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The relationship between accessory corpus luteum maintenance and pregnancy-specific protein B in pregnant Japanese Black cows

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Abstract

In pregnant cows, regression of accessory corpus luteum (ACL) was more frequently observed in ACL contralateral to the original CL than ACL ipsilateral to the original CL. To investigate the factors related to ACL regression, CL characteristics and plasma pregnancy-specific protein B (PSPB) levels were evaluated in pregnant Japanese Black cows with human chorionic gonadotropin (hCG)-induced ACL formation. The cows were assigned to the control (n=10) or the hCG treatment group (n=50) on the day of embryo transfer (ET) (Day 0 = Estrus; Days 6–8 = Day ET). Cows treated with hCG were divided into: ipsilateral ACL maintenance (ipsi-maint, n = 25), contralateral ACL maintenance (contra-maint, n = 17), and contralateral ACL regression (contra-regr, n = 8). Characteristics of CL and PSPB were evaluated from Days 35 to 63. On Day 35, no difference was observed in the volume and blood flow area of ACL between the contra-maint and contra-regr groups. Plasma progesterone (P_4) concentrations were higher in hCG-treated cows than in untreated cows, regardless of ACL regression. During the experiment, P_4 concentrations decreased in the contra-regr group, while those unchanged in the ipsi-maint, contra-maint, and control groups. Higher PSPB levels were observed in the hCG-treated groups than in the control group. Compared to the ACL maintenance group, delayed PSPB recovery was observed in the contra-regr group. Additionally, PSPB concentration in the ipsi-maint group was greater than that in the contra-maint group. This study provides insights into the relationship between ipsilateral ACL maintenance and PSPB in pregnant cows.

Key Words: accessory corpus luteum maintenance, CL characteristics, Japanese Black cows, PSPB

Introduction

In cattle, progesterone (P_4), produced by the corpus luteum (CL), is crucial for initiating and sustaining pregnancy[4,37)]. The establishment and maintenance of pregnancy in cattle are the most important factors in herd economy. During early pregnancy, P_4 supports conceptus development by promoting endometrial gland development and uterine immune tolerance, as well as inhibiting myometrial contractions[31,40)]. Insufficient P_4 levels during early pregnancy may result in embryonic mortality or pregnancy loss[49)].

Human chorionic gonadotropin (hCG), a

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hormone with LH-like activity, induces ovulation in the dominant follicle[18,19,25,31]. In commercial cattle reproduction, ACL formation is induced, via hCG administration, to increase plasma P_4 levels during the early post-estrus period[8,18,19]. Administration of hCG on Days 5 to 7 post-estrus increases and decreases in plasma P_4 and estradiol-17β (E_2) concentrations, respectively; and increases embryo survival, thus improving conception rates after artificial insemination (AI)[38,42] and embryo transfer (ET)[32,44]. To induce the formation of an accessory CL (ACL), hCG and gonadotropin-releasing hormone (GnRH) are administered during early post-estrus[3,18,29].

Based on the location of the first-wave dominant follicle, the ACL is formed ipsilaterally or contralaterally to the original CL. When ACL was induced via hCG or GnRH treatment during early post-estrus, ACL regression occurs in lactating dairy cows[3,29] or beef cows[19] during pregnancy. Regression of ACL primarily occurs in the ACL that is contralateral to the original CL[3,19,29]. The contralateral ACL is located on the opposite side of the gravid horn. However, there was no clear explanation for this phenomenon. Recently, paper reported that cows experiencing early ACL regression before Day 26 exhibited lower circulating pregnancy specific protein B (PSPB) concentrations than those with ipsilateral ACL maintenance[29]. PSPB, also known as pregnancy-associated glycoprotein-1 (PAG1), is synthesized by mononucleate and binucleate trophoblastic cells at the placenta-uterine interface and is secreted into the maternal blood[5,43]. PSPB plays a multifaceted role in regulating reproductive processes. PSPB has a luteotrophic effect directly on the luteal tissue[10,11] or indirectly through the endometrial tissue[9,45,47]. PSPB stimulates the secretion of PGE2 from endometrial tissue and luteal cells from non-pregnant cows, while not affecting P_4 synthesis in vitro[10,48]. Additionally, on Days 60 and 90 of ovine pregnancy, PSPB increases both PGE2 and P_4 secretion from caruncular/placental tissue[47]. PGE2 is known as the luteotrophic factor[2].

