Variability of mammary blood flow in lactating Holstein-Friesian cows during the first twelve weeks of lactation

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ABSTRACT

The goals of the present study were to measure mammary blood flow volume (BFV) during the first 12 wk of lactation in dairy cows by using color Doppler sonography and to determine what affects the mammary blood flow. Forty cows were examined via color Doppler sonography on d 1, 7, 14, 28, 56, and 84 after parturition (d 0). The total BFV (BFV_{total}) to the 4 mammary glands was calculated by measuring time-averaged maximum velocities (TAMV) and cross-sectional areas (A) of the left and right pudendoepigastric trunks via transrectal color Doppler sonography. Because there were no significant differences in A, TAMV, and BFV between the right and left pudendoepigastric trunks, the means of A and TAMV, and the BFV_{total} of both trunks were used for calculations. The intraindividual and interindividual variability of repeated BFV measures quantified by intraclass correlation coefficients were 96 and 98%, respectively. The BFV_{total} ranged from 19.9 to 27.9 L/min, with a mean of 22.3 ± 4.9 L/min. Interindividual differences in BFV values were attributable to variations in A and TAMV. The interindividual variability of the BFV_{total}, which was determined using the coefficients of variation of the BFV_{total} on individual days, ranged from 16 to 28%. All the cows had similar changes in the BFV_{total} during the study. Changes in BFV_{total} were not correlated with changes in the mean of A, but there was a good correlation between changes in BFV_{total} and in the mean of TAMV ($r = 0.94$). The BFV_{total} was highest on d 1 of lactation, decreased 28% by d 7, and remained at this level until d 28. By d 56, the BFV_{total} had increased by 15% compared with d 14 and by 10% compared with d 28. The BFV_{total} on d 84 was significantly different from all other days except d 56. There were moderate correlations between daily milk yield and BFV on individual days ($0.24 < r < 0.35$). In conclusion, Doppler sonography is a reliable method for determining blood

flow in the pudendoepigastric trunk of cows. There is great variability in mammary blood flow among cows and in BFV.

Key words: mammary blood flow, milk yield, Doppler, lactation number

INTRODUCTION

The arterial blood flow to the bovine mammary gland plays a key role in providing substrates necessary for milk synthesis (Davis and Collier, 1985; Davis et al., 1988; Prosser et al., 1996). Numerous studies (Kronfeld et al., 1968; Kensinger et al., 1983; Gorewit et al., 1989; Thivierge et al., 2000; Piccione et al., 2004b) have investigated mammary blood flow, but mostly for short periods and in a small number of cows, and most of the investigations have used invasive techniques (Kensinger et al., 1983; Gorewit et al., 1989; Thivierge et al., 2000). Kensinger et al. (1983) studied the variability of mammary blood flow by implanting electromagnetic blood flow transducers around the right external pudendal artery of 4 Holstein cows during their dry periods. In this study, the heterogeneity was greatest for cows and days. The time of day that measurements were taken had no detectable influence on mammary blood flow and the coefficients of variation were low, with a range of 0.63 to 1.51% for 17 estimates. The authors of that study suspected that blood flow volume (BFV) might be associated with the previous milk yield or lactation number; however, definitive proof of this was not possible because of the small number of cows. Kronfeld et al. (1968) and Linzell (1974) have presented the fact that mammary blood flow and milk yield are strongly correlated. Kronfeld et al. (1968) measured the mammary blood flow by the antipyrine absorption method in normal, fasted, and ketonic cows. The calculated values of mammary blood flow (mg/min) were closely related to milk production. In another study, Gorewit et al. (1989) used 4 open Holstein cows to validate a transit-time ultrasonic blood flow metering system for measuring arterial blood flow in the mammary gland. The ultrasonic transducers were surgically placed around the right external pudendal artery, and in 2 of the cows, electromagnetic flow transducers were also

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implanted. The values obtained with the transit-time ultrasonic flow meter were in agreement with those obtained with the electromagnetic blood flow transducer, which gave a flow rate of 1.23 to 2.23 L/min in cows with a milk yield of 8 to 10 kg/d. Noninvasive measurement of mammary blood flow in cows was first done by Piccione et al. (2004b) using Doppler sonography in 7 Bruna Italiana cows, which were a mean of 5 yr old. The maximum systolic and diastolic blood flow velocities in the external pudendal artery were measured in 3 different lactation phases on d 40, 100, and 210 postpartum and in the dry period, 290 d postpartum. The study found no significant difference in blood flow velocity among the various stages of lactation. The BFV was not determined.

