Relationship between the side of pregnancy and side of subsequent ovarian activity during the early postpartum period in lactating dairy cows

Hiromi KUSAKA1), Hiroshi MIURA1), Motohiro KIKUCHI1) and Minoru SAKAGUCHI1)

Abstract. After parturition, the ovary ipsilateral to the side of previous pregnancy exhibits lower functional activity than that exhibited by the contralateral ovary. The local inhibitory effects of the corpus luteum of the previous pregnancy, and/or the presence of a previous gravid uterine horn, may induce the ipsilateral suppression of folliculogenesis. We examined the influence of the side of previous pregnancy on ovulation and folliculogenesis, until completion of the third postpartum ovulation. The ovaries of 30 Holstein cows were scanned by ultrasonography, through the three postpartum ovulation sequences. No significant differences in the development of growing follicles, 5–8 mm in diameter, were detected between ipsilateral and contralateral ovaries. However, the total number of dominant follicles emerging ipsilaterally before the second postpartum ovulation were less than those emerging contralaterally (25 vs. 75%), and both the first and second ovulation occurred less frequently on the ipsilateral versus contralateral side (23 vs. 77% and 27 vs. 73%, respectively). Sequential observation in this study clearly indicated that the influence of the side of previous pregnancy persisted until the second postpartum ovulation, and this affected postpartum dominant follicle selection and ovulation, but not the development of growing follicles.

Key words: Corpus luteum, Follicle, Ovulation, Pregnancy, Uterus

The growth of a single large or dominant ovarian follicle begins around 10–14 days postpartum [1, 2], and 80% of the first postpartum ovulations in lactating dairy cows occur after ≤ 4 follicular waves [3]. Although the first ovulation in lactating dairy cows classically occurs 14 to 21 days postpartum [4], in modern dairy cows, the average interval from parturition to first ovulation is probably delayed 10 days or more [3, 5], after which normal ovarian follicular dynamics are restored in most cows. This usually occurs after the second postpartum ovulation [3]. It is generally accepted that high-producing dairy cows typically experience negative energy balance (EB), generally associated with atypical ovarian activities, such as double ovulation [6] and anovulatory repeated follicular waves [7]. Compared to the follicular dynamics during the normal estrous cycle, there is limited information available as to the process of follicular development during the early postpartum period, particularly in modern dairy cows [8].

The existence of activity imbalance between the ovaries during the early postpartum period has also been reported, in terms of the frequency of early postpartum ovulation [4, 9, 10]. These early studies have reported that the ovary ipsilateral to the side of previous pregnancy exhibits lower functional activity than the contralateral ovary; this bias towards the ovulation side is remarkable near parturition, and maintained for 30–35 days postpartum [4, 10]. Whilst these early results, obtained via transrectal palpation examination, may not be highly accurate, the results of more recent studies obtained using transrectal ultrasonography clearly show a similar bias towards ovarian activity during the early postpartum period [11–13]. Given that previous and recent studies have focused primarily on the first postpartum ovulation (monitoring it once), the duration of imbalance in ovarian activities is currently unclear. Furthermore, few studies have focused on the influence of the side of previous pregnancy on postpartum follicular wave emergence or follicular development in each ovary [14].

A large follicle in the ovary ipsilateral to the gravid horn has some beneficial effects on reproductive performance exerted via follicular estradiol secretion. The presence of a follicle > 8-mm in diameter in the ipsilateral ovary is associated with a shorter calving to conception interval [15]. Moreover, ovulation in the ipsilateral ovary prior to insemination is associated with an improved conception rate [16]. These studies were conducted with the hypothesis that an increase in estradiol secretion from the dominant follicle (DF) in the ipsilateral ovary locally enhances uterine involution; however, this hypothesis has not yet been accepted [17]. Very limited information on the influence of postpartum ovarian activity on the reproductive traits of cows is available [12, 15, 16]. There are currently no studies on the relationship between postpartum ovarian activity imbalance and dairy cow productivity traits, including body condition and milk composition.

As already mentioned, our previous results demonstrated that most lactating dairy cows regain normal ovarian follicular dynamics...
after the second postpartum ovulation [3]. We re-analyzed this dataset to confirm the influence of the side of previous pregnancy on folliculogenesis and ovulation throughout the three postpartum ovulation sequences. The reproductive and productive traits in relation to ovulation imbalance in cows were also examined.

