Uterine and Ovarian Blood Flows Measured with Transrectal Doppler Sonography during the Estrous Cycle in Cows

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Uterine and Ovarian Blood Flows Measured with Transrectal Doppler Sonography during the Estrous Cycle in Cows

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Summary

Uterine and ovarian blood flows through the estrous cycle in cows were determined using transrectal color Doppler sonography, and the relationship between the change in uterine and ovarian circulations and the diameter of the ovulatory follicle (OF), area of corpus luteum (CL) and concentrations of plasma progesterone were investigated. Three non-lactating Deutsch Fleckvieh cows with a mean estrous cycle of 20.8±3.3 days and 5.7±1.5 years old were used. The sizes and locations of follicles and CL, blood flows in uterine and ovarian arteries were determined with color Doppler sonography, and plasma progesterone (P) concentrations were assayed over two estrous cycles. The uterine and ovarian arteries bearing the CL during the luteal phase (Day 0 to 15) and OF during follicular phase (Day -5 to -1) were referred to as dominant artery (Day 0 = ovulation). OF emerged on Day 9.2±3.3 (15.4±0.9 mm). Maximum area of CL was observed on Day 9.5 ±0.4 (525.1±100.6 mm^2). Plasma P showed maximum concentrations 3.8±0.7 ng/ml on Day 12. Mean pulsatility index (PI) values for the uterine artery during the luteal phase (2.5±0.3) were significantly higher than those of follicular phase (2.0±0.2). In contrast, mean time averaged maximum velocity (TAMV) values for the uterine artery during the follicular phase (21.8±2.3 cm/sec) were significantly higher than those of the luteal phase (17.5±1.8 cm/sec). Peak TAMV values were recorded on Day -4 (24.1±1.7 cm/sec) in the dominant uterine artery and on Day -3 (25.2±1.4 cm/sec) in the non-dominant artery. Mean PI values for the non-dominant ovarian artery (4.3±0.3) were significantly higher than those for the dominant artery (3.7±0.3) during the luteal phase, but there was no difference between mean PI values for each of the ovarian arteries (3.8±0.2, 3.8±0.1, respectively) during the follicular phase. Mean TAMV values during the luteal phase were significantly different between the dominant (11.8±0.9 cm/sec) and non-dominant ovarian arteries (9.4±1.0 cm/sec), but there was no difference between mean TAMV values for each ovarian artery (11.8±0.4 cm/sec, 11.4 ±0.8 cm/sec, respectively) during the follicular phase. Peak TAMV values were recorded on Day 11 (12.8±1.3 cm/sec) in the dominant artery and on Day -3 (12.2±1.1 cm/sec) in the non-dominant artery. Mean PI values for both uterine arteries during the follicular phase increased significantly compared with those of the luteal phase. In ovarian arteries, PI and TAMV values showed a

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difference between dominant and non-dominant arteries dependent on CL existence.

Key words: cow, color Doppler sonography, estrous cycle, ovarian blood flow, uterine blood flow

Introduction

In the field of animal reproduction, ultrasonography has been used as a non-invasive method to observe the female reproductive tract [12, 16]. Color Doppler sonography has been established as a clinical method in human medicine for assessing placental circulation, in order to predict retardation of fetal growth [7, 8]. In cows, blood flow of the uterine artery and ovarian artery has been investigated using a surgically implanted Doppler ultrasonic probe or an electromagnetic blood flow probe [10, 17]. In a recent study, the measurement of uterine and ovarian blood flows with noninvasive transrectal Doppler sonography was shown to be possible [4]. This research enabled further investigations about uterine and ovarian blood flows during the estrous cycle or early pregnancy, and intra corpus luteum (CL) blood flow around the luteolysis [1, 4, 9, 10, 18].

In cows [4, 10], mares [3, 5] and sows [11], cyclic uterine blood flow patterns were elucidated. It has been speculated that variations in the levels of estrogen (E) and progesterone (P) regulate the rhythmic changes in blood flow in these animals. Variations in E and P also affect the regulation of ovarian blood flow [17]. Additionally, it is reported that ovarian blood flow showed significant difference between the ipsilateral and contra lateral to the CL in the single ovulatory animals [5, 9, 11].

