NOTE

Theriogenology

Changes in the C-reactive protein and 13,14-dihydro-15-keto-prostaglandin F$_{2\alpha}$ concentrations of uterine lavage samples after the administration of povidone-iodine in cows

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ABSTRACT. Changes in the C-reactive protein (CRP) and 13,14-dihydro-15-keto-prostaglandin F$_{2\alpha}$ (PGFM) concentrations of uterine lavage fluid were examined in cows given an intrauterine povidone-iodine (PI) infusion. The mean polymorphonuclear leukocyte (PMN) ratios (the ratio of PMN to total cells) and CRP concentration of uterine lavage fluid on the day after the treatment were significantly ($P<0.05$) greater in the PI infusion group (PMN: 53.0 ± 32.7%, CRP: 50.2 ± 32.3 ng/mL) than in the non-treatment control group (PMN: 7.9 ± 21.9%, CRP: 17.2 ± 5.9 ng/mL), whereas there was no significant difference in the mean PGFM concentration between the two groups. The present findings suggest that the uterine CRP level is a useful biomarker of local uterine inflammation in cows.

KEYWORDS: bovine uterine lavage, C-reactive protein, inflammation, 13,14-dihydro-15-keto-prostaglandin F$_{2\alpha}$, povidone-iodine

The intrauterine administration of povidone-iodine (PI) is an effective treatment for clinical/subclinical endometritis [5, 8] and repeat breeding syndrome in cows [1]. Previous studies have shown that the intrauterine infusion of iodine in the early luteal phase induced early luteal regression and shortened the length of the inter-estrus intervals in cycling cows [7, 9]. However, intrauterine PI treatment may cause necrotizing endometritis and the release of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) in cows [4, 10], suggesting that such iodine treatment induces inflammatory responses by endometrial cells and that PGF$_{2\alpha}$ is synthesized and released at some point during endometrial recovery. It is also known that peripheral measurements of 13,14-dihydro-15-keto-prostaglandin F$_{2\alpha}$ (PGFM), a metabolite of PGF$_{2\alpha}$, provide an accurate measure of endometrial PGF$_{2\alpha}$ secretion [11, 12].

The uterine lavage technique is often used to monitor large sections of the intrauterine environment in the veterinary clinical field. Recently, we reported for the first time that C-reactive protein (CRP), which is generally used as a systemic inflammatory biomarker, was present in uterine lavage samples from cows [14]. The uterine lavage fluid concentration of CRP decreased in parallel with the frequency of polymorphonuclear leukocytes (PMN), but not with changes in plasma CRP levels, from 3 to 6 weeks postpartum in dairy cows. These findings imply that uterine lavage fluid contains local biochemical factors that may be useful for evaluating uterine status, and that CRP of uterine lavage samples provides an index for diagnosing inflammatory status from the biochemical point of view, in addition to the cytological examination by PMN measurement in cows.

The present study was undertaken to investigate the effects of endometrial inflammation induced by intrauterine PI infusions on the concentrations of CRP and PGFM in uterine lavage samples collected from cows. A previous study showed a relationship between the PGFM concentrations in uterine fluid and endometritis in postpartum dairy cows. In that study, the uterine concentration of PGFM decreased, with the sharpest decline seen until 1–2 weeks postpartum [6]. However, to the best of our knowledge, no previous studies have determined the levels of PGFM in uterine lavage samples from cows. This study examined the usefulness of measuring CRP as a local biomarker for detecting endometrial inflammation and the response of uterine lavage PGFM levels to uterine irritation by PI.

This study was carried out between January and June 2019. In this study, the effects of PI-I on uterus were evaluated under the
condition of the luteal phase in parous cows. To collect data from the non-pregnant parous cows at a wide range of postpartum elapsed time, 14 lactating Holstein cows, which were maintained at commercial dairy farms in Chiba Prefecture, Japan, and 2 non-lactating Holstein cows, which were maintained at the dairy farm of Tokyo University of Agriculture and Technology in Tokyo, Japan, were used. After the latest parturition, it had been clinically confirmed that none of the cows had any general reproductive problems. All procedures were approved by the Committee for the Use and Care of Animals at Tokyo University of Agriculture and Technology (#30-126).