**Materials and Methods**

**Animals**

The Animal Care and Use Committee of the National Agricultural Research Center for Hokkaido Region approved the experimental protocol. Data presented in this study were collected from 30 lactating (22 primiparous and 8 multiparous) Holstein cows that calved between October 1999 and June 2001, at the National Agricultural Research Centre for Hokkaido Region (Sapporo, Japan). Data from 20 cows were eliminated from a previously reported dataset consisting of 50 cows [3] due to double ovulations [6], follicular cysts [18], or missing follicle size values. During the first 10 weeks after calving, primiparous and multiparous cows were housed in a free-stall barn and tie-stall barn, respectively. All the cows were subsequently housed in the same free-stall barn. Throughout the experimental period, the cows were fed a diet that met all maintenance, growth, and lactation requirements in accordance with the Japanese feeding standards (Agriculture, Forestry and Fisheries Research Council Secretariat, 1999). During the summer (May to September), cows were pastured for 3–4 h/day, and the amount of food was reduced to meet the nutritional requirements during this period. Cows were milked twice daily (at 0900 h and 1900 h), and the milk yield was recorded. Average daily milk yield was calculated for the period covering 7–70 days postpartum, and total milk yield records were corrected for a 305-day lactation period. Milk composition (fat, protein and lactose) was also evaluated monthly, using a mid-infrared spectrometry method (MilkoScan, Foss Japan).

**Measurement of body condition scores**

Average body condition scores (BCS), based on a 5-point scale (where 1 = thin and 5 = fat) [19], were assigned weekly by 2 or 3 independent observers, from 1-week peripartum to 10-weeks postpartum.

**Ultrasound examinations**

The ovaries and uterine horn of each cow were monitored using a real-time linear array ultrasound scanner (SSD-620; ALOKA, Tokyo, Japan) equipped with a 5-MHz rectal probe (UST-580U-5; ALOKA) [3]. Observations were initiated 6–8 days after parturition and continued until the third postpartum ovulation, at intervals of 2–3 days (3 times per week). The location of the corpus luteum (CL) of the previous pregnancy (PCL) was confirmed at the first observation. Follicles ≥ 5 mm in diameter were measured, and diagrams of their relative positions were drawn on each examination day [20]. The maximum number of follicles 5–8 mm in diameter during a follicular wave was used as an index of the development of growing follicles. The largest follicle, growing to a diameter of > 10 mm and at least 2 mm larger than other follicles, was designated the DF [21]. To determine the postpartum interval between each ovulation, ovaries were examined daily by transrectal palpation, when regular ultrasound examinations were not scheduled. Measurements of the endometrial diameter of the previous gravid horn were taken at the base of each horn (approximately 5 cm anterior to the uterine body). Uterine cross-sectional images were used to calculate the mean endometrial diameter and confirm uterine health.

**Estrus detection and artificial insemination**

All cows were observed twice daily for at least 30 min before milking. Those exhibiting standing estrus, determined with the aid of a heatmount detector (Kamar, Steamboat Springs, CO, USA) or mounting activity accompanied by other symptoms, such as vaginal mucous discharge and swelling of the vulva, were determined to be in estrus. After a voluntary waiting period of postpartum 45 days, the cows in estrus were artificially inseminated by trained inseminators using frozen-thawed semen from bulls, in which normal fertility had been confirmed. Conception and the sequential gravid horn were confirmed by detection of a fetal heartbeat, using ultrasonography at 35–40 days after each artificial insemination (AI). As no severe reproductive dysfunction was diagnosed during the experimental periods, no hormonal treatments were given to any of the cows. After AI, ovaries were examined daily by transrectal palpation until ovulation was confirmed. Cows that did not have a positive pregnancy diagnosis within the period of this study (first 180 days in milk) were assumed to have conceived 21 days after their last unsuccessful service [22]; thus, adjusted days open were used as a measure of final fertility.

**Terminology**

The intervals between parturition and first ovulation, first and second ovulation, and second and third ovulation were termed PPI, POI-1, and POI-2, respectively. There were two intraovarian relationships, depending on the location of the DF and PCL, as previously described using dairy heifer [23]: 1) a contralateral relationship, in which the DF and PCL were in different ovaries, and 2) an ipsilateral relationship, in which the DF and PCL were in the same ovary (Fig. 1). Given that more than half of the cows had ovulations in the ovary contralateral to the PCL at both the first and second ovulations, 30 cows were divided into two groups to analyze uterine endometrial involution and compare reproductive and productive performance. The cows with contralateral relationships at both the first and second ovulations were defined as Group-CC (n = 17; 11 primiparous and 6 multiparous); the others were defined as Group-NCC (n = 13; 11 primiparous and 2 multiparous) and included three combinations of two ovulation sequences: contralateral - ipsilateral (n = 6), ipsilateral - contralateral (n = 5) and ipsilateral - ipsilateral (n = 2).

