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—Original Article—

# Effects of human chorionic gonadotropin treatment after artificial inseminations on conception rate with the first follicular wave dominant follicle in the ovary ipsilateral to the corpus luteum in lactating dairy cows

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**Abstract.** We examined the effect of human chorionic gonadotropin (hCG) treatment 5 days after artificial insemination (AI) on conception rate when the first-wave dominant follicle (DF) in the ovaries was either ipsilateral or contralateral to the corpus luteum (CL) in lactating dairy cows. 577 cows from 4 dairy farms were divided into the following two groups 5 days after AI using transrectal ultrasonography: (1) the ipsilateral group (IG; n = 348), in which the DF was ipsilateral to the CL, and (2) the contralateral group (CG; n = 229), in which the DF was contralateral to the CL. IG and CG were further subdivided into two groups: non-treatment groups, which received no treatment (IG, n = 220; CG, n = 128), and hCG treatment group, that was administrated 1500 IU hCG 5 days after AI (IG, n = 143; CG, n = 86). Pregnancy was diagnosed by rectal palpation or transrectal ultrasonography from 53 to 67 days after AI. Conception rate was significantly ($P < 0.01$) higher in the hCG treatment group of IG (40.6%) than in the non-treatment group of IG (21.4%); however, there was no difference in the non-treatment (51.7%) and hCG treatment (43.0%) groups of CG. Parity, farm, days in milk at AI, interaction between the farm and hCG treatment and interaction between the farm and location of the first-wave DF and CL did not affect conception rate. Our results suggest that conception rate can be improved by administrating hCG only to cows with the first wave DF ipsilateral to the CL.

**Key words:** Conception rate, Corpus luteum, Cow, First follicular wave dominant follicle, Human chorionic gonadotropin (hCG)

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The fertility of lactating dairy cows has been decreasing worldwide over the past few decades [1]. The decline in fertility has been attributed to various factors, such as lower estrous detection, housing systems, herd size, and metabolic and disease state [2, 3]; however, one of the main cause of low conception rates in high-producing dairy cows is in low plasma progesterone ($P_4$) concentrations after artificial insemination (AI) [4, 5].

Human chorionic gonadotropin (hCG) has a luteinizing hormone-like effect in cattle [6]. In the early luteal phase (Day 5-7), hCG induces the ovulation of the dominant follicle (DF) of the first follicular wave and the formation of an accessory corpus luteum (CL), with a subsequent increase in plasma $P_4$ concentrations in cattle [7]. On the basis of the increased plasma $P_4$ concentrations, several trials of hCG treatment after AI in the early luteal phase of lactating dairy cows have been performed to increase the conception rates [8]. However, the effects of hCG administration on fertility are not consistent between studies [9, 10]. We thought that there must be a factor that relates the effect of hCG treatment to conception rate.

We previously reported that the development of the first-wave DF in the ovary ipsilateral to the CL was associated with reduced conception rates in lactating dairy cows [11], suggesting that the first-wave DF that develops ipsilateral to the CL has a negative local effect on fertility. On this basis, removing the first-wave DF that develops ipsilateral to the CL using hCG treatment could eliminate the detrimental effects on fertility, and thereby, possibly increase conception rates. To date, no studies have investigated the effects of hCG treatment on conception rates by comparing the first-wave DF that is ipsilateral and contralateral to the CL in lactating dairy cows.

The objective of this study was to evaluate the effect of hCG treatment 5 days after AI on the conception rate in ipsilateral and contralateral situations.

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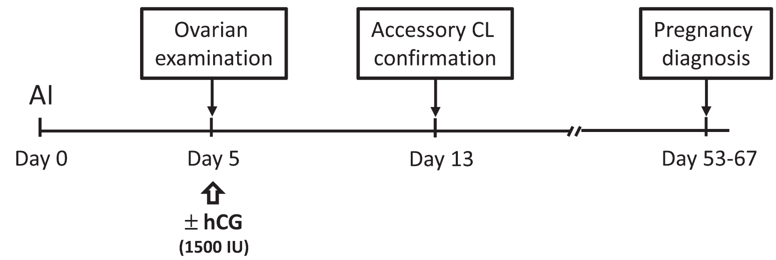


**Fig. 1.** Schematic diagram of the experimental model. The day of artificial insemination (AI) was defined as Day 0. In all of the lactating dairy cows, the position of the first follicular wave dominant follicle (the first-wave DF) and corpus luteum (CL) was confirmed at Day 5 using ultrasonography. hCG (1500 IU) was treated to hCG treatment group on Day 5. Accessory CL, which was formed after ovulation of the first-wave DF, was confirmed on Day 13 using ultrasonography only at Farm D. Pregnancy diagnosis was performed on Day 53–67 by rectal palpation or trans-rectal ultrasonography.