The objective of the present study was to examine mammary blood flow for the first time in a greater number of cows and more frequently during the first 12 wk of lactation by using transrectal Doppler sonography. In addition, the association between mammary blood flow and milk yield was investigated. This study could be the groundwork for later studies, such as investigations of the influence of mammary blood flow on udder health.

MATERIALS AND METHODS

Cows

Forty multiparous, lactating German Holstein cows were examined during the first 12 wk postpartum. Of the 40 cows, 18 were in their second lactation, 19 were in their third, and 3 were in their fourth. The mean age of the cows was 4.1 yr (range, 2.8 to 5.3 yr), and they weighed an average of 592 kg (minimum: 478; maximum: 783 kg) at 1 d postpartum. The milk yield could not be determined on the first day because the calves were still nursing their dams.

The cows were part of the herd at the research farm of the Institute of Animal Nutrition, Friedrich-Loeffler Institute, in Brunswick and were housed in a free stall with slatted floors and straw-bedded cubicles. The feed consisted of corn and grass silage and was formulated for a daily production of 10 kg of milk; for every 2 kg of additional milk production, the cows received 1 kg of a dairy grain mix via transponder feeding.

Study Design

The cows underwent clinical and color Doppler sonographic examinations on d 1, 7, 14, 28, 56 ± 3 , and 84 ± 3 after parturition (d 0) and were weighed 1 d after calving. Milk yield was recorded on d 7, 14, 28, 56 ± 3 , and 84 ± 3 .

Clinical Examination

Before each examination, the cows underwent a physical examination, including a visual inspection and palpation of the mammary gland. Milk samples from each quarter were assessed macroscopically, the pH was determined using indicator paper, and a California Mastitis Test was carried out.

Color Doppler Sonography

Evaluation of mammary blood flow was carried out by measuring the BFV in the right and left pudendoepigastric trunks by Doppler sonography. The caudal epigastric and external pudendal arteries branch from the pudendoepigastric trunk and are the main arterial blood supplies of the mammary gland (Waibl et al., 1996). After passing through the inguinal canal, the external pudendal artery runs dorsocaudally to the base of the mammary gland and branches into the cranial and caudal mammary arteries, which supply the cranial and caudal halves of the gland (Bragulla and König, 1999).

The pudendoepigastric trunk was located transrectally by first imaging the abdominal aorta, which is situated dorsally and is easily located with the ultrasound transducer. The external iliac artery branches from the aorta at approximately the level of the sixth lumbar vertebra. It runs ventrally over the internal lumbar musculature and then along the iliac shaft in a craniomedial direction. The external iliac artery was followed along the iliac shaft ventrally to the point of transition of the artery into the femoral artery and where the deep femoral artery branches off. This bifurcation was located in the ventral third of the iliac shaft, approximately 3 to 6 cm dorsal to the pelvic floor. The femoral artery disappeared from view in the limb musculature, and the deep femoral artery was imaged running ventrally. A few centimeters after the first bifurcation and approximately at the level of the pelvic floor, the deep femoral artery branched into the medial circumflex femoral artery and the pudendoepigastric trunk. The pudendoepigastric trunk was imaged approximately 2 cm after this bifurcation, which was cranioventral to the pelvic floor, at an angle that facilitated blood flow measurement.

The transrectal Doppler ultrasound examinations were carried out between 0800 and 1200 h and lasted approximately 20 min in each cow. Doppler measurements were done in pulsed-wave mode (Figure 1) with a Toshiba SSA 370 A Version K instrument equipped with a 7.00-MHz microconvex probe (Toshiba Co., Tokyo, Japan). Blood flow waveforms were obtained at an interrogation angle of 20 to 60° between the Doppler ul-

Figure 1. Color Doppler sonogram of the right pudendoepigastric trunk on d 84 postpartum in pulsed-wave mode. The Doppler wave of the pudendoepigastric trunk is seen on the left and the pudendoepigastric trunk and Doppler window are seen on the right (color version available in the online pdf).

trasound beam and the flow direction. The observations were displayed live and were recorded on videotapes.