**Statistical analyses**

All statistical analyses were performed using JMP statistical software (JMP Statistics and Graphics guide, ver. 11.0; SAS Inst., Cary, NC). The frequencies of contralateral and ipsilateral relationships between DF and PCL were analyzed using the Distribution platform. This analytical technique assumed a null hypothesis of an equal distribution (1:1) for contralateral versus ipsilateral comparison. The mean maximum numbers of growing follicles between each interval to postpartum ovulation were analyzed using one-way analyses of variance (ANOVA) and Tukey’s multiple comparisons.
test. Pregnancy rates between Groups -CC and -NCC were compared using the Chi-square test ($\chi^2$). Other measurements were compared using Student’s $t$-test. Differences were considered significant at a probability of $P < 0.05$; data are presented as the mean ± SD.

Results

Overall reproductive performance, milk yield and ovarian activity

Table 1 summarizes the means for reproductive performance, milk yield and postpartum ovarian activity for all 30 cows investigated. No significant differences were detected in reproductive traits between the parity groups, but the milk yield in multiparous cows was greater than in primiparous cows ($P < 0.05$), and the mean lactation number of all the cows was 1.6 ± 1.2. No significant differences were detected in the parameters reflecting postpartum ovarian activity between the parity groups. As one of the cows conceived before her third ovulation, 89 ovulations and 169 waves from parturition to third ovulation (side of the previous gravid horn; right side n = 17, left side n = 13) were analyzed.

| Reproductive performance, milk yield and ovarian activity of postpartum dairy cows differing in parity | Primiparous (n = 22) | Multiparous (n = 8) | Total |
|---|---|---|---|
| **Reproductive performance** | | | |
| Days to First detected estrus | 45.9 ± 18.2 | 59.6 ± 15.6 | 49.5 ± 18.4 |
| First service | 63.9 ± 10.9 | 66.6 ± 10.7 | 64.6 ± 10.7 |
| Pregnancy rate of first service (%) | 40.9 (n = 9) | 50.0 (n = 4) | 43.3 (n = 13) |
| Pregnancy rate (%) | 86.4 (n = 19) | 87.5 (n = 7) | 86.7 (n = 26) |
| Services per pregnancy | 2.1 ± 1.4 | 1.8 ± 1.0 | 2.0 ± 1.3 |
| Adjusted days open * | 84.0 ± 24.9 | 90.0 ± 36.6 | 85.6 ± 27.9 |
| **Milk yield, kg** | | | |
| Daily (7–70 days) | 29.6 ± 3.7 a | 39.8 ± 6.7 b | 32.3 ± 6.5 |
| Peak (week) | 32.6 ± 4.0 a | 43.0 ± 7.8 b | 35.3 ± 7.0 |
| 305-day | 7,802 ± 1,010 a | 10,372 ± 2,088 b | 8,487 ± 1,768 |
| **Ovarian activity** | | | |
| Days of First ovulation | 23.0 ± 11.0 | 28.0 ± 12.9 | 24.7 ± 11.5 (10–52) |
| Second ovulation | 39.3 ± 8.9 | 43.6 ± 14.7 | 40.5 ± 10.6 (25–70) |
| Third ovulation | 60.7 ± 8.1 | 64.4 ± 10.7 | 61.6 ± 8.7 (45–79) |
| Duration of PPI | 23.0 ± 11.0 | 28.0 ± 12.9 | 24.7 ± 11.5 |
| IOI-1 | 15.9 ± 5.6 | 15.6 ± 5.0 | 15.8 ± 5.3 |
| IOI-2 | 21.3 ± 4.5 | 24.6 ± 4.4 | 22.1 ± 4.6 |
| Number of follicular waves in PPI | 1.7 ± 1.2 | 2.1 ± 1.1 | 1.8 ± 1.2 |
| IOI-1 | 1.6 ± 0.7 | 1.6 ± 0.7 | 1.6 ± 0.7 |
| IOI-2 | 2.3 ± 0.6 | 2.3 ± 0.5 | 2.3 ± 0.6 |

PPI, postpartum intervals to first ovulation; IOI-1, first intraovulatory interval; IOI-2, second intraovulatory interval. * Cows without a positive pregnancy diagnosis by 180 DIM were assigned days open value equal to 21 days after their last unsuccessful service. a, b Values with different symbols are significantly different ($P < 0.05$).
Development of growing follicles

The mean maximum number of growing follicles gradually increased from parturition to the third ovulation, and became significantly larger during IOI-2 compared to PPI (P < 0.05); no significant differences were detected between the ovaries with and without PCL (Table 2).

