Impact of Ovarian and Uterine Conditions on Some Diagnostic Tests Output of Endometritis in Postpartum High-Yielding Dairy Cows

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Contents
The effect of ovarian predominating structures and uterine condition on the result of some diagnostic tools for the evaluation of endometritis was studied in postpartum (pp) Holstein–Friesian dairy cows (n = 58). Endometrial cytology (EC) and the evaluation of vaginal mucus by vaginoscopy or Metricheck were performed weekly from week 3 to 7 pp. The ovarian studies involved the predominating structures including cystic follicles with plasma progesterone (P4; more or <1 ng/ml; >23 mm), corpus luteum (CL), pre-ovulatory follicles (10–23 mm) and small follicles (<10 mm). The uterine conditions comprised uterine involution, tonicity and fluid in uterus (FIU) regarding echogenicity extent. During week 5, the percentage of polymorphonuclear neutrophils (PMN%) was higher (p < 0.05) in animals with pre-ovulatory follicles (mean ± SEM, 26.3 ± 7.6%) than animals having CL (11.0 ± 3.6%). In cystic ovaries, during week 5, PMN% was higher (p < 0.05) in follicular cysts with low progesterone (P4 < 1 ng/ml; 9.3 ± 2.6%) than those with high P4 (P4 ≥ 1 ng/ml; 1.5 ± 1.1%). Moreover, PMN% was higher (p < 0.01) in animals with non-involved uterus (11.5 ± 7.4%) than those with involved uterus (2.7 ± 0.6%) during week 7 pp. The animals that had abnormal mucus determined by Metricheck was higher in animals with atonic uterus than those with tonic uterus during week 6 (82.6% vs 51.5%; p < 0.05) and 7 (71.4% vs 25.7%; p < 0.01) pp. In addition, by vaginoscopy, the prevalence of animals with abnormal discharge showing small follicles (100%, 5/5) during week 3 pp and pre-ovulatory follicles (40.0%, 8/20) during week 5 pp was higher (p < 0.05) when compared to those having CL during week 3 (33.3%; 1/3) and week 5 pp (7.7%; 2/26), respectively. In conclusion, endometrial cytology, Metricheck and vaginoscopy were influenced by the predominating various ovarian structures and uterine condition in early pp high-yielding dairy cows.

Introduction
Infection of the female genital tract is common among mammals and economically important in humans and cattle (Ross 2002; Sheldon et al. 2006). Endometritis in cows causes significant economic losses because of decreased reproductive performance, increased feed intake per lactation, reduced milk yield and increased culling rate (Borsberry and Dobson 1989; LeBlanc et al. 2002). Both pathogenic and non-pathogenic bacteria can enter the uterus at the time of parturition, can rapidly multiply (Usmani et al. 2001) and can cause metritis in more than 40% of dairy cows within a week of parturition (Sheldon et al. 2009). These infections of the genital tract cause infertility by interrupting uterine and ovarian function with consequent extended days to first service and days to pregnancy (Sheldon et al. 2009).

Clinical endometritis is defined in cattle as the presence of a purulent uterine discharge detectable in the vagina 21 days or more postpartum (pp) or mucopurulent discharge detectable in the vagina after 26 days pp (Sheldon et al. 2006). Visual exploration of such vaginal contents is achieved by vaginoscope (McDougall 2001; LeBlanc et al. 2002), gloved hand (Williams et al. 2005) and Metricheck device (McDougall et al. 2007; Pleticha et al. 2009; Senosy et al. 2009). Moreover, inflammation of the endometrium with the absence of abnormal vaginal discharge and subsequent marked reduction in reproductive performance is referred to subclinical endometritis. This type of inflammation is characterized by polymorphonuclear neutrophils (PMNs) surpassing between 5% of cells (Gilbert et al. 2005; Santos et al. 2009), 8% of cells (Barlund et al. 2008) and 10% of cells (Kasimanickam et al. 2004) in samples collected by flushing the uterine lumen or by endometrial cytobrush.

It has long been accepted that no single method of diagnosis of endometritis has high sensitivity and specificity owing to the absence of a ‘gold standard’. It is possible that certain factors cause false-negative or false-positive diagnoses such as the effect of cycle stage on endometrial neutrophilia. Indeed, these factors both ovarian and uterine factors could affect test sensitivity and specificity. However, the influence of ovarian predominating structures (follicles or corpora lutea) and uterine condition including uterine fluid content, uterine involution and uterine tonicity on the results of some diagnostic techniques including endometrial cytology (EC), Metricheck and vaginoscopy was controversial and not clearly investigated during different periods pp in high-producing dairy cows. It is imperative to establish the impact of the ovary and uterus on the diagnostic merit of such techniques in evaluating uterine condition during pp period to improve the efficiency of such methods and consequently improve the reproductive management of apparently normal cattle with no history of periparturient complications.

