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—Original Article—

Influence of ipsilateral coexistence of the first wave dominant follicle and corpus luteum on ovarian dynamics and plasma sex steroid hormone concentrations in lactating dairy cows treated with human chorionic gonadotropin

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Abstract. We examined the effect of human chorionic gonadotropin (hCG) treatment 5 days after estrus on ovarian dynamics and plasma progesterone (P_4) and estradiol (E_2) concentrations when the first-wave dominant follicle (DF) was ipsilateral or contralateral to the corpus luteum (CL) in lactating dairy cows. Seventy cows were divided into two groups: (1) ipsilateral group (IG; n = 37), in which the first-wave DF was ipsilateral to the CL, and (2) contralateral group (CG; n = 33), in which the first-wave DF was contralateral to the CL. IG and CG were further subdivided into two groups: non-treatment group (IG, n = 18; CG, n = 19), and hCG treatment group: administrated 1500 IU of hCG 5 days after estrus (IG, n = 19; CG, n = 14). Blood sampling and ovarian examination were performed at 3, 5, 7, 9, 11, and 13 days after estrus. Mean diameter of the first-wave DF on Day 9 tended ($P = 0.067$) to be larger in IG than in CG in the non-treatment group. Mean diameter of CL and plasma P_4 and E_2 concentrations did not differ between IG and CG in the non-treatment and hCG treatment groups. Accessory CL development did not differ between IG and CG in the hCG treatment group. Our findings indicate that CL development and plasma P_4 and E_2 concentrations were not affected by the existence of the first-wave DF; however, first-wave DF development was affected by the existence of a CL in the same ovary.

Key words: Corpus luteum, First follicular wave dominant follicle, Human chorionic gonadotropin (hCG), Ovarian dynamics, Progesterone

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The first follicular wave emerges immediately after estrus, and the first-wave dominant follicle (DF) undergoes atresia during day 8 to 10 of the estrous cycle in a two-wave pattern in cattle; therefore, most first-wave DFs are not ovulated [1]. To clarify the manner of development of the dominant follicle, the first-wave DF has been extensively examined in previous studies [2–4]. However, the effects of the first-wave DF on fertility in cattle have not been examined in detail.

Previously, we have reported that the development of the first-wave DF ipsilateral to the corpus luteum (CL) was associated with reduced conception rates in lactating dairy cows [5] and the conception rate could be improved by administrating human chorionic gonadotropin (hCG) only to the cows having the first-wave DF ipsilateral to the CL at 5 days after artificial insemination (AI) [6]. However, we could not clarify the mechanism underlying the low fertility when the first-wave DF was ipsilateral to the CL and the physiological mechanisms of increasing conception rate by hCG treatment with the first-wave DF ipsilateral to the CL at 5 days after AI.

The dynamics of DF growth and the hormonal milieu during first-wave DF development have been well studied [7]. However, the dynamics of DF growth, CL growth and plasma progesterone (P_4) and estradiol (E_2) concentrations between the first-wave DF ipsilateral or contralateral to the CL in lactating dairy cows are not well understood. According to a previous study, lactating dairy cows that were diagnosed pregnant have higher P_4 concentrations during the early luteal phase after AI compared with that of non-pregnant cows [8]. The effect of plasma E_2 concentration on conception rate during the early luteal phase has not been explored thoroughly. Therefore, it is hypothesized that if the plasma P_4 or E_2 concentrations were different between the ipsilateral and the contralateral locations of the first-wave DF and the CL during the early luteal phase without or with hCG treatment, we could clarify causes of lower conception rate in cows having the first-wave DF ipsilateral to the CL and increasing conception rate.

In this study, we aimed to compare the dynamics of DF growth, CL growth, and plasma P_4 and E_2 concentrations between the ipsilateral and contralateral locations of the first-wave DF and the CL without or with hCG treatment at 5 days after estrus, and to evaluate the effect of ovarian structures and systemic steroid hormones on the mechanism underlying the difference in conception rate between the first-wave DF ipsilateral to the CL.

