Journal of Reproduction and Development, Vol. 52, No. 1, 2006

—Research Note—

Effect of the Dominant Follicle Aspiration before or after Luteinizing Hormone Surge on the Corpus Luteum Formation in the Cow

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Abstract. Luteinizing hormone (LH) surge and follicle rupture act as trigger to start corpus luteum (CL) formation. Thus, we aimed to investigate whether a dominant follicle that has not been exposed to an LH surge can become a functional CL. For this purpose, follicular fluid from the dominant follicles (DF) of cows was aspirated before or after a GnRH-induced LH surge, and subsequent CL formation was observed. Holstein cows were divided into four groups as follows: Luteal phase, a DF was aspirated 7 days after GnRH injection; Pre-LH surge, a DF was aspirated 42 h after $PGF_{2\alpha}$ injection during the mid luteal phase; Post-LH surge, a DF was aspirated 24 h after GnRH injection following $PGF_{2\alpha}$; and Intact follicle, ovulation was induced by GnRH injection after $PGF_{2\alpha}$. Observation of morphological changes in the aspirated follicle using color Doppler ultrasonography and blood sampling was performed on Days 0, 3, 6, and 9 (Day 0=follicle aspiration). CL formation following DF aspiration was observed only in the Post-LH surge group. In both the Luteal phase and Pre-LH surge groups, however, none of the cows showed local blood flow at the aspirated site or CL formation. Luteal blood flow area, CL volume, and plasma progesterone concentration in the Post-LH surge group were no different from those in the Intact follicle group. The present results clearly demonstrate that rather than follicle rupture, it is the LH surge that is essential for CL formation in cows.

Key words: Corpus luteum formation, Cow, Follicle aspiration, LH surge

(J. Reprod. Dev. 52: 129–135, 2006)

It is well established that preovulatory luteinizing hormone (LH) surge acts as a trigger for the successive processes of follicular luteinization, ovulation, and corpus luteum (CL) formation by inducing morphological, endocrinological, and biochemical changes in the preovulatory follicle [1]. One of the most important events of the ovulatory process is follicle rupture. Breakdown of the basement membrane accompanied by follicle rupture permits invasion of theca cells and thecal microcapillary into the avascular granulosa layer, contributing to structural and functional parts in CL formation [1, 2]. Several studies indicate that blocking the endogenous LH surge using GnRH antagonist resulted in delay or suppression of ovulation and CL formation in various species [3–5].

Accepted for publication: October 5, 2005
Published online: November 17, 2005
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Little is known concerning whether a follicle without spontaneous rupture or unexposed to the LH surge has the full CL formation ability *in vivo* because CL formation takes place from a ruptured follicle after ovulation, and a follicle that has not been exposed to the LH surge cannot ovulate [6]. The aims of present study were 1) to determine the impact of follicular rupture after LH surge on CL formation in cows by aspirating the follicular fluid (FF) of preovulatory follicle, and 2) to examine the effect of the LH surge on subsequent CL formation by aspirating dominant follicles at different stages of the bovine estrous cycle. Furthermore, we also determined the local blood flow surrounding the developing CL as an index of structural and functional development of the CL using color Doppler ultrasonography, since our previous study using the transrectal color Doppler ultrasonography technique in cows demonstrated that the blood flow surrounding the CL gradually increases with CL development in parallel with plasma progesterone (P_4) level [7].

Materials and Methods

Animals and experimental design

This experiment was carried out at the Field Center of Animal Science and Agriculture, Obihiro University, Japan. The experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro University. Holstein cows were kept under the normal management program of the Field Center, as described previously [7]. Figure 1 shows a timetable for the treatment of each experimental group. All cows were received 500 μg of a prostaglandin (PG) $F_{2\alpha}$ analogue (cloprostenol [estrumate]; Sumitomo Pharm. Co., Osaka, Japan) at the mid stage of the estrous cycle. The cows with induced luteolysis were assigned randomly to the following experimental groups. Follicle aspiration was performed at the luteal phase; a new follicular wave and ovulation of the dominant follicle was induced by a GnRH analogue (Fertirelin acetate 100 μg; [Conceral]; Takeda Pharm. Co., Osaka, Japan) injected 48 h after $PGF_{2\alpha}$, and then a dominant follicle was aspirated 7 days after GnRH injection (Luteal phase, n=5). Follicle aspiration was performed at the follicular phase before LH surge; a dominant follicle was aspirated 42 h after an injection of $PGF_{2\alpha}$ (Pre-LH surge, n=5). Follicle aspiration was performed at the follicular phase after LH surge; GnRH was injected 48 h after $PGF_{2\alpha}$ to induce an LH surge. A dominant follicle was aspirated 24 h after GnRH injection (Post-LH surge, n=4). To compare the luteal formation of the treatment groups with CL development after ovulation, ovulation of Intact follicles was induced by injection of GnRH 48 h after $PGF_{2\alpha}$ (Intact follicle, n=5). The day of follicle aspiration (Luteal phase, Pre-LH surge, and Post-LH surge) or ovulation (Intact follicle) was designated as Day 0.