The objective of this study was to investigate the influence of ovarian structures (follicles or corpora lutea) and uterine conditions (uterine fluid, uterine tone and uterine involution) on the results of some diagnostic tests for endometritis including EC, Metricheck and vaginoscopy during the pp period in high milking cows.
Materials and methods

Animals

A herd of 198 Holstein adult cows was used in this study from the National Livestock Breeding Center, Iwate Station, Morioka, Japan. Cows were housed in free stalls, fed a diet formulated according to standard guidelines and machine-milked twice a day. Cows with no history of a caesarean section, retained foetal membranes, dystocia, or acute mastitis were used to avoid any direct effect of disease on uterine condition. Fifty-eight Holstein-Friesian dairy cows (mean ± SD, parity = 2.6 ± 0.8; age = 4.5 ± 1.0 year) were randomly selected and used throughout the study. These cows calved between May 2007 and May 2008, and their milk production over 305 days was 10 259 ± 1 318 kg. The animals did not receive any antimicrobial or hormonal treatments during the study period.

Study design

All cows were examined five times on a weekly basis starting from 3-week pp by one veterinarian. During the examination, cows were examined by vaginoscope, Metricheck (Simcro Tech Ltd., Hamilton, New Zealand), EC and transrectal US, consecutively during weeks 3 (17.7 ± 1.7 DIM; mean ± SD), 4 (24.7 ± 1.8 DIM), 5 (31.8 ± 1.7 DIM), 6 (38.7 ± 1.6 DIM) and 7 (45.8 ± 1.8 DIM) after normal calving.

Endometrial cytology (EC)

Endometrial cytology was carried out according to Senosy et al. (2009). Endometrial cytological samples were collected by rotating the brush while in contact with the uterine body wall. The brush was retracted into the stainless steel tube prior to removal from the uterus. The stainless steel device was washed with antiseptic solution (0.05% benzalkonium chloride) between uses. The brushes with the connector were sterilized by formaldehyde gas sterilization and neutralization equipment (Holl Steri 130; Asukamedical Co., Ltd., Osaka, Japan) before use.

The slides of EC were prepared by rolling the brush on a clean glass microscope slide for each sample and fixed with absolute methanol cytostatic for 5 min (Ahmadi et al. 2006). Fixed slides were stained with a modified Giemsa stain for 20 min, washed in distilled water and dried. Cytological assessment was used to determine the percentage of PMN% by counting a minimum of 200 cells of PMN and endometrial cells at ×400 magnification for quantitative assessment of endometrial inflammation. The EC slides were assessed on two different days to make the two EC slides reading independent on each other and not affected by the preceding one.

Evaluation of vaginal mucus by Metricheck

Vaginal mucus was collected by Metricheck, as previously described (Senosy et al. 2009). The material within the concave surface of the device and/or adherent to the convex surface was scored according to Sheldon et al. (2006) on a 0–3 scale (score 0 = clear or translucent mucus, score 1 = mucus containing of white or off-white pus, score 2 = discharge containing <50% purulent material and score 3 = discharge containing ≥50% purulent material). The Metricheck device was sterilized by gas sterilizer before use.

Vaginal examination

Vaginoscopy was performed to detect any discharge from the cervical os or on the floor of the vagina and classified according to McDougall et al. (2007) into three categories: dry vagina or clear mucus, mucopurulent and purulent discharge without odour.