The objective of this study was to examine uterine and ovarian circulations throughout the estrous cycle in cows using transrectal color Doppler sonography, and to determine the relationship between the change in uterine and ovarian circulations and the diameter of the ovulatory follicle, area of the corpus luteum and concentrations of plasma progesterone.

Materials and Methods

Animals

Three non-lactating Deutsch Fleckvieh cows with a mean estrous cycle length of 20.8±3.3 days (range, 18 to 27 days) were used in this study. Their mean age was 5.7±1.5 years old (range, 4 to 7 years old).

Observations of ovarian dynamics

Each cow was observed with transrectal Doppler examinations by the same operator over two estrous cycles (total of 6 estrous cycles). The sizes and locations of follicles (>5mm in diameter) and corpus luteum (CL >10mm in diameter) in the ovaries were recorded using ultrasound examinations. The last day on which the dominant follicle (DF) was observed was designated as Day -1, while the first day when the ovulatory follicle (OF) disappeared was designated as Day 0. The follicular diameter was calculated with the average of height (H) and width (W) at the apparent maximal image. The area (A) of the CL was calculated by freezing the image at its maximum size and calculated from H and W using the following equation: $A\ (mm^2) = 0.5\ H \times 0.5\ W \times \pi$

Measurement of Uterine and Ovarian Blood Flows

Uterine and ovarian blood flows were investigated both in the left and the right laterals. The uterine artery was examined based on the method published previously [4]. The uterine artery can be found within the mesometrium as a movable arterial vessel. Near its origin from the umbilical artery, the uterine artery can be visualized with the color Doppler technique. The ovarian artery appears as a conglomerate adjacent to the ovary. This conglomerate can be followed proximately close to its origin from the aorta, and it was at this position that blood flows were observed by pulsed Doppler function. The left and the right sides of the uterine and ovarian arteries bearing the CL during diestrus or the OF during estrus, respectively, were referred to as dominant arteries, while the contra lateral sides were referred to as non-dominant arteries.

Doppler measurements were performed using a Toshiba SSH-140A ultrasound device (Toshiba Medical Co., Tokyo, Japan), equipped with a 7 MHz micro convex probe. In order to eliminate the signals from moving tissue and vessel wall movements in the path of the Doppler ultrasound pulse, the high pass filter was set at 100 Hz. All of the blood flow velocity waveforms were obtained at an interrogation angle between the Doppler sound beam and blood flow from 30 to 60 degrees. Each observations was recorded on videotapes.

At least two similar consecutive flow velocity waveforms with maximum end-diastolic frequency shift were used to calculate the blood flows. Pulsatility index (PI) and time averaged maximum velocity (TAMV) were measured to reflect a change in blood flows. PI is calculated as the ratio of difference between the peak systolic frequency shift (PSF) and the end diastolic frequency shift (EDF) to time average maximum frequency shift (TAMF): PI = (PSF-EDF) / TAMF. TAMV is calculated from TAMF: $TAMV = TAMF \times c / 2F \times \cos \alpha$ (c = ultrasound program speed, F = transmitted wave frequency, α = angle between the ultrasound beam and the blood flow). PI and TAMV values of two uniformed consecutive pulse waves were averaged.

Hormone Assay

Blood samples were taken after each color Doppler examination. Plasma was separated within 30 minutes and frozen at -20℃ until assay. Plasma progesterone (P) concentration was measured by enzyme immunoassay as published previously [14, 15]. Progesterone was measured after extraction in residue of 20 μl plasma per well using monoclonal antibody. All intra- and inter- assay variations were < 10%.