Emergence of the dominant follicle and occurrence of ovulation

The number of total DFs with contralateral and ipsilateral relationships with PCLs during PPI, IOI-1 and IOI-2 are summarized in Fig. 2. The total emergence of ipsilateral DFs was less than contralateral DFs (ipsilateral versus contralateral; 34% (n = 57) vs. 66% (n = 112)), and deviated from a 1:1 ratio (P < 0.01). Emergence of an ipsilateral DF during PPI was less frequent than a contralateral DF (24% (n = 13) vs. 76% (n = 41)), and ipsilateral DFs during IOI-1 were less frequent than contralateral DFs (27% (n = 13) vs. 73% (n = 36)); both significantly deviated from a 1:1 ratio (P < 0.01).

The summarized emergence of an ipsilateral DF before the second postpartum ovulation also deviated from a 1:1 ratio (ipsilateral vs. contralateral; 25% (n = 26) vs. 75% (n = 77), P < 0.01), but this deviation disappeared during IOI-2 (ipsilateral vs. contralateral; 47% (n = 31) vs. 53% (n = 35)). The mean interval between calving and emergence of the first DF in the ipsilateral ovary to the PCL was significantly longer than in the contralateral ovary (ipsilateral vs. contralateral; 9.5 ± 2.5 vs. 7.5 ± 1.7, P < 0.05); however, no such delay was observed during IOI-1 and IOI-2. The frequency of first ovulations after various numbers of follicular waves were: 56% after one wave (ipsilateral vs. contralateral; 10% (n = 4) vs. 46% (n = 13)), 20% after 2 waves (ipsilateral vs. contralateral; 7% (n = 2) vs. 13% (n = 4)), and 23% after ≥ 3 waves (ipsilateral vs. contralateral; 7% (n = 2) vs. 13% (n = 4)).

Fig. 2. Number of total dominant follicles emerged with contralateral and ipsilateral relationships to PCL during PPI, IOI-1 and IOI-2, and the ratio of the contralateral relationship in each ovulation interval. PCL, corpus luteum of previous pregnancy; PPI, postpartum intervals to first ovulation; IOI-1, first intraovulatory interval; IOI-2, second intraovulatory interval; CON, contralateral relationship in which the DF and PCL are in different ovaries; IPSI, ipsilateral relationship in which the DF and PCL are in the same ovary. * Frequency of the waves in which the contralateral and ipsilateral relationship with PCL significantly deviates from the 1:1 ratio (P < 0.01).

Table 2. Mean maximum number of growing follicles per wave in the ovary with and without PCL during PPI, IOI-1 and IOI-2

| Intervals | Ovary       | Mean of both the ovaries |
|-----------|-------------|--------------------------|
|           | With PCL    | Without PCL              |
| PPI       | 3.59 ± 1.99 | 3.98 ± 1.90              |
| IOI-1     | 4.08 ± 2.02 | 4.27 ± 2.11              |
| IOI-2     | 4.36 ± 1.91 | 4.61 ± 2.82              |