After collection of all data, the Doppler calculations were made off-line using 2 similar consecutive flow-velocity waveforms. The time-averaged maximum velocity (**TAMV**) was calculated from the time-averaged maximum frequency shift over the cardiac cycle by using the following equation:

$$\text{TAMV (cm/s)} = \frac{\text{TAMF} \times c}{2F \times \cos \alpha},$$

with c being the ultrasound propagation speed, F being the transmitted wave frequency, and α being the angle between the ultrasound beam and the direction of blood flow (Figure 2). The TAMV values of 2 uniform consecutive pulse waves were averaged 3 times at a interval of 1 min. In addition, the cross-sectional areas (**A**) of both pudendoepigastric trunks were determined at the same place the blood flow measurements were done. At each examination, the mean of 3 measurements was calculated from digitized 2-dimensional grayscale images of the vessels. The BFV was then computed according to the following formula:

$$\text{BFV (mL/min)} = \text{TAMV (cm/s)} \times 60 \times A \text{ (cm}^2\text{)},$$

with TAMV being the time-averaged maximum velocity of blood flow in the pudendoepigastric trunk and A being the cross-sectional area of the pudendoepigastric trunk.

Statistical Analysis

Statistical analysis was carried out using SAS 9.1 and StatView 5.0 (SAS Inst. Inc., Cary, NC). The Shapiro-Wilk test was used to test for normality of milk yield, TAMV, A , and BFV. Means and standard errors were calculated for all of the above-mentioned variables. Blood flow variables for the left and right pudendoepigastric trunks were compared using Pearson correlation coefficients and the paired Student's t -test. Comparisons were made between cows in lactation 2 (**L2**, $n = 18$) and cows in lactation 3 or 4 (**L >2**, $n = 22$) by using Student's t -test for independent samples. Because only 3 cows were in their fourth lactation, the cows from the third and fourth lactations were combined. The effects of the day after parturition and cow on blood flow variables and milk yield were tested by

ANOVA for repeated measures. Comparison-wise differences between the days were analyzed using multiple paired *t*-tests. Estimation of the variance components was used to compare the influence of cow and day of lactation on milk yield and blood flow variables. Analysis of covariance was used to analyze the interaction between BFV and milk yield separately for each lactation number. Intra- and interobserver reproducibility values of Doppler measurement results were expressed as coefficients of variation and intraclass correlation coefficients (ICC). Levels of $P < 0.05$ were defined as statistically significant, and levels of $0.05 \leq P < 0.1$ were defined as trends.

RESULTS

Clinical Findings

On d 14, 56, and 84, only 39 of the 40 cows were examined. On d 1, only 36 cows were examined, and on d 7 and 28, only 37 cows were examined. The reason for these failures was that in 12 of the examinations, macroscopic changes in the milk were seen in at least one quarter. The changes included watery secretions and the presence of clots of various sizes. Furthermore, one cow had parturient paresis on d 1, one cow had severe diarrhea on d 7, and another died on d 63. Equipment failure accounted for the remaining missing examinations.

None of the evaluated cows had acute mastitis with visible changes in milk secretion on any of the examination days. In the most of the cases, the pH was 6.6 and the cell count was less than 1,000,000 cells. In 21% of the cases, the pH was >6.6 , and in 19% of the examinations, the cell count was greater than 1,000,000 cells. Day 1 was excluded from this consideration because pH values and cell counts are influenced by the colostrum.

Milk Yield

The milk yield increased by 34% from d 7 to 56 ($P < 0.05$; Table 1). By d 84, the milk yield had decreased by 4% ($P < 0.05$) compared with d 56. In cows in L >2 , milk yield was 16% higher on d 7 and 14 ($P < 0.05$) than milk yield in cows in L2 (data not shown). There was no difference in milk yield between the 2 lactation groups ($P > 0.05$) on d 28, 56, and 84.