Statistical Analysis

Statistical analysis was carried out using the Statview statistical software package (Abacus Concepts, Inc., Berkeley, CA). The data of follicular diameter and CL area, uterine and ovarian blood flows, plasma P concentrations were indicated as the mean ± sd, and compared using student's *t*-test. Changes in follicular diameter, CL area, blood flow parameters and plasma P concentrations were compared using Pearson's correlation coefficient. To facilitate comparison of each parameter, the estrous cycle was divided into the following two stages: the luteal phase ranging from Day 0 to 15 and the follicular phase ranging from Day -5 to -1 (Day 0 = ovulation).

Results

Ovarian Dynamics

Number of follicular waves of the six estrous cycles was two waves in 4 cycles and three waves in 2 cycles. Emergence of the first wave DF (> 5 mm) was observed on Day -1.0±0.6 and that of the

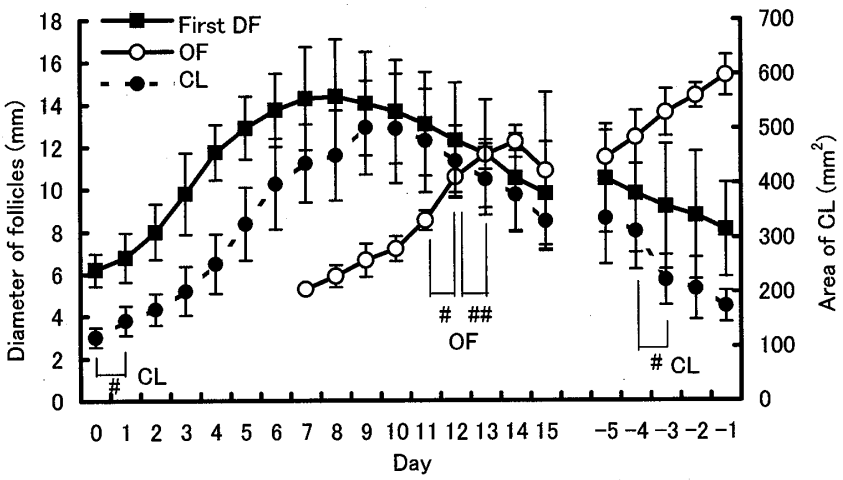

Figure 1. Follicular and corpus luteum dynamics during the estrous cycle.
: Significant difference between days ($p<0.05$).
##: Significant difference between days ($p<0.01$).

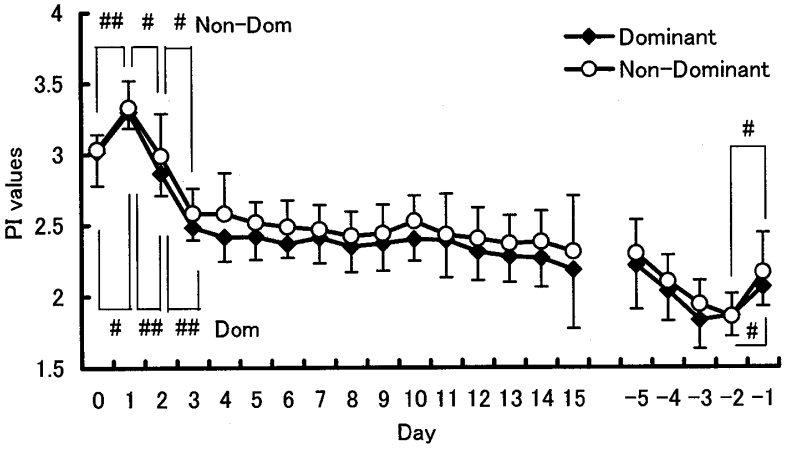

Figure 2. PI values for the uterine arteries during the estrous cycle.
: Significant difference between days ($p<0.05$).
##: Significant difference between days ($p<0.01$).

OF on Day 9.2±3.3 (Fig. 1). Maximum diameter of the first wave DF was 14.7±2.3 mm (Day 7.7 ±1.4) and maximum diameter of the OF was 15.4±0.9 mm. Maximum area of the CL was observed on Day 9.5 ±0.4 (525.1±100.6 mm^2).