Furthermore, placental development differs between gravid and non-gravid horn[16,33]. On Day 38 of gestation, placental plates were present exclusively in the gravid horn, with none found in the non-gravid horn[27]. In the pattern of blood flow to the uterine of pregnant cows, blood flow to the gravid horn significantly increases between Days 14 and 18, whereas blood flow in the contralateral non-gravid horn remains constant, and later, from Days 25 to 30, blood flow to the gravid horn further increases, while the contralateral uterine artery blood flow temporarily decreases and then increases[15,34]. Thus, after Day 35, the blood flow to the gravid horn remains higher than that in the non-gravid horn[34]. Taken together, in the gravid horn with well placental development and higher functional blood flow supply might lead to high secretion of PSPB in circulation. Therefore, PSPB secretion may differ between gravid horn and non-gravid horn which could be used as a candidate factor to maintain the ipsilateral ACL during gestation.

Several studies have evaluated the change in CL characteristics that occurs before and after maternal recognition, and reported a decrease in size, blood flow, and P_4 secretion in the regressing CL following failure of maternal recognition[3,12,31]. Thus, ACL characteristics may be related to the occurrence of regression or maintenance. However, to date, no study has evaluated the characteristics of the ACL in relation to its location with the gravid horn, which may be related to the occurrence of regression.

In dairy cattle, approximately half of all pregnancy losses was initiated by CL regression[12]. In addition, early contralateral ACL regression before Day 33 increased pregnancy loss[29]. Therefore, clarification of the mechanism behind ACL regression during pregnancy could shed light on the processes leading to luteal regression that precedes pregnancy loss. Understanding the mechanism of CL regression during pregnancy may contribute to mitigate the risk of pregnancy loss.

Therefore, in this study we aimed to: (1) evaluate the CL characteristics (CL volume, blood flow area (BFA), and plasma P_4,) and (2) measure the PSPB in pregnant Japanese Black cows with ACL formation, following hCG administration.

Materials and Methods

Animals

This study was conducted on a commercial farm in Shihoro-cho, Hokkaido, Japan, from April 2022 to September 2023. The Japanese Black cows (n = 111) were housed in barns and fed corn silage and feed concentrate. The experimental protocol is illustrated in Figure 1. This study was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine, Japan (Approval number: 20-217).

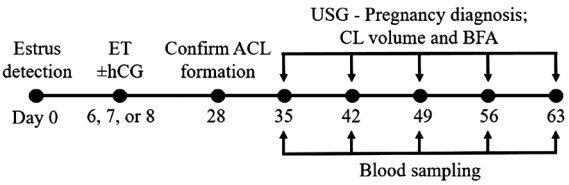

Fig. 1. Protocol of the treatments and samplings.
On the day of ET, the cows were treated with hCG 1,500 IU or not treated (control group).
On Day 28, the formation and location (ipsilateral or contralateral to original CL) of ACL were confirmed. From Days 35 to 63, CL characteristics, CL regression and pregnancy status were recorded, and blood samples collected for P4 and PSPB measeument.
hCG = human chorionic gonadotropin;
USG = Ultrasonography; BFA = Blood flow area.

Synchronization the recipient cows

The estrous cycles of the cows were synchronized by administration of a 12-day vaginal insert containing 1.9 g P_4 (CIDR 1900; Zoetis JP, Tokyo, Japan) and 2 mg estradiol benzoate (Ovahormone® injection; ASKA Animal Health Co., Ltd., Tokyo, Japan). In addition, 500 μg cloprostenol (Estrumate; MSD Animal Heath, Merck & Co., Inc., Rahway, NJ, USA) was administered on the day of CIDR removal. Estrus was detected 2 d after CIDR removal. The day of estrus was set as Day 0.

