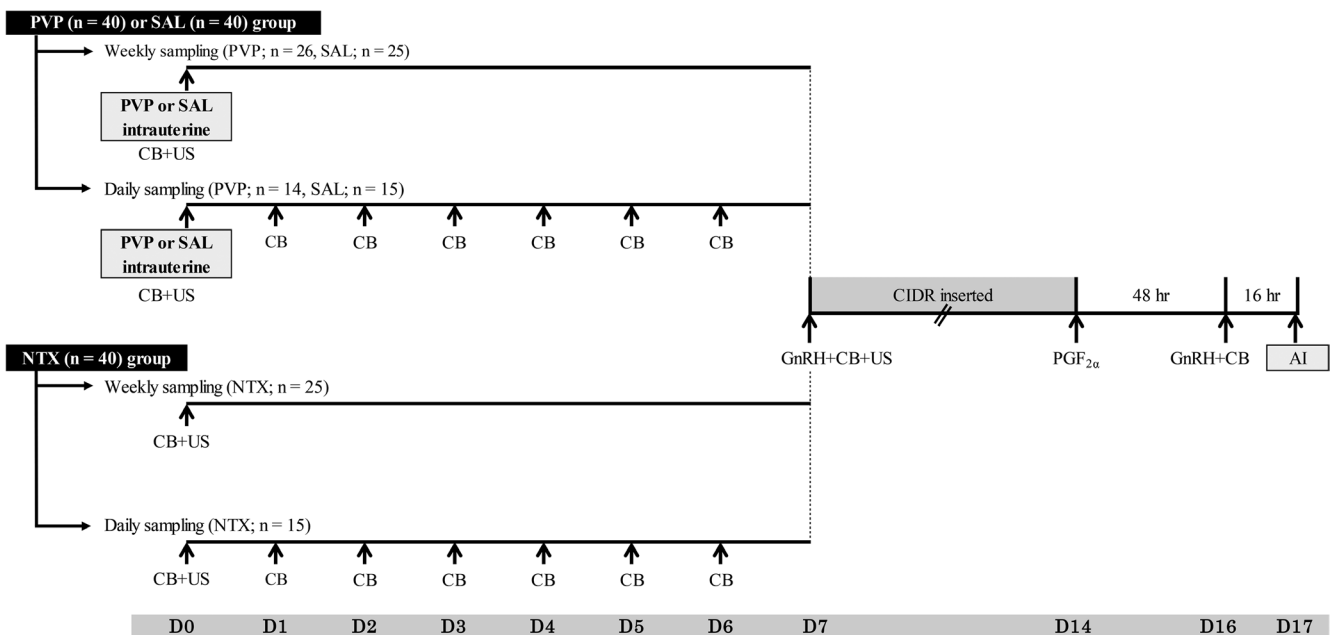


Fig. 4. Schematic diagram of a timed AI protocol consisting of intrauterine infusion of PVP-I followed by CIDR insertion for 7 days in lactating dairy cows (Reproduced with permission of the Japanese Society of Veterinary Science from Yoshida *et al.* J Vet Med Sci 82: 926–934, 2020 [83]). Animals were assigned to receive intrauterine infusion of povidone-iodine (PVP-I) or physiological saline (SAL) at 5 weeks \pm 3 days postpartum (D0), followed by a subsequent CIDR-synch timed artificial insemination (AI) protocol 7 days later. PVP, Intrauterine infusion of 2% PVP-I; SAL, Intrauterine infusion of SAL; NTX, No treatment; PGF_{2α} (prostaglandin F_{2α}), 500 μ g cloprostenol; CIDR, Controlled internal drug release insert containing 1.9 g progesterone; GnRH (gonadotrophin-releasing hormone), 100 μ g fertirelin acetate; CB, Cytobrush; US, Ultrasonography of the ovaries and uterus.

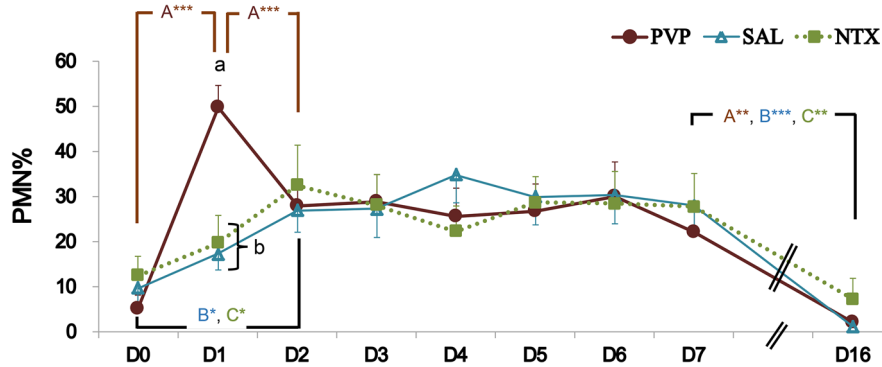


Fig. 5. Changes in the percentage of polymorphonuclear neutrophils (PMN%). (Reproduced with permission of the Japanese Society of Veterinary Science from Yoshida *et al.* *J Vet Med Sci* 82: 926–934, 2020 [83]). PMN% in endometrial samples collected for 8 consecutive days after intrauterine infusion of povidone-iodine (PVP-I) (PVP: n = 14), physiological saline (SAL: n = 15), or no treatment (NTX: n = 15) in postpartum dairy cows. Letters A, B, and C reflect the differences between days in the PVP, SAL, and NTX groups, respectively. a–b: Different letters indicate differences between groups on D1 ($P < 0.001$); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

after PVP-I infusion into the uterus.

Recently, a high prevalence of multi-antimicrobial (including third-generation cephalosporin)-resistant *E. coli* in the uterine lumen of cows with metritis and/or endometritis has been reported [84, 85]. Likewise, *T. pyogenes* isolates derived from postpartum dairy cows with metritis harbored a high prevalence of several different antibiotics [86]. Cephalosporin is the treatment of choice for bovine uterine diseases in many parts of the world. Antibiotic resistance of microbes surely affects the clinical outcome of treatment with cephalosporin, and therefore, may warrant an alternative treatment [85]. Antibiotic resistance has become a serious concern for both animal and human health. Therefore, as an alternative to antibiotics, PVP-I should shed light on endometritis therapy in cows, although it has been available for a long time.

Conclusions

A portion of the pathophysiology and some of the predisposing factors for bovine endometritis have been clarified through the cytobrush technique and the development of therapeutic protocols during the early postpartum period. Prepartum management to prevent glucose deficiency, prompt diagnosis to identify causative agents and levels of intrauterine inflammation, and appropriate treatment to minimize the emergence of antimicrobial resistance should be beneficial for tackling endometritis and improving reproductive performance and productivity in bovine herds.

Conflict of interests: The authors declare no conflicts of interest associated with this manuscript.

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