Uterine Blood Flows

There was high correlation ($r = 0.97$; $p<0.01$) between PI values of the dominant and non-dominant uterine arteries during the luteal phase (Fig. 2). Mean PI values during the luteal phase showed no difference between dominant (2.5±0.3) and non-dominant arteries (2.6±0.3, Table 1). During the follicular phase, there was a significant correlation in PI values ($r = 0.92$; $p<0.01$) between the dominant and non-dominant uterine arteries, and mean PI values showed no difference between each uterine artery (2.0±0.2, 2.1±0.2). Mean PI values during the luteal phase were higher than those of the follicular phase in the dominant and non-dominant uterine arteries, respectively

Table 1. Comparison of mean PI and TAMV values between dominant and non-dominant uterine and ovarian arteries during luteal and follicular phases.

Uterine blood flows

	Dominant	Non-Dominant	P value
Mean PI value			
Luteal phase (90) [1]	2.5±0.3 [2]	2.6±0.3 [2]	0.39
Follicular phase (30) [1]	2.0±0.2	2.1±0.2	0.52
Mean TAMV value (cm/sec)			
Luteal phase (90) [1]	17.6±1.8 [2]	17.5±1.8 [2]	0.88
Follicular phase (30) [1]	21.7±2.4	21.9±2.3	0.88

Ovarian blood flows

	Dominant	Non-Dominant	P value
Mean PI value			
Luteal phase (90) [1]	3.7±0.3	4.3±0.3 [2]	< 0.01
Follicular phase (30) [1]	3.8±0.1	3.8±0.2	0.78
Mean TAMV value (cm/sec)			
Luteal phase (90) [1]	11.8±0.9	9.4±1.0 [2]	< 0.01
Follicular phase (30) [1]	11.8±0.4	11.4±0.8	0.45

1) Number of values determined.
2) Significant difference between luteal phase and follicular phase ($p<0.01$).

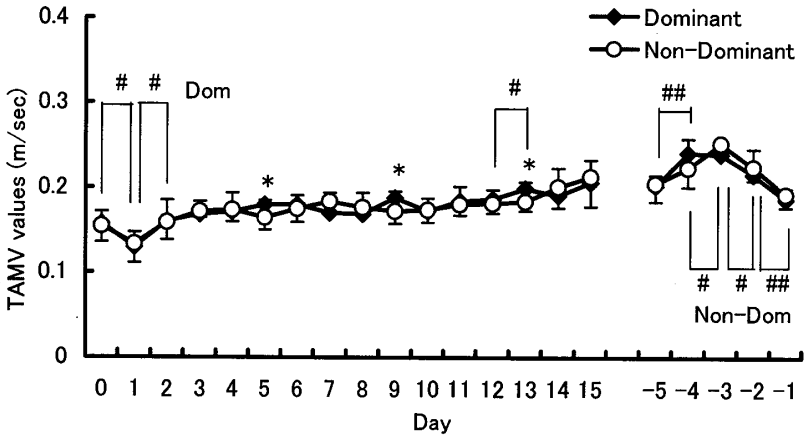

Figure 3. TAMV values for uterine arteries during estrous cycle.
* : Significant difference between dominant and non-dominant arteries ($p<0.05$).
\# : Significant difference between days ($p<0.05$).
\## : Significant difference abetween days ($p<0.01$).

($p<0.01$). In the dominant uterine artery, the highest PI value was observed on Day 1 (3.3±0.1) and the lowest on Day -3 (1.8±0.2). The highest PI value was observed on Day 1 (3.3±0.2) and the lowest on Day -2 (1.9±0.2) in the non-dominant uterine artery.