Embryo transfer (ET) and hCG treatment

The cows were subjected to ET on Day 6, 7, or 8. A skilled technician transferred frozen-thawed embryos from Japanese Black cows into the uterine horn ipsilateral of the ovary with the original CL. All cows subjected to ET were confirmed to have an active CL on the day before ET, using transrectal ultrasonography (MyLab One Vet, Esaote Europe B.V., Maastricht, Netherlands) that was performed by a single operator. On the day of ET, the cows were randomly assigned into two groups, hCG (1,500 IU; Gestron1500; Kyoritsu Seiyaku, Tokyo, Japan) treatment (n = 86) and no treatment (n = 25) groups.

Ovarian examination and pregnancy diagnosis by ultrasound

To confirm the formation of ACL (≥15 mm in diameter), ovarian structure was observed on Day 28 using ultrasonography (MyLab One Vet). The locational relationship between the original CL and the ACLs (ipsilateral or contralateral) was determined. CL dimensions and luteal BFA were measured on Days 35, 42, 49, 56, and 63. The CALIPER function of the ultrasonography device was used to measure the major (length) and minor (width) axes of the CL. The CL volume was calculated using the following formula: $V=4/3 \times \pi \times R^3$, with radius (R) calculated using the formula R= (length/2+ width/2)/2. In cases in which the CL had a fluid-filled cavity, the cavity volume was calculated and subtracted from the total volume of the CL[39]. Blood flow surrounding the CL was assessed using color Doppler ultrasonography (MyLab One Vet). Luteal BFA was measured using ImageJ (version 1.52, National Institute of Health, Bethesda, MD, USA), developed at the U.S. National Institutes of Health (https://imagej.nih.gov/ij/), as previously decribed[21]. Pregnancy was initially diagnosed on Day 35 by observing an amniotic vesicle containing an embryo with a detectable heartbeat. Cows identified as pregnant on Day 35 underwent pregnancy status re-evaluation on Days 42, 49, 56, and 63. Data was collected from 60 pregnant cows, including untreated (n = 10) and hCG-treated (n = 50) cows.

Plasma P_4 measurement

Blood samples were collected on Days 35, 42, 49, 56, and 63, from the coccygeal vein or artery, into evacuated heparinized tubes. The tubes were immediately placed on ice. The blood samples were

Table 1. Comparison of volume and blood flow area (BFA) between original CL and accessory CL on Day 35.

Group	n	CL	Volume (cm^3)	BFA (cm^2)
Control	10	Original	4.8 ± 0.3^{x}	2.2 ± 0.2^{x}
Ipsi-maint	25	Original	$6.5 \pm 0.5^{a, y}$	$3.5 \pm 0.1^{a, y}$
		Accessory	$3.8 \pm 0.3^{b, m}$	$1.6 \pm 0.1^{b, m}$
Contra-maint	17	Original	$6.3 \pm 0.5^{a, y}$	$3.2 \pm 0.1^{a, z}$
		Accessory	$3.3 \pm 0.1^{b, n}$	$1.4 \pm 0.1^{b, n}$
Contra-regr	8	Original	$6.2 \pm 0.2^{a, y}$	$3.2 \pm 0.1^{a, z}$
		Accessory	$3.2 \pm 0.1^{b, n}$	$1.3 \pm 0.1^{b, n}$

[a,b] Different letters indicate differences between the original and accessory CL ($P < 0.05$).
[x,y,z] Different letters indicate differences in the original CL among the groups ($P < 0.05$).
[m,n] Different letters indicate differences in the accessory CL among groups ($P < 0.05$).
The data was presented as the mean ± SEM.

centrifuged at 2,000 × g for 15 min at 4 ºC (within 2 h from sampling) and plasma components were separated and stored at –30 ºC until further use in the hormone assay. Plasma P_4 concentrations were determined, in duplicate, using competitive double-antibody enzyme immunoassay (EIA), as previously described[28]. The P_4 assays were performed after extraction with diethyl ether. The P_4 recovery rate was 92.7%. The EIA standard curve ranged from 0.05–50 ng/ml. The intra- and inter-assay coefficients of variation (CV) were 7.7% and 8.6%, respectively.