PPI, postpartum intervals to first ovulation; IOI-1, first intraovulatory interval; IOI-2, second intraovulatory interval; PCL, corpus luteum of previous pregnancy. a, b Values with different symbols are significantly different (P < 0.05).
Distributions of the first, second, and third ovulation days in cows with contralateral and ipsilateral relationships between the ovulatory follicle and PCL are shown in Fig. 3. The total occurrence of ipsilateral ovulations was less than contralateral ovulations (ipsilateral vs. contralateral; 34% (n = 29) vs. 66% (n = 60)), deviating from a 1:1 ratio (P < 0.01). The occurrence of ipsilateral ovulations during the first ovulation sequence was less frequent than contralateral ovulations (23% (n = 7) vs. 77% (n = 23)), and ipsilateral ovulations during the second ovulation sequence were less frequent than contralateral ovulations (27% (n = 8) vs. 73% (n = 22)); both significantly deviated from a 1:1 ratio (P < 0.01). The summarized occurrence of ipsilateral ovulations before the second postpartum ovulation deviated from a 1:1 ratio (ipsilateral versus contralateral; 25% (n = 15) vs. 75% (n = 45), P < 0.01). At the third ovulation, these deviations disappeared in accordance with the emergence of DF during IOI-2 (ipsilateral versus contralateral; 48% (n = 14) vs. 52% (n = 15)). No significant differences were detected in the mean intervals from parturition to the first, second, or third ovulations between the two intraovulatory relationships (contralateral versus ipsilateral; 24.3 ± 12.1 vs. 26.0 ± 10.0, 39.9 ± 10.7 vs. 42.1 ± 10.9, and 63.3 ± 8.7 vs. 59.8 ± 8.8, respectively).

**Uterine endometrial involution**

Table 3 shows the changes in endometrial diameter from the previous gravid horn to first ultrasonography scan in Group-CC, in which cows had only contralateral ovulation at both the first and second ovulation sequences, and Group-NCC, in which cows had corresponding ipsilateral ovulation(s). Whilst a significant difference was detected in the measured values between the two groups at second ovulation (P < 0.05), the ratio of the measured values to the initiation values showed no differences between the three ovulation sequences. No severe abnormal findings to the endometrium were detected in any cow via sequential scanning.

**Table 3.** Changes in uterine endometrial diameter of previous gravid horn in the two groups

| Group | CC (n = 17) | NCC (n = 13) |
|-------|-------------|--------------|
| **Endometrial diameter (mm)** | | |
| At the start | 56.7 ± 6.4 | 53.2 ± 11.6 |
| First ovulation | 31.5 ± 8.8 | 27.5 ± 10.2 |
| Second ovulation | 26.5 ± 5.2 a | 22.6 ± 4.8 b |
| Third ovulation | 26.9 ± 6.0 | 24.7 ± 4.4 |
| **Ratio to the value at the start (%)** | | |
| First ovulation | 56.3 ± 0.2 | 53.0 ± 0.2 |
| Second ovulation | 46.9 ± 0.1 | 44.2 ± 0.1 |
| Third ovulation | 44.4 ± 0.0 | 48.4 ± 0.0 |
| **Day of measurement** | | |
| At the start | 6.8 ± 1.0 | 6.9 ± 1.1 |
| First ovulation | 23.7 ± 12.3 | 25.9 ± 10.8 |
| Second ovulation | 40.3 ± 11.9 | 40.7 ± 9.2 |
| Third ovulation | 61.1 ± 9.4 a | 62.2 ± 8.2 b |

Group-CC, the cows with contralateral relationship between PCL and ovulatory follicle at both the first and the second ovulation; Group-NCC, cows excluded from Group-CC. a,b Values with different symbols are significantly different (P < 0.05).
Reproductive and productive traits

A summary of the reproductive and productive traits between Groups -CC and -NCC is shown in Table 4. There were no significant differences between the two groups in terms of reproductive traits, milk yields, or BCS changes during the experimental period. At 1-week peripartum, the average scores of Groups -CC and -NCC were 3.38 and 3.35, respectively; both subsequently decreased (Fig. 4). The BCS from parturition to 10-weeks postpartum in Groups -CC and -NCC ranged from 3.20 to 2.86 and 3.19 to 2.77, respectively.

For milk composition, the mean values for milk fat and protein concentration during the 4 months of postpartum ranged from 3.6 to 4.1% and 3.0 to 3.3% for Groups -CC and -NCC, respectively; there were no significant differences between the two groups (Fig. 5A and 5B). However, lower milk lactose concentrations were recorded in the cows in Group-CC at 30 and 60 days postpartum, compared to the cows in Group-NCC (P < 0.05, Fig. 5C).

Discussion

Our sequential observations indicate that the side of previous pregnancy has no effect on the development of growing follicles, as observed within the experimental period. However, the emergence of ipsilateral DFs and occurrence of postpartum ipsilateral ovulation were less frequent, and were sustained until the second ovulation was completed, 22–56 days postpartum. This postpartum imbalance in ovarian activity was not associated with the reproductive and productive performance of cows, except for milk lactose concentration.