There was a significant correlation in TAMV values between the dominant and non-dominant uterine arteries during the luteal phase ($r = 0.88$; $p<0.01$) and follicular phase ($r = 0.87$; $p<0.05$, Fig. 3). Mean TAMV values showed no difference between the dominant (17.6±1.8 cm/sec) and non-dominant uterine arteries (17.5±1.8 cm/sec) during the luteal phase, and also no difference during the follicular phase (21.7±2.4 cm/sec, 21.9±2.3 cm/sec, Table 1). Mean TAMV values during the

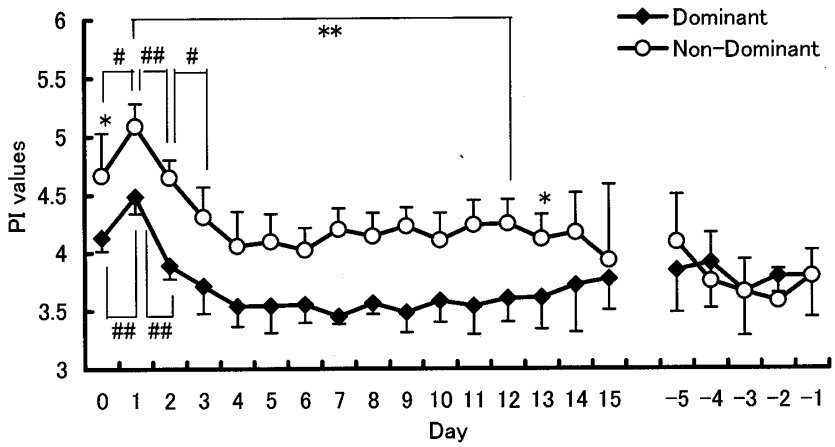

Figure 4. PI values for ovarian arteries during estrous cycle.
* : Significant difference between dominant and non-dominant arteries ($p<0.05$).
** : Significant difference between dominant and non-dominant arteries ($p<0.01$).
\# : Significant difference between days ($p<0.05$).
\#\# : Significant difference between days ($p<0.01$).

follicular phase were higher than those of the luteal phase in the dominant and non-dominant uterine arteries, respectively ($p<0.01$). In the dominant uterine artery, the highest TAMV value was observed on Day -4 (24.1 ± 1.7 cm/sec) and the lowest on Day 1 (13.0 ± 1.7 cm/sec). The highest TAMV value was observed on Day -3 (25.2 ± 1.4 cm/sec) and the lowest on Day 1 (13.3 ± 2.3 cm/sec) in the non-dominant artery. There was a significant difference in TAMV values between dominant and non-dominant uterine arteries on Days 5, 9 and 13 ($p<0.05$).

Ovarian Blood Flows

There was a significant correlation ($r = 0.87$; $p<0.01$) in PI values between the dominant and non-dominant ovarian arteries during the luteal phase, but no relationship was found during the follicular phase ($r = 0.41$; $p>0.27$, Fig. 4). During the luteal phase, mean PI values for the dominant ovarian artery (3.7 ± 0.3) were significantly higher than those for the non-dominant ovarian artery (4.3 ± 0.3, $p<0.01$, Table 1). In contrast, mean PI values during the follicular phase showed no difference between dominant (3.8 ± 0.1) and non-dominant (3.8 ± 0.2) ovarian arteries. Mean PI values for the non-dominant ovarian artery during the luteal phase were significantly higher than those of the follicular phase ($p<0.01$), but mean PI values for the dominant ovarian artery showed no difference. There was significant difference in PI values between dominant and non-dominant ovarian arteries from Day 0 to Day 13 (Day 0, 13: $p<0.05$; Day 2 to 12: $p<0.01$). The highest PI value was observed on Day 1 (4.5 ± 0.1) and the lowest on Day 7 (3.4 ± 0.1) in the dominant ovarian artery. In contrast, the highest PI value was observed on Day 1 (5.1 ± 0.2) and the lowest on Day -2 (3.6 ± 0.3) in the non-dominant ovarian artery.