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Materials and Methods

Animals and management

Postpartum lactating Holstein cows (n = 70: postpartum day of estrus, 114.8 ± 42.9; parity, 2.1 ± 1.2; milk production, 37.0 ± 8.9 kg/day; body condition score [9], 2.8 ± 0.4; means ± SD) at the Field Science Center of Obihiro University in Hokkaido, northeast Japan, were used. The trial was conducted from December 2013 to February 2015. Cows underwent regular estrous cycles and were clinically healthy during the breeding period. Cows that experienced reproductive or metabolic diseases, such as metritis, ketosis, hypocalcemia, or displaced abomasum, were excluded. The cows were kept in a freestall barn under the normal management program of the Field Science Center of Obihiro University, and were fed a TMR diet that included corn silage, grass silage, soybean meal, corn grain, and concentrate, along with free access to water. All cows were milked twice daily in a parallel parlor. The experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro University.

Study design

Spontaneous estrus was detected by visual observation, examination of tail paint, and rectal palpation to confirm the preovulatory follicle and regressed CL. After the estrus was detected, ovulation of preovulatory follicle was confirmed at every 24 h using rectal palpation or transrectal ultrasonography. We defined the day of ovulation as Day 1. After confirming the ovulation, the cows were randomly assigned to either the control (non-treatment) or the hCG treatment group. The cows in the non-treatment group (n = 37) were not administrered hCG, whereas those in the hCG treatment group (n = 33) were intramuscularly administered 1500 IU of hCG (Gestron1500, Kyoritsu Seiyaku, Tokyo, Japan) on Day 5. In addition, we examined CL and first-wave DF, to determine the largest follicle in the ovaries on Day 5 using transrectal ultrasonography; the location of the first-wave DF was confirmed to be either ipsilateral [ipsilateral group (IG): non-treatment, n = 18; hCG treatment, n = 19] or contralateral [contralateral group (CG): non-treatment, n = 19; hCG treatment, n = 14] to the CL. We only used the cows with single CL and no co-dominant follicle in this experiment; no co-dominant follicle means there was only one follicle which size had > 10.0 mm on Day 5. In the hCG treatment group, ovarian examination was performed on Day 7 to confirm the evacuation of the first-wave DF to evaluate the ovulation of the first-wave DF. The schematic diagram of study design is shown in Fig. 1

Ovarian examination

Ovarian examinations were performed using a transrectal ultrasonic device equipped with a 5.0-MHz linear transducer (HS-101V, Honda Electronics, Toyohashi, Japan). The locations of the first-wave DF and CL of all the cows were confirmed on Day 5. In non-treatment group, the mean diameters of the first-wave DF and CL were determined on Day 3, 5, 7, 9, 11, and 13. In the hCG treatment group, the mean diameter of the CL was determined on Day 3, 5, 7, 9, 11, and 13; the mean diameter of the first-wave DF was determined on Day 3 and 5; and the mean diameter of the accessory CL was determined on Day 7, 9, 11, and 13.

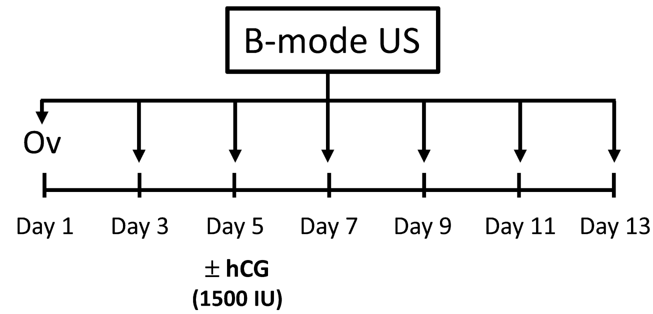

Fig. 1. Schematic diagram of experimental model. Ovulation day was defined as Day 1. In all lactating dairy cows, the position of the first follicular wave dominant follicle (the first-wave DF) and the corpus luteum (CL) was confirmed at Day 5 using ultrasonography. Human chorionic gonadotropin (hCG) (1500 IU) was administered in hCG treatment group at Day 5, and ovulation of the first-wave DF was confirmed at Day 7 using ultrasonography. The mean diameter of the first-wave DF and CL were measured on Day 3, 5, 7, 9, 11, and 13. In addition, blood samples for hormone assays were collected on Day 3, 5, 7, 9, 11, and 13 from tail vein. B-mode US = trans-rectal ultrasonography.

