The Journal of Veterinary Medical Science

NOTE

Theriogenology

Profile of uterine flush lipid mediators in cows with subclinical endometritis: pilot study

Toko MAEHARA[1,2], Takeshi OSAWA[3], Go KITAHARA[3], Hiroshi SATOH[1], Takahisa MURATA[2,4,5]*

[1]Cooperative Veterinary Pharmacology and Toxicology, Cooperative Department of Veterinary Medicine Faculty of Agriculture, Iwate University, Iwate, Japan
[2]Animal Radiology, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan
[3]Theriogenology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[4]Veterinary Pharmacology, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan
[5]Food and Animal Systemics, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan

J Vet Med Sci
86(5): 518–523, 2024
doi: 10.1292/jvms.23-0450

Received: 30 October 2023
Accepted: 8 March 2024
Advanced Epub:
25 March 2024

ABSTRACT. Subclinical endometritis affects reproductive outcomes and causes economic losses in dairy cows, thus, it is important to understand disease progression mechanisms and develop diagnostic procedures for better disease management. We measured the levels of 146 lipid mediators in uterine flush samples using lipid chromatography-mass spectrometry. We detected 25 lipid mediators in the uterine flush of both the control and subclinical endometritis cows; 15 of the 25 lipid mediators were AA-derived metabolites. Among the AA-derived metabolites, cyclooxygenase (COX)-generated mediators were the most abundant. Specifically, levels of 11β-13,14-dihydro-15-keto prostaglandin (PG) $F_{2\alpha}$, PGE_2, PGA_2, 13-hydroxyoctadecadienoic acid, and PGD_1 were elevated in all the cows with subclinical endometritis. This study may provide new insights for the management of subclinical bovine endometritis.

KEYWORDS: cattle, lipid mediator, subclinical endometritis, uterine

Endometritis is an inflammatory disease that affects the functional lining of the endometrium and is a major cause of reproductive disorders and economic loss, with an estimated incidence of 35–50% [5, 26]. Although the pathogenesis of endometritis is not fully understood, impaired uterine immunity is a common factor in high-producing dairy cows. The epithelium of the uterus becomes damaged during calving, which can promote inflammation and increase susceptibility to bacterial infections [27]. Approximately 80–100% of cows have bacterial infections in their uterus within the first two weeks after calving. Immune responses usually restore damage and prevent further infections; however, prolonged damage or additional infections can lead to endometritis. There are two types of endometritis: clinical and subclinical endometritis.

Clinical endometritis is characterized by a foul-smelling and purulent vaginal discharge originating in the uterus. Clinical endometritis is diagnosed when there is purulent uterine discharge, a cervix with a diameter of 7.5 cm 20 days postpartum, or discharge containing more than 50% white mucopurulent materials in the vagina 26 days postpartum [18]. In contrast, subclinical endometritis does not exhibit these symptoms, which delays its treatment. However, subclinical endometritis also results in low milk production and reproductive performance; therefore, early diagnosis and treatment are crucial. Many reports have shown that cows with >5% polymorphonuclear cells (PMN) between 21 and 62 days postpartum have subclinical endometritis [10, 11, 19].

Polyunsaturated fatty acids (PUFAs) are bioactive lipid mediators produced from essential fatty acids. PUFAs can be divided into two families: omega-3 and omega-6. Omega-3 PUFAs include docosahexaenoic acid (DHA), eicosatetraenoic acid (EPA), and arachidonoyl ethanolamine (AEA), while omega-6 PUFAs include arachidonic acid (AA) and dihomo-γ-linolenic acid (DGLA). PUFAs can be metabolized into various lipid mediators through the activation of cyclooxygenase (COX), lipoxygenases (LOX), and cytochrome P450 (CYP450), or through non-enzymatic oxidation. PUFAs play crucial roles in various physiological processes.

