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FULL PAPER

Theriogenology

# Intrauterine infusion of povidone-iodine: Its effect on the endometrium and subsequent fertility in postpartum dairy cows

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**ABSTRACT.** This study aimed to describe the duration of inflammation after intrauterine infusion of polyvinylpyrrolidone-iodine (povidone-iodine, PVP-I), determine the effect of PVP-I infusion on the subsequent fertility, and evaluate the histopathology of the endometrium in dairy cows. In Experiment 1, 120 lactating clinically healthy Holstein-Friesian cows at 5 weeks postpartum (W5) were equally divided into three groups: intrauterine infusion of 2% PVP-I (PVP), saline (SAL), and no treatment (NTX). Endometrial cytology was performed daily from D0 (W5) to D7 to determine the percentage of polymorphonuclear cells (PMN%) in 44 of the 120 cows. All cows received timed artificial insemination at D17. In Experiment 2, 25 cows were randomly classified into sacrifice at 24 hr or 48 hr after 2% PVP-I infusion (PVP24 and PVP48), and 24, 48, 72, or 96 hr after SAL infusion (SAL24; SAL48; SAL72; SAL96), or no treatment (NTX). Histopathology was performed on the uterus of each cow. In Experiment 1, PMN% was greater in PVP ($P<0.05$) than in SAL and NTX, on D1, but decreased to a level similar to that of the other groups by D2. Conception rate was higher ($P<0.05$) in PVP cows compared to SAL and NTX cows. In Experiment 2, stratified columnar epithelium in the uterus disappeared in PVP24 and SAL24. The epithelium was regenerated in PVP48, SAL72, and SAL96, but not in SAL48. In conclusion, the results of the study suggest that PVP-I induces transient uterine inflammation, promotes regeneration of endometrial epithelial cells and improves fertility.

**KEY WORDS:** cattle, cytology, histopathology, polymorphonuclear neutrophils, povidone-iodine

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Normal uterine involution during the puerperal period is essential for efficient reproductive performance in dairy cows. During the postpartum period, the uterus becomes contaminated with a wide range of bacteria. Uterine involution is usually resolved by 5 weeks postpartum, although it is common that bacteria may still be present in the bovine uterus [29]. Subclinical endometritis is defined as inflammation of the endometrium without purulent or mucopurulent vaginal discharge. The incidence of subclinical endometritis at 3 to 6 weeks postpartum has been reported to be between 12% and 38% [2, 6, 11].

Cytology of the endometrium is an acceptable diagnostic technique used for the detection of subclinical endometritis and polymorphonuclear neutrophils (PMNs) are used to monitor uterine inflammation. The uterine lavage [16] or cytobrush [6, 10] techniques can be used to collect PMNs in order to evaluate endometrial cytology. Cows with an endometrial PMN percentage (PMN%) equal to or greater than 6, at 4–6 weeks postpartum, have lower reproductive performance [2]. The cytobrush technique has been chosen as the reference for cytological diagnostic examination because of its practicability [6, 31].

Bovine practitioners choose intrauterine infusion of polyvinylpyrrolidone-iodine (povidone-iodine, PVP-I) as a common treatment option for endometritis; reproductive performance improves after intrauterine infusion of PVP-I in cows with endometritis [14]. Moreover, bovine practitioners often just assume that cows have subclinical endometritis and infuse PVP-I into a healthy uterus, without making a proper diagnosis in the field. Additionally, some veterinarians administer PVP-I in the uterus as a preventive medication. However, two reports have shown no positive effects associated with intrauterine infusion of PVP-I on subsequent reproductive performances, including the first artificial insemination (AI) conception rate, and pregnancy rate at 180 days postpartum and days open [12, 21]. PVP-I is a broad-spectrum microbicide with potency to inactivate bacteria, fungi, protozoans, and several viruses [13, 28]. PVP-I has clinically been used as a disinfectant for antisepsis of the skin, mucous membranes, and wounds. Unlike antibiotics, and other antiseptic substances, no acquired resistance or cross-resistance has been

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observed after more than 150 years of use [3, 15, 25]. The benefits of using PVP-I include low treatment costs and no milk withdrawal period. A study has shown that uterine infusion of 1% PVP-I caused inflammation of the endometrium in mares [23]. However, the degree and duration of endometrium inflammation, after intrauterine infusion of PVP-I, and the conception rate after subsequent timed AI in healthy cows without clinical endometritis remain unknown.

Here we observed endometrial changes after intrauterine infusion of PVP-I in postpartum cows without clinical endometritis regardless of PMN%. In the present study, two experiments were conducted to address three objectives. Experiment 1 was conducted to (1) investigate the duration of inflammation after intrauterine infusion of PVP-I, and to (2) determine the effect of PVP-I infusion on the subsequent conception rate after timed AI in postpartum dairy cows. Experiment 2 was conducted to (3) evaluate histopathology of the bovine endometrium after intrauterine infusion of PVP-I.

## MATERIAL AND METHODS

### *Experiment 1*

*Animals*: Between February 2015 and December 2016, 120 lactating Holstein-Friesian cows [age: 2.8 ± 1.4 years (mean ± standard deviation; SD)] at 5 weeks postpartum (W5, 35 ± 3 days after parturition), housed in tie stalls at 12 commercial dairy farms in Gunma prefecture, Japan, were studied. All 120 cows had unassisted calving, dystocia, retained placental or clinical endometritis, and no treatment history after calving or during the study period.