The 14 lactating cows were randomly assigned to the PI infusion (PI-I, 2−5 years of age, 39−68 days postpartum) or control (CONT, 2−8 years of age, 54−91 days postpartum) group, and the 2 non-lactating cows (8 and 10 years of age, more than a year postpartum) were assigned to both the PI-I and CONT groups; i.e., they were utilized twice. Two non-lactating cows were utilized first in the PI-I group and then in the CONT group, respectively. They were kept untreated for one estrous cycle and were confirmed normal length of the estrous cycle and clinical normality of the genital tract by a vaginal examination and transrectal palpation before they were assigned to the CONT group. All cows were confirmed to have functional corpus lutea in their ovaries at the time of the uterine lavage procedure. The functional corpus luteum was defined by transrectal palpation as a distinct firm mass having an ovulation papilla or crown protruding from the surface within the ovarian stroma. The PI-I group received 50 mL of 2% PI (Iwaki Seiyaku Co., Ltd., Tokyo, Meiji Seika Co., Ltd., Tokyo, Japan), which was administered into the uterine body through an intruterine injector for cows. On the day after the PI infusion, the uterus was lavaged using a balloon catheter (Nipro, Osaka, Japan) by infusing 50 mL of sterilized saline, as described previously [14]. Briefly, the uterus was massaged after the infusion of saline and was then retracted so that the fluid (37.6 ± 6.2 mL) could be collected in a centrifuge tube without negative pressure aspiration. The CONT group only underwent uterine lavage, which was conducted in the same way as in the PI-I group, after the confirmation of the presence of a functional corpus luteum. The lavage samples were centrifuged at 760 × g for 5 min; the supernatant was removed and stored at −20°C until it was subjected to the CRP and PGFM assays; and the remaining pellet, which was used for the cytological examination, was aspirated and smeared onto a glass slide and allowed to air-dry. All slides were fixed and stained using Diff-Quick (Sysmex International Reagents Co., Ltd., Kobe, Japan). Cytological assessments were performed by examining 100 cells with ×10 and ×40 objective lenses to determine the ratio of PMN to total cells, including white blood cells and epithelial cells, (the PMN ratio) using previously reported procedures [3].

The concentrations of CRP and PGFM in the uterine lavage samples were quantified using duplicate aliquots and enzyme immunoassay (EIA) kits for CRP (C-reactive protein ELISA kit for bovine, Cloud-Clone Corp., Katy, TX, USA) and PGFM (13,14-dihydro-15-keto-PGF2α EIA kit, Arbor Assays, Ann Arbor, MI, USA). The assay sensitivity and intra-assay coefficient of variation were 2.4 ng/mL and 3.3% for CRP, respectively, and were 46.2 pg/mL and 2.9% for PGFM, respectively. Both assays were conducted according to the manufacturer’s instructions.

Data are expressed as the mean ± SD and analyzed using the KaleidaGraph™ 5.0 (Synergy Software, Reading, PA, USA). The Mann-Whitney U-test was used to detect significant differences between mean values. The correlation between PMN and CRP was analyzed by Pearson’s correlation coefficient. P-values of <0.05 were considered to indicate statistically significant differences.

The PMN ratios and CRP and PGFM concentrations of the uterine lavage samples are shown in Table 1. The mean PMN ratio was significantly greater in the PI-I group than in the CONT group. The mean CRP concentration of uterine lavage fluid was significantly higher in the PI-I group (50.2 ± 32.3 ng/mL) than in the CONT group (17.2 ± 5.9 ng/mL). The uterine lavage fluid PGFM concentrations showed marked inter-individual variation in both groups (range: 230.8–3,637.6 pg/mL for CONT and 75.6–4,020.1 pg/mL for PI-I), and there was no significant difference between the mean values for the two groups. The correlation between the uterine lavage fluid CRP concentration and the PMN ratio is depicted in Fig. 1. There was no significant correlation between the CRP concentration of uterine lavage fluid and the PMN ratio (r=−0.249).