## Materials and Methods

*Animals and management*

Postpartum lactating Holstein cows (n = 555) at four dairy farms (Farm A, n = 199; Farm B, n = 202; Farm C, n = 66; Farm D, n = 88) in Hokkaido, northeast Japan, were used. The trial was conducted from April 2009 to February 2015. Cows underwent regular estrous cycles and were clinically healthy during the breeding period. Cows that experienced reproductive or metabolic diseases were excluded. The cows from Farm A, B and D were kept in a free-stall barn, and those at Farm C were kept in tie-stall barn. The cows were fed a total mixed rations diet consisting of corn silage, grass silage, soybean meal, corn grain, and concentrate, with free access to water. All cows were milked twice daily. The mean annual milk production at Farm A, B, C and D during the study period were 9,850 kg, 9,300 kg, 12,500 kg, and 12,100 kg, respectively. Artificial insemination was initiated 60 days after parturition. The experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro University.

*Experimental design*

In total, 599 AIs were performed for the lactating dairy cows [postpartum day of AI, 125.4 ± 62.6 (Farm A, 148.5 ± 79.2; Farm B, 117.1 ± 55.1; Farm C, 105.7 ± 38.9; Farm D, 113.6 ± 42.3); parity: 2.4 ± 1.5 (Farm A, 2.6 ± 1.4; Farm B, 2.3 ± 1.5; Farm C, 2.4 ± 1.4; Farm D, 2.2 ± 1.4); means ± SD]. Cows were inseminated after estrus and estrus was detected by visual observation, examination of the tail paint, and rectal palpation to confirm the preovulatory follicle and regressed CL (day of AI = Day 0). After AI, we examined the CL and first-wave DF to determine the largest follicle (> 8.0 mm) in the ovaries, on Day 5 using transrectal ultrasonography equipped with a 5.0 MHz linear transducer (HS-101V, Honda Electronics, Toyohashi, Japan). At this point, if a cow had no dominant follicles, the size of the largest follicle < 8.0 mm, or a cow with two CLs, in which the cows had double ovulation, the cow was excluded from the study; there were no cows with co-dominant follicles. Two cows did not develop the first-wave DF, and 20 lactating cows had double ovulation; therefore, we excluded 22 cows from the experiment on Day 5. The cows were randomly assigned to either the non-treatment group or hCG treatment group at Day 5. The cows in the non-treatment group (n = 363) were not administrated hCG after AI, whereas those in the hCG treatment group (n = 214) were intramuscularly administrated 1500 IU hCG (Gestron1500, Kyoritsu Seiyaku, Tokyo, Japan) on Day 5. In addition, the location of the first-wave DF in the ovary was confirmed to be either ipsilateral [ipsilateral group (IG): non-treatment, n = 220; hCG treatment, n = 128] or contralateral [contralateral group (CG): non-treatment, n = 143; hCG treatment, n = 86] to the CL. In the hCG treatment group from Farm D, ovarian examination was performed on Day 13 to check for the formation of the accessory CL for the evaluation of ovulation of the first-wave DF. Pregnancy was determined using transrectal ultrasonography or rectal palpation from Day 53 to 67. If estrus was detected before the pregnancy diagnosis, we concluded that the cow was not pregnant. Figure 1 shows the experimental timeline.

*Pregnancy diagnosis*

Positive pregnancy diagnosis by ultrasonography was made by the presence a fetus with a heart-beat. Positive pregnancy diagnosis by palpation was made by membrane slip or palpation of the amniotic vesicle. Conception rate was calculated by dividing the number of pregnant cows by the total number of cows enrolled in the study.