All clinical and laboratory protocols received ethical approval from the Institutional Review Board for Animal Experiments at the University of Miyazaki, Japan.

*Experimental design*: The 120 cows were randomly divided into three groups, intrauterine infusion of PVP-I (PVP, n=40), intrauterine infusion of physiological saline (SAL, n=40), and no infusion (no treatment: NTX, n=40). Intrauterine infusions were performed at W5 (Day 0; D0) irrespective of the estrous cycle. Endometrial cytology samples were collected immediately before intrauterine infusion at W5 (D0), D7 and D16 (Weekly sampling: Fig. 1). Cows with ≥6% of PMN on D0 were diagnosed as having subclinical endometritis. Additional endometrial smear samples were collected daily for the first 8 days, from D0 to D7, from a subset of cows (Daily sampling: n=44; PVP, n=14; SAL, n=15; NTX, n=15). The required sample size was calculated based on the pre-planned analysis of these subsets.

*Intrauterine treatment*: Fifty milliliters m*l* of PVP-I (2% Povidone Iodine, Isodine Animal®; DS Pharma Animal Health Co., Ltd., Osaka, Japan) or SAL (Terumo Physiological Saline; Terumo Corp., Tokyo, Japan) were infused into the uterus of PVP and SAL groups, respectively. Before intrauterine infusion, the perineum and vulva were disinfected and dried with paper towels. The intrauterine injector (Intrauterine injector for cow, Nakahara-Type; Fujihira Kogyo Inc., Tokyo, Japan) was inserted through the cervix in order to reach the internal uterine orifice, and a syringe plunger was pushed. A rectal massage was performed immediately

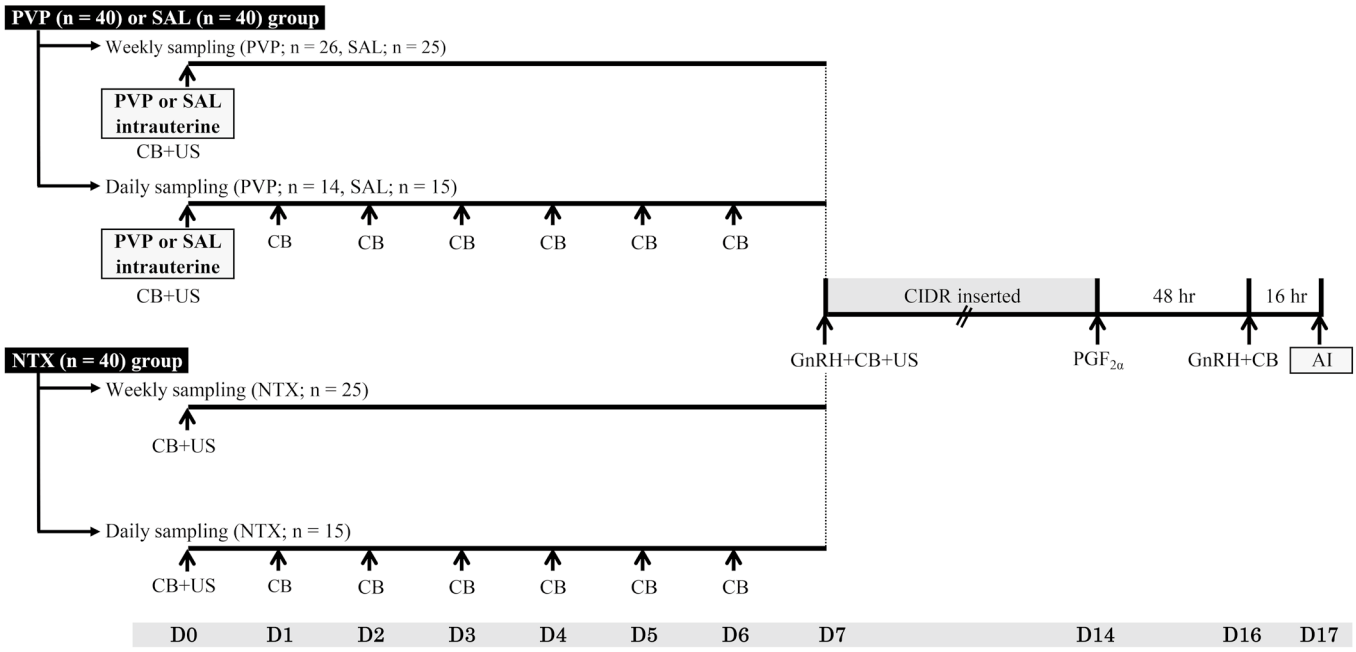


**Fig. 1.** Schematic diagram of Experiment 1 in lactating dairy cows. Animals were assigned to receive intrauterine infusion of povidone-iodine (PVP-I) or physiological saline (SAL) at 5 weeks ± 3 days postpartum (D0), followed by subsequent CIDR-synch timed artificial insemination (AI) protocol 7 days later. PVP: Intrauterine infusion of 2% PVP-I; SAL: Intrauterine infusion of SAL; NTX: No treatment; $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$): 500 $\mu$g cloprostenol; CIDR: Controlled internal drug release insert containing 1.9 g progesterone; GnRH (gonadotrophin releasing hormone): 100 $\mu$g fertirelin acetate; CB: Cytobrush; US: Ultrasonography of the ovaries and uterus.

after infusion, to ensure that a sufficient amount of PVP reached the tip (distal part) of the right and left uterine horns.