There was a high correlation ($r = 0.69$; $p<0.01$) between TAMV values for the dominant and non-dominant ovarian arteries during the luteal phase, but no relationship was found during the follicular phase ($r = 0.51$; $p=0.21$, Fig. 5). Mean TAMV values in the dominant ovarian artery (11.8 ± 0.9 cm/sec) were significantly higher than those in the non-dominant ovarian artery (9.4 ± 1.0 cm/sec) during the luteal phase ($p<0.01$, Table 1). On the other hand, mean TAMV values during the follicular phase showed no difference between the dominant and non-dominant ovarian arteries (11.8 ± 0.4 cm/sec, 11.4 ± 0.8 cm/sec). Mean TAMV values during the follicular phase were significantly

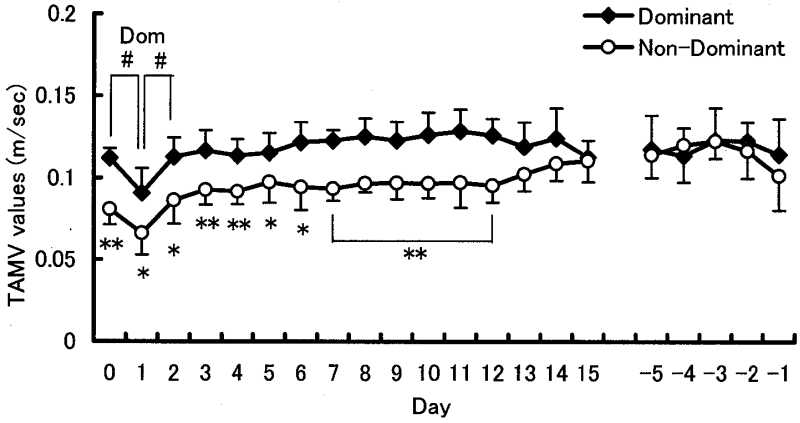

Figure 5. TAMV values for ovarian arteries during the estrous cycle.
* : Significant difference between dominant and non-dominant arteries ($p<0.05$).
** : Significant difference between dominant and non-dominant arteries ($p<0.01$).
\# : Significant difference between days ($p<0.05$).

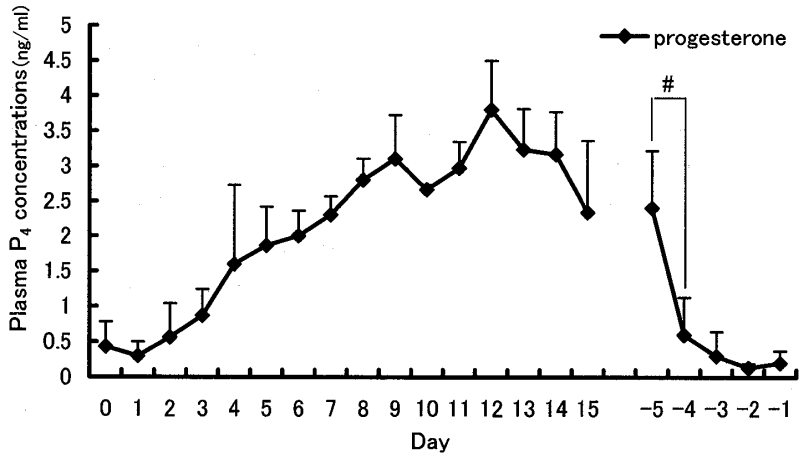

Figure 6. Plasma progesterone concentrations during the estrous cycle.
\# : Significant difference between days ($p<0.05$).

higher than those of the luteal phase in non-dominant ovarian artery ($p<0.01$), but there was no difference in TAMV values between the luteal phase and the follicular phase. TAMV values were significantly different between both ovarian arteries from Day 0 to Day 12 (Days 0, 3, 4, 7 to 12: $p<0.05$; Days 1, 2, 5, 6: $p<0.01$). The highest TAMV value was observed on Day 11 (12.8 ± 1.3 cm/sec) and the lowest on Day 1 (9.1 ± 1.5 cm/sec) in the dominant ovarian artery. In contrast, the highest TAMV value was observed on Day -3 (12.2 ± 1.1 cm/sec) and the lowest on Day 1 (6.6 ± 1.3 cm/sec) in the non-dominant ovarian artery.