Transrectal palpation and ultrasonographic examination

Transrectal palpation was performed to assess the position, size and consistency of the uterus and cervix according to Studer (1983). The genital tracts were monitored using a real-time B-mode mobile US unit with a 5/7.5-MHz linear array transducer (Agroscan; ECM Noveko International, Inc., Angoulême, France). The US equipment was supplied with image freeze and electronic calliper functions for taking measurements. Reproductive tract measurements included uterine horn size at the base of the horn, ovarian status, presence and echogenic nature (echogenic or non-echogenic) of fluid in uterus (FIU). The uterus was considered involuted when the difference between pregnant and non-pregnant horns became <10 mm, while non-involuted uterus when the difference between both horns was ≥10 mm (Kask et al. 2003). Follicles were defined as non-echogenic rounded structures with a clear demarcation between the follicular wall and antrum. A corpus luteum (CL) was defined as a grainy echogenic structure that had a well-defined border with the less echogenic ovarian stroma, and in some corpora lutea, there was a non-echodense lacuna (Sheldon et al. 2002). The maximum diameter of each structure was measured using the electronic calliper. When the image of the structure being scanned was not circular, the diameter was estimated by averaging two dimensions at 90° (Sheldon and Dobson 2000). The animal considered ovulated, ovulated group (OVL group), when an ovulatory follicle (10–23 mm) detected in the preceding examination disappeared with the formation of ovulation depression and assured by the formation of a CL in the subsequent examination. Non-ovulated group (NOVL group) included the predominating ovarian structures on both ovaries either anovulatory follicle (10–23 mm; persist for at least two consecutive examinations), cystic follicles (>23 mm diameter; persist for 2 weeks or more) or small follicles (<10 mm; the largest follicle on the ovaries with the absence of lutein tissue).

At the end of the experimental period (week 7), cows were grouped according to uterine involution (involuted and non-involuted uterus groups), uterine tonicity (tonic and flaccid or atonic uterus groups), ultrasonographic character of FIU (pus, non-echoic fluid and no FIU groups), the predominating ovarian structure at the time of examination into OVL group with the formation of
CL and progesterone > 1.0 ng/ml and NOVL group. NOVL group was subdivided into animals possessing follicles 10–23 mm, follicles < 10 mm and follicles >23 mm groups. Based on plasma P4 concentration, animals possessed follicles > 23 mm were allocated into cystic follicles with plasma P4 level < 1 ng/ml and cystic follicles with plasma P4 level ≥1 ng/ml.

**Blood sampling and progesterone assay**

Blood samples were collected weekly (from weeks 3 to 7) from the coccygeal vessels, into heparinized vacuum tubes and transported on ice to the laboratory. Plasma was separated by centrifugation at 2000 × g for 15 min, harvested and stored at −20°C until assayed for progesterone.

Plasma progesterone concentrations were measured using a time resolved-fluorescence immunoassay (TR-FIA) kit (DELFIA Progesterone Reagents; Wallac Oy, Turku, Finland) according to the manufacturer’s protocol and a previous modified method (Senosy et al. 2009). Assay sensitivity was 0.22 ng/ml, and a 50% effective dose (ED50) was 1.4 ng/ml. Intra- and inter-assay coefficients of variation were 5.2 and 10.4%, respectively.

**Statistical analysis**

The principal points of the study were PMN%, vaginal mucus scores by Metricheck and vaginoscopy, FIU, uterine involution and uterine toxicity in all animals during 3, 4, 5, 6 and 7 week pp and the interaction of these factors on the diagnosis of endometritis by cytology, Metricheck and vaginoscopy. The general linear models ANOVA for repeated measures (SPSS Version 16.0; SPSS, Inc., Chicago, IL, USA) was used for determining the main effects of each group and week, and their interaction. If a significant effect of time or a significant interaction was determined, a Fisher’s least significant difference (LSD) test was used as a post hoc analysis to locate mean differences among groups within weeks and among weeks within groups. The prevalence of cases that had a score of one or higher by Metricheck, mucopurulent or purulent discharge by vaginoscopy, was tested using the Chi-square and Fisher’s exact probability tests. Data are presented as the mean ± SEM. Probability values of < 0.05 were considered significant.

**Results**

**Uterine and ovarian conditions as diagnosed by ultrasonography**

Uterine conditions (involution, contents and tonicity) and ovarian predominating structures (CL, follicles of 10–23 mm, follicles < 10 mm and cystic follicles > 23 mm) in the study animals during different weeks pp are presented in Table 1.

**Endometrial cytology**

The PMN% decreased gradually with increased days pp in all groups except those that had pus in the uterus, follicles of 10–23 mm and those that had cystic follicles (Tables 2 and 3). During week 5 pp, PMN% was significantly higher (p < 0.05) in animals that had follicles of 10–23 mm in diameter when compared to that had CL while it was not different from that of cystic follicles with both low P4 (P4 < 1 ng/ml) and high P4 (P4 ≥ 1 ng/ml, Table 2). Moreover, PMN% was significantly higher (p < 0.05) in cystic follicles with low P4 than that of cystic follicles with high P4 during week 5 pp. During week 6 pp, PMN% was significantly higher (p < 0.05) in the animals that had cystic follicles with low P4 than that of animals having CL or follicles of 10–23 mm, while it was not different from that of animals possessing cystic follicles with high P4 or follicles < 10 mm (p > 0.05; Table 2). The PMN% was higher (p < 0.01) in the animals having non-involuted uterus during week 7 than that having uterine involution. There was no significant difference in PMN% between different uterine content conditions either echogenic, non-echogenic or no fluid in the uterus during different weeks pp.

