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—Original Article—

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

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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

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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

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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, IOI-1, and IOI-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 the 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

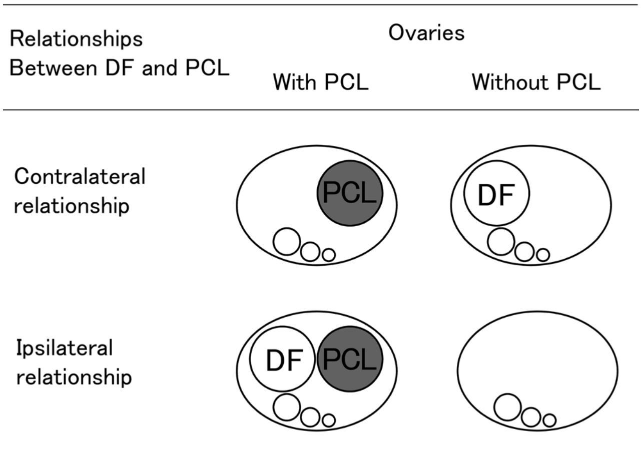

Fig. 1. Illustration of the spatial association between contralateral and ipsilateral intraovarian patterns as described previously by Ginther and Hoffman (2016). Terminology for two relationships describing the location between the dominant follicle (DF) and corpus luteum of previous pregnancy (PCL): contralateral relationship in which the DF and PCL are in different ovaries; ipsilateral relationship in which the DF and PCL are in the same ovary.

test. Pregnancy rates between Groups -CC and -NCC were compared using the Chi-square test (χ^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.

Table 1. 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 ($P < 0.05$).

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 (Total number of waves)
	With PCL	Without PCL	
PPI	3.59 ± 1.99	3.98 ± 1.90	3.79 ± 1.94 [a]
IOI-1	4.08 ± 2.02	4.27 ± 2.11	4.17 ± 2.06 [ab]
IOI-2	4.36 ± 1.91	4.61 ± 2.82	4.48 ± 2.40 [b]

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).

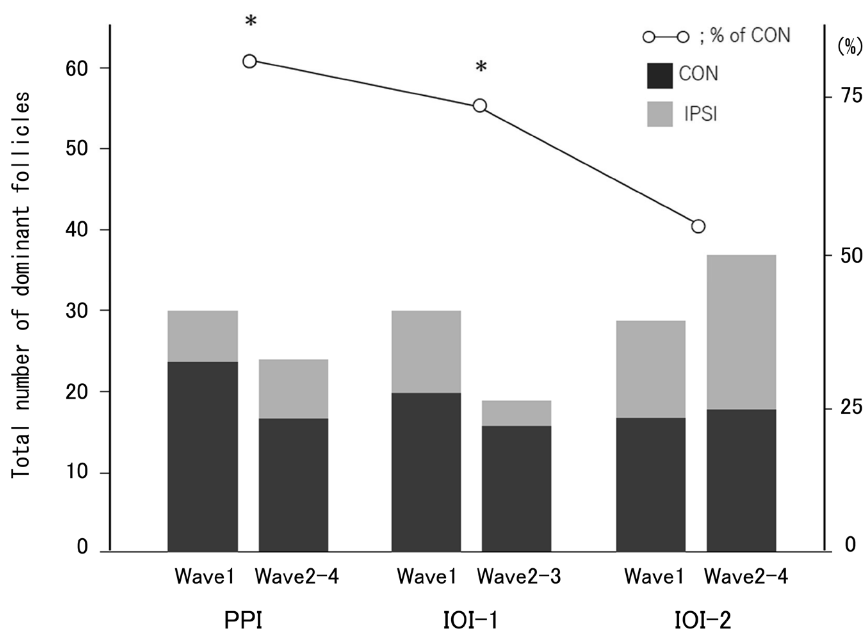

Fig. 2. Number of total dominant follicles emerged with contralateral and ipsilateral relationships to PCL during PPI, IOI-1 and IOI-2, and the ratio of the contralateral relationship in each ovulation interval. PCL, corpus luteum of previous pregnancy; PPI, postpartum intervals to first ovulation; IOI-1, 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).

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;

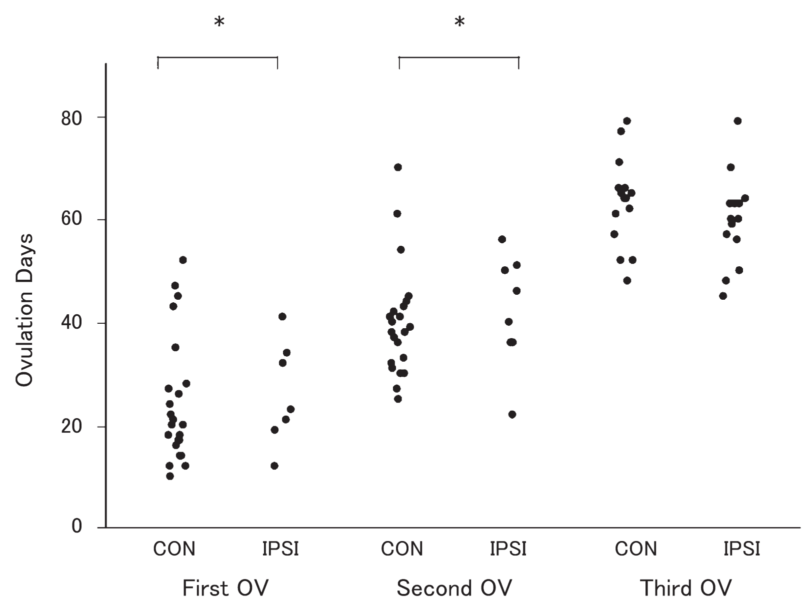

Fig. 3. Distribution of the first, second and third ovulation days for all the cows with contralateral and ipsilateral relationships between the ovulatory follicle and PCL. PCL, corpus luteum of previous pregnancy; OV, ovulation; CON, contralateral relationship in which the ovulatory follicle and PCL are in the different ovaries; IPSI, ipsilateral relationship in which the ovulatory follicle and PCL are in the same ovary * Frequency of ovulations in which the contralateral and ipsilateral relationship to the PCL significantly deviates from a 1:1 ratio ($P < 0.01$).