*Correspondence to: Murata T: amurata@mail.ecc.u-tokyo.ac.jp, Animal Radiology, Graduate School of Agricultural and Life Science, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

Previous studies have shown that the production levels of lipid mediators varies depending on the type and stage of disease. For example, urinary prostaglandin (PG) $F_{2\alpha}$ and its metabolites are elevated in feline idiopathic cystitis in cats [30], and some arachidonic acid-derived lipid mediators in clinical mastitic milk are increased compared to health controls [12].

Inflammatory uterine diseases, such as metritis and endometritis, contribute to poor reproductive performance in cows. Therefore, early diagnosis and appropriate treatment are crucial. Various methods are used to diagnose endometritis, including visual assessment of cervical and vaginal discharge, ultrasonography, vaginal examination, counting the population of PMNs in uterine flush, and bacterial culture of uterine swabs [5, 21, 31]. However, subclinical endometritis is often overlooked because it shows no clinical symptoms, and diagnostic methods are often neither common nor accurate [18]. Additionally, the pathogenesis of endometritis is not fully understood, which contributes to delays in diagnosis and treatment. Thus, the development of new diagnostic methods for endometritis and understanding its pathogenesis are crucial. We hypothesized that the production of pro-inflammatory mediators, such as PGs and thromboxane, and their metabolites is increased in the uterine flush of patients with subclinical endometritis compared to that in normal uterine status. Conversely, levels of anti-inflammatory mediators, such as Lipoxin A4 and Resolvin D1, are decreased in the uterine flush of endometritis compared to those with normal uterine status. In this study, we investigated the lipid profile of uterine flush samples collected from dairy cows with subclinical endometritis using liquid chromatography-mass spectrometry (LC-MS/MS).

All experiments and animal care were conducted in accordance with the institutional guidelines of the Animal Care Committee of the University of Tokyo and University of Miyazaki. Holstein cows were housed at the Miyazaki Prefecture Livestock Public Corporation and Field Science Center at the university. Uterine flush samples were collected from 3–13 weeks postpartum, specifically in September and October, 2017. Twelve cows were used in the study and were divided into two groups: normal uterine status (shown as a control) (n=5) and subclinical endometritis (n=7). To determine which cows had subclinical endometritis, diagnostic criteria were used according to previous studies [6].

Individuals with PMNs were more than 18% at 3–4 weeks postpartum, equal to or more than 6% at 5 weeks postpartum, and 4% at 8 weeks postpartum, indicating subclinical endometritis. Cows with less than 18%, 6%, and 4% PMNs at 3, 5, and 8 weeks postpartum were considered controls. Cows with subclinical endometritis did not exhibit clinical symptoms such as fever or foul-smelling discharge; therefore, we defined these seven samples as subclinical endometritis in this study. The body condition score (BCS) of the cows was estimated from 1 to 5 and rated in 0.25 increments, according to a previous study [7]. However, the BCS of the two animals in the control and subclinical endometritis groups was not recorded because of insufficient data collection (control ID 3 and 4). There was no significant difference in the BCS of the control and subclinical endometritis cows, which ranged between 2.25 and 2.75 (Table 1, P=0.62; t-test was performed using values from samples other than not applicable (NA)). The ovarian status of the individual cows was assessed using ultrasonography (Supplementary Table 1).

Uterine flush samples were collected by intrauterine infusion of sterile saline solution (30 mL) using a sterile plastic infusion pipette, as previously described [11]. Uterine flush samples were collected for cytological assessment and measurement of lipid mediators. Cytological assessments were performed as described previously [33]. PMN% in the uterine flush was calculated as the number of polymorphonuclear neutrophils relative to a total of 300 nucleated cells at ×400 magnification using Diff-Quik (Sysmex Co., Kobe, Japan) stained slides. The uterine flush samples were stored at −80°C until measurement. Samples were centrifuged at 250 × g for 20 min at 4°C and the supernatant was used for analysis. Samples (400 µL) were diluted with distilled water (550 µL) and mixed with internal standard solution (50 µL, Supplementary Table 2). The mixed solution was applied to a solid-phase extraction cartridge (OASIS). After application, the cartridges were washed with distilled water and hexane, and eluted with 100% methanol. Samples were injected into the LC-MS/MS (8030 Shimadzu, Kyoto, Japan), and 146 lipid mediators were measured and analyzed using the LC-MS/MS Method Package version 2 with LabSolutions software (Shimadzu) according to the manufacturer's instructions. Each metabolite was identified on the basis of its retention time and selected reaction-monitoring ion transitions. An internal standard was used for each group. Data are presented as ratios relative to the internal standard. The level of each metabolite was calculated as the peak area of each metabolite per peak area of the internal standard. We calculated the ratio to internal standard as follows: intensity

