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NOTE

Theriogenology

Double ovulation rate of the first follicular wave follicles is higher in the first follicular wave dominant follicle in the ovary contralateral to the corpus luteum treated with human chorionic gonadotropin five days after estrus in lactating dairy cows

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ABSTRACT. We examined the effect of human chorionic gonadotropin (hCG) treatment on double ovulation rate of first-wave follicles five days after estrus in lactating dairy cows. Cows were divided into two groups: 1) Ipsilateral group (IG; n=35), in which the first-wave dominant follicle (DF) was ipsilateral to the corpus luteum (CL), and 2) Contralateral group (CG; n=30), in which the first-wave DF was contralateral to the CL five days after estrus, then 1,500 IU of hCG was administrated. Double ovulation rate was significantly higher in the CG (26.7%) than in the IG (2.9%). This study demonstrate that the double ovulation rate of first-wave follicles was higher in the first-wave DF located in the contralateral ovary to the CL.

KEY WORDS: corpus luteum, cow, double ovulation, first follicular wave dominant follicle, human chorionic gonadotropin (hCG)

Human chorionic gonadotropin (hCG) has a luteinizing hormone-like effect in cattle [9]. In the early luteal phase (Days 5–7), hCG induces the ovulation of the first follicular wave (first-wave) dominant follicle (DF) and accessory corpus luteum (CL) formation, with a subsequent increase in plasma progesterone concentrations in cattle [10].

We previously reported that the development of the first-wave DF in the ipsilateral ovary to the CL was associated with reduced conception rates in lactating dairy cows [7], and conception rates can increase by only administrating hCG to cows presenting a first-wave DF ipsilateral to the CL [8]. Based on these results, when we used hCG treatment for ovulation of the first-wave DF five days after artificial insemination for improving fertility in lactating dairy cows, confirming the location of first-wave DF and CL in the ovaries was important. However, there was little information on the effect of hCG treatment on ovulation rates of the first-wave DF, which was ipsilateral or contralateral to the CL in lactating dairy cows.

The study aimed to compare the effects of hCG treatment, five days after estrus, on the ovulation and double ovulation rates of the first-wave DF that was ipsilateral and contralateral to the CL in lactating dairy cows.

Sixty-five postpartum lactating Holstein dairy cows [postpartum day of estrus: 120.7 ± 40.6, parity: 2.1 ± 1.3, milk production: 34.5 ± 8.0 kg/day, body condition score (BCS): 2.8 ± 0.3; means ± SD] at the Field Science Center of Obihiro University, Hokkaido, Northeast Japan, were used. Scoring of BCS was referenced from Ferguson *et al*. [3]. The trial was conducted from December 2013 to February 2015. All experimental cows underwent regular estrous cycles and were clinically healthy during the breeding period. Cows that experienced reproductive or metabolic diseases were excluded. The cows were kept in a freestall barn under the normal management program of the Field Science Center of Obihiro University. Cows were fed a total mixed ration diet comprised of corn silage, grass silage, soybean meal, corn grain, and concentrate, 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

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Estrus was detected by visual observation, examination of tail paint, and rectal palpation to confirm the preovulatory follicle and regressed CL. Ovulation was confirmed 24 hr after detecting estrus using rectal palpation or transrectal ultrasonography equipped with a 5.0-MHz linear transducer (HS-101V, Honda Electronics, Toyohashi, Japan). We defined the day of ovulation as Day 1. On Day 5, we examined CL and first-wave DF, to determine the largest follicle (>10.0mm) in the ovaries using transrectal ultrasonography. The location of the first-wave DF in the ovary was confirmed to be either ipsilateral [ipsilateral group (IG): n=35, postpartum day of estrus: 120.2 ± 40.1, milk production: 36.0 ± 8.2 kg/day, BCS: 2.8 ± 0.3; means ± SD] or contralateral [contralateral group (CG): n=30, postpartum day of estrus: 121.5 ± 41.8, milk production: 32.8 ± 7.6 kg/day, BCS: 2.9 ± 0.3] to the CL. We used only cows with single CL and no co-dominant follicle; no co-dominant follicle means there was only one follicle which size had >10.0 mm. Fifteen-hundred IU hCG (Gestron1500, Kyoritsu Seiyaku, Tokyo, Japan) was intramuscularly administrated on Day 5. Ovarian examination was performed on Day 13 to check the accessory CL for evaluating ovulation of the first-wave DF. Mean diameters of the first-wave DF and CL on Day 5 were determined from stored images from ultrasonograph.

The sizes of the first-wave DF and CL were analyzed between groups (IG vs. CG) by unpaired Student's *t*-test. One-way ANOVA was used for the determining the group effects (IG with single ovulation vs. CG with single ovulation vs. CG with double ovulation), and Tukey' HSD test as a multiple comparison test was used to detect significant differences among groups. The ratio of double ovulations was analyzed as a dependent variable (single ovulation; 0, double ovulation; 1). Independent variables for the logistic regression model were milk production (<34 kg, ≥34 kg), parity (1, 2, ≥3), BCS (<3, ≥3), days in milk at estrus (<120 days, ≥120 days), and location of the first-wave DF and the CL (IG, CG). Statistical analysis was performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), 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 frequently used in biostatistics [6]. Statistical significance was declared for $P<0.05$. Data were presented as mean ± SEM.