Pregnancy specific protein B assay

PSPB was evaluated using blood samples collected on Days 35, 42, 49, 56, and 63 by a commercial ELISA kit (CSB-E13353B; CUSABIO, Houston, TX 77054, USA). The ELISA standard curve covered a range of 0–24 ng/ml. The intra- and inter-assay CVs were 7.8% and 8.5%, respectively.

Experimental design

•ACL regression

To investigate whether ACL maintenance or regression is related to the location of the original CL, the occurrence of ACL regression was evaluated weekly from Days 35 to 63. According to a previous study[19,39], in present study, CL regression was defined when the decreased volume was >20% of CL volume on Day 35.

•Characteristics of CL

To assess whether CL characteristics are related to ACL regression, CL volume, BFA, and plasma P_4 levels were analyzed. Based on the occurrence of ACL regression, the cows were divided into 4 groups: 1) untreated cows (control; n = 10), 2) cows with ipsilateral ACL maintenance (ipsi-maint; n = 25), 3) cows with contralateral ACL maintenance (contra-maint; n = 17), and 4) cows with contralateral ACL regression (contra-regr; n = 8). There were no animals with ipsilateral ACL regression. The CL characteristics were compared among four groups.

•Profile of the plasma PSPB concentrations

To investigate whether PSPB affects ACL regression, changes in plasma PSPB concentrations within each group were analyzed and compared.

Statistical analysis

The rates of ACL regression from Days 35 to 63 were compared between the ipsilateral and contralateral groups using the chi-square test. The original CL volume, ACL volume, BFA, plasma P_4, and PSPB concentrations were compared among the groups using a two-way repeated measures ANOVA. *Post hoc* analysis was performed using the Tukey's Games-Howell test. Statistical differences were considered significant at probabilities less than 5% ($P < 0.05$). All statistical analyses were performed using IBM SPSS for Windows version 22 (IBM Corp., Armonk, New York, United States).