**Evaluation of vaginal mucus character by Metricheck**

The prevalence of animals that had a score of one or more is presented in Figs 1 and 2. The ovarian structures did not affect the prevalence of animals that had a score of one or more during different weeks pp. (Figs 1a and 2a). The prevalence of animals with turbid mucus, as detected by Metricheck, possessing different ovarian structures decreased gradually with the advancement of days pp.

The prevalence of animals having a score of one or more was significantly higher in animals with atonic uterus (82.6%, 19/23; 71.4%, 15/21) than those with atonic uterus (51.4%, 18/35; 25.7%, 9/35) during weeks 6 (p < 0.05) and 7 (p < 0.01), respectively (Fig. 1b).

| Table 1. The uterine and ovarian condition as diagnosed by ultrasonography during different weeks postpartum (n = 58) |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Uterine involution             | Week 3 n (%)    | Week 4          | Week 5          | Week 6          | Week 7          |
| Involved                       |                 |                 |                 |                 |                 |
| Corpus luteum                 | 21 (36.2)       | 47 (81.0)       | 47 (81.0)       | 51 (87.9)       | 52 (92.9)       |
| Non-involved                   | 37 (63.8)       | 11 (19.0)       | 11 (19.0)       | 7 (12.1)        | 4 (7.1)         |
| Uterine contents               |                 |                 |                 |                 |                 |
| No fluid in uterus             | 15 (25.9)       | 34 (58.6)       | 34 (58.6)       | 38 (65.5)       | 42 (75)         |
| Non-echogenic fluid            | 36 (62.1)       | 21 (36.2)       | 24 (41.4)       | 20 (34.5)       | 14 (25)         |
| Purulent                       | 7 (12.1)        | 3 (5.1)         | 0               | 0               | 0               |
| Uterine toxicity               |                 |                 |                 |                 |                 |
| Tonic uterus                   | 29 (50.0)       | 38 (65.5)       | 39 (67.2)       | 35 (60.3)       | 35 (62.5)       |
| Atonic uterus                  | 29 (50.0)       | 20 (34.5)       | 19 (32.8)       | 23 (39.7)       | 21 (37.5)       |
| Ovarian structures             |                 |                 |                 |                 |                 |
| Ovulated group                 |                 |                 |                 |                 |                 |
| Corpus luteum                 | 3 (5.2)         | 18 (31.0)       | 26 (44.8)       | 35 (60.3)       | 35 (62.5)       |
| Non-ovulated group             |                 |                 |                 |                 |                 |
| Follicles 10–23 mm             | 44 (75.9)       | 24 (41.4)       | 20 (34.5)       | 16 (27.6)       | 16 (28.6)       |
| Follicles < 10 mm              | 5 (8.6)         | 3 (5.2)         | 1 (1.7)         | 2 (3.4)         | 0 (0.0)         |
| Follicles > 23 mm              | 6 (10.3)        | 13 (22.4)       | 11 (19.0)       | 5 (8.6)         | 5 (8.9)         |
| P4 < 1 ng/ml                   | 6 (10.3)        | 13 (22.4)       | 7 (63.6)        | 3 (60.0)        | 3 (60.0)        |
| P4 ≥ 1 ng/ml                   | –               | –               | 4 (36.4)        | 2 (40.0)        | 2 (40.0)        |

*Two animals were culled because of the replacement system of the farm.

Ovulated group: Animals possessed anovulatory follicle that disappeared and assured by subsequent CL formation.

Non-ovulated group: Animals possessed predominating structure on the ovaries either persistent anovulatory follicles, follicular cysts or small follicles with the absence of lutein tissue.
Table 2. PMN\% (Mean ± SEM) during different weeks postpartum according to ovarian status and uterine involution

<table>
<thead>
<tr>
<th>Weeks</th>
<th>OVL group</th>
<th>NOVL group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CL</td>
<td>Follicles 10–23 mm</td>
</tr>
<tr>
<td>3</td>
<td>21.4 ± 12.2</td>
<td>29.4 ± 3.7</td>
</tr>
<tr>
<td>4</td>
<td>16.4 ± 5.4</td>
<td>32.0 ± 6.4</td>
</tr>
<tr>
<td>5</td>
<td>11.0 ± 3.6a</td>
<td>26.3 ± 7.6b</td>
</tr>
<tr>
<td>6</td>
<td>6.8 ± 1.9a</td>
<td>5.2 ± 1.4a</td>
</tr>
<tr>
<td>7</td>
<td>3.2 ± 1.0</td>
<td>4.1 ± 1.7</td>
</tr>
</tbody>
</table>

Values with different superscript within the same row are significant, ab: p < 0.05, ac: p < 0.05, cd: p < 0.01.