Table 1. Sample list of individual cows

	ID	Postpartum week	BCS	PMN%
Control	1	7	2.75	0
	2	6	2.25	0.5
	3	4	NA	1.2
	4	13	NA	3.9
	5	8	2.25	2.6
Subclinical endometritis	1	9	2.5	30.0
	2	9	2.5	30.0
	3	11	2.5	6.7
	4	10	2.25	11.0
	5	3	2.75	16.0
	6	3	NA	24.2
	7	4	NA	17.2

NA=not applicable.

of the objective component × (intensity of internal standard substance in standard mixture)/(intensity of internal standard substance in the sample). The non-detectable sample was calculated as 0, according to a previous report [14].

Data are presented as mean ± SEM. Statistical analysis were conducted as follows: first, the Shapiro-Wilk test was conducted to assess whether the distribution was normal. Subsequently, for samples displaying a normal distribution, the F-test was employed to examine equal variances, the Student's *t*-test was used for data with equal variances, and the Kolmogorov–Smirnov test was applied for data with unequal variances. Nonparametric tests were employed for samples that did not exhibit a normal distribution, and comparisons between two groups (Mann-Whitney *U* test) were conducted to detect test values. Statistical significance was set at $P<0.05$. Undetected samples were included in the statistical analyses with a value of 0.

Twenty-five lipid mediators were detected in the uterine flush using LC-MS/MS. Lipid mediators detected in more than 80% of the uterine flushes in both the control and subclinical endometritis samples are shown in Table 2 and Supplementary Fig. 1. PUFAs, AA, AEA, and oleoylethanolamide (OEA) were detected, whereas EPA, linoleic acid (LA), and DGLA were not detected in either the control or subclinical endometritis uterine flush. Although the OEA level ($P=0.034$) was significantly lower in the control uterine flush group than in the subclinical endometritis group, the levels of other PUFAs did not differ between the control and subclinical endometritis groups (Table 2).

Eleven COX- (Supplementary Fig. 1A, shown by big dots) and two LOX- metabolites (Supplementary Fig. 1B, shown by big dots), as well as one CYP metabolite (Supplementary Fig. 1C, shown by big dots) of AA, were detected in both control and subclinical endometritis uterine flushes (summarized data are shown in Table 2). PGD_2 metabolite,11β-13,14-dihydro-15-keto $PGF_{2\alpha}$ and 13, 14-dihydro-15keto-PGD_2 were not detected in control uterine flush but were detected in approximately 85% of cows with subclinical endometritis (Table 2 and Supplementary Fig. 1A). PGD_2, PGE_2, $PGF_{2\alpha}$, and their metabolites were detected both in the fluid of control and subclinical endometritis cows. The levels of PGE_2 and its metabolite PGA_2 were also increased in the subclinical endometritis uterine flush compared to those in the control. 11β-13,14-dihydro-15keto $PGF_{2\alpha}$, PGD_2 metabolite, tended to be higher in subclinical endometritis uterine flushes, compared with control uterine flushes ($P=0.055$).