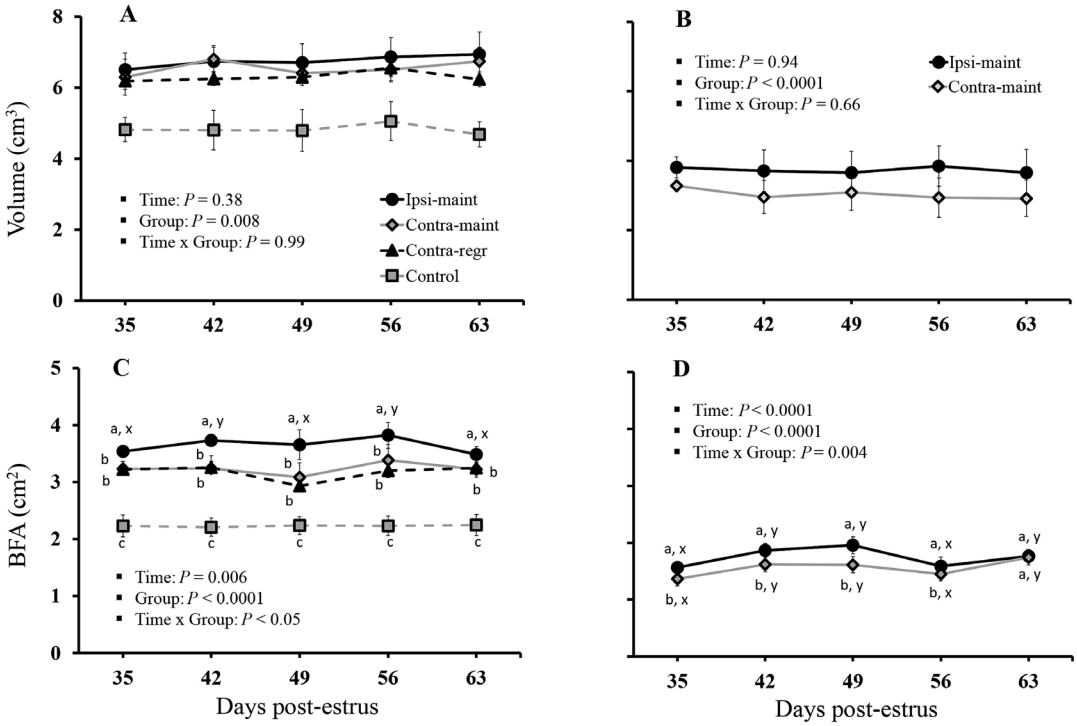

Fig. 2. Characteristics of CLs in four groups, control, ipsi-maint, contra-maint, contra-regr from Days 35 to 63. Luteal tissue volume of original CL (A) and accessory CL (B). BFA of original CL (C) and accessory CL (D).
[a, b, c)] indicate significant differences between specific groups ($P < 0.05$).
[x, y)] indicate significant differences between Days 35, 42, 49, 56, and 63 ($P < 0.05$).

Results

ACL regression

ACL regression was not observed in cows with an ipsilateral ACL. In cows with a contralateral ACL, ACL regression was greater (32%, 8 out 25, $P < 0.01$). In the ACL regression group, all cows showed regression from Days 35 to 42; the volume of the regressed ACL was less than 0.9 cm^3.

Characteristics of CL

The original CL volume and BFA were smaller in the control group than in the hCG-treated group (ipsi-maint, contra-maint, and contra-regr) on Day 35 (Table 1). The ACL volume and BFA in the ipsi-maint group were larger and higher than those contra-maint and contra-regr groups on Day 35 ($P < 0.05$; Table 1). There was no difference in the ACL volume or BFA between the contra-maint and contra-regr groups on Day 35. In all the groups, no changes in volumes were observed both in the original CL and ACL volumes during the experimental period (Fig. 2A, 2B). In the contra-maint, contra-regr, and control groups, no changes were observed in the original CL BFA during the experimental period (Fig. 2C). The original CL BFA in the contra-maint and contra-regr groups was lower than that in the ipsi-maint group but higher than that in the control group ($P < 0.05$, Fig. 2C). The ACL volume in the contra-maint group were consistently lower than that in the ipsi-maint group, during the experimental period ($P < 0.05$; Fig. 2B). The BFA in the contra-maint group were consistently lower than that in the ipsi-maint group from Days 35 to 56 ($P < 0.05$; Fig. 2D).

Plasma P_4 levels in the ipsi-maint, contra-maint, and control groups were consistent throughout the experimental period (Fig. 3). There was no significant difference in the circulating P_4 levels between the ipsi-maint and the contra-maint groups. In the contra-regr group, P_4 levels

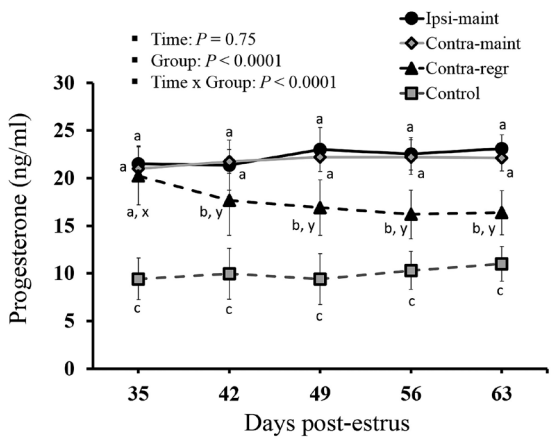

Fig. 3. Concentration plasma P_4 in four groups, control, ipsi-maint, contra-maint, contra-regr from Days 35 to 63.
[a, b, c)] indicate significant differences between specific groups ($P < 0.05$).
[x, y)] indicate significant differences between Days 35, 42, 49, 56, and 63 ($P < 0.05$).