Table 1. The effects of intrauterine povidone-iodine infusion on the polymorphonuclear leukocyte ratio and C-reactive protein and 13,14-dihydro-15-keto-prostaglandin F2α levels in uterine lavage samples

| Group | PMN (%) | CRP (ng/mL) | PGFM (pg/mL) |
|-------|---------|-------------|--------------|
| CONT  | 7.9 ± 21.9 | 17.2 ± 5.9 | 1,424.9 ± 1,178.9 |
| (n=9) | (0−62) | (6.8−27.0) | (230.8−3,637.6) |
| PI-I  | 53.0 ± 32.7* | 50.2 ± 32.3* | 1,516.8 ± 1,605.9 |
| (n=9) | (12.4−120.0) | (75.6−4,020.1) | |

Mean ± SD. *, P<0.05 vs. CONT. 1) Range of values. CONT: control group, PI-I: povidone-iodine infusion group, PMN: polymorphonuclear leukocyte ratio, CRP: C-reactive protein, PGFM: 13,14-dihydro-15-keto-prostaglandin F2α.

Fig. 1. Associations between intrauterine C-reactive protein (CRP) concentrations and polymorphonuclear leukocyte ratios (PMN) in the povidone-iodine infusion (●) and the non-treatment (○) groups.
Previous studies showed that the histological appearance of the endometrium had markedly changed by 24 hr after the intrauterine infusion of iodine solution [9, 10]. Destructive damage to the surface epithelium as well as edema, vacuolization, and cellular destruction of the adjacent endometrium were observed in cows given 2% Lugol’s solution during the estrous cycle [9]. Considering these findings, it is likely that the increase in the PMN ratio seen on the day after the PI infusion in the present study was due to endometrial inflammation induced by the iodine solution. Our previous study showed that uterine lavage fluid CRP levels decreased during the uterine involution process in postpartum dairy cows [14]. In that study, the CRP concentrations of uterine lavage samples obtained at 3 weeks (presumed to be during uterine involution) and 6 weeks (presumed to be after uterine involution) postpartum were similar to those of the PI-I and CONT groups, respectively, in the present study. The present findings support our previous suggestion that the CRP concentration of uterine lavage fluid could be utilized as a local biomarker for evaluating uterine inflammation in cows. They also suggest that in cows the normal (non-inflammatory) CRP level for uterine lavage samples collected under the current methodological conditions is around 20 ng/mL.

The present results are similar to our previous findings in that there was no significant correlation between the uterine lavage fluid CRP concentration and PMN ratio [14]. The CRP in the peripheral circulation is primarily synthesized by hepatocytes, but it is also synthesized by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes [13]. Inflammatory factors other than inflammatory cells, such as PMN, may also be involved in the production and release of CRP into the uterine lumen from the endometrium, where smooth muscle cells and macrophages are abundant. It is likely that the correlation between the CRP and PMN is influenced by the pathological conditions of uterine inflammation and/or experimental conditions. The combined approach of the cytological (PMN) and biochemical (CRP) examinations may provide a significant indication for diagnosing clinical and subclinical inflammatory diseases of the uterus.

To the best of our knowledge, this is the first study to demonstrate that PGFM can be detected in uterine lavage samples from cows. PGFM is rapidly metabolized to PGFM, and the uterine lavage fluid PGFM level is assumed to reflect PGFM production in the endometrium [12]. When an iodine solution is infused into the uterus during the early luteal phase, transient inflammation occurs in the endometrium within 24 hr, and the lifespan of the cow’s corpus luteum is shortened [7]. However, in the present study, no significant increase in the PGFM concentration of uterine lavage fluid was observed in the PI-I group. A previous study suggested that PGFM production after iodine treatment occurs during endometrial repair [10]. Grunert et al. [2] reported that in cows endometrial repair began 3 to 4 days after the administration of intrauterine iodine solution, and epithelial regeneration was nearly complete by the 5th day. It is suggested that intrauterine PGFM production is not increased at 24 hr after the administration of PI. Alternatively, it should be noted that marked inter-individual variation in uterine lavage fluid PGFM concentrations was seen in both groups. This may have been because of the differences in the stage of the luteal phase, as the temporal relationship between days after ovulation and PI infusion was not determined in this study. It is known that PGFM can be secreted in a pulsatile fashion, and its level alters according to the stage of the estrous cycle [12].

In conclusion, the present study showed that the intrauterine CRP level increased after a PI infusion and supports our previous suggestion that the measurement of CRP levels in uterine lavage samples is a novel clinically useful biochemical examination for detecting uterine inflammation in cows. No effect of the intrauterine administration of PI on PGFM production was seen in the uterine lavage fluid collected the day after treatment, suggesting that CRP is more informative than PGFM as an index for evaluating acute inflammatory status in cows.

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