Early studies have suggested that the ovary ipsilateral to the side of previous pregnancy exhibits lower functional activity than the contralateral ovary. These studies focused on the occurrence of postpartum ovulation only once during the early postpartum period, using the transrectal palpation technique [4, 9, 10]; the results indicate that ipsilateral ovulation occurs in 20–33% of ovulations and that this imbalance is detectable either up until 20 days postpartum [9] or between 30 and 35 days postpartum [4, 10]. The persistence of this ovulation-side imbalance, called the “carry-over effect”, was thought to depend on the interval from parturition. In our study, a 25% ipsilateral occurrence was observed in the first and second postpartum ovulations (Fig. 3), consistent with the results of these previous studies. However, our results clearly indicate that the

Table 4. Reproductive performance and milk yield of the cows in the two groups

| Traits                           | Group          |
|----------------------------------|----------------|
|                                  | CC (n = 17)    | NCC (n = 13) |
| Days to                          |                |
| First detected estrus            | 47.9 ± 16.3    | 51.7 ± 21.3  |
| First service                    | 64.6 ± 11.5    | 64.7 ± 10.1  |
| Pregnancy rate of first service (%)| 41.2 (n = 7)   | 46.2 (n = 6) |
| Pregnancy rate (%)               | 94.1 (n = 16)  | 76.9 (n = 10)|
| Services per pregnancy           | 2.1 ± 1.4      | 2.0 ± 1.2    |
| Adjusted days open*              | 89.4 ± 30.6    | 80.7 ± 24.1  |
| Milk yield, kg                   |                |
| Daily (7–70 days)                | 33.0 ± 1.6     | 31.4 ± 1.8   |
| Peak (week)                      | 35.9 ± 1.7     | 34.6 ± 2.0   |
| 305-day                          | 8,573 ± 436    | 8,375 ± 498  |

Abbreviations: See Table 3. * Cows without a positive pregnancy diagnosis by 180 DIM were assigned days open value equal to 21 days after their last unsuccessful service.
Persistence of this ovulation side imbalance does not depend on the interval days from parturition, but on the ovulation number. This “carry-over effect” was exerted at both the first and second postpartum ovulations, but not at the third.

Using ultrasonography, a recent study indicated that follicular recruitment activity, evaluated by the number of follicles > 4 mm in diameter, is lower in the ipsilateral than contralateral ovary, resulting in less frequent emergence of postpartum first ipsilateral DFs and occurrence of ipsilateral ovulation [14]. These results, limiting the first postpartum dominant follicular wave, suggest an ipsilateral inhibitory effect on postpartum ovarian activity shortly after parturition. Our results, obtained from sequential examinations over an extended experimental period, from the emergence of the first postpartum follicular wave to third postpartum ovulation (Fig. 2), demonstrate a lower frequency of ipsilateral ovulation due to a reduced emergence of ipsilateral DFs until the second postpartum ovulation; however, no such imbalance was detected in the development of growing follicles throughout the experimental period (Table 2). It is postulated that the increase in the number of growing follicles until the third ovulation might reflect a recovery in ovarian activity during the postpartum period, consistent with the number of follicular waves before ovulation [3]. Thus, the present study clearly demonstrates that the side of previous pregnancy exerts suppressive effects on the emergence of follicular waves and occurrence of ovulation, but not on the development of growing follicles. These effects were confirmed, not only before the first postpartum ovulation, but also up until the second postpartum ovulation was completed.

Several studies indicate that the presence of a large follicle in the ovary ipsilateral to the side of previous pregnancy improves reproductive performance [15–17]. In the present study, the number of cows that experienced ipsilateral ovulation was too small to analyze the effect of the presence of a large follicle in the ipsilateral ovary on fertility. No difference in reproductive performance was observed between cows with and without contralateral ovulation at both the first and second ovulation sequences (Table 4). Thus, ovulation imbalance during the early postpartum period might not have a major effect on the subsequent reproductive performance of lactating cows; however, this requires verification in future studies among a larger number of animals.

With respect to milk yield and BCS, no significant differences were detected between the two groups, except for a lower milk lactose concentration (Fig. 5C). A recent study has indicated the utility of milk content data as indices of EB and ovarian activity [24]. The synthesis of milk lactose depends on glucose supplied to the mammary glands, and its concentration may reflect the energy status of lactating cows, as it is synthesized almost entirely from plasma glucose. Our results suggest that cows with contralateral ovulation at both the first and second postpartum ovulations were in a state of negative EB from 30–60 days postpartum, during which most of the second ovulations occurred. We speculate that changes in milk lactose concentrations might reflect minute negative EB of early postpartum cows; hence, a difference in milk lactose concentration could potentially be a sensitive indicator for ovarian activity compared to milk yield and BCS. However, as the present study is the first to report a relationship between ovarian activity imbalance and productive performance, these results require verification in future studies among a larger number of animals.