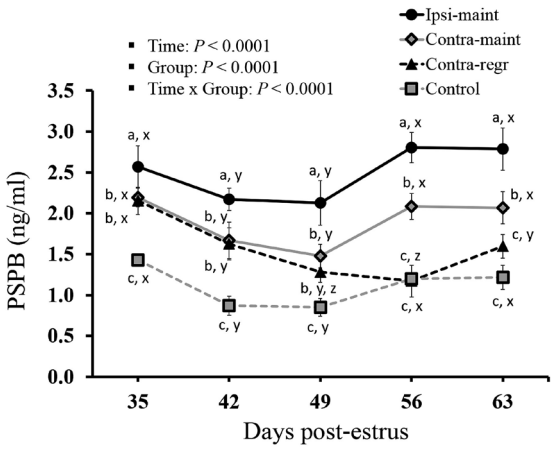

Fig. 4. Concentration plasma PSPB in four groups, control, ipsi-maint, contra-maint, contra-regr from Days 35 to 63.
[a, b, c)] indicate significant differences between specific groups ($P < 0.05$).
[x, y, z)] indicate significant differences between Days 35, 42, 49, 56, and 63 ($P < 0.05$).

decreased from Days 35 to 42 ($P < 0.01$) and were maintained from Days 42 to 63. Plasma P_4 levels in the contra-regr group were lower than that in ipsi-maint and contra-maint groups from Days 42 to 63, but higher in the control group during the experimental period.

Profile of the plasma PSPB concentrations

Throughout the experimental period, the highest, median, and lowest PSPB levels were observed in the ipsi-maint, contra-maint, and control groups, respectively ($P < 0.05$; Fig. 4). In these groups, the PSPB level decreased from Days 35 to 42, was maintained until Day 49, increased from Days 49 to 56, and remained constant until Day 63. In the contra-regr group, the PSPB level continually decreased from Days 35 to 49, remained constant until Day 56, and then increased from Days 56 to 63. From Days 56 to 63, PSPB levels in the contra-regr group were lower than that in the contra-maint group ($P < 0.05$).

Discussion

The present study showed that regression occurred in the contralateral ACL of hCG-treated Japanese Black cows. Contralateral ACL regression has been observed in previous studies that used GnRH to induce ACL formation[3,29)]. Additionally, a study on hCG-treated crossbred beef heifers reported a high occurrence of contralateral ACL regression[19)]. Taken together, these results indicate that contralateral ACL regression is a common phenomenon in cattle, independent of parity and hormonal treatment used to induce ACL formation.

In the present study, the original CL volume was significantly higher than the ACL volume in hCG-treated cows. However, the mean luteal tissue volume was similar between the original CL and the GnRH-induced ACL, in pregnant lactating cows[30)]. A previous study showed that in heifers treated with hCG or GnRH on Day 5, P_4 significantly increases with hCG treatment, not GnRH treatment[40)]. GnRH induces only an LH surge and does not stimulate the function of the original CL[7)]. Because hCG has longer LH-like

and luteotrophic activity[26,31], the development and function of the original CL may be stimulated in hCG-treated cows. Therefore, in the present study, the original CL volume and BFA in the hCG-treated cows were larger and higher, respectively, than that in the control group. Upon stimulation of the original CL function, P_4 levels increased rapidly. Pulsatile secretion of LH, which supports the development of CL, is inhibited by high P_4 levels[40], resulting in smaller volume and lower BFA in the ACL compared to original CL.