Blood sampling

Blood samples were collected into heparinized tubes from the tail vein on Day 3, 5, 7, 9, 11, and 13 in both non-treatment and hCG treatment groups. Plasma was separated by centrifugation (2000 × *g* for 20 min at 4°C) and frozen at −30°C until analysis of P_4 and E_2 concentrations.

Plasma P_4 concentration assay

Plasma P_4 concentrations were determined in duplicate using a double-antibody enzyme immunoassay (EIA), according to previously established EIA procedures [10]. Assays were performed after extraction with diethyl ether. The recovery rate of P_4 was 85%. The standard curve ranged from 0.05 to 50 ng/ml. The ED50 of the assay was 2.4 ng/ml. The intra- and inter-assay CVs were 5.8% and 10.5% at 10.5 ng/ml.

Plasma E_2 concentration assay

Plasma E_2 concentrations on Day 5 were determined in duplicate using a double-antibody EIA kit (Serum 17β-Estradiol Enzyme Immunoassay Kit, Arbor Assays, MI, USA). Assays were performed after extraction with diethyl ether. All samples were concentrated 10 times for measuring. The recovery rate of E_2 was 80%. The standard curve ranged from 3.75 to 120 pg/ml. The ED50 of the assay was 12.4 pg/ml and the intra-assay CV was 12.5% at 15 pg/ml.

Statistical analysis

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequency used in biostatistics [11].

All data were confirmed to follow a normal distribution and were analyzed by F-test or Bartlett test to verify homogeneity of variances. We used two-way ANOVA with repeated measures to determine the main effects of group (IG *vs*. CG) and day (days from ovulation day) and their interaction to assess the sequential data of the mean diameter of the first-wave DF in the non-treatment group and the mean diameter of the first-wave DF and accessory CL in the hCG treatment group. The mean diameter of CL and plasma P_4 concentration were evaluated using three-way ANOVA with repeated measures to determine the main effects of group (IG *vs*. CG), treatment (non-treatment *vs*. hCG treatment) and day (days from ovulation day) and their interactions to assess sequential data. When a significant interaction was detected, Holm test, as a multiple comparison test, was used to detect significant differences among the groups within days and among the days within groups. Plasma E_2 concentrations at Day 5 were analyzed by two-way ANOVA to determine the main effects of group (IG *vs*. CG) and treatment (non-treatment group *vs*. hCG treatment group) and their interaction.

A P value of < 0.05 indicated a significant difference, a P value of < 0.1 indicated a tendency. Data are presented as mean ± SEM.

Results

Mean diameter of the first-wave DF in the non-treatment group

Analysis of the mean diameter of the first-wave DF in the non-treatment group is shown in Fig. 2. The group effect (P < 0.05), day effect (P < 0.001), and the group by day interaction (P < 0.05) were significant. The mean diameter of the first-wave DF on Day 9 tended to be greater in IG than that in CG (P = 0.067).

Mean diameter of the CL in the non-treatment and the hCG treatment groups

Analysis of the mean diameter of CL in the non-treatment and the hCG treatment groups is shown in Fig. 3. The day effect was significant (P < 0.001). There were no differences in the group effect, the treatment effect, the group by day interaction, the group by treatment interaction, the day by group interaction, and the group by treatment by day interaction.

Mean diameter of the first-wave DF in the hCG treatment group

The day effect was significant (P < 0.05). There were no differences in the group effect and the group by day interaction (data not shown).