Plasma Progesterone Concentrations

Plasma P showed a maximum concentration of 3.8 ± 0.7 ng/ml on Day 12 and a minimum concentration 0.1 ± 0.1 ng/ml on Day -2 (Fig. 6). There was a significant correlation between plasma P concentrations and the area of the CL ($r = 0.80$; $P<0.01$). During the luteal phase, plasma P concentrations were correlated with TAMV values in both uterine and ovarian arteries (uterine artery: $r = 0.72$, $p<0.01$; ovarian artery: $r = 0.75$, $p<0.01$), but there was no correlation during the

follicular phase (uterine artery: r = 0.22, p = 0.64; ovarian artery: r = −0.17, p = 0.81).

Discussion

A normal estrous cycle is about 21 days (length: 18 to 24 days), and the wave of follicular growth involves the synchronous development of follicles with 2 or 3 follicular waves per estrous cycle [12]. Our study showed similar results regarding the range and number of waves per estrous cycle. These results show that measurement of uterine and ovarian arterial blood flow using transrectal color Doppler sonography did not affect the follicular dynamics in cows.

In the present study, there was no difference between the CL or OF bearing uterine artery (dominant) blood flow and the contra lateral uterine artery (non-dominant) blood flow during the estrous cycle. PI of the uterine arteries showed the lowest values in 2 or 3 days before ovulation and peak values following the day of ovulation. These results are in agreement with those of other studies, which investigated the RI value of the uterine artery during the estrous cycle in cows and mares [4, 5]. Other studies have reported that blood flows between pregnant and non-pregnant uterine horn arteries showed significant difference in 16 days after insemination [2, 9]. In the present study, PI and TAMV values of both uterine arteries showed no difference in the estrous cycle, that is, the existence of the CL or OF has no influence on uterine arterial blood flow. It is reported that the uterine blood velocity showed positive correlation with estradiol-17 β and negative correlation with progesterone in the estrous cycle [9]. Additionally, Ford et al. [10] reported that the uterine arterial blood flow is controlled by the plasma estrogen concentrations. The results from our study where plasma P concentrations were correlated with TAMV values in both uterine arteries ($r = 0.76$; $p<0.01$) suggested that uterine blood flow is controlled by plasma progesterone concentrations.

PI values for the non-dominant ovarian artery showed a similar change to those for the uterine arteries. In contrast, PI values for the dominant ovarian artery showed a similar change like the uterine artery during the follicular phase, but PI values for the dominant ovarian artery showed significant lower level than those for the non-dominant artery during the luteal phase (Days 0 to13). These lower PI values during the luteal phase are in agreement with another study on ovarian arterial blood flow during the estrous cycle in mares [5]. On the other hand, TAMV values in the dominant artery during the luteal phase (Days 0 −12) were significantly higher than those for the non-dominant artery. These results showed that the existence of the CL has a great influence on ovarian arterial blood flow. The higher blood velocity in the dominant ovarian artery during the luteal phase is estimated to be the effect of the corpus luteum acting as a low impedance shunt [9, 13]. In our study, the peak TAMV value was observed on Day 12, and was synchronized exactly with the day of peak plasma progesterone concentrations. These results also showed that the existence of the CL and plasma progesterone concentrations affect dominant ovarian arterial blood velocity. Wise et al. [3] reported that measurements of ovarian arterial blood flow using electromagnetic blood flow probes showed a higher level during the luteal phase and the lower level before ovulation. However, mean TAMV values in the dominant ovarian artery showed no difference between the luteal and follicular phases in our study. These discrepancies may be derived from the differences of the method and the point in the ovarian artery used to obtain the blood flows.

In this study, uterine and ovarian blood flows were successfully measured using non-invasive color Doppler sonography. The results showed the relationship between dominant ovarian arterial blood flow and the existence of the CL. Further investigation is necessary to clarify the nature of this relationship, and in order to develop clinical applications for increasing fertility.

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