Two LOX metabolites (Supplementary Fig. 1B, indicated by big dots), one CYP metabolite (Supplementary Fig. 1C, indicated by big dots), and non-enzymatic oxidized products of AA (Supplementary Fig. 1D, indicated by big dots) were detected in both the control and subclinical endometritis uterine flushes (summarized data are shown in Table 2). However, the levels of these lipid mediators did not differ significantly between the two groups (Table 2). One EPA- (Supplementary Fig. 1E shown by big dot), two AEA- (Supplementary Fig. 1F shown by big dot), and two DGLA-(Supplementary Fig. 1H shown by big dot) derived metabolites did not differ between the control and subclinical endometritis uterine flushes (summarized data are shown in Table 2). The level of the LOX metabolite of LA, 13-hydroxyoctadecadienoic acid (HODE), was significantly increased in the uterine flush of cows with subclinical endometritis compared to that in control cows ($P=0.049$) (Table 2 and Supplementary Fig. 1G shown by big dots).

In this study, we detected 25 lipid mediators in the uterine flush of patients with control or subclinical endometritis. The most frequently detected lipid mediators were the 15 derived from AA. Other lipid mediators detected included one EPA-, two AEA-, two LA-, and DGLA-derived metabolites. Levels of the PGD_2 metabolite,11β-13,14-dihydro-15-keto $PGF_{2\alpha}$ tended to be elevated in subclinical endometritis. The level of OEA was significantly higher in uterine flush samples from cows with subclinical endometritis than in those from control cows.

The plasma levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) in cows with uterine infection were higher than in uninfected cows [4]. $PGF_{2\alpha}$ metabolizes to PGFM in the lung [25]. In our study, $PGF_{2\alpha}$, PGE_2, PGD_2, 11β-13,14-dihydro-15keto-$PGF_{2\alpha}$ and 13,14-dihydro-15keto-PGD_2 were detected in the uterine flush, with increased levels observed in cows with subclinical endometritis. However, PGFM and 11,15-dioxo-9α-hydroxy-,2,3,4,5-tetranorprostan-1,20-dioic acid (PGDM) were not detected in the uterine flush. PGFM, 13,14-dihydro-15-keto-PGE_2 (PGEM), and PGDM are the final metabolites of PGs that can be detected in urine and plasma. The detection of prostaglandin metabolites in urine and serum samples may lead to further elucidation of the pathogenesis of endometritis and subsequently, to the development of more sensitive diagnostic methods.

PMNs are a diagnostic criterion for endometritis. In this research, the diagnostic criteria for subclinical endometritis were PMNs equal to or more than 6% after 5 weeks postpartum and 4% after 8 weeks postpartum. Cows with subclinical endometritis showed no clinical signs, such as fever, foul-smelling, or purulent discharge in the vagina. A previous report indicated that the production of $PGF_{2\alpha}$, PGE_2, and LTB_4 was elevated in endometrial cells isolated from cows with clinical endometritis compared to controls [2]. They also indicated that the production of $PGF_{2\alpha}$ and PGE_2, but not LTB_4, was significantly increased in endometrial cells of subclinical endometritis with a high number of PMNs (>18%) compared to a low number of PMNs (<18%). The levels of PGs and TXs are often increased at inflammatory sites, and these mediators are used as inflammatory biomarkers. Pro-inflammatory mediators such as the PGD_2 metabolite 11β-13,14-dihydro-15keto, PGE_2, and its metabolite PGA_2 were increased in uterine flush with subclinical endometritis, which suggests the presence of endometrial inflammation.

13-HODE is derived from LA. HODE generation increases under conditions of increased oxidative stress such as atherosclerosis and diabetes [22, 29]. Reactive oxygen species (ROS) are thought to play a role in the development of endometritis. 13-HODE promotes chemotactic activity in isolated bovine PMNs [13] and the expression of cell adhesion molecules [9]. Subclinical endometritis promotes oxidative stress and 13-HODE production. 13-HODE may be one of the factors that exacerbate subclinical endometritis by increasing PMNs activation and expression of adhesion molecules. In this study, we did not measure the concentration of 13-HODE or focus on the ROS. Further investigation of the role of 13-HODE in endometritis is required to better understand the pathogenesis of this condition. OEA is a known ligand of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α). Several studies have indicated that OEA exerts anti-inflammatory effects in various animal models. Administration of OEA has been shown to reduce inflammation in lipopolysaccharide-induced macrophage activation of asthma using animal models, and obesity in humans [17, 23,