It has been suggested that the suppression of folliculogenesis in the ovary ipsilateral to the previous gravid horn is induced by the local inhibitory effects of the ipsilateral PCL and/or gravid uterine horn [4, 9–11]. Progesterone secreted from the PCL strongly inhibits the growth of the DF in both ovaries until parturition [25]. Luteolysis of the CL during the normal estrous cycle is rapid, whereas following parturition is prolonged [26], and until 35 days postpartum, physical remnants of PCL cells can be histologically detectable [27]. In addition to the effect of the PCL, inhibitory effects of the previous gravid horn on ovarian activities have been suggested, given that one study demonstrated that postpartum ovarian activity imbalance could be detected, even after PCL removal by prostaglandin (PG) F₂α.

![Fig. 5. Changes in milk fat (A), protein (B) and lactose (C) concentrations (%) for 4 months postpartum: Group-CC (○), and Group-NCC (■). Vertical bars show SD. * Values differ significantly between the two groups (P < 0.05). Group-CC, cows with contralateral relationship between the corpus luteum of the previous pregnancy and ovulatory follicle at both the first and second ovulation; Group-NCC, cows excluded from Group-CC.](image-url)
administration before parturition [14]. The counter current vascular communication between the uterus and ovary [28] may contribute to the inhibitory effect of the gravid horn on ipsilateral ovarian activity. This intra-organ communication is dependent on both sides of the reproductive tract, and larger amounts of secretions from the involuting uterus may be observed [11]; however, the exact inhibitory mechanisms are still unknown.

The results of the present study suggest that ovulation-side imbalance does not influence uterine regression, as indicated by ratings of endometrial diameter changes (Table 3). Morphological changes in the endometrium in the gravid horn may not reflect cytological disturbances. Sheldon et al. [13] reported that a uterine bacterial infection could disturb the hormonal interactions that control normal cyclical ovarian function, and that imbalances in postpartum ovarian activity could be detected even after PCL removal before parturition [14]. Our results indicate that the effect of the previously pregnant side on ovarian activity lasted until completion of the second postpartum ovulation, occurring after 35 days postpartum (Fig. 3). Thus, we hypothesize that factor(s) other than the PCL of ovaries in the previously pregnant side and gravid horn induce an imbalance in ovarian activity until the second ovulation postpartum, in a synergistic manner, and that ovarian factors other than the PCL exist prior to parturition. Further studies are required to understand the mechanisms that lead to imbalances in ovarian activity during the early postpartum period.

In conclusion, the sequential observations in this study clearly indicate that the side of previous pregnancy affects postpartum DF selection and ovulation, but not the development of growing follicles; these effects persist until the second postpartum ovulation. Postpartum ovarian activity imbalance was not associated with the reproductive and productive performance of cows, except for milk lactose concentration, probably related to postpartum EB status.