Blood Flow Variables

The intraindividual reproducibility had an ICC of 96% for BFV, and the interindividual reproducibility had an ICC of 98% for BFV. The correlation between A and BFV was $0.60 < r \leq 0.67$ ($P < 0.05$) on d 1, 7, and 28 and was $0.46 < r \leq 0.50$ ($P < 0.05$) on d 14, 56,

Figure 2. Schematic representation of a Doppler wave with maximum systolic (S), minimum diastolic (M), end diastolic (D), and time-averaged maximum frequency shift (TAMF) during one cardiac cycle.

and 84. The correlation between TAMV and BFV was $0.53 < r \leq 0.74$ ($P < 0.05$) on d 1, 7, 14, and 84 and was $0.37 < r \leq 0.43$ ($P < 0.05$) on d 28 and 56.

There were no significant differences in A, TAMV, or BFV between the right and left pudendoepigastric trunks ($P > 0.05$). Thus, further analyses used the means of A (A_m), the means of TAMV ($TAMV_m$), and the total combined BFV (BFV_{total}) of both trunks.

The A_m value for the entire examination period was $2.08 \pm 0.03 \text{ cm}^2$. Compared with d 7, A_m had increased ($P < 0.05$) 3% by d 56 and had increased 8% by d 84 (Table 1). There were no other significant differences in the A_m values of individual examination days ($P > 0.05$). The coefficients of variation for interindividual differences in the A_m ranged from 15% on d 84 to 25% on d 1. Cows in the L >2 group had higher A_m values than cows in the L2 group at all sampling times ($P < 0.05$): the A_m was 27% higher on d 1, 28% higher on d 7, 24% higher on d 14, 22% higher on d 28, 21% higher on d 56, and 12% higher on d 84. The A_m values remained constant in the L >2 group throughout the study period ($P > 0.05$), whereas the values of the L2 group remained constant until only d 28 ($P > 0.05$). By d 84, the values had increased by 17% compared with d 1 and by 14% compared with d 28 ($P < 0.05$). The A_m values on d 84 were 9% higher than on d 56 in the L2 group ($P > 0.05$).

The overall $TAMV_m$ was $91.0 \pm 1.4 \text{ cm/s}$ for the study period. The $TAMV_m$ was highest on d 1 and had decreased 27% by d 7 ($P < 0.05$), after which time the value did not change, except for d 56 ($P > 0.05$; Table 1). On d 56, $TAMV_m$ had increased by 7% compared with d 14 and by 4% compared with d 28, respectively ($P < 0.05$). The coefficients of variation for interindividual differences in $TAMV_m$ ranged from 15 to 22%. In the L2 group, the $TAMV_m$ was 14% higher on d 56

Figure 3. Correlations between total blood flow volume (BFV, in L/min) and milk yield (MY, in kg) of cows in the second (blue, L2) and cows in the third and fourth (red, L3 and L4) lactation on d 56 postpartum (color version available in the online pdf).

($P > 0.05$). At all other sampling times, there were no significant differences between the 2 lactation groups ($P > 0.05$).

There was no correlation between changes in A_m and BFV_{total} ($P > 0.05$). In contrast, there was a high correlation between the changes in $TAMV_m$ and BFV_{total} ($r = 0.94$; $P < 0.05$).

The BFV_{total} (Table 1) ranged from 19.9 to 27.9 L/min, with a mean of 22.3 ± 0.4 L/min and was distinctly higher on d 1 of the lactation than on any other sampling days ($P < 0.05$). By d 7, the BFV_{total} had decreased by 28% ($P < 0.05$) and remained at this level on d 14 and 28 ($P > 0.05$). By d 56, the BFV_{total}

had increased by 12% compared with d 14 and by 9% compared with d 28 ($P < 0.05$). The BFV_{total} on d 84 differed significantly from all other sampling days, except for d 56 ($P < 0.05$).