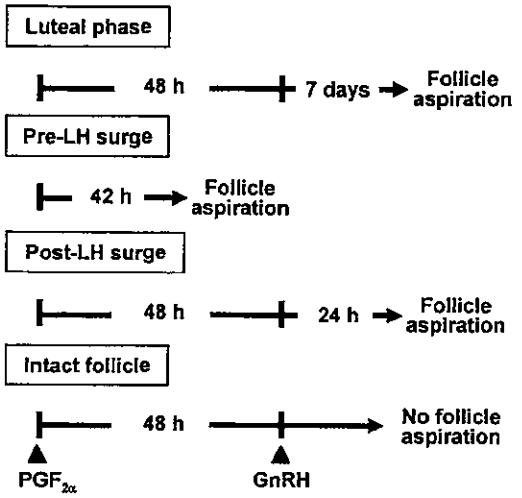

Fig. 1. Timetable of the treatment for follicular aspiration in each group.

Follicle aspiration and ultrasonography

The dominant follicle of each cow was aspirated by transvaginal ultrasound-guided follicle aspiration. For ultrasound guidance of the aspiration needle, an ultrasound scanner (SSD-5500, ALOKA Co., Ltd., Tokyo, Japan) was used that was equipped with a 7.5 MHz transvaginal convex transducer (UST-M15-21079, ALOKA Co., Ltd.) with an attached stainless steel needle guide. Before follicle aspiration, the cows received caudal epidural anesthesia, 5 ml of 2% lidocaine ([Xylocaine]; AstraZeneca Co., Osaka, Japan), to prevent straining, and then their vulva and perineal area were cleaned. The transvaginal convex transducer was inserted into the vagina, and the ovary containing the dominant follicle was positioned next to the transducer face by rectal manipulation so that the targeted follicle was

displayed on the needle path of the monitor. An 18-gauge single-lumen stainless steel needle connected to a 5 ml disposable syringe was pushed into the needle guide and inserted into the antrum of the follicle through the vaginal wall. If the dominant follicle could not be identified by ultrasound, all follicles with a diameter >8 mm were aspirated. The FF was collected into a 1.5 ml plastic tube, brought to the laboratory in ice water, and centrifuged to remove follicular debris. The FF samples were kept at −30 C until hormone determination. Ultrasound scanning and blood sampling were performed every three days between Day 0 and Day 9. To examine morphological changes after follicle aspiration, the ovaries were scanned by transrectal ultrasonography using an ultrasound scanner (SSD-5500, ALOKA Co., Ltd.) equipped with a 7.5-MHz convex transducer (UST-995-7.5, ALOKA Co., Ltd.) according to the standard procedure described previously [8]. Color signals were used to generate images of blood flow with a velocity greater than 2 mm/s. Scan records (images) were stored on the magneto-optical (MO) disk drive of a personal computer, and the colored areas were quantified using the NIH Image program (Version 1.62), as described previously [8].

Hormone determinations

Blood samples were collected by caudal venipuncture just prior to each ultrasound scan, and plasma was obtained by centrifugation and stored as previously described [9]. At the end of the experiment, all plasma and FF samples were analyzed in the same assay in duplicate using a second antibody enzyme immunoassay (EIA). Hormone assays were performed after diethyl ether extraction as described previously [10]. EIA for P_4 was described previously by Miyamoto *et al.* [11]. The standard curve for P4 ranged from 0.05 to 50 ng/ml, and the ED_{50} of the assay was 1.1 ng/ml. The intra- and interassay coefficients of variations (CVs) were 4.7 and 6.5%, respectively. The recovery rate of P_4 (1 ng) added to 1 ml plasma samples was 95 %. EIA for PGE_2 is described elsewhere [12]. The standard curve ranged from 0.02 to 20 ng/ml, and the ED_{50} of the assay was 1.8 ng/ml. The intra- and interassay CVs were 9.5% and 12.0%, respectively.

Statistical analysis

Plasma concentrations of P_4, PGE2 concentration in FF, the blood flow area within the CL and CL volume measured at different time points were expressed as means ± SEM. The data were examined by repeated-measures ANOVA followed by Scheffe's F-test as a multiple comparison test with time as the variable tested. Differences were considered significant at $P<0.05$.