Mean diameter of the accessory CL in the hCG treatment group

The accessory CL was formed in all cows of the hCG treatment group. Analysis of the mean diameter of the accessory CL in the hCG treatment group is shown in Fig. 4. The day effect was significant (P < 0.001). There were no differences in the group effect and the group by day interaction.

Plasma P_4 concentration in the non-treatment and the hCG treatment groups

Analysis of the plasma P_4 concentration in the non-treatment and the hCG treatment groups is shown in Fig. 5. The day effect (P < 0.001), and the treatment by day interaction (P < 0.01) were significant, and the treatment effect was tendency (P < 0.1). There were no differences in the group effect, the group by day interaction, the group by treatment interaction, and the group by treatment by day interaction.

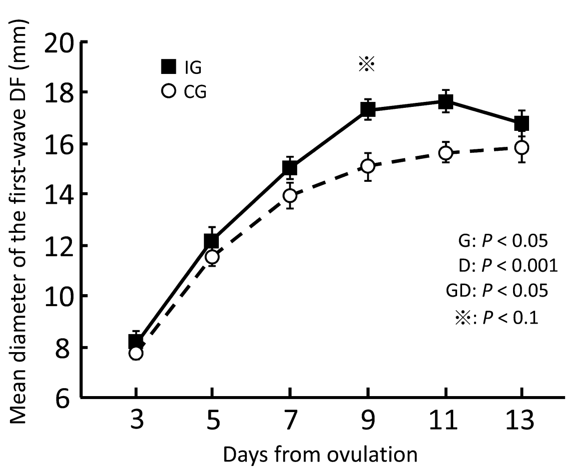

Fig. 2. Comparative changes of mean diameter of the first follicular wave dominant follicle (the first-wave DF) in ipsilateral group (IG) and contralateral group (CG) in the non-treatment group between Day 3 to Day 13. Day 1 = the day of ovulation. Effect of the group (G) (P < 0.05), day (D) (P < 0.001) and their interaction between group and day (GD) (P < 0.05). The asterisk denotes difference between IG and CG on Day 9.

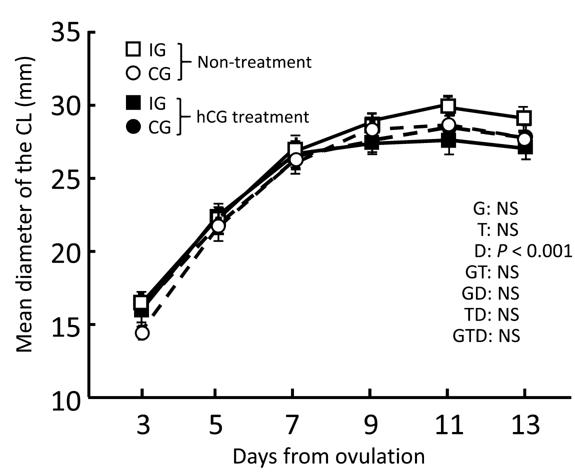

Fig. 3. Comparative changes of mean diameter of the corpus luteum (CL) in ipsilateral group (IG) and contralateral group (CG) in the non-treatment group and the human chorionic gonadotropin (hCG) treatment group between Day 3 to Day 13. Day 1 = the day of ovulation. Effect of the day (D) (P < 0.001).

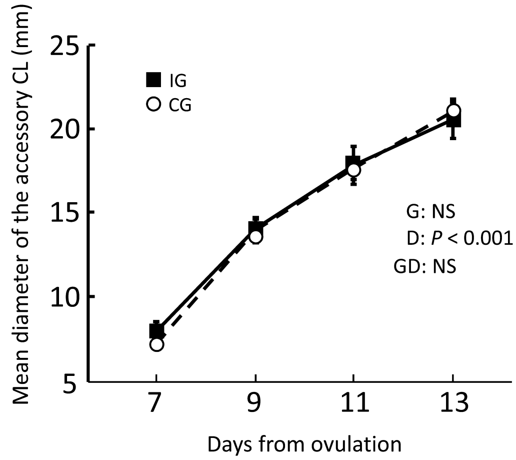

Fig. 4. Comparative changes of mean diameter of the accessory corpus luteum (CL) in ipsilateral group (IG) and contralateral group (CG) in the human chorionic gonadotropin (hCG) treatment group between Day 7 to Day 13. Day 1 = the day of ovulation. Effect of the day (D) (P < 0.001).