3% (n = 1) *vs.* 20% (n = 6)).

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 were 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 also 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 relationships; 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	62.2 ± 8.2

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 ($P < 0.05$).

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.

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

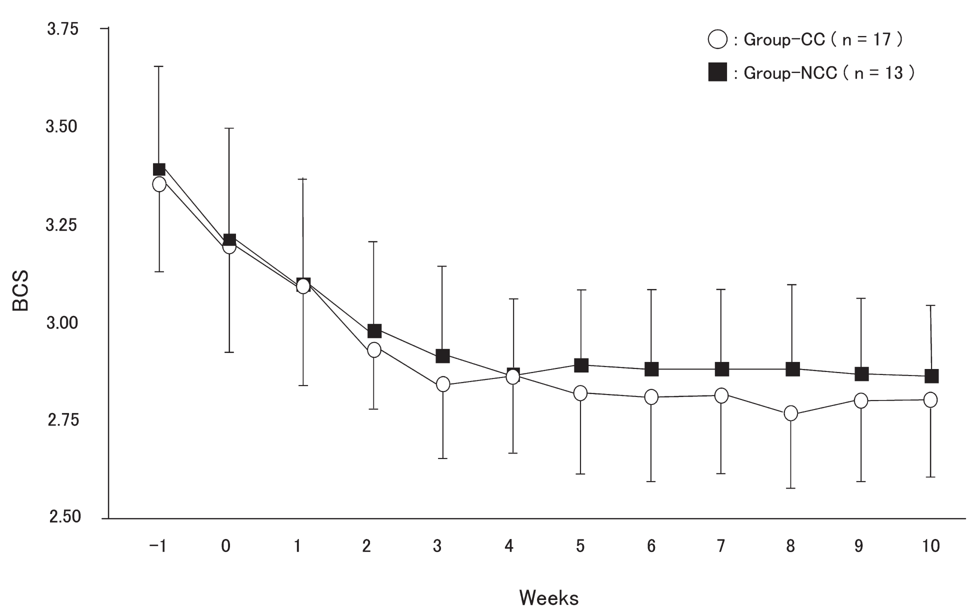

Fig. 4. Changes in BCS from 1-week peripartum to 10-week postpartum: Group-CC (○), and Group-NCC (■). Vertical bars show SD. BCS, body condition scores; Group-CC, cows with contralateral relationship between the corpus luteum of the previous pregnancy and ovulatory follicle at both the first and second ovulation; Group-NCC, cows excluded from Group-CC.

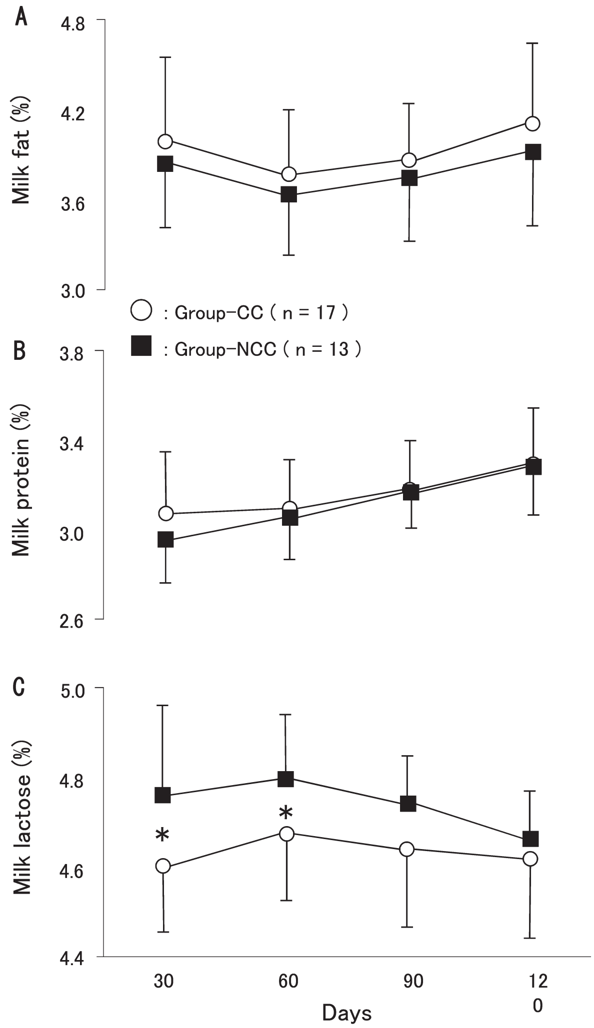

Fig. 5. Changes in milk fat (A), protein (B) and lactose (C) concentrations (%) for 4 months postpartum: Group-CC (○), and Group-NCC (■). Vertical bars show SD. * Values differ significantly between the two groups ($P < 0.05$). Group-CC, cows with contralateral relationship between the corpus luteum of the previous pregnancy and ovulatory follicle at both the first and second ovulation; Group-NCC, cows excluded from Group-CC.

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) $F_{2\alpha}$

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.

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