References

1. Savio JD, Boland MP, Hynes N, Roche JF. Resumption of follicular activity in the early post-partum period of dairy cows. J Reprod Fertil 1990; 88: 569-579. [Medline] [CrossRef]
2. McDougall S, Burke CR, MacMillan KL, Williamson NB. Patterns of follicular development during periods of anovulation in pasture-fed dairy cows after calving. Res Vet Sci 1995; 55: 212-216. [Medline] [CrossRef]
3. Sakaguchi M, Sasamoto Y, Suzuki T, Takahashi Y, Yamada Y. Postpartum ovarian follicular dynamics and estrous activity in lactating dairy cows. J Dairy Sci 2004; 87: 2114-2121. [Medline] [CrossRef]
4. Marion GB, Gier HT. Factors affecting bovine ovarian activity after parturition. J Anim Sci 1968; 27: 1621-1626. [CrossRef]
5. Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 2001; 84: 1277-1293. [Medline] [CrossRef]
6. Kusaka H, Miura H, Kikuchi M, Sakaguchi M. Incidence of double ovulation during the early postpartum period in lactating dairy cows. Theriogenology 2017; 91: 98-103. [Medline] [CrossRef]
7. Lucy MC. Mechanisms linking nutrition and reproduction in postpartum cows. Reprod Suppl 2003; 61(Suppl): 415-427. [Medline]
8. Beam SW, Butler WR. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J Reprod Fertil Suppl 1999; 54(Suppl): 411-424. [Medline]
9. Saiduddin S, Riesen JW, Tyler WJ, Casale LE. Some cause-ovary effects of pregnancy on post-partum ovarian function in the cow. J Dairy Sci 1967; 50: 1846-1847. [Medline] [CrossRef]
10. Foote WD, Peterson DW. Relationships between side of pregnancy and side of subsequent ovarian activities in beef and dairy cattle. J Reprod Fertil 1968; 16: 415-421. [Medline] [CrossRef]
11. Nation DP, Burke CR, Rhodes FM, MacMillan KL. The inter-ovarian distribution of dominant follicles is influenced by the location of the corpus luteum of pregnancy. Anim Reprod Sci 1999; 56: 169-176. [Medline] [CrossRef]
12. Sheldon IM, Noakes DE, Dobson H. The influence of ovarian activity and uterine involution determined by ultrasonography on subsequent reproductive performance of dairy cows. Theriogenology 2000; 54: 409-419. [Medline] [CrossRef]
13. Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H. Influence of uterine bacterial contamination after parturition on ovarian follicle selection and follicle growth and function in cattle. Reproduction 2002; 123: 837-845. [Medline] [CrossRef]
14. Sheldon IM, Noakes DE, Dobson H. Effect of the regressing corpus luteum of pregnancy on ovarian folliculogenesis after parturition in cattle. Biol Reprod 2002; 66: 266-271. [Medline] [CrossRef]
15. Bonnett BN, Martin SW, Meek AH. Associations of clinical findings, bacteriological and histological results of endometrial biopsy with reproductive performance of postpartum dairy cows. Prev Vet Med 1993; 15: 205-220. [CrossRef]
16. Bridges PJ, Taft R, Lewis PE, Wagner WR, Immek KE. Effect of the previously gravid uterine horn and postpartum interval on follicular diameter and conception rate in beef cows treated with estradiol benzoate and progesterone. J Anim Sci 2000; 78: 2172-2176. [Medline] [CrossRef]
17. Sheldon IM, Noakes DE, Rycroft AN, Dobson H. The effect of intratrauterine administration of estradiol on postpartum uterine involution in cattle. Theriogenology 2003; 59: 1357-1371. [Medline] [CrossRef]
18. Sakaguchi M, Sasamoto Y, Suzuki T, Takahashi Y, Yamada Y. Fate of cystic ovarian follicles and the subsequent fertility of early postpartum dairy cows. Vet Rec 2006; 159: 197-201. [Medline] [CrossRef]
19. Edmonson AJ, Lean JJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989; 72: 68-78. [CrossRef]
20. Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J Reprod Fertil 1988; 83: 663-671. [Medline] [CrossRef]
21. Siriois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127: 916-925. [Medline] [CrossRef]
22. Smith MC, Wallace JM. Influence of early post partum ovulation on the re-establishment of pregnancy in multiparous and primiparous dairy cattle. Reprod Fertil Dev 1998; 10: 207-216. [Medline] [CrossRef]
23. Gunther OJ, Hoffmann MM. Interactions of side (left and right ovary) with the number of follicles per ovary and with the intravarian relationships between dominant follicle and corpus luteum in heifers. Theriogenology 2016; 86: 907-913. [Medline] [CrossRef]
24. Reksen O, Havrevoet O, Gröhn YT, Bolstad T, Waldmann A, Ropstad E. Relationships among body condition score, milk constituents, and postpartum luteal function in Norwegian dairy cows. J Dairy Sci 2002; 85: 1406-1415. [Medline] [CrossRef]
25. Gunther OJ, Kot K, Kolick L, Martin S, Wittbank MC. Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. J Reprod Fertil 1996; 108: 271-279. [Medline] [CrossRef]
26. Dufour JJ, Ray GL. Distribution of ovarian follicular populations in the dairy cow within 35 days after parturition. J Reprod Fertil 1985; 73: 229-235. [Medline] [CrossRef]
27. Sawyer HR. Structural and functional properties of the corpus luteum of pregnancy. J Reprod Fertil Suppl 1995; 49(Suppl): 97-110. [Medline] [CrossRef]
28. Gunther OJ. Internal regulation of physiological processes through local vasoarterial pathways: a review. J Anim Sci 1974; 39: 550-564. [Medline] [CrossRef]