*Collection of endometrial samples for cytology*: Endometrial cytology samples were collected from the 120 cows using a cytobrush device (Metribrush; Fujihira Kogyo Inc., Tokyo, Japan). Once the cytobrush reached the cervix, the plastic sleeve was drawn back and the brush was moved forward and rolled onto the endometrium of the uterine body. The cytobrush was then rotated onto two clean microscope slides. Immediately after sample collection, cells were fixed with cytofixative alcohol spray (Cytokeep II; Alfresa Pharma Co., Osaka, Japan), stained with Diff-Quik stain (Sysmex Co., Kobe, Japan), washed with distilled water, and air-dried. A total of 300 nucleated cells were counted under a microscope at ×400 magnification to determine PMN%.

*Ultrasonography*: Ultrasound images of ovarian status (presence of the corpus luteum, CL, and follicles), and uterine status (fluid in the lumen and echogenicity of the endometrium), were obtained on D0 and D7 by transrectal ultrasonography (HS101V scanner equipped with a 5 MHz liner transducer; Honda Electronics Co., Ltd., Toyohashi, Japan). The stage of the estrous cycle, or ovarian status, at the time of starting timed AI on D7, was determined by the assessment of ovarian structure on D0 and D7 by ultrasonography as follows: CL of 20 mm or greater in diameter detected on D0 or D7 (presence of CL); and only follicles and/or CL of less than 20 mm in diameter on D0 and D7 (no CL) [8].

*Timed AI protocol and reproductive performance*: All 120 cows received 100 $\mu$g fertirelin acetate (GnRH, 2 m*l* Consultan®; ASKA Animal Health Co., Ltd., Tokyo, Japan) on D7, and CIDR, containing 1.9 g progesterone (CIDR1900®; Zoetis Japan, Tokyo, Japan), in the vagina for 7 days from D7. The device was removed on D14, and 500 $\mu$g cloprostenol, a prostaglandin $F_2\alpha$ analog ($PGF_{2\alpha}$, 2 m*l* Resipron-C®; ASKA Animal Health Co., Ltd.), was administered immediately after the removal of the device. A second GnRH was administered 48 hr after $PGF_{2\alpha}$ administration (Fig. 1). Timed AI was performed 16–20 hr after the second GnRH administration in all groups. Pregnancy was diagnosed by transrectal ultrasonography at 45 ± 5 days after timed AI. The conception rate was calculated for each group in animals with or without subclinical endometritis, and in animals with or without CL on D0. Additionally, the overall pregnancy rate within 150 days postpartum was calculated for each group. After the timed AI, second and subsequent inseminations were performed after estrus detection.

*Blood sampling*: To examine biochemical parameters, and determine progesterone concentrations, blood samples were collected from a subset of cows (44 cows) on D7, immediately prior to CIDR insertion into the vagina. Samples were collected from the coccygeal vein into standard vacuum blood collection tubes without anticoagulant (Venoject® VJ-P100Ad01 vacuum tubes; Terumo, Tokyo, Japan). Each blood sample was allowed to clot for 30 min at room temperature and subsequently kept on ice for 2 hr until centrifugation. Serum was separated by centrifugation at 2,000 × *g* for 10 min and frozen at −20°C until analysis. Serum non-esterified fatty acid (NEFA), total cholesterol (T-Cho), and total protein (TP) concentrations were determined using a biochemical blood analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Serum progesterone concentrations were determined using a VIDAS assay kit (bioMérieux Japan Ltd., Tokyo, Japan).

*Statistical analysis*: Statistical analysis was performed using a statistical program file (ystat 2013 for Windows/Macintosh; Igaku Tosho Shuppan, Tokyo, Japan). Differences in PMN% across the three groups or within group were evaluated by non-parametric permutation multivariate analysis of variance (PERMANOVA). Analysis of variance and $\chi^2$ procedures, or Fisher's exact test, were used to determine the effects of treatment on conception rate. Results are expressed as either the mean ± SEM or a percentage. $P<0.05$ was considered significant.

### *Experiment 2*

*Animals*: A total of 25 clinically healthy lactating Holstein-Friesian cows [Age: 3.8 ± 0.4 years (mean ± SD), Days postpartum: 343 ± 111 (mean ± SD)] were included in the experiment. Selection criteria included a normal estrous cycle and a normal uterine and cervix status, identified by transrectal ultrasonography, but planned to be culled due to low milk production.

The cows were divided into three groups to compare the histopathology of uterine tissues: after intrauterine infusion of 50 m*l* of 2% PVP-I (PVP; n=10), 50 m*l* of physiological saline (SAL; n=12), or no infusion (no treatment: NTX, n=3). Cows were sacrificed 24 hr (PVP24: n=5; SAL24: n=3) and 48 hr (PVP48: n=5; SAL48: n=3) after PVP-I or SAL intrauterine infusion. As epithelium regeneration was not observed in the SAL group after 48 hr, additional samples for histopathology were collected 72 hr (SAL72: n=3) and 96 hr (SAL96: n=3) after SAL intrauterine infusion.