Results

In both the Luteal phase and Pre-LH surge groups, dominant follicles were punctured and FF was collected from all cows. In one of the four cows in the Post-LH surge group, a dominant follicle was punctured, but FF was not collected.

Follicular fluid PGE_2 concentration

A higher concentration of PGE_2 in the FF of the Post-LH group proves that the follicles had already been exposed to the LH surge [10]. The concentrations of PGE_2 in the FF of each group are shown in Fig. 2. The follicular fluid PGE_2 concentration in the Post-LH surge group was significantly higher than for the Luteal phase and Pre-LH surge groups ($P<0.05$).

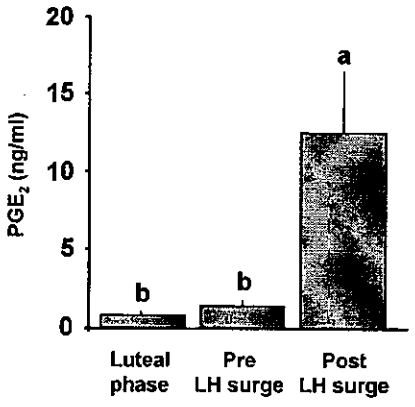

Fig. 2. Concentration of PGE_2 in the follicular fluid from follicles aspirated for the Luteal phase (n=5), Pre-LH surge (n=5), and Post-LH surge (n=3) groups. a, b: Values with different letters are significantly different ($P<0.05$).