The analyses of covariance showed that the regression lines for the different parities were not significantly different ($P > 0.05$) for the respective times of assessment. The intersections of the regression lines with the y-axis of groups L2 and L >2 were significantly different ($P < 0.05$; Figure 3) only on d 56. In all other cases, the intersections with the y-axis did not differ ($P > 0.05$). The correlation between daily milk yield and BFV_{total} on individual sampling days was moderate ($0.24 < r < 0.35$). Differences in BFV_{total} of cows in the L2 and L >2 groups were seen only at the beginning of lactation and on d 28 (Table 1; $P < 0.05$). In the L >2 group, the BFV_{total} was 25% higher on d 1, 29% higher on d 7, and 15% higher on d 28 compared with group L2 ($P < 0.05$); there were no significant differences between the 2 groups on d 14, 56, and 84 ($P > 0.05$).

DISCUSSION

The results of intraindividual and interindividual variation in repeated measures showed that transectal Doppler sonography is a useful method with high reproducibility for measurement of BFV in the pudendoepigastric trunk. Determination of the exact cross-sectional area of a blood vessel via Doppler sonography is difficult in vessels with a relative small diameter (Dudwiesus, 1995). The pudendoepigastric trunk had a relative large mean cross-sectional area of 2.07 ± 0.40 cm², which corresponded to a diameter of

Table 1. Milk yield (MY, kg/d) and total blood flow volume (BFV, L/min) per MY (BFV/MY) on d 7, 14, 28, 56, and 84 of lactation (d 0 = parturition) in all cows and means of area (A, cm²), the time-averaged maximum velocity (TAMV, cm/s), and BFV in the left and right pudendoepigastric trunks during the first 84 d of lactation in all cows, in cows in the second lactation (L2), and in cows with more than 2 lactations (L >2)¹

Item	Day 1	Day 7	Day 14	Day 28	Day 56	Day 84
MY						
All	—	25.6 $\pm$ 0.9 ^a	29.8 $\pm$ 0.8 ^b	31.8 $\pm$ 1.1 ^{bd}	34.3 $\pm$ 1.0 ^c	33.0 $\pm$ 1.2 ^d
A						
All	202.6 $\pm$ 8.4 ^{ab}	200.2 $\pm$ 7.1 ^a	200.3 $\pm$ 6.3 ^a	198.2 $\pm$ 6.1 ^{ab}	210.5 $\pm$ 6.4 ^b	219.0 $\pm$ 5.2 ^c
L2	169.4 $\pm$ 7.3 ^{a,A}	166.0 $\pm$ 7.3 ^{a,A}	171.5 $\pm$ 7.7 ^{a,A}	175.2 $\pm$ 6.0 ^{a,A}	184.5 $\pm$ 7.8 ^{b,A}	203.8 $\pm$ 8.0 ^{b,A}
L >2	232.3 $\pm$ 10.8 ^{a,B}	230.6 $\pm$ 5.4 ^{a,B}	224.9 $\pm$ 5.5 ^{a,B}	224.1 $\pm$ 6.3 ^{a,B}	232.8 $\pm$ 6.7 ^{a,B}	231.9 $\pm$ 5.7 ^{a,B}
TAMV						
All	116.0 $\pm$ 3.7 ^a	85.0 $\pm$ 3.0 ^{bc}	84.3 $\pm$ 3.0 ^b	86.8 $\pm$ 2.3 ^b	89.9 $\pm$ 2.3 ^c	88.0 $\pm$ 2.7 ^{bc}
L2	117.2 $\pm$ 4.8 ^a	85.2 $\pm$ 4.0 ^b	91.7 $\pm$ 2.8 ^b	90.3 $\pm$ 3.2 ^b	97.4 $\pm$ 3.0 ^{c,A}	94.6 $\pm$ 3.8 ^{bc}
L >2	114.9 $\pm$ 5.2 ^a	84.8 $\pm$ 4.5 ^b	78.0 $\pm$ 4.6 ^b	82.8 $\pm$ 3.2 ^b	83.5 $\pm$ 2.9 ^{b,B}	82.5 $\pm$ 3.5 ^b
BFV						
All	27.9 $\pm$ 1.3 ^a	20.2 $\pm$ 0.9 ^{bc}	19.9 $\pm$ 0.8 ^b	20.4 $\pm$ 0.6 ^b	22.3 $\pm$ 0.6 ^{cd}	23.0 $\pm$ 0.8 ^d
L2	23.7 $\pm$ 1.2 ^A	16.6 $\pm$ 0.8 ^A	18.6 $\pm$ 0.7 ^A	18.8 $\pm$ 0.7	21.4 $\pm$ 0.9	23.1 $\pm$ 1.3
L >2	31.8 $\pm$ 1.8 ^B	23.4 $\pm$ 1.3 ^B	21.1 $\pm$ 1.4 ^B	22.2 $\pm$ 0.9	23.1 $\pm$ 0.7	22.9 $\pm$ 1.0
BFV/MY						
All	—	0.81 $\pm$ 0.04 ^a	0.68 $\pm$ 0.03 ^{bc}	0.66 $\pm$ 0.03 ^c	0.66 $\pm$ 0.02 ^{bc}	0.71 $\pm$ 0.03 ^{ab}