*Histopathology*: After sacrificing the cows, and confirming that there was no abnormality in gross appearance, the uterine horn was incised and fixed in 10% buffered formalin. Uterine tissue samples were collected from five cross-sectional parts: the uterine body and two sections from the right and left uterine horns each. The uterine horns were divided into three equal parts, with two of the three parts (the horn tip and base) sampled. Tissue samples were embedded and sectioned at 5 $\mu$m thickness. Sections were stained with hematoxylin and eosin (HE), and subsequently examined under a light microscope.

Microscopic observations were performed to assess if exfoliation of the endometrial epithelial cells was present. After exfoliation of the endometrial epithelium was confirmed, observation of at least one of the following three characteristics was considered as undergoing the process of epithelial cell regeneration: (i) a squamous epithelium; (ii) hyperplasia; and (iii) a stratified columnar epithelium. Endometrial epithelial cells were observed under high magnification (×400) in 10 fields of view. Of the three characteristics, squamous epithelium and hyperplasia were regarded as a regenerated image. Infiltration of neutrophils was described in four categories: none (no inflammatory infiltrate, average of <3 PMN per 400X microscopic field); slight (3–6 PMN per 400X field); moderate (7–10 PMN per 400X field); and high (>10 PMN per 400X field in the five cross-sectional parts) [7]. The ratio of the number of fields at which epithelial exfoliation or regenerated images, and infiltration of neutrophils in the lamina propria, were observed in the 10 fields was determined; the average of the ratios in each of the five cross-sectional parts was calculated.

*Statistical analysis*: Statistical analysis was performed using the statistical program file (ystat 2013 for Windows/Macintosh; Igaku Tosho Shuppan, Tokyo, Japan). Analysis of variance and $\chi^2$ procedures were used to perform comparisons of the epithelial regeneration between groups. Results are expressed as a percentage. $P<0.05$ was considered significant.

## RESULTS

*Experiment 1*

The percentage of cows with subclinical endometritis did not differ across the three groups (PVP: 25.0%, SAL: 30.0%, NTX: 27.5%). Therefore, the results on the profile of PMN%, reproductive performance, and blood biochemical analysis in the three treatment groups were compared regardless of PMN% on D0.

*Comparison of PMN% on D0, D7, and D16 in the three groups*: PMN% on D0, D7, and D16 were similar (D0: $P=0.344$, D7: $P=0.268$, D16: $P=0.132$) across the three treatment groups (PVP: 5.7 ± 2.1, 10.5 ± 3.0 and 4.3 ± 1.8, SAL: 8.2 ± 2.5, 15.8 ± 3.7 and 2.5 ± 1.1, NTX: 8.8 ± 2.1, 15.1 ± 3.8 and 7.8 ± 2.5).

*Comparison of daily profiles of PMN% from D0 to D7 in the three groups*: In the subsets of the three groups, a significant increase ($P=0.0001$) in PMN% from D0 to D1 was observed in the PVP group (Fig. 2). In contrast, no such increase was observed in the SAL and NTX groups. PMN% in the PVP group (48.8 ± 5.8) on D1 was higher ($P=0.0001$, vs SAL; $P=0.0009$, vs NTX) than the other two groups (SAL: 17.2 ± 3.6, NTX: 19.7 ± 6.1). On D2, PMN% decreased in the PVP group ($P=0.0001$), having a level similar to those in the SAL and NTX groups. PMN% increased in the SAL and NTX groups (SAL: $P=0.01$, NTX: $P=0.02$) by D2. Average PMN% decreased from D7 to D16 in all three groups (PVP: $P=0.003$, SAL: $P=0.0001$, NTX: $P=0.002$, Fig. 2).

*Reproductive performance*: The conception rate after timed AI was greater in the PVP group than the SAL ($P=0.01$) and NTX ($P=0.04$) groups (Table 1). There was no difference in the conception rate between cows with CL in the PVP and other groups. Among the cows without CL, the conception rate of the PVP group was greater ($P=0.047$) than that of the SAL group. There was no difference in the pregnancy rate between the three groups within 150 days postpartum.

*Blood biochemical analysis*: A significant difference was not found in NEFA, T-Cho, or TP concentrations between the PVP,

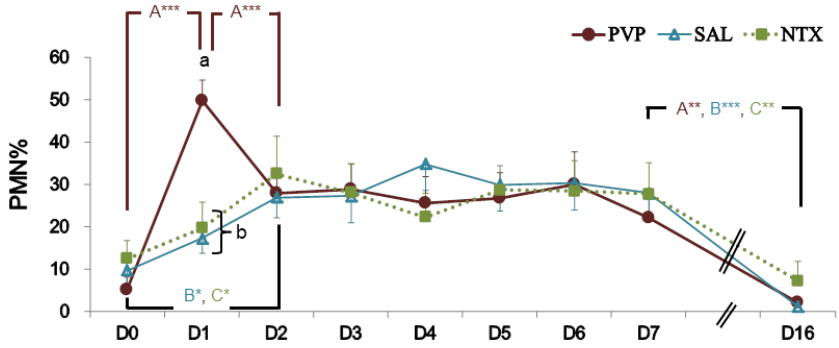


**Fig. 2.** Changes in the percentage of polymorphonuclear neutrophils (PMN%). PMN% in endometrial samples collected for 8 consecutive days after intrauterine infusion of povidone-iodine (PVP-I) (PVP: n=14), physiological saline (SAL: n=15), or no treatment (NTX: n=15) in postpartum dairy cows. Letters A, B, and C reflect differences between days in PVP, SAL and NTX groups, respectively. a–b: different letters reflect differences between groups on D1 ($P<0.001$); *$P<0.05$, **$P<0.01$, ***$P<0.001$.