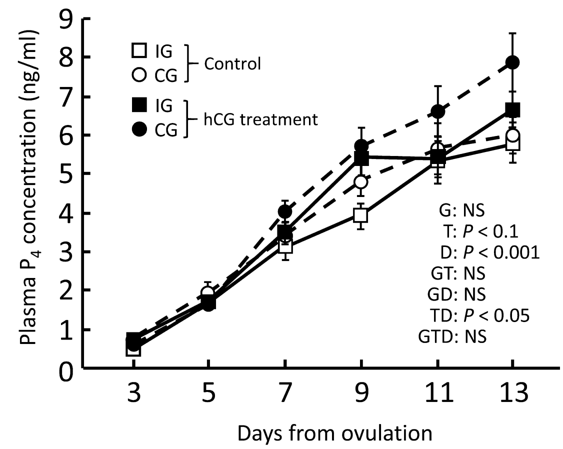

Fig. 5. Comparative changes of plasma P_4 concentration in psilateral group (IG) and contralateral group (CG) in the non-treatment group and the human chorionic gonadotropin (hCG) treatment group between Day 3 to Day 13. Day 1 = the day of ovulation. Effect of the day (D) (P < 0.001), the treatment (T) (P < 0.1), and the treatment by day interaction (TD) (P < 0.05).

Table 1. Plasma estradiol (E_2) concentration of ipsilateral group (IG) and contralateral grooup (CG) in the non-treatment and the human chorionic gonadotropin (hCG) treatment groups on Day 5 [5]

	Non-treatment group [3]		hCG treatment group [4]	
	IG [1]	CG [2]	IG	CG
Plasma E_2 concentration (pg/ml)	2.9 ± 0.3	2.9 ± 0.2	2.7 ± 0.3	3.0 ± 0.1

[1] IG = The first-wave DF located ipsilateral to the corpus luteum (CL). [2] CG = The first-wave DF located contralateral to the CL. [3] Non-treatment = Cows were not treated with hCG. [4] hCG treatment = Cows were treated with hCG (1500 IU) at 5 days from ovulation. [5] Day 5 = Ovulation day was defined as Day 1.

Plasma E_2 concentration in the non-treatment and hCG treatment groups

Analysis of the plasma E_2 concentration in the non-treatment and hCG treatment groups is shown in Table 1. There were no differences in the group effect, the treatment effect, and the group by treatment interaction.

Discussion

In this study, we investigated the dynamics of the development of the first-wave DF, CL, and accessory CL and the plasma P_4 and E_2 concentrations in lactating dairy cows treated or untreated with hCG 5 days after estrus, and with a first-wave DF that was ipsilateral or contralateral to the CL. The mean diameter of the first-wave DF tended to be greater in IG than that in CG in the non-treatment group. However, there were no significant differences in the mean diameter of CL, accessory CL, plasma P_4 concentration and plasma E_2 concentration between IG and CG with or without hCG treatment 5 days after estrus.