NA: No animal had the corresponding structure; OVL group: Animals that ovulated and form CL; NOVL group: Animals that did not ovulate and the follicles persist for at least 10 days without the formation of CL.

PMN, polymorphonuclear neutrophils.

Table 3. PMN\% (Mean ± SEM) during different weeks postpartum according to uterine contents and tonicity

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Uterine contents (FIU)</th>
<th>Uterine tonicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pus</td>
<td>NEF</td>
</tr>
<tr>
<td>3</td>
<td>38.8 ± 10.8</td>
<td>24.5 ± 3.4</td>
</tr>
<tr>
<td>4</td>
<td>39.7 ± 20.3</td>
<td>20.5 ± 4.6</td>
</tr>
<tr>
<td>5</td>
<td>NA</td>
<td>19.7 ± 6.3</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>7</td>
<td>NA</td>
<td>5.3 ± 2.5</td>
</tr>
</tbody>
</table>

FIU, fluid in uterus; PMN, polymorphonuclear neutrophils.
NA: No animal had the corresponding structure; NEF: Animals possessed non-echogenic fluid in the uterus.

There was no significant difference among the animals having a score of one or more either in the animals with no FIU, with pus or with non-echoic fluid during different weeks pp (Fig. 1c).

Evaluation of vaginal mucus character by vaginoscopy

The prevalence of animals that had mucopurulent or purulent vaginal mucus is presented in Figs 2 and 3. The prevalence of animals that had abnormal mucus discharge was significantly (p < 0.05) higher in the animals having non-echoic fluid in the uterus (77.8%, 28/36) than those that had no FIU (46.7%, 7/15) during week 3 pp (Fig. 3c). During week 3 pp, the percentage of animals with abnormal vaginal discharge was higher (p < 0.05) in animals having non-echoic fluid in the uterus (77.8%, 28/36) than those that had no FIU (46.7%, 7/15) during week 3 pp (Fig. 3c).

The prevalence of animals that had abnormal vaginal discharge was significantly higher (p < 0.05) in animals
that had not completed uterine involution (54.5%, 6/11) than those that had completed uterine involution (14.9%, 7/47) during week 4 pp (Fig. 3d).

Discussion

The main goal of the present study was to investigate the effects of ovarian status and uterine condition during the pp period on the outcome of the diagnostic trials of endometritis in high-producing dairy cows. During the puerperium, the uterus of almost all cows is contaminated, and a mild, non-pathological form of endometritis develops (Anderson et al. 1985; Klucinski et al. 1990) characterized by migration of neutrophil granulocytes and other inflammatory cells into the endometrium and the uterine cavity (Lewis 1997; BonDurant 1999; Sheldon 2004; Sheldon and Dobson 2004). In the present study, PMN% decreased with increased days pp. That result is in accordance with many earlier studies (Gilbert et al. 2005; Senosy et al. 2009). During week 5, the PMN% was higher in animals with follicles of 10–23 mm in diameter than those possessed CL. Moreover, PMN% was higher in follicular cysts with low progesterone than that with higher progesterone as the cysts with high progesterone were luteinized and their steroidogenic activity was shifted to progesterone. It is generally believed that oestrogen and progesterone hormones, varying concentrations in relation to the different stages of the reproductive cycle, play immune-modulating roles which have deportment on the progress and clearance of uterine infections (Lewis 2003). Estradiol synthesized by follicles >10 mm and follicular cysts with low progesterone when compared to cysts with high progesterone has a beneficial effect on uterine function (Ireland et al. 1984). Consequently, the number of PMN in the uterus is elevated during oestrus or after treatment with estradiol (Subandrio et al. 2000). On the other hand, during week 6, PMN% is higher in the animals possessing anovulatory follicles (>23 mm) with low P4 than that of follicles of 10–23 mm and animals showed CL. Although we have not determined estradiol level in peripheral circulation, our results may support
earlier reports concluding that a higher PMN% accompanied higher estradiol (Subandrio et al. 2000). Furthermore, earlier reports concluded that cows with genital tract bacterial infections had slower growth of dominant follicles and less likely to ovulate (Sheldon et al. 2002; Williams et al. 2007).