**Table 1.** Conception rate with timed artificial insemination (AI), and reproduction performance after timed AI in 120 dairy cows

| Group | PVP (n=40) % (n/n) | SAL (n=40) % (n/n) | NTX (n=40) % (n/n) |
|---|---|---|---|
| Conception rate at timed AI | 27.5 (11/40)[a)] | 7.5 (3/40)[b)] | 10.0 (4/40)[b)] |
| 44 cows (daily sampling) | 42.9 (6/14) | 13.3 (2/15) | 6.7 (1/15) |
| 76 cows (weekly sampling) | 19.2 (5/26) | 4.0 (1/25) | 12.0 (3/25) |
| Presence or absence of CL in ovaries from D0 to D7 | | | |
| With CL | 23.5 (4/17) | 11.1 (2/18) | 10.5 (2/19) |
| Without CL | 30.4 (7/23)[a)] | 4.6 (1/22)[b)] | 9.5 (2/21) |
| Pregnancy rate at 150 DIM | 45.0 (18/40) | 32.5 (13/40) | 32.5 (13/40) |

PVP: intrauterine infusion of 2% povidone-iodine (PVP-I); SAL: intrauterine infusion of physiological saline; NTX: no treatment; CL: corpus luteum; D0: 5 weeks ± 3 days postpartum; D7: 7 days after D0; DIM: days in milk. Values marked with different superscripts within a row differ significantly ($P<0.05$).

SAL, and NTX groups (NEFA: 282.5 ± 140.4, 278.8 ± 168.3, and 323.0 ± 216.0 Eq/*l*; T-Cho: 188.8 ± 58.8, 190.6 ± 53.1, and 176.7 ± 42.3 mg/d*l*; TP: 8.0 ± 0.5, 7.6 ± 0.5, and 8.2 ± 0.9 g/d*l*, respectively).

*Experiment 2*

Histological examination, when observing the 10 fields per section of PVP24 cows (n=5), revealed an average of 81.3% disappearance of stratified columnar epithelial cells (Table 2 and Fig. 3, PVP24-a). In some fields of view of group PVP24 (18.7%), squamous metaplasia was observed (Table 2 and Fig. 3, PVP24-b). Moderate infiltration of neutrophils was observed in the lamina propria of the PVP24 group (Fig. 3, PVP24-c). Stratified columnar epithelial cells disappeared in all fields of view of group SAL24 (Fig. 4, SAL24). Slight infiltration of neutrophils observed in the lamina propria in SAL24 cows was lower than those in PVP24. Infiltration of neutrophils in the lamina propria was not observed in the NTX group. In all samples, neutrophil infiltration was confined to the lamina propria and no further infiltration of neutrophils was observed. Epithelial cells regenerated in the uterine body and horns of the PVP48 group (Fig. 3, PVP48). The types of regeneration images observed in PVP48 cows were as follows: squamous epithelium, 4.7%; hyperplasia, 65.7%; and stratified columnar epithelium, 42.7% (Table 2). In contrast, epithelium regeneration was not observed in any cow in the SAL48 group (Fig. 4, SAL48), ($P$=0.0001). However, the epithelium was regenerated in the SAL72 and SAL96 groups (Fig. 4). Stratified columnar epithelial cells were observed in the NTX group (Fig. 3, NTX).

## DISCUSSION

In the present study, we observed that infusion of PVP into the uterus caused a transient inflammation of the endometrium and an increased timed AI conception rate in postpartum dairy cows. To the best of our knowledge, this is the first report to demonstrate the daily profile of PMN% in dairy cows treated with intrauterine infusion of PVP-I in the early postpartum period.

To evaluate changes in the degree of endometrial inflammation, PMN% was measured every 24 hr for 8 days. A previous study showed that intrauterine infusion of PVP-I decreased PMN% within two weeks in cows with endometritis [18]. In the present study, the PVP group had transient inflammation the day after infusion that was reduced after 48 hr. The response of the endometrium was considered to be an acute inflammatory response. In women undergoing assisted reproductive techniques, endometrial scratch injury (ESI) and local endometrial injury (LEI) improve pregnancy rates after *in vitro*-fertilized embryo transfer [22, 26]. The rationale for performing ESI and LEI is to trigger local acute inflammation, through the release of cytokines and growth factors. Similarly, intrauterine infusion of PVP-I in the present study may have caused acute inflammation. PVP-I infusion has been shown to irritate the endometrium in rats [1] and therefore, it is speculated that PVP-I may enhance local defense mechanisms.