Table 2. Lipid profile of endometritis and control cows and lipid mediators detected in more than 80% of uterine flush in either control or endometritis

Substrate enzyme		Lipid mediator	Normal (N=5)	Endometritis (N=7)	*P* value
			Ratio to Internal Standard		
			Detection rate (%)		
AA			0.030 ± 0.054	0.23 ± 0.30	0.20
			100	100	
	COX				
		6-keto-$PGF_{1\alpha}$	0.032 ± 0.029	0.034 ± 0.022	0.92
			100	100	
		6,15-diketo-13, 14-dihydro-$PGF_{1\alpha}$	0.0035 ± 0.0048	0.0013 ± 0.0012	0.87
			80	100	
		11β-13,14-dihydro-15-keto $PGF_{2\alpha}$	0	2.56 ± 2.12	0.055
			0	85.7	
		11-HETE	0.42 ± 0.54	0.85 ± 1.08	0.43
			80	85.7	
		13,14-dihydro-15keto-PGD_2	0	2.44 ± 4.73	0.012
			0	85.7	
		20-hydroxy-$PGF_{2\alpha}$	0.0027 ± 0.0038	0.0024 ± 0.0025	0.93
			100	85.7	
		PGA_2	0.024 ± 0.049	17.02 ± 36.82	0.017
			40	100	
		PGD_2	0.16 ± 0.18	84.3 ± 204.7	0.20
			100	100	
		PGE_2	0.050 ± 0.064	1.26 ± 1.79	0.03
			100	100	
		$PGF_{2\alpha}$	0.79 ± 1.63	1.96 ± 3.55	0.51
			80	85.7	
		TXB_2	0.060 ± 0.077	0.52 ± 0.53	0.167
			80	85.7	
AA	LOX				
		12-HETE	0.78 ± 1.17	0.94 ± 1.17	0.81
			80	85.7	
		15-HETE	0.093 ± 0.107	0.157 ± 0.187	0.51
			80	85.7	
	CYP				
		20-HETE	0.050 ± 0.072	0.51 ± 0.73	0.20
			40	100	
	Non-OX				
		8-iso-PGE_2	0.34 ± 0.35	8.09 ± 17.5	0.35
			100	100	
EPA	LOX				
		12-HEPE	0.10 ± 0.13	0.48 ± 0.60	0.13
			80	85.7	
AEA			1.63 ± 3.23	1.91 ± 2.54	0.50
			60	100	
		PGE_2-EA	0.0044 ± 0.0062	0.0046 ± 0.0043	0.97
			60	100	
		$PGF_{2\alpha}$-EA	0.010 ± 0.012	0.011 ± 0.0098	0.86
			100	100	
OEA			0.070 ± 0.10	0.23 ± 0.097	0.034
			100	100	
LA	LOX				
		9-HODE	0.0078 ± 0.018	0.31 ± 0.31	0.093
			40	100	
		13-HODE	0.095 ± 0.15	0.50 ± 0.42	0.049
			100	100	
DGLA	COX				
		PGD_1	0.0080 ± 0.017	4.95 ± 11.1	0.017
			20	100	
		PGE_1	0.059 ± 0.096	3.22 ± 8.1	0.46
			100	100	

AA, arachidonic acid; AEA, arachidonoyl ethanolamine; COX, cyclooxygenase; CYP, cytochrome; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; EPA, eicosatetraenoic acid; HEPE, hydroxyicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LOX, lipoxygenases; OEA, oleoylethanolamide; PG, prostaglandin.