Ovulation of the first-wave DF occurred in every cow after hCG treatment. Logistic regression showed that the double ovulation rate of the first-wave follicles was significantly higher in CG (26.7%) than in IG (2.9%) (Table 1). Ovulation of the subordinate follicle occurred more frequently in the ovary contralateral to the CL than in the ovary ipsilateral to the CL in CG (IG; Contralateral to the CL vs. Ipsilateral to the CL: 0 vs. 1, CG; Contralateral to the CL vs. Ipsilateral to the CL: 7 vs. 1). Milk production, parity, days in milk at estrus, and BCS were not associated with the double ovulation ratio of the first-wave follicles after hCG treatment. Mean diameter of the first-wave DF (IG: 12.3 ± 0.3 mm, CG: 11.7 ± 0.3 mm) and CL (IG: 22.9 ± 0.5 mm, CG: 22.8 ± 0.5 mm) were not different between IG and CG. Mean diameter of the first-wave DF in CG with double ovulation (10.5 ± 0.4 mm) was significantly smaller than IG (12.3 ± 0.3 mm) and CG (12.1 ± 0.3 mm) with single ovulation. Mean diameters of the CL were not different among groups (IG with single ovulation; 23.0 ± 0.4 mm, CG with single ovulation; 22.6 ± 0.6 mm, CG with double ovulation; 23.6 ± 1.0 mm).

The double ovulation rate of the first-wave follicles was significantly higher in CG than in IG. Deviation of the DF occurs two to three days after follicular wave emergence [5]. The DF, which reaches 8.0 to 10.0 mm, expresses LH receptors between 2 to 4 days after emergence of the follicular wave [1]. However, there are no reports that comparing the timing of the deviation or the size of DF at the time of deviation between IG and CG. In this study, the size of the largest follicle reached >10.0 mm five days after estrus in IG and CG, and the mean diameter of the first-wave DF was not different between IG and CG on Day 5. Therefore, we speculated that the largest follicle must be the DF and the subordinate follicle must have been atretic on day of hCG treatment. However, when we analyzed the mean diameter of the first-wave DF separated by IG with single ovulation, CG with single ovulation and CG with double ovulation, the mean diameter of the first-wave DF in CG with double ovulation was significantly smaller than IG and CG with single ovulation. From these results, the growth rate of the first-wave DF with CG in some cows was slower, and it was presumed that the deviation timing was delayed, and the subordinate follicle was not atretic five days after estrus. Therefore, the higher double ovulation rate of the first-wave follicles in the CG compared with those in the IG might be caused by increased ovulation of the subordinate follicle in the first-wave in the CG with a slower growth rate of the first-wave DF. From previous research, the percentage of the wall of the dominant follicle with color Doppler signals of blood flow was higher during follicle development in IG than in CG in heifer [2]. Moreover, blood-flow resistance with the most prominent color-Doppler signal just before entering the ovary was lower in IG than in CG in heifers during five days after ovulation [4]. These results showed that blood flow perfusion to the ovary with the first-wave DF could be higher in IG than CG. It is speculated that the cause of slower development of the first-wave DF in the CG of some cows was lower perfusion of blood flow to the DF, which might lead to delay the timing of deviation. However, we could not clarify the reason for slower development of the first-wave DF in some cows with CG. Previous research showed that the size of the first-wave DF located adjacent to the CL is larger than separated (the distance of the first-wave DF and the CL was greater than 2.0 mm) [4], and this means position or locational relationship

Table 1. Double ovulation ratio of the first-wave follicles between ipsilateral group (IG) and contralateral group (CG)

	IG[1]	CG[2]	*P*-value
Double ovulation rate (%)	2.9 (1/35)	26.7 (8/30)	<0.05

[1]IG = Ipsilateral group. The first-wave DF located ipsilateral to the CL. [2]CG = Contralateral group. The first-wave DF located contralateral to the CL.

between DF and CL affects the characteristics of follicle development. If the same phenomenon occurred in the first-wave DF and subordinate follicles, the position of the first-wave DF and subordinate follicles might affect the growth rate of each follicle.

Further research is needed to verify the differences in the timing of deviation of the first-wave and of the gene expressions, such as LH receptor expression, of the largest follicle and subordinate follicle between IG and CG. In addition, we need to evaluate the detailed position and dynamics of the first-wave DF and subordinate follicles in the ovary of CG.

In conclusion, present study demonstrates that the double ovulation rate of the first-wave DF is higher in the first-wave DF located contralateral to the CL than in the first-wave DF located ipsilateral to the CL. However, the ovulation rate is not different whether the first-wave DF is ipsilateral or contralateral to the CL in lactating dairy cows.

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