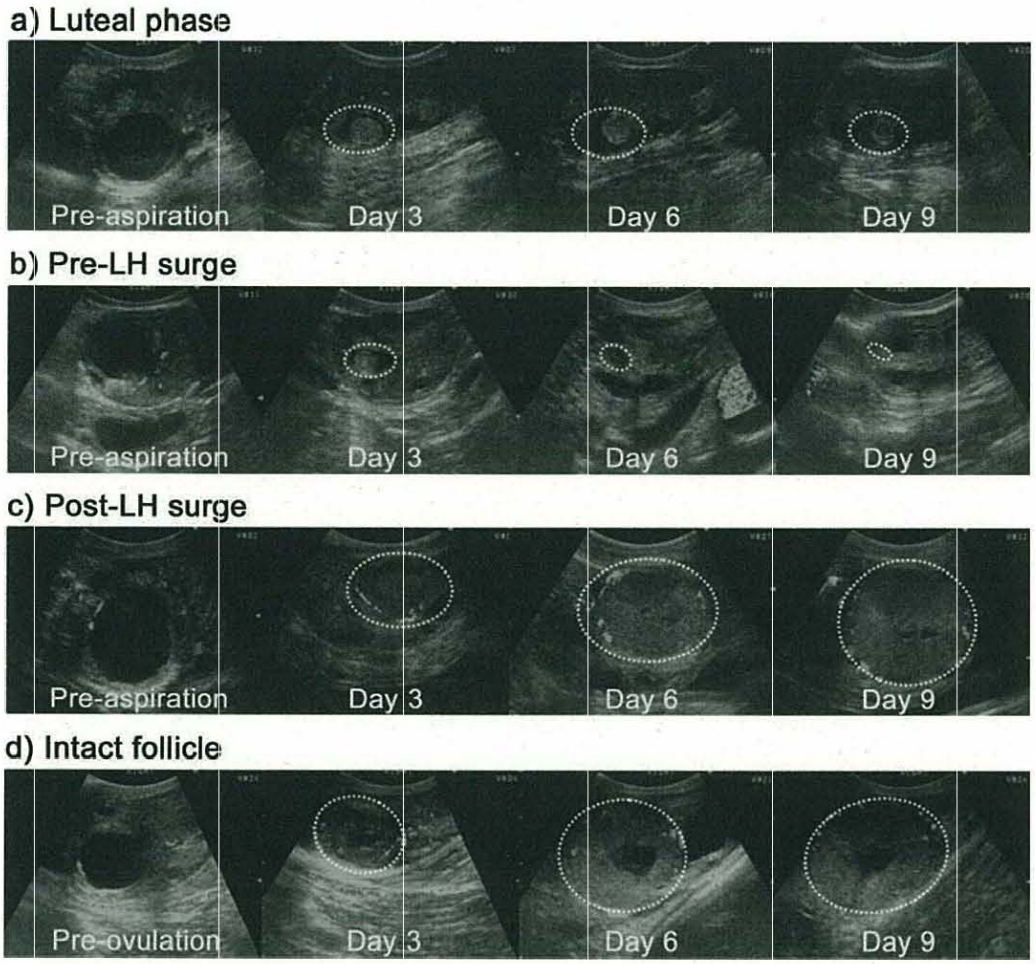

Fig. 3. Images of the effect of dominant follicle aspiration performed for a) Luteal phase, b) Pre-LH surge, c) Post-LH surge, and d) Intact follicle cows. The site of the aspirated or ovulated dominant follicle in each group is surrounded by a white dotted line. Color signals were used to generate images in which blood flow with a velocity greater than 2 mm/sec can be located as colored areas. Forward flow is usually presented in red and reverse flow in blue. None of the cows showed CL formation or visible blood flow in either the Luteal phase or Pre-LH surge groups. However, the Post-LH surge group showed CL formation, with visible blood flow at the site of the aspirated dominant follicle. There was no difference in blood flow area and CL size between the Post-LH surge and Intact follicle groups.

Ultrasound observations after follicle aspiration or ovulation

After follicle aspiration, no cows showed CL formation or visible blood flow in either the Luteal phase or Pre-LH surge group (Figs. 3a and b). However, all the cows in the Post-LH surge group showed CL formation, with visible blood flow at the site of the aspirated dominant follicle (Fig. 3c). In the Luteal phase group, the CL that was present at the beginning of the experiment was still present between Day 0 and Day 9. The Intact follicle group showed CL formation, with visible blood flow after ovulation (Fig. 3d).

Changes in the plasma P_4 concentration

The changes in plasma P_4 concentration for each group are shown in Fig. 4. The Luteal phase group retained a high plasma P_4 concentration throughout the experiment. This was due to the presence of a mature CL at the time of follicle aspiration. In the Pre-LH surge group, the plasma P_4 concentration remained at the basal level from Day 0 to Day 9. In

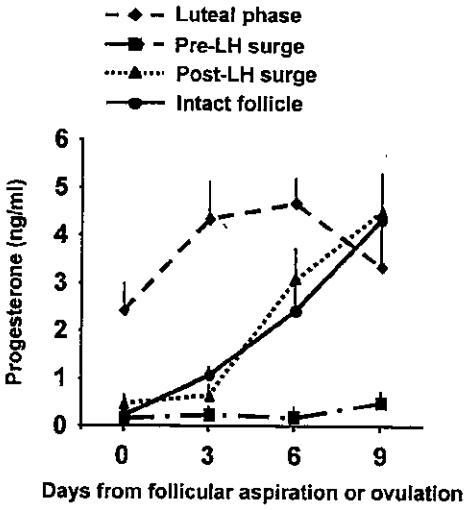

Fig. 4. Changes in the plasma concentration of progesterone of cows after follicular aspiration for the Luteal phase (n=5), Pre-LH surge (n=5), Post-LH surge (n=4) groups, and after ovulation for the Intact follicle group (n=5). Data shown as the mean ± SEM of each time period.

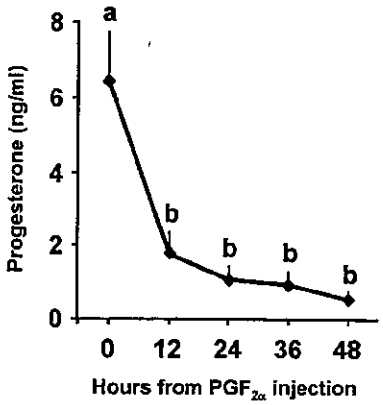

Fig. 5. Changes in the plasma concentration of progesterone after $PGF_{2\alpha}$ injection in the Post-LH surge group cows. Data shown as the mean ± SEM of each time period (n=4). a, b: Values with different letters are significantly different (P<0.05).

the Post-LH surge and Intact follicle groups, the plasma P_4 concentrations significantly increased from Day 0 to Day 9 along with CL development (P<0.05). On Day 6 and Day 9, the Pre-LH surge group had lower P_4 concentrations than the other three groups (P<0.05). Injection of a luteolytic dose of $PGF_{2\alpha}$ in the Post-LH surge group on Day 14 resulted in an acute decrease in the plasma P_4 level in all the cows (Fig. 5).

Changes in the CL volume and luteal blood flow area

As shown in Fig. 6, the newly formed CL in the Post-LH surge group showed no significant differences in terms of CL volume (Fig. 6a) and luteal blood flow area (Fig. 6b) compared with CL formed after ovulation in Intact follicle group. Both CL volume and luteal blood flow area increased in parallel, accompanying CL formation throughout the experimental period.