Previous studies that compared the size of the first-wave DF ipsilateral and contralateral to the CL in heifers have shown complex results [12]. The first-wave DF and CL are greater in IG than those in CG when adjacent (the distance between the walls of the DF and CL is less than 3.0 mm); however, the size of the first-wave DF is greater in CG than that in IG and the size of the CL does not differ between IG and CG when separated (the distance between the walls of the DF and CL is more than 3.0 mm) from each other [12]. In this study, we used lactating dairy cows, where the results showed that the first-wave DF was greater in IG than that in CG; and that the mean diameter of the CL, plasma P_4 concentration, and plasma E_2 concentration on Day 5 did not differ between IG and CG. To the best of our knowledge, this is the first study comparing the characteristics of the first-wave DF, CL, plasma P_4 concentration and plasma E_2 concentration between IG and CG in lactating dairy cows. A previous study in heifers [13] using color Doppler ultrasonography demonstrated that the blood flow signal in the follicular wall was greater in IG than that in CG. This result indicates that the vascular perfusion in the follicular wall of the first-wave DF is greater in IG than in CG. Blood supply to the follicle is closely related to follicular growth [14]. Therefore, it has been speculated that the greater vascular perfusion of the first-wave DF in IG might lead to greater growth. To evaluate the activity of the first-wave DF in IG and CG, we measured plasma E_2 concentration on Day 5. Although the size of the first-wave DF constantly increases from 1 to 11 days after estrous cycle [2], the follicular [2] and plasma E_2 concentration [15] were decreased from 5 to 8 days after estrous cycle. Therefore, we hypothesized that the time for measuring the plasma E_2 concentration was better on Day 5 to compare the activity of the first-wave DF between IG and CG. The plasma E_2 concentration was not different between IG and CG; therefore, it is speculated that the activity of the first-wave DF was not different between IG and CG. However, it is required to evaluate the blood flow perfusion and follicular E_2 concentration on Day 5 in lactating dairy cows. Further investigaton is needed to confirm these differences in blood flow perfusion and the activity of steroidogenesis between IG and CG of the first-wave DF.

In the hCG treatment group, the mean diameter of the CL and accessory CL and plasma P_4 concentration did not differ between IG and CG. These results indicated that the accessory CL did not affect the development of CL and P_4 production, regardless of whether the accessory CLs were formed ipsilateral or contralateral to the CL in the ovary. A previous study showed that the hCG treatment at 5 days after ovulation increases plasma P_4 concentration; however, they did not compare the plasma P_4 concentration between IG and CG [16]. To the best of our knowledge, the present study is the first to report that CL development and plasma P_4 concentration were not affected by the location of the first-wave DF.

The physiological mechanisms of low fertility and increased conception rate in IG after hCG administration at 5 days after estrus were not completely clarified in a previous study [6]. According to a previous study, lactating dairy cows that were diagnosed pregnant have higher P_4 concentrations from 6 to 8 days after AI compared with that in non-pregnant cows [8]; however, there were no differences in plasma P_4 concentration, CL size and the accessory CL size between IG and CG from Day 3 to Day 13 in IG and CG of the non-treatment and hCG groups in the present study. On the basis of these results, low fertility in IG appears to be unrelated to the lower function of CL in IG than in CG and the increased conception rate in IG cows administrated with hCG was not caused by higher plasma P_4 concentration compared with CG cows administrated with hCG. It is reported that endometrial P_4 concentration is higher in ipsilateral to the ovary with the CL [17]. Since the hCG treatment in IG induced an accessory CL formation in the same side of the ovary with original CL, the P_4 concentration in the oviduct and uterine horn ipsilateral to the CL might be higher and have a positive effect on fertility compared with IG without hCG treatment. Another possibility is that the E_2 secreted from the first-wave DF might have locally affected the ipsilateral uterine horn or oviduct and changed the function of the reproductive tract. Additionally, since the ovulation of the first-wave DF in IG due to hCG treatment could eliminate the detrimental effects on fertility, the conception rate increased in IG. Further research is warranted to confirm the local locational effect of the first-wave DF on uterus and oviduct function.

In conclusion, the results of this study demonstrated that the development of the first-wave DF tended to be greater in IG than in CG without hCG treatment. There were no differences between IG and CG in CL growth, plasma P_4 concentration, and plasma E_2 concentration at 5 days after estrus with or without hCG treatment. Moreover, accessory CL development did not differ between IG and CG with hCG treatment at 5 days after estrus.

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