*Statistical analysis*

Binary logistic regression was applied. Pregnancy status was analyzed as a dependent variable (not pregnant; 0, pregnant; 1). The independent variables were parity (1, 2 and ≥ 3), farm (Farm A, Farm B, Farm C, Farm D), days in milk at AI (< 120 days, ≥ 120 days), location (IG, CG), treatment (non-treatment, hCG treatment) and the interaction between the location of the first-wave DF and CL in the ovaries and treatment, the interaction between the farm and treatment, and the interaction between the farm and the location of the first-wave DF and CL were incorporated into the logistic regression model. When the interaction was significant, conception rates between the groups (IG *vs*. CG) in the non-treatment group and hCG treatment group and conception rates between the treatment group (non-treatment *vs*. hCG treatment) in IG and CG were assessed by chi-square test with Holm's adjustment.

Statistical significance was declared for $P < 0.05$. Data are presented as the mean ± standard error of the mean (SEM). All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical

**Table 1.** Factors that affected the conception rate (%)

| Factor | Class | Conception rate | P-value |
|---|---|---|---|
| Location | IG [1] | 28.4 (99 / 348) | P < 0.001 |
| | CG [2] | 48.5 (111 / 229) | |
| Treatment | non-treatment [3] | 33.3 (121 / 363) | P < 0.01 |
| | hCG treatment [4] | 41.6 (89 / 214) | |
| Location × Treatment | IG × non-treatment | 21.4 (47 / 220) [a] | P < 0.001 |
| | IG × hCG treatment | 40.6 (52 / 128) [b] | |
| | CG × non-treatment | 51.7 (74 / 143) [b] | |
| | CG × hCG tretment | 43.0 (37 / 86) [b] | |

[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. [3] non-treatment = Cows were not treated hCG. [4] hCG treatment = Cows were treated hCG (1500 IU) at five days post AI. [a, b] P < 0.01.

**Table 2.** Factors that did not affect the conception rate (%)

| Factor | Class | Conception rate | P-value |
|---|---|---|---|
| Location | 1 | 33.7 (67 / 199) | N.S. |
| | 2 | 32.9 (51 / 155) | |
| | ≥ 3 | 41.3 (92 / 223) | |
| Farm | Farm A | 33.2 (63 / 190) | N.S. |
| | Farm B | 34.4 (67 / 195) | |
| | Farm C | 33.9 (21 / 62) | |
| | Farm D | 45.4 (59 / 130) | |
| Days in milk at AI | < 120 days | 34.1 (109 / 320) | N.S. |
| | ≥ 120 days | 39.3 (101 / 257) | |
| Farm × Location | Farm A × IG [1] | 25.9 (30 / 116) | N.S. |
| | Farm B × IG | 24.2 (30 / 124) | |
| | Farm C × IG | 28.9 (11 / 38) | |
| | Farm D × IG | 40.0 (28 / 70) | |
| | Farm A × CG [2] | 44.6 (33 / 74) | |
| | Farm B × CG | 52.1 (37 / 71) | |
| | Farm C × CG | 41.7 (10 / 24) | |
| | Farm D × CG | 51.7 (31 / 60) | |
| Farm × Treatment | Farm A × non-treatment [3] | 31.7 (39 / 123) | N.S. |
| | Farm B × non-treatment | 31.9 (44 / 138) | |
| | Farm C × non-treatment | 27.0 (10 / 37) | |
| | Farm D × non-treatment | 43.1 (28 / 65) | |
| | Farm A × hCG treatment [4] | 35.8 (24 / 67) | |
| | Farm B × hCG treatment | 40.4 (23 / 57) | |
| | Farm C × hCG treatment | 44.0 (11 / 25) | |
| | Farm D × hCG treatment | 47.7 (31 / 65) | |

[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. [3] non-treatment = Cows were not treated hCG. [4] hCG treatment = Cows were treated hCG (1500 IU) at five days post AI.

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 [12].

## Results

*Location of the first-wave DF and CL, hCG treatment and interaction of the location of the first-wave DF and CL and hCG treatment on pregnancy outcomes*

The logistic regression revealed that location (P < 0.001), treatment (P < 0.01) and their interaction (P < 0.001) were significantly associated with the conception rate (Table 1). In IG, conception rate was higher in hCG treatment group than non-treatment group (P < 0.01). In CG, conception rate was not different between hCG treatment group and non-treatment group. In non-treatment group, conception rate of IG was lower than that of CG (P < 0.01). In hCG treatment group conception rate was not different between IG and CG.