24, 32]. There are reports that PGD_1 suppresses vascular permeability and atopic dermatitis [5, 8], and PGD_1 activates Peroxisome Proliferator-Activated Receptors (PPAR)-γ [34]. PPAR-γ is expressed on the bovine endometrial tissues [28] and activation of PPAR-γ with agonist decreases endometrial stromal cells-derived production of inflammatory cytokines. Surprisingly, the level of OEA and PGD_1 was significantly higher in the uterine flush of cows with subclinical endometritis than in control cows. It is possible that the production of OEA and PGD_1 may inhibit the further exacerbation of inflammation. Elucidation of the functions of OEA and PGD_1 in subclinical endometritis may lead to a better understanding of its pathogenesis. There are several methods for estrus detection in cows, such as monitoring behavior, body temperature, and smell in the perineal region [1, 15, 20]. Proper estrus detection can improve conception rates. $PGF_{2\alpha}$ regulates development and function of the corpus luteum, and PGE_2 is increased during the late-luteal phase followed by a decrease during regression [3]. The production levels of lipid mediators such as 15-KETE and oxylipin in the corpus luteum of cows are altered as the estrous cycle progressed [16]. These studies indicate that PUFAs are involved in reproduction. Profiling the kinetics of PUFAs may help understand not only uterine diseases but also the estrus, reproduction, and physiology of cows.

This study has some limitations. First, the samples were only collected in Miyazaki, one of the 47 prefectures of Japan. The production and metabolism of lipid mediators depend on several factors, such as diet, stress, and breeding environment; therefore, samples should be obtained from a variety of regions and seasons. Second, sampling was conducted at 4–13 weeks in this study. Adjusting the sampling timing, such as weeks postpartum, may reveal more significant differences. Nevertheless, our data indicated that the levels of pro-inflammatory mediators such as PGD_2 metabolite 11β-13,14-dihydro-15-keto $PGF_{2\alpha}$, PGE_2 and its metabolite PGA_2 and LA-metabolite LOX-derived 13-HODE, and anti-inflammatory mediators such as DGLA-metabolite COX-derived PGD_1 and OEA were elevated in subclinical endometritis. This pilot study may provide new insights into the future management of bovine subclinical endometritis.

CONFLICT OF INTEREST. Authors declare no Conflict of Interests for this article.

ACKNOWLEDGMENTS. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (20H05678 to T.M.), an Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) from the Japan Science and Technology Agency (JST, JPMJTR22UF), the Terumo Life Science Foundation, the Research Support Program of SEKISUI CHEMICAL Co., Ltd., and the Tojuro Iijima Foundation for Food Science and Technology (to T.M.). and the Ito Foundation to TO.