In the present study, PMN% was significantly higher in animals with non-involved uterus than animals with uterine involution during week 7 pp. Earlier reports stated that uterine involution is completed within 4–5 weeks pp (Kask et al. 2003). In the present study, some animals had delayed uterine involution beyond week 7 pp. Uterine involution is delayed owing to bacterial infection (Usmani et al. 2001), which is consequently accompanied by a higher percentage of PMN.

Many reports have stated that there was no association between PMN% and the nature of uterine fluid analysed by ultrasonography (Kasimanickam et al. 2004). Earlier reports concluded that the agreement between EC and US as a diagnostic tool of endometritis was very low (Kasimanickam et al. 2004), suggesting that EC and US were measuring two different causative factors; a cellular response in the case of EC and a clearance response in the case of FIU (Kasimanickam et al. 2004).

Examination with the Metricheck device resulted in a higher apparent prevalence of endometritis than that detected by vaginoscopy based on the degree of turbidity of vaginal mucus. This result coincides with a previous study comparing the Metricheck device with the use of a speculum (McDougall et al. 2007; Pleticha et al. 2009; Serosy et al. 2009). In the present work, the ovarian structures had no effect on the outcome of degree of turbidity of vaginal mucus by Metricheck while uterine tonicity played a major role in the prevalence of animals with turbid mucus. The prevalence of animals with turbid mucus was higher in animals with a flaccid uterus than with atonic uterus. This finding concurred with previous reports (Runciman et al. 2008) studying the possible factors associated with turbid vaginal discharge finding that a flaccid uterus was associated with positive vaginal discharge but not associated with tonic or contracted uterus. The process of uterine clearance and involution is regulated by myometrial contractility and resultant elimination of bacterial infections (Usmani et al. 2001).

Examination of the vaginal contents for the presence of purulent material is the most accurate tool for diagnosis of endometritis (Breitzlaff 1987; Sheldon and Noakes 1998; LeBlanc et al. 2002). During week 5 pp, factors that influenced the diagnostic outcome achieved using vaginoscopy were the predominating structures including the follicular structures of 10–23 mm and anovulatory follicles. Higher prevalence of animals with turbid mucus was higher in animals possessed small follicles when compared to animals ovulated and formed CL during week 3 pp. This condition could be explained by earlier study reporting that animals restoring ovarian cyclicity within 21 days pp had decreased prevalence of uterine infection when compared to non-cyclic animals (Galvão et al. 2009). Moreover, the high percentage of animals that had turbid vaginal mucus in the case of preovulatory follicles (from 10 to 23 mm) when compared to CL is because of higher oestrogen secretion that was elevated above normal pro-oestrous concentrations (Hatler et al. 2003) as estradiol concentration increased with greater follicular size in anovulatory cows (Yoshioka et al. 1998). Higher oestrogen secretion increases the uterine endometrium secretion that flushes any contamination of the uterus. Uterine involution could influence the outcome of vaginoscopy performed during week 4 pp. A higher percentage of animals with abnormal vaginal discharge with non-involved uteri may be because of abnormalities in myometrial contraction resulting in retaining bacterial infection inside the uterus (Usmani et al. 2001).

Taken together, the outcome of endometrial cytological examination could be influenced by predominating ovarian structures during weeks 5 and 6. Moreover, higher EC during week 7 in the non-involved uterus may be because of uterine infection that delayed uterine involution. Vaginoscopic examination results were influenced by ovarian structures during week 3 and 5, while it was influenced by the state of uterine condition (uterine involution) during week 4. Furthermore, the outcome of vaginal examination by Metricheck was affected by uterine condition during weeks 6 and 7 pp.

In conclusion, the results of EC, Metricheck and vaginoscopy were affected by the predominating ovarian structures and uterine condition in early pp high-yielding dairy cows. Further investigations are needed on a large number of animals and different herds.

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Conflicts of interest
None of the authors have any conflict of interest to declare.

Author contributions
Serosy W carried out the research work, statistics and analysis of hormones and wrote the manuscript and the corresponding author. Tanoeika N and Uchiza M collected the data of the animals of the study and blood sampling. Osaka T put the design of the experiment and revised the manuscript and aided in the facilities of the work. Izaike Y revised the manuscript.

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