^{a-d}Within rows, values with different superscripts are different ($P < 0.05$).

^{A,B}Within columns, values with different superscripts differ between L2 cows and L >2 cows ($P < 0.05$).

¹Data are presented as means $\pm$ SE.

approximately 1.62 cm. Burns and Jaffe (1985) showed that a 1-mm error in measuring the diameter of a 10-mm blood vessel can lead to a 20% error in calculating the cross-sectional area. In contrast, making a 1-mm error when measuring the diameter of a 16-mm blood vessel leads to only a 12% error in calculating the cross-sectional area.

Although in other studies the BFV in wk 4 to 24 of lactation in one-half of the mammary gland ranged from 1.23 to 7.02 L/min (Davis et al., 1988; Gorewit et al., 1989; Delamaire and Guinard-Flament, 2006), the BFV in our study ranged from 19.9 to 27.9 L/min for the whole udder. Thus, the BFV in our study was 30 to 91% higher compared with the studies mentioned. The external pudendal artery, which is the main arterial blood supply of the mammary gland, and the caudal epigastric artery, which supplies the rectus abdominus and the abdominal oblique muscles (Waibl et al., 1996), originate from the pudendoepigastric trunk. Because the muscles supplied by the caudal epigastric artery are relatively small, the blood flow in this artery is very small compared with that in the external pudendal artery. Thus, the disparity between our BFV results and those reported in other studies cannot be attributed to using the pudendoepigastric trunk instead of the external pudendal artery. A more likely explanation is the difference in the methods used for measuring BFV. Most of the previous studies in cows used electromagnetic blood flow transducers placed around the external pudendal artery (Kensinger et al., 1983; Davis and Collier, 1985; Davis et al., 1988; Gorewit et al., 1989; Delamaire and Guinard-Flament, 2006). Meschia (1989) pointed out that this technique can restrict the blood vessels and hinder their expansion, thus leading to an underestimation of the actual BFV. Another important difference is that our study measured BFV during peak lactation in Holstein cows, which had milk yields higher than those reported in other studies (Davis et al., 1988; Gorewit et al., 1989). The mean milk production in our study was 31.2 kg/d, compared with 7.9 kg/half mammary gland per day in Jersey cows (Davis et al., 1988) and 8 to 10 kg/half mammary gland per day in Holstein cows (Gorewit et al., 1989).

Compared with other methods of measuring BFV, color Doppler sonography is noninvasive and can therefore be used in clinical studies with a large number of cows. Our novel study investigated the interindividual variability of mammary blood flow in Holstein cows. The dairy herd used consisted of cows that were representative of the German Holstein breed with respect to size and milk production (Arbeitsgemeinschaft Deutscher Rinderzüchter, 2005). The coefficients of variation ranged from 17 to 21% for milk production, from 15 to 25% for A_m , from 15 to 22% for TAMV, and from 16

to 28% for blood flow variables. Thus, individual variation was relatively high for all the variables. Delamaire and Guinard-Flament (2006) reported coefficients of variation of 19% for milk yield and 11% for BFV in a study of 4 cows. In studies with 4 to 6 goats (Thompson and Thomson, 1977; Fleet and Peaker, 1978; Davis et al., 1979; Henderson and Peaker, 1980), the coefficients of variation for milk yield varied from 17 to 27% and those for BFV varied from 15 to 61%. The coefficient of variation for the cross-sectional area of the blood vessel was 12% in 10 goats (Christensen et al., 1989). The coefficients of variation of mean systolic and diastolic blood flow velocities were 16 and 30%, respectively, in 5 goats that were milked twice daily (Piccione et al., 2004a).