In our study, we found that the ACL volume and BFA were higher in the ipsi-maint group than that in the contra-maint group. Similarly, the original CL BFA was higher in the ipsi-maint group than that in the contra-maint group. These results suggested that the differences in BFA of CL might be related to the differences in blood supply to the ovary with CL. In a previous study, uterine blood flow in the gravid horn was higher than that in the non-gravid horn during the second month of gestation[30,34]. In addition, the formation of multiple CLs in the ovaries increased the blood flow in the uterine arteries[20]. Previous study showed that blood flow of the ipsilateral uterine artery through an anastomosis with the ovarian artery contribute to the blood flow to the ovary bearing the CL, and this blood flow increased during luteal phase[14]. The study using the cow with unilateral uterine horn aplasia showed that the formation of the CL increases the blood flow in the ovarian arteries regardless the presence of uterine horn[1]. Taken together, these studies indicated that the presence of CL increase the blood supply to the ovary by increasing in blood flow through the uterine artery and the ovarian artery. Therefore, blood flow supply to ovary and CL might be higher in the ipsi-maint group than that in the contra-maint and contra-regr groups. It is possible that high blood flow in the ovary resulted in a higher BFA in the original CL and ACL in the ipsi-maint group. Since the increase in blood flow support the increase in CL volume between Days 26 and 60[34], an increase in the BFA led to a larger original CL and ACL in the ipsi-maint group.

The present study aimed to investigate whether ACL characteristics are related to maintenance or regression. Our results showed that there were no differences in the volume and BFA of the ACL between contra-maint and contra-regr groups on Day 35 of gestation. Furthermore, there was no significant difference in plasma P_4 levels among the hCG-treated cows on Day 35. These findings showed no differences in ACL characteristics between the contra-maint and contra-regr groups. Therefore, ACL regression may not be caused by CL characteristics. It can be hypothesized that the luteotrophic or luteolytic factors, provided by the blood supply, may be related to ACL maintenance or regression.

An increase in blood flow to the uterus on the gravid horn was previously reported between Days 16 and 50[34]. In addition, the elevation of blood flow was maintained between Days 40 and 60[41]. Moreover, in a model of superovulation in dairy cows, an association was observed between blood flow in the uterine arteries and the formation of multiple CLs in ovaries[20]. The placenta is more developed in the gravid horn[15,33]; therefore, it is thought that the amount of PSPB produced by the placenta is also greater in the gravid horn. Previous studies indicated that increases in the secretion function of placenta and trans-placental exchange could be explained primarily by the increase of uterine blood flow[35,36]. Consequently, in the ipsi-maint group, higher blood flow to the gravid horn, induced by the presence of the ACL, may have caused an increase in the production of PSPB in the placenta and result in higher transfer into blood circulation. Therefore, the highest level of PSPB was observed in the ipsi-maint group. In contrast, in the contra-maint and contra-regr groups, blood flow to the gravid horn were lower than that in the ipsi-maint group. In addition, the presence of the contralateral ACL may have increased the blood flow in the non-gravid horn which may induce PSPB production. These may explain why PSPB concentrations were at intermediate levels in the contralateral ACL groups and at the lowest levels in the control group.

