Technical note: Development and evaluation of a standardized technique to assess blood perfusion in teats of dairy cows using power Doppler ultrasonography

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ABSTRACT

Ample research has described the assessment of dimensional changes for different teat traits, whereas diagnostic techniques to reliably assess blood circulation in teats of dairy cows are limited. Here, we describe the development and evaluation of a scanning technique to quantify blood flow in teats of dairy cows using power Doppler ultrasonography. In 2 consecutive trials, 384 teat scans [trial 1, $n = 256$ (sagittal plane, $n = 128$; transverse plane, $n = 128$); trial 2, $n = 128$ (transverse plane)] from 16 cows were obtained by the same 2 operators. Perfusion intensity from single images (trial 1) and video images (trial 2) were assessed using a commercially available software program. Intraclass correlation coefficients (ICC) and concordance correlation coefficients (CCC) were used to assess interoperator reproducibility (agreement between measurements performed by different operators) and intraoperator repeatability (agreement between measurements performed by the same operator). In trial 1, interoperator ICC and CCC indicated poor agreement ($\text{ICC} \leq 0.26$, $\text{CCC} \leq 0.26$). Intraoperator ICC and CCC demonstrated poor agreement between duplicate measurements within operators ($\text{ICC} \leq 0.19$, $\text{CCC} \leq 0.19$). Modifications after trial 1 included (1) a different ultrasound device, (2) analysis of video clips rather than single images, (3) restriction to 1 sectional plane (i.e., transverse), and (4) a scanning sequence such that repeated scans within operators were measured one after another. Through these modifications, intraoperator repeatability in trial 2 yielded fair to good agreement, with intraoperator ICC and CCC over both operators ranging from 0.44 to 0.70 and from 0.57 to 0.69, respectively, whereas interoperator ICC and CCC showed poor agreement (ICC

$= 0.35$, $\text{CCC} = 0.34$). We conclude that repeatable measurements of blood perfusion intensity of teats in dairy cows can be attained with power Doppler ultrasonography. Power Doppler ultrasonography is a suitable tool to quantify slow flow in small vessels and may be an acceptable diagnostic technique to assess changes in blood circulation that result from machine milking in teats of dairy cows, although further research is necessary to validate this hypothesis.

Key words: angiography, perfusion intensity, repeatability, reproducibility, ultrasound

Technical Note

Mechanical forces that are applied during machine milking create stress to the teat, which can cause congestion and edema of the teat tissue (Hamann and Mein, 1990). These machine milking-induced short-term changes are thought to decrease the teats' defense mechanisms and to be associated with increased susceptibility to new intramammary infections (Neijenhuis et al., 2001; Paulrud et al., 2005). In addition, short-term changes are considered to diminish animal well-being (Hillerton et al., 2002). Multiple research groups have developed diagnostic techniques to quantify teat tissue changes that occur during machine milking. These include a 2-dimensional vision-based measuring device (Zwertvaegher et al., 2013), a translucent measuring ruler (Guarín and Ruegg, 2016), a modified spring-loaded caliper (Hamann and Mein, 1990), radiography (McDonald, 1975), and B-mode ultrasonography (USG; Neijenhuis et al., 2001). These techniques continue to provide invaluable information that enhances our understanding and helps improve the milk harvesting process. However, their assessment is restricted to dimensional changes of different teat traits; no direct measurements of blood flow alterations in the teat tissue are obtainable. Consequently, knowledge about changes in teat blood circulation as induced by machine

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milking is scarce. Limited information regarding the microcirculation in the teat skin using laser Doppler flowmetry dates back more than 2 decades (Persson, 1991; Hamann et al., 1994). Other methods used to indirectly determine blood circulation include different techniques of measuring teat skin temperature (Eichel, 1992; Ordolff, 2000; Paulrud et al., 2005). Doppler USG is used to detect the presence, direction, intensity, and character of blood flow in vessels. Its physical principle is based on the fact that the frequency of a wave that is reflected from a moving object is modified by a characteristic frequency shift, which is determined by the object's velocity relative to the wave source; a phenomenon that was first described by Doppler (1842) and is called the "Doppler effect." Different modes of Doppler USG that have been increasingly used in bovine medicine include pulsed-wave Doppler USG (Starke et al., 2011; Müller et al., 2017), color Doppler USG (Pinaffi et al., 2017; Siqueira et al., 2019), and power Doppler USG (Bollwein et al., 2010; Brozos et al., 2012).