REFERENCES

1. Ali AS, Jacinto JGP, Mönchemyer W, Walte A, Gentile A, Formigoni A, Mammi LME, Csaba Bajcsy Á, Abdu MS, Kamel MM, Ghallab ARM. 2022. Estrus detection in a dairy herd using an electronic nose by direct sampling on the perineal region. *Vet Sci* **9**: 9. [Medline]
2. Barański W, Łukasik K, Skarżyński D, Sztachańska M, Zduńczyk S, Janowski T. 2013. Secretion of prostaglandins and leukotrienes by endometrial cells in cows with subclinical and clinical endometritis. *Theriogenology* **80**: 766–772. [Medline] [CrossRef]
3. Berisha B, Schams D, Rodler D, Sinowatz F, Pfaffl MW. 2018. Changes in the expression of prostaglandin family members in bovine corpus luteum during the estrous cycle and pregnancy. *Mol Reprod Dev* **85**: 622–634. [Medline] [CrossRef]
4. Del Vecchio RP, Matsas DJ, Fortin S, Sponenberg DP, Lewis GS. 1994. Spontaneous uterine infections are associated with elevated prostaglandin F_2alpha metabolite concentrations in postpartum dairy cows. *Theriogenology* **41**: 413–421. [Medline] [CrossRef]
5. Denis-Robichaud J, Dubuc J. 2015. Determination of optimal diagnostic criteria for purulent vaginal discharge and cytological endometritis in dairy cows. *J Dairy Sci* **98**: 6848–6855. [Medline] [CrossRef]
6. Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ. 2010. Definitions and diagnosis of postpartum endometritis in dairy cows. *J Dairy Sci* **93**: 5225–5233. [Medline] [CrossRef]
7. Ferguson JD, Galligan DT, Thomsen N. 1994. Principal descriptors of body condition score in Holstein cows. *J Dairy Sci* **77**: 2695–2703. [Medline] [CrossRef]
8. Flower RJ, Kingston WP. 1975. Proceedings: Prostaglandin D1 inhibits the increase in vascular permeability in rat skin produced by prostaglandin E1, E2 and D2. *Br J Pharmacol* **55**: 239P–240P. [Medline]
9. Friedrichs B, Toborek M, Hennig B, Heinevetter L, Müller C, Brigelius-Flohé R. 1999. 13-HPODE and 13-HODE modulate cytokine-induced expression of endothelial cell adhesion molecules differently. *Biofactors* **9**: 61–72. [Medline] [CrossRef]
10. Gabler C, Drillich M, Fischer C, Holder C, Heuwieser W, Einspanier R. 2009. Endometrial expression of selected transcripts involved in prostaglandin synthesis in cows with endometritis. *Theriogenology* **71**: 993–1004. [Medline] [CrossRef]
11. Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* **64**: 1879–1888. [Medline] [CrossRef]
12. Hayashi A, Fujii S, Nakamura T, Kobayashi K, Sakatani M, Endo M, Takahashi T, Murata T. 2019. Production of lipid mediators in mastitic milk of cow. *Anim Sci J* **90**: 999–1007. [Medline] [CrossRef]
13. Henricks PA, Engels F, van der Vliet H, Nijkamp FP. 1991. 9- and 13-hydroxy-linoleic acid possess chemotactic activity for bovine and human polymorphonuclear leukocytes. *Prostaglandins* **41**: 21–27. [Medline] [CrossRef]
14. Herbers J, Miller R, Walther A, Schindler L, Schmidt K, Gao W, Rupprecht F. 2021. How to deal with non-detectable and outlying values in biomarker research: Best practices and recommendations for univariate imputation approaches. *Compr Psychoneuroendocrinol* **7**: 100052. [Medline] [CrossRef]
15. Higaki S, Darhan H, Suzuki C, Suda T, Sakurai R, Yoshioka K. 2021. An attempt at estrus detection in cattle by continuous measurements of ventral tail base surface temperature with supervised machine learning. *J Reprod Dev* **67**: 67–71. [Medline] [CrossRef]
16. Hughes CHK, Bosviel R, Newman JW, Pate JL. 2019. Luteal lipids regulate progesterone production and may modulate immune cell function during the estrous cycle and pregnancy. *Front Endocrinol (Lausanne)* **10**: 662. [Medline] [CrossRef]
17. Kwon EK, Choi Y, Yoon IH, Won HK, Sim S, Lee HR, Kim HS, Ye YM, Shin YS, Park HS, Ban GY. 2021. Oleoylethanolamide induces eosinophilic airway inflammation in bronchial asthma. *Exp Mol Med* **53**: 1036–1045. [Medline] [CrossRef]

18. LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J Dairy Sci* **85**: 2223–2236. [Medline] [CrossRef]
19. Madoz LV, Giuliodori MJ, Jaureguiberry M, Plöntzke J, Drillich M, de la Sota RL. 2013. The relationship between endometrial cytology during estrous cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows. *J Dairy Sci* **96**: 4333–4339. [Medline] [CrossRef]
20. Marquez HJ, Ambrose DJ, Schaefer AL, Cook NJ, Bench CJ. 2021. Evaluation of infrared thermography combined with behavioral biometrics for estrus detection in naturally cycling dairy cows. *Animal* **15**: 100205. [Medline] [CrossRef]
21. Nazhat SA, Kitahara G, Kozuka N, Mido S, Sadawy M, Ali HE, Osawa T. 2018. Associations of periparturient plasma biochemical parameters, endometrial leukocyte esterase and myeloperoxidase, and bacterial detection with clinical and subclinical endometritis in postpartum dairy cows. *J Vet Med Sci* **80**: 302–310. [Medline] [CrossRef]
22. Nocella C, Cammisotto V, Fianchini L, D'Amico A, Novo M, Castellani V, Stefanini L, Violi F, Carnevale R. 2018. Extra virgin olive oil and cardiovascular diseases: benefits for human health. *Endocr Metab Immune Disord Drug Targets* **18**: 4–13. [Medline] [CrossRef]
23. Overton HA, Babbs AJ, Doel SM, Fyfe MC, Gardner LS, Griffin G, Jackson HC, Procter MJ, Rasamison CM, Tang-Christensen M, Widdowson PS, Williams GM, Reynet C. 2006. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab* **3**: 167–175. [Medline] [CrossRef]
24. Payahoo L, Khajebishak Y, Asghari Jafarabadi M, Ostadrahimi A. 2018. Oleoylethanolamide supplementation reduces inflammation and oxidative stress in obese people: a clinical trial. *Adv Pharm Bull* **8**: 479–487. [Medline] [CrossRef]
25. Piper PJ, Vane JR, Wyllie JH. 1970. Inactivation of prostaglandins by the lungs. *Nature* **225**: 600–604. [Medline] [CrossRef]
26. Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, Ayres H, Marsola RS, Martinez N, Thatcher WW, Santos JE. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. *J Dairy Sci* **96**: 5682–5697. [Medline] [CrossRef]
27. Sheldon IM, Williams EJ, Miller AN, Nash DM, Herath S. 2008. Uterine diseases in cattle after parturition. *Vet J* **176**: 115–121. [Medline] [CrossRef]
28. Socha BM, Łupicka M, Szczepańska AA, Korzekwa AJ. 2017. PPAR expression throughout the oestrous cycle in the bovine endometrium. *Theriogenology* **100**: 88–94. [Medline] [CrossRef]
29. Szczuko M, Kotlęga D, Palma J, Zembroń-Łacny A, Tylutka A, Gołąb-Janowska M, Drozd A. 2020. Lipoxins, RevD1 and 9, 13 HODE as the most important derivatives after an early incident of ischemic stroke. *Sci Rep* **10**: 12849. [Medline] [CrossRef]
30. Takenouchi S, Kobayashi Y, Shinozaki T, Kobayashi K, Nakamura T, Yonezawa T, Murata T. 2022. The urinary lipid profile in cats with idiopathic cystitis. *J Vet Med Sci* **84**: 689–693. [Medline] [CrossRef]
31. Williams EJ, Fischer DP, Pfeiffer DU, England GC, Noakes DE, Dobson H, Sheldon IM. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* **63**: 102–117. [Medline] [CrossRef]
32. Yang L, Guo H, Li Y, Meng X, Yan L, Dan Zhang, Wu S, Zhou H, Peng L, Xie Q, Jin X. 2016. Oleoylethanolamide exerts anti-inflammatory effects on LPS-induced THP-1 cells by enhancing PPARα signaling and inhibiting the NF-κB and ERK1/2/AP-1/STAT3 pathways. *Sci Rep* **6**: 34611. [Medline] [CrossRef]
33. Yoshida R, Kitahara G, Osawa T. 2020. Intrauterine infusion of povidone-iodine: Its effect on the endometrium and subsequent fertility in postpartum dairy cows. *J Vet Med Sci* **82**: 926–934. [Medline] [CrossRef]
34. Yu K, Bayona W, Kallen CB, Harding HP, Ravera CP, McMahon G, Brown M, Lazar MA. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J Biol Chem* **270**: 23975–23983. [Medline] [CrossRef]