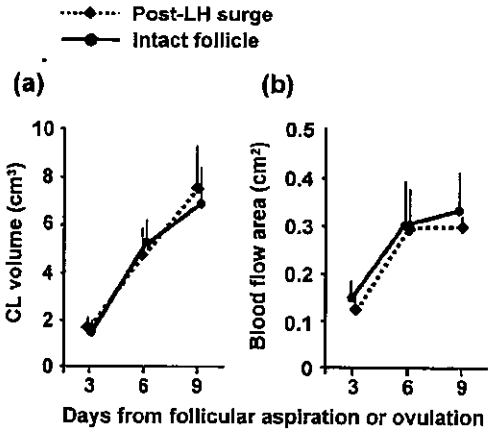

Fig. 6. Changes in (a) corpus luteum volume and (b) luteal blood flow area during corpus luteum development in the Post-LH surge and Intact follicle groups. Data shown as the mean ± SEM of each time period (n=4 in the Post-LH surge group, n=5 in the Intact follicle group).

Discussion

In the Post-LH surge group, PGE_2 concentration in the FF was higher than in the Luteal and Pre-LH surge groups, indicating that the follicles of the Post-LH group had already been exposed to the LH surge [10]. After dominant follicle aspiration, CL formation was observed only in the Post-LH group. The profiles of changes in plasma P_4 concentration, CL volume, and luteal blood flow area in these cows did not differ from those for the Intact follicle group. In addition, in the Post-LH group, the plasma P_4 concentrations decreased after $PGF_{2\alpha}$ injection. The findings in this study are as follows:

1) Formation of a functional CL occurs without spontaneous follicle rupture; and 2) a follicle that has not been exposed to an LH surge does not have the ability to develop a CL.

Follicle rupture involves a number of structural changes within the follicular wall that are closely related to extracellular matrix degradation, tissue remodeling, and cellular migration associated with a local inflammatory reaction [1, 2, 13]. In the present study, forcible removal of the FF in the Post-LH group before spontaneous follicle rupture was successful for formation of a morphologically and functionally normal CL. The progress of structural changes within the follicular wall in the Post-LH group at time of follicular aspiration could not be determined in our study. However, our results clearly indicate that spontaneous follicle rupture is not necessary for formation of a functional CL.

It has been reported that inhibition of follicle rupture by injection of PGE_2 synthesis inhibitor or indomethacin into preovulatory follicle just before LH surge does not alter luteal function in the following luteal phase of cattle [14], sheep [15], and monkeys [16]. In addition, injection of LH into sheep and rabbit preovulatory follicles induced luteinization and P_4 secretion without ovulation [17]. Therefore, it is likely that once a follicle is exposed to LH surge, removal of the FF and follicle rupture at ovulation is not required for CL formation.

In the present study, the formation of a functional CL was observed only when the dominant follicle was exposure to the LH surge (Post-LH surge and Intact follicle groups), however, none of the cows in both the Luteal phase and Pre-LH surge groups showed either local blood flow at the aspirated site or CL formation. The luteinization process begins shortly after the LH surge and is characterized by transformation of follicular cells into luteal cells, which is accompanied by distinct changes in the expression of steroidogenic enzymes to increase P_4 biosynthesis [1, 18]. A previous report showed that the mechanism for P_4 release from the CL did not fully develop when the granulosa cells (GC) were removed from the preovulatory follicle after the LH surge; therefore, luteinized GC makes a substantial contribution to the output of P_4 by the CL [19]. In addition, CL formation is characterized by highly active vascularization and angiogenesis [20]. GC treated with LH showed an increase in the release of P_4 and angiogenic factors, such as vascular endothelial growth factor, suggesting a direct action of LH on GC to stimulate luteal angiogenesis as well as luteinization [21]. Suppression of the endogenous LH surge by GnRH antagonist treatment inhibits both the increase of P_4 secretion and early luteal angiogenesis in the macaque [22]. Taken together, luteinization and luteal angiogenesis stimulated by LH surge is essential for CL formation accompanied with vascularization.

In conclusion, exposure of the follicle to LH surge, rather than follicle rupture, is the most important determinant of functional CL formation accompanied by vasculature in cows. The present results also demonstrate that a follicle that has not been exposed to LH surge lacks the ability to develop a CL.

Acknowledgments

The authors thank Dr. K. Okuda, Okayama University, Japan, for the P_4 antiserum. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), and the 21st Century COE Program (A-1) of Ministry of Education, Culture, Sports, Science and Technology, Japan. T. J. A. was a postdoctoral fellow supported by JSPS and Inoue Foundation. M.M. was a postdoctoral fellow supported by the 21st Century COE Program. K.G.H is supported by JSPS Research Fellowships for Young Scientists.

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