**Table 2.** Histological evaluation of uteri samples collected 24 hr after intrauterine infusion of polyvinylpyrrolidone (PVP-I) or physiological saline (SAL), 48 hr after infusion of PVP-I or SAL, 72 hr or 96 hr after infusion of SAL, and no infusion (NTX)

| Hours after infusion | 24 hr | | 48 hr | | 72 hr | 96 hr | |
|---|---|---|---|---|---|---|---|
| | PVP (n=5) | SAL (n=3) | PVP (n=5) | SAL (n=3) | SAL (n=3) | SAL (n=3) | NTX (n=3) |
| Disappeared of epithelium cell % (n/n) | | | | | | | |
| Uterus body | 96 (48/50) | 100 (30/30) | 0 | 100 (30/30) | 0 | 0 | 0 |
| Uterine horn bases (right and left) | 75 (75/100) | 100 (60/60) | 0 | 100 (60/60) | 0 | 0 | 0 |
| Uterine horn tips (right and left) | 73 (73/100) | 100 (60/60) | 0 | 100 (60/60) | 0 | 8.3 (5/60) | 0 |
| Average % | 81.3 | 100 | 0[a)] | 100[b)] | 0 | 2.8 | 0 |
| Squamous epithelium % (n/n) | | | | | | | |
| Uterus body | 4 (2/50) | 0 | 10 (5/50) | 0 | 6.7 (2/30) | 0 | 0 |
| Uterine horn bases (right and left) | 25 (25/100) | 0 | 3 (3/100) | 0 | 0 | 0 | 0 |
| Uterine horn tips (right and left) | 27 (27/100) | 0 | 1 (1/100) | 0 | 0 | 0 | 0 |
| Average % | 18.7 | 0 | 4.7[a)] | 0[b)] | 1.3 | 0 | 0 |
| Hyperplasia % (n/n) | | | | | | | |
| Uterus body | 0 | 0 | 62 (31/50) | 0 | 33.3 (10/30) | 73.3 (22/30) | 0 |
| Uterine horn bases (right and left) | 0 | 0 | 69 (69/100) | 0 | 91.7 (55/60) | 31.7 (19/60) | 0 |
| Uterine horn tips (right and left) | 0 | 0 | 66 (66/100) | 0 | 63.3 (38/60) | 51.7 (31/60) | 0 |
| Average % | 0 | 0 | 65.7[a)] | 0[b)] | 62.8 | 52.2 | 0 |
| Stratified columnar epithelium % (n/n) | | | | | | | |
| Uterus body | 0 | 0 | 58 (29/50) | 0 | 0 (0/30) | 26.7 (8/30) | 100 (30/30) |
| Uterine horn bases (right and left) | 0 | 0 | 29 (29/100) | 0 | 8.3 (5/60) | 8.3 (5/60) | 100 (60/60) |
| Uterine horn tips (right and left) | 0 | 0 | 41 (41/100) | 0 | 36.7 (22/60) | 36.7 (22/60) | 100 (60/60) |
| Average % | 0 | 0 | 42.7[a)] | 0[b)] | 11.3 | 23.9 | 100 |

PVP: intrauterine infusion of 2% povidone-iodine (PVP-I); SAL: intrauterine infusion of physiological saline; NTX: no treatment. Values marked with different superscripts within a row differ significantly ($P$<0.05). †Percentage of endometrial epithelial cell morphology at 10 random different fields at high magnification (×400) for each excised section. The morphology of epithelial cells is classified into: squamous epithelium, hyperplasia, and stratified columnar epithelium.