It should be noted that all the cows maintained their original CL and pregnancy

throughout the experimental period. Original CL was even maintained in the control group, which had the lowest concentrations of PSPB in circulation. If PSPB circulation supports the maintenance of the CL, the ACL should be maintained in the contralateral ACL group with moderate PSPB concentrations. However, the results of this study showed that ACL regression occurred more frequently in the contralateral ACL group. Therefore, maintenance or regression of the ACL may not be regulated by PSPB in circulation. It is assumed that luteotrophic factors related PSPB, might work on ACL maintenance through local communication between contralateral ACL and non-gravid uterine horn. PSPB stimulates the secretion of PGE2 from endometrial tissue and placenta[45,47], which pass through the venoarterial pathway to maintain the CL[23]. In this way, PSPB indirectly maintain contralateral ACL by PGE2 through utero-ovarian pathway. On the other hand, PSPB may directly reach to ACL through the venoarterial pathway, maintain ACL by stimulation of PGE2 secretion from luteal tissue as luteotrophic factor[48]. However, it is unclear whether PSPB can pass through blood vessels, because PSPB has a large molecular weight (66 kDa)[22]. Previous study observed uterine vein isolated from pregnant rat have high permeability to large molecule (70 kDa dextran)[6]. Therefore, PSPB may be provided through the local utero-ovarian pathway from the uterus to the CL, which may support CL maintenance. Consequently, high PSPB released from a well-developed placenta in the gravid horn might help to maintain the ipsilateral ACL by direct and/or indirect luteotrophic effects. In contrast, lower PSPB or luteotrophic factors from non-gravid horn may not be sufficient to maintain the contralateral ACL. Furthermore, the day of filling the allantochorion into the non-gravid horn had a higher variation than gravid horn[34]. Thus, in the non-gravid horn, there might be variation of the uterine blood flow, which cause differences in the supply of luteotrophic factors from uterus to the contralateral ACL. Consequently, in some cows, the contralateral ACL regressed, while none in others.

A noteworthy the elevation of PSPB levels occurs at the end of the first month of gestation whereas it decreases during the latter half of the second month[13,17,22]. In addition, a decrease in PSPB levels after Day 33 of gestation was reported in cows subjected to hCG treatment and in untreated control groups[12]. Changes in PSPB levels in ipsi-maint, contra-maint and control groups in our study, were consistent with previous reports. In the contra-regr group, PSPB continually decreased until Day 56 and was significantly lower than that in the contra-maint group on Day 63. Delayed PSPB recovery was observed in the contra-regr group compared to the ipsi-maint, contra-maint, and control groups. ACL regression may reduce the uterine blood flow in the non-gravid horn, which may change PSPB production from the placenta in the non-gravid horn. A previous study has reported high PSPB levels during the second month of gestation in pregnant lactating cows with high P_4 concentrations after hCG treatment[12]. A study was conducted on pregnant cows on Day 39 of gestation, wherein they received an intramuscular injection of PGF2α[17]. The results indicated that the concentration of PSPB decreased after plasma P_4 decreased. In the present study, in the contra-regr group, a decrease in P_4 levels was observed concurrently with ACL regression on Day 42. Reduced P_4 levels were maintained from Days 42 to 63. In the other groups, no changes in P_4 concentration were observed during the experimental period. Therefore, the change in P_4 levels in the contra-regr group may have contributed to slower PSPB recovery. Our study suggests that P_4 concentration are related to changes in plasma PSPB levels during the second month of gestation.

The present study, similar to previous studies, showed that the contralateral ACL was susceptible to regression, but pregnancy loss was not observed. Previous studies have shown that pregnancy loss is higher when ACL regression occurs early in pregnancy[29]. This suggests that there is a risk of pregnancy loss when hCG administration is applied to promote conception rate. However, in this previous study, pregnancy diagnosis was performed on the Day 26 post-AI using only PSPB

measurement without confirmation of an alive fetus[29]. Thus, it is unclear whether regression of the ACL is the cause of pregnancy loss. In present study, the fetus was detected on the Day 35 post-estrus, indicating that pregnancy loss might not occur even the ACL regresses after the fetus detection. Furthermore, 75% of ACL regression occurs after the first month of pregnancy[30]. When hCG administration is applied to promote conception rate, although regression of ACL occurs during pregnancy, it can be proposed that this regression does not increase the risk of pregnancy loss.

In conclusion, we observed a higher occurrence of regression contralateral ACL than ipsilateral ACL in Japanese Black cows. No significant difference was observed in the volume and BFA of the ACL between the contra-maint and contra-regr groups. In addition, in our study the ipsi-maint group exhibited higher PSPB levels than the contra-maint and contra-regr groups. Plasma P_4 concentrations were consistent in the ACL maintenance groups, during the experimental period, but decreased in the regression group. Our findings provide insight into the relationship between ipsilateral ACL maintenance and PSPB in pregnant cows.

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