*Parity, farm, days in milk at AI, interaction of the farm and hCG treatment and interaction of the farm and location of the first-wave DF and CL on pregnancy outcomes*

The logistic regression revealed that parity, farm, days in milk at AI, the interaction of the farm and hCG treatment and the interaction of the location of the first-wave DF and CL and farm were not significantly associated with the conception rate (Table 2).

*Formation rate and location of the accessory CL in IG and CG treated by hCG on Day 13 in Farm D*

Treatment of hCG at Day 5 induced formation of an accessory CL in all cows at Farm D (Table 3). In addition, the accessory CL was formed ipsilateral to the CL in IG of all cows, on the contrary, the accessory CL formed contralateral to the CL in CG of all cows (Table 3).

**Table 3.** Formation rate (%) and location of the accessory corpus luteum (CL) in the IG and CG treated with hCG on Day 13 at Farm D

| Location of accessory CL | IG [1] | CG [2] |
|---|---|---|
| Ipsilateral to CL | 100 | 0 |
| | (35 / 35) | (0 / 30) |
| Contralateral to CL | 0 | 100 |
| | (0 / 35) | (30 / 30) |

[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.

## Discussion

This study investigated the effect of hCG treatment 5 days after AI on the conception rate between the first-wave DF that was ipsilateral and contralateral to the CL in lactating dairy cows. The results showed that the hCG treatment improved the conception rate in IG but not in CG.

Previous studies have shown mixed results regarding the effect of hCG treatment 5 days after AI on conception rate [9, 10]; various factors are involved in the effect of hCG treatment on fertility [8]. However, no studies have investigated the effects of hCG treatment on the conception rate, by comparing the first-wave DF that is ipsilateral and contralateral to the CL in the ovaries of lactating dairy cows. To the best of our knowledge, we report for the first time that hCG treatment 5 days after AI had a beneficial effect on the conception rate in lactating dairy cows with the first-wave DF that was ipsilateral to the CL (there was no beneficial effect when contralateral to the CL). Based on these results, an improvement of conception rate could be achieved by administering hCG only to the cows with the first-wave DF ipsilateral to the CL. In addition, there were no interaction between the farm and hCG treatment or between the farm and the location of the first-wave DF and CL on conception rate in Experiment 2; therefore, the effect of hCG treatment on conception rate between IG and CG might not be affected by the feeding environment.

In this experiment, parity, days in milk at AI, and farm factors were not affected as determined by multivariable logistic regression analysis. In previous research, the factors of parity and days in milk at AI did not affected the conception rate [13], on the contrary, another study showed that the factors of parity and farm did affect the conception rate [8, 14]. From these results, the effect of these factors on conception are different among studies. However, these studies did not examine about the factor of the location of the first-wave DF and CL. From the present study, the location of the first-wave DF and CL greatly influenced conception rate; therefore when we used a multivariable logistic regression analysis, the factors of parity, days in milk at AI, and farm were not observed to affect conception rate in the present study.

The physiological mechanisms of increasing conception rate by hCG treatment in IG were not completely clarified in this study. In the previous study, lactating dairy cows that were diagnosed pregnant have higher $P_4$ concentrations from 6 to 8 days after AI compared to non-pregnant cows [5]. In another previous study, plasma $P_4$ concentration was higher in IG than CG at four days after ovulation in heifers [15], however there was no difference between IG and CG at four days after ovulation in lactating dairy cows (unpublished data). Although we need further research to determine whether the plasma $P_4$ concentration was different between IG and CG, higher conception rate in CG compared to IG might be not caused by a greater plasma $P_4$ concentration in CG than IG.

Based on these results, the cause of low fertility in IG appeared not to be related to the lower function of CL in IG. Estradiol ($E_2$) concentration in the oviduct ipsilateral to the preovulatory follicle is higher than in that contralateral to the preovulatory follicle in bovine [16]. It is possible that $E_2$ secreted from the first-wave DF might have locally affected the same side of the uterine horn or oviduct and affected the function of the reproductive tract, and because 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. On the contrary, it has been reported that the $P_4$ concentration in the endometrial tissue is higher in ipsilateral to the ovary with the CL [17]. Therefore, because hCG treatment in IG had an accessory CL and original CL in the same side of the ovary, 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 with no hCG treatment. Hence, in the present study, we could not evaluate the purely local negative effect of the first-wave DF on fertility. Further research is warranted to verify the locational effect of the first-wave DF on fertility following follicle aspiration after AI.