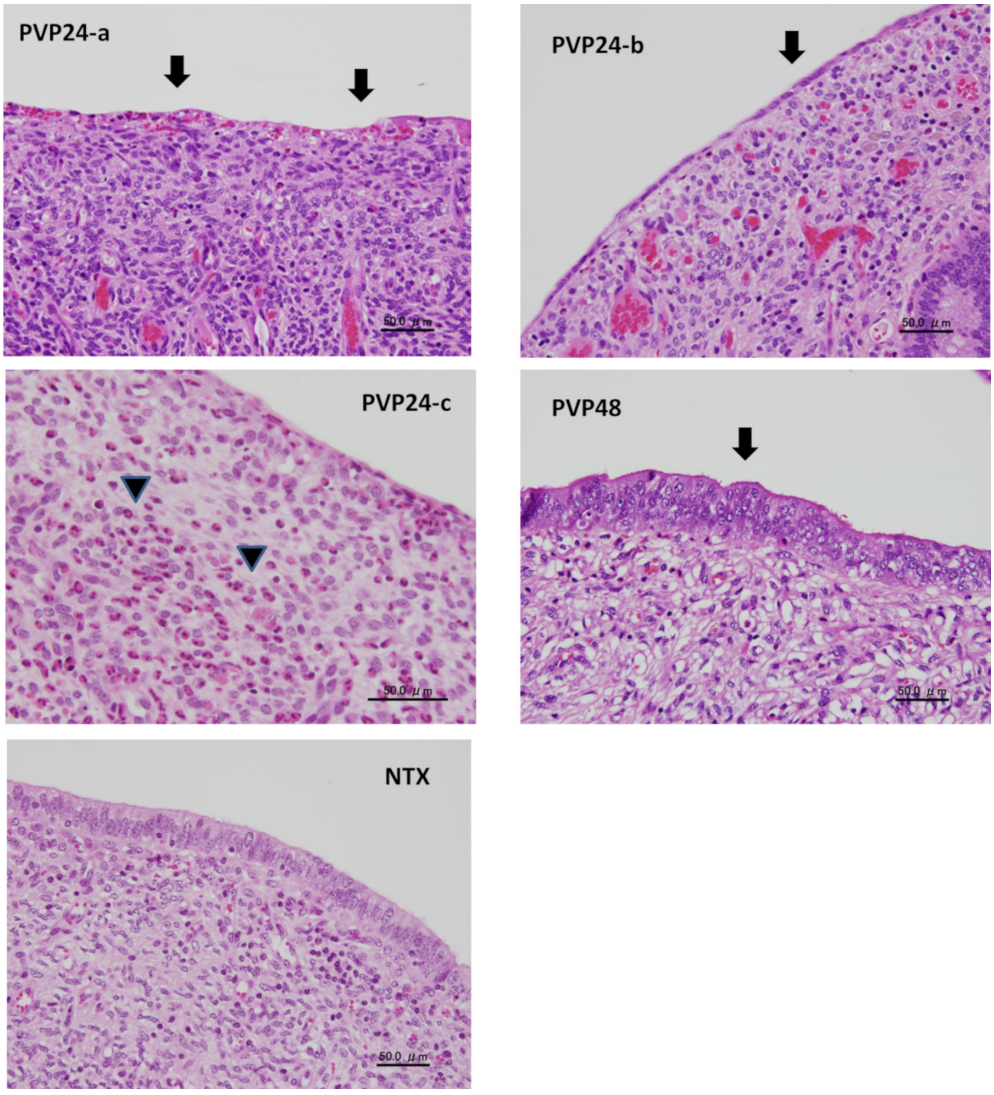


**Fig. 3.** Representative images of hematoxylin and eosin (HE)-stained cross-sections of the epithelium. PVP24-a: Disappearance of endometrial epithelial cells (arrows) 24 hr after intrauterine infusion of povidone-iodine (PVP-I). PVP24-b: Squamous metaplasia (arrow) observed in some PVP24 group cows. PVP24-c: Moderate infiltration of neutrophils in the lamina propria (arrowheads). PVP48: Hyperplasia of epithelial cells (arrow) was regenerated 48 hr after intrauterine infusion of PVP-I. NTX (No treatment): Columnar epithelium in the untreated group. Scale bar=50.0 $\mu$m.

PVP-I is a broad-spectrum microbicide with potency to inactivate bacteria, fungi, protozoans, and several viruses. PVP-1 is known for its fast-acting bactericidal activity *in vitro*. A bactericidal effect has been observed within 15 sec of contact against bacteria in the oral cavity [9]. One caveat of the present study is that microbiological analyses were not performed. The fast-acting bactericidal activity of PVP-I may be related to the transient inflammatory response of the endometrium, which was observed in the present study, and should be clarified in future studies.

PMN% increased from D0 to D7 not only in the PVP group, but also in the SAL and NTX groups. Although the cytobrush technique is advantageous for diagnosis, red blood cell contamination has been reported [24]. It has not been previously reported that the use of a cytobrush adversely affects reproductive performance in cattle. While uterine biopsies have been shown to affect subsequent reproductive performance [5], endometrial cytology using a cytobrush four times, at a 3-day interval in one estrous cycle, did not affect PMN% throughout the estrous cycle in the cow [17]. However, studies have not been carried out to elucidate if daily sampling by a cytobrush, for several days, affects the endometrial environment in cattle. Although potential false positive, and/or false negative, diagnoses of subclinical endometritis via cytobrush should be taken into consideration, the present results suggest that daily insertion of a cytobrush into the uterus may cause continuous stimulation of the endometrium, resulting in temporary inflammation.

The PVP group had a higher conception rate than the other two control groups. We considered that a transient inflammatory response in the uterus, induced by PVP-I, may have promoted early regeneration of epithelial cells, resulting in the improved