The BFV was highest on the first day of lactation, which was in agreement with the results of Reynolds (1969), who used continuous thermodilution to study BFV in mammary circulation in goats. In that study, mammary blood flow began to increase markedly 2 to 3 d before parturition. Shortly after parturition, BFV was 180% higher than 20 to 14 d before parturition, but by 1 wk postpartum, the BFV had decreased by approximately 44%. In our study, the BFV had decreased by 26% at 7 d postpartum compared with the first day. Reynolds reasoned that the high mammary BFV in the peripartum period was due to nutrient requirements for milk synthesis and to redistribution of blood from the uterus to the mammary gland. However, no studies directly substantiate that hypothesis.

At the beginning of lactation, the mammary blood flow was mainly a function of the lactation number. This was in agreement with a study by Kensinger et al. (1983), in which BFV was associated with lactation number in 4 dry cows. The findings of our study indicate that cows with higher lactation numbers have larger blood vessels. The reason the BFV of the 2 lactation groups approximated each other at the end of the study period was that the vessel diameter and blood flow velocity measured in the 2 groups became similar. In the cows with $L > 2$, the blood flow velocity and cross-sectional area of the measured artery remained unchanged during the study period, whereas in cows with 1 or 2 lactations, these variables increased.

On individual sampling days, there were moderate correlations between the TAMV and BFV and between the A and BFV. This means that BFV cannot be determined reliably in individual cows by using the blood flow velocity or the cross-sectional area alone. However, when changes in blood flow variables were considered during the study period, it was evident that the cross-sectional area remained relatively constant and that there was a high correlation between the relative TAMV and BFV variations. Thus, individual

variations in the blood supply of the mammary gland are due to differences in vessel diameter as well as TAMV, whereas changes in blood flow during lactation are caused primarily by changes in TAMV. Similar results were reported in a study that measured blood flow in the milk vein in 4 goats (Nielsen et al., 1990). The diameter of the vessel remained unchanged during lactation; thus, it appeared that changes in BFV were associated with changes in TAMV.

Although cows with $L > 2$ had a higher BFV to d 14, there was only a moderate correlation between BFV and milk yield. In contrast, some authors have found a clear association between the 2 variables (Kronfeld et al., 1968; Kensinger et al., 1983; Davis et al., 1988; Delamaire and Guinard-Flament, 2006). Kronfeld et al. (1968) measured the mammary blood flow by the antipyrine absorption method, and they presented a linear regression between BFV and milk yield in 21 calculated values. Measurements using a transducer placed around the external pudendic artery in 4 dry cows (Kensinger et al., 1983) revealed that the cow with the lowest milk yield in the previous lactation also had the lowest blood flow. In contrast, the cow with the highest milk yield in the previous lactation had the highest BFV. Another study of 4 Holstein cows showed that extending the milking interval from 8 to 24 h resulted in a reduction in milk yield and blood flow rate in the external pudendal artery (Delamaire and Guinard-Flament, 2006). On the other hand, studies with goats showed that the ratio of mammary blood flow to milk yield decreased as milk yield increased (Linzell, 1974; Nielsen et al., 1990). It was thought that high-producing dairy goats might be able to use mammary blood and the nutrient supply more efficiently than low-producing goats (Nielsen et al., 1990). Thompson and Thomson (1977) investigated the effect of mild cold exposure in 5 goats and found that the milk yield decreased by 20%, whereas the BFV and oxygen consumption remained unchanged. Moreover, at the onset (Davis et al., 1979) and cessation (Fleet and Peaker, 1978) of lactation in the goat, mammary blood flow was more closely correlated with oxygen consumption than with the rate of milk secretion or glucose uptake.

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