In conclusion, the present study of lactating dairy cows demonstrated that hCG treatment 5 days after AI had a beneficial effect on fertility only when the first-wave DF developed ipsilateral to the CL in the ovary, and not when the first-wave DF developed contralaterally.

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## References


1. **Lucy MC.** Reproductive loss in high-producing dairy cattle: where will it end? *J Dairy Sci* 2001; **84**: 1277–1293. [Medline] [CrossRef]
2. **López-Gatius F.** Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows. A review. *Theriogenology* 2012; **77**: 1029–1041. [Medline] [CrossRef]
3. **Shahinfar S, Page D, Guenther J, Cabrera V, Fricke P, Weigel K.** Prediction of insemination outcomes in Holstein dairy cattle using alternative machine learning algorithms. *J Dairy Sci* 2014; **97**: 731–742. [Medline] [CrossRef]
4. **Mann GE, Lamming GE.** Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001; **121**: 175–180. [Medline] [CrossRef]
5. **Lopes AS, Butler ST, Gilbert RO, Butler WR.** Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim Reprod Sci* 2007; **99**: 34–43. [Medline] [CrossRef]
6. **Price CA, Webb R.** Ovarian response to hCG treatment during the oestrous cycle in heifers. *J Reprod Fertil* 1989; **86**: 303–308. [Medline] [CrossRef]
7. **Schmitt EJ, Diaz T, Barros CM, de la Sota RL, Drost M, Fredriksson EW, Staples CR, Thorner R, Thatcher WW.** Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *J Anim Sci* 1996; **74**: 1074–1083. [Medline] [CrossRef]
8. **Nascimento AB, Bender RW, Souza AH, Ayres H, Araujo RR, Guenther JN, Sartori R, Wiltbank MC.** Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. *J Dairy Sci* 2013; **96**: 2873–2882. [Medline] [CrossRef]
9. **Santos JE, Thatcher WW, Pool L, Overton MW.** Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *J Anim Sci* 2001; **79**: 2881–2894. [Medline] [CrossRef]
10. **Hanlon DW, Jarratt GM, Davidson PJ, Millar AJ, Douglas VL.** The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. *Theriogenology* 2005; **63**: 1938–1945. [Medline] [CrossRef]
11. **Miura R, Haneda S, Kayano M, Matsui M.** Short communication: Development of the first follicular wave dominant follicle on the ovary ipsilateral to the corpus luteum is associated with decreased conception rate in dairy cattle. *J Dairy Sci* 2015; **98**: 318–321. [Medline] [CrossRef]
12. **Kanda Y.** Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452–458. [Medline] [CrossRef]
13. **López-Gatius F.** Feeling the ovaries prior to insemination. Clinical implications for improving the fertility of the dairy cow. *Theriogenology* 2011; **76**: 177–183. [Medline] [CrossRef]
14. **Garcia-Ispierto I, Roselló MA, De Rensis F, López-Gatius F.** A five-day progesterone plus eCG-based fixed-time AI protocol improves fertility over spontaneous estrus in high-producing dairy cows under heat stress. *J Reprod Dev* 2013; **59**: 544–548. [Medline] [CrossRef]
15. **Ginther OJ, Siddiqui MA, Baldrighi JM, Hoffman MM.** Effect of intraovarian proximity between dominant follicle and corpus luteum on dimensions and blood flow of each structure in heifers. *Theriogenology* 2014; **82**: 875–883. [Medline] [CrossRef]
16. **Wijayagunawardane MP, Miyamoto A, Cerbito WA, Acosta TJ, Takagi M, Sato K.** Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* 1998; **49**: 607–618. [Medline] [CrossRef]
17. **Takahashi H, Haneda S, Kayano M, Matsui M.** Differences in progesterone concentrations and mRNA expressions of progesterone receptors in bovine endometrial tissue between the uterine horns ipsilateral and contralateral to the corpus luteum. *J Vet Med Sci* 2016; **78**: 613–618. [Medline] [CrossRef]