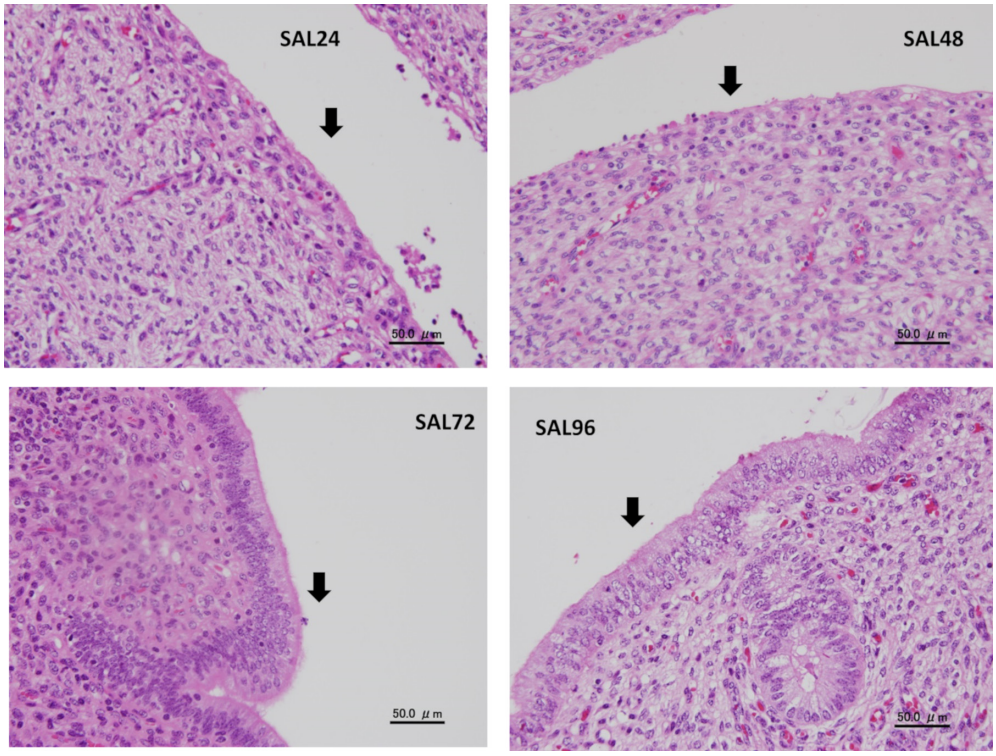


**Fig. 4.** Representative images of hematoxylin and eosin (HE)-stained cross-sections of the epithelium. SAL24: Disappearance of endometrial epithelial cells (arrow) 24 hr after intrauterine infusion of physiological saline (SAL). SAL48: Disappearance of endometrial epithelial cells (arrow) 48 hr after intrauterine infusion of SAL. SAL72: Hyperplasia of epithelium cells (arrow) was regenerated 72 hr after intrauterine infusion of SAL. SAL96: Hyperplasia of epithelium cells (arrow) was regenerated 96 hr after intrauterine infusion of SAL. Scale bar=50.0 $\mu$m.

conception rate. However, even the conception rate of 27.5% observed in the PVP group was not as high as previous reports including those employing CIDR-synch (30.2%) [27], and CIDR-synch after a resynchronization protocol (51.3%) [4]. Possible reasons for the relatively low conception rate may be that the timed AI program, in the present study, was initiated regardless of the stage of the estrous cycle or ovarian status, and the first AI was performed relatively early in the postpartum period. Murugavel *et al*. [19] performed a resynchronization protocol and timed AI at 56 days postpartum, and the conception rate was 24.4%. Therefore, the conception rate of 27.5% in the PVP group is comparable. The timed AI program, in which intrauterine infusion of PVP-I was performed 5 weeks postpartum, with insemination conducted 52 days postpartum, resulted in a higher conception rate compared with control groups. However, there was no difference in the overall pregnancy rate within 150 days postpartum among the three groups. Daily intrauterine infusions of Lugol's solution prolonged and shortened the estrous cycle [20], but a single PVP-I did not affect the estrous cycle. The CIDR-synch and timed AI program applied in all three groups, in the present study, may have accelerated resumption of estrous cycles. Such an effect may have surpassed the positive effect of a single PVP-I infusion, although the PVP group had a higher conception rate by the first (timed) AI. Blood chemical parameters in the PVP group were the same as those in the control groups. Therefore, the nutritional status of the three groups was considered to be similar, and was not a reason for the higher conception rate in the PVP group. Rather, PVP-I may have ameliorated the intrauterine environment due to its effect on endometrium regeneration. However, a limitation in the present study is that a low number of animals per group (low power) was used. Further research, using a larger sample size, is warranted to evaluate other variables associated with conception rate in a multivariable model.

The results of Experiment 2 showed the disappearance of epithelial cells, and infiltration of neutrophils in the lamina propria, within 24 hr after PVP infusion. Epithelial cells disappeared 20 hr after intrauterine infusion of Lugol's solution, and regeneration of epithelial cells was observed 2 days after infusion [20]. However, the degree of neutrophil infiltration was moderate, not high, in the lamina propria. This does not seem to fit with the cytobrush results of Experiment 1, in which a substantial increase in PMN% was observed 24 hr after PVP infusion. We conjecture that such contradictory findings may be due to observational time differences between the endometrial smear and histological tissue samples. That is to say, peak inflammation in the lamina propria may have been reached before 24 hr post-infusion of PVP-I, and the inflammation may have decreased to some extent by the time the cytobrush samples were taken. Alternatively, cytobrush samples may have included neutrophils that migrated into the endometrium, and appeared out of the basement membrane of the endometrium as epithelial exfoliation occurred.

We expected that the disappearance of epithelial cells would only be observed in the PVP-I group. However, epithelial cells also

disappeared in the SAL group. A previous study, on nasal septal mucosal injury, showed that after injecting saline (pH 6.1) into the nasal cavity, and aspirating the liquid 30 min later, the mucosal epithelium did not disappear. When using distilled (pH 6.6) and tap (pH 7.4) water with different pH values, mucosal epithelial cells disappeared 4 hr after infusion [30]. One possible reason for why the epithelial cells detached from the endometrium, could be that the physiological saline remained in the uterus for a certain period after infusion, causing the pH in the uterus to increase. Epithelial cells regenerated in the PVP48, SAL72, and SAL96 groups, but not in the SAL48 group. In inflammatory lung disease, transmigration of human neutrophils contributes to epithelial injury and β-catenin is activated; β-catenin regulates epithelial repair [32]. After PVP-I administration, neutrophil infiltration in the subcutaneous tissue may have promoted regeneration of endometrial epithelial cells. These results observed in Experiment 2 are presumed to support the results of Experiment 1, in which the conception rate was higher in the PVP-I group than in the other groups. Future studies are needed to elucidate the relationship between epithelial regeneration and neutrophil infiltration in cows with endometritis, and in healthy cows, after PVP-I infusion into the uterus.

In conclusion, intrauterine infusion of PVP-I induces transient inflammation in the uterus, promotes the regeneration of endometrial epithelial cells, and improves reproductive performance.

CONFLICT OF INTEREST. The authors declare no conflicts of interest associated with this manuscript.

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