Pulsed-wave Doppler USG displays quantitative information as a time-velocity waveform on a 2-dimensional graph. Because it allows for assessment of specific velocities of a sampled area with specific size at a localized site (i.e., sample volume or gate), pulsed-wave Doppler is most suitable for evaluation of specific vessels. Color and power Doppler USG apply a color scale on a wider region of interest and superimpose this information on a simultaneously acquired 2-dimensional B-mode image. Color Doppler USG obtains mean flow velocity (i.e., speed and direction of motion). In contrast, power Doppler USG displays only the intensity or power of the Doppler shift, which is determined by the concentration of red blood cells; directional information is not preserved. Compared with color Doppler, power Doppler USG has higher sensitivity for slow flow rates and flow in small vessels, is independent of the angle of insonation, and has no aliasing. Conversely, power Doppler is more sensitive to motion artifacts (Mattoon and Nyland, 2015). A further application of Doppler USG could be the assessment of milking machine-induced alterations in teat blood circulation of dairy cows. Such knowledge could contribute to our understanding of how blood flow is affected by current machine milking practices and how potential changes relate to teat defense mechanisms and susceptibility to new intramammary infection. The overall objective of this study was to develop a scanning technique for assessment of blood flow intensity of dairy cows' teats using Doppler USG. Because we anticipated slow flow rates through a reportedly intertwined system of small blood vessels with numerous arterio-venous shunts (Hamann and Burvenich, 1994), we considered power Doppler USG to be most suitable, for the aforementioned reasons.

To evaluate the precision of measurements obtained, we set out to determine interoperator reproducibility (agreement between measurements performed by different operators) and intraoperator repeatability (agreement between measurements performed by the same operator) of different scanning techniques.

This study was conducted between October 2018 and January 2019 at the Teaching Dairy Barn of the College of Veterinary Medicine, Cornell University (Ithaca, NY). All procedures were approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2017-0046). Cows ($n = 152$) were housed in 2 freestall pens with recycled sand bedding and fed a TMR consistent with NRC (2001) requirements. The average (mean $\pm$ SD) projected 305-d mature-equivalent milk yield was $11,980 \pm 2,522$ kg and ranged from 4,708 to 17,145 kg. Cows were milked 3 times per day at 0400, 1100, and 1900 h in a double-10 parallel milking parlor (P2100, DeLaval International AB, Tumba, Sweden). The vacuum pump (7.5 kW) was regulated by a variable frequency drive and set to supply a receiver operator vacuum of 45 kPa. The milking unit was composed of the cluster MC70 (DeLaval International AB) and a milking liner with a square barrel shape (ProSquare DPX2, IBA, Millbury, MA). The pulsators (Delatron, DeLaval International AB) were set to a pulsation rate of 60 cycles/min, a ratio of 70:30, and a side-to-side alternating pulsation. These settings resulted in pulsation phases of 123 ms (a-phase), 572 ms (b-phase), 99 ms (c-phase), and 206 ms (d-phase), and an average claw vacuum during peak milk flow period of 37.9 kPa. The automatic cluster removers were set to remove the units at a milk flow of 1.4 kg/min with a 0-s delay and a vacuum decay time of 2.3 s. To facilitate sonographic imaging and diminish potential confounding of blood perfusion by periparturient udder edema, inflammation, or injury, eligible cows had to be at least 50 DIM and free of clinical mastitis for the last 4 wk, and must have had no udder abnormalities such as nonlactating quarters or teat injuries. In addition, cows must have had records of proper ease of handling based on the herd manager's experience.

In trial 1, a convenience sample of 32 teats (8 cows) was selected according to McBride (2005), who suggested a sample of ≥ 25 for assessment of concordance correlation coefficient and to allow for potential attrition of 1 cow. Cows were in their first ($n = 6$), second ($n = 1$), or fourth ($n = 1$) lactation and between 115 and 315 DIM. Power Doppler USG was performed with a portable ultrasound device (Sonosite Edge II, Sonosite Inc., Bothell, WA) and a 5- to 10-MHz linear-array transducer (L52; Sonosite Inc.). The optimal imaging modes were identified before the study and kept consistent throughout the trial. The arterial exam type with

a depth of 42 mm was used. For the B-mode settings, the medium resolution (Optimize: Gen), dynamic range of 3, brightness of 10, and up/left orientation were chosen. The color power Doppler (CPD) mode settings were as follows: flow sensitivity and wall filter were set to medium, steering angle setting of the color region of interest (ROI_{USG}) was set to 0, and the pulse repetition frequency was set to 4,902 Hz. These settings were selected in accordance with recommendations by Mattoon and Nyland (2015). That is, flow sensitivity, pulse repetition sensitivity, and wall filter were selected based on the attempt to find the best compromise among (1) ability to detect low flow, (2) sensitivity to motion artifacts, and (3) penetration depth. We tested multiple combinations of these settings until maximum image quality was achieved. Because no scale for gain settings in B- or CPD-mode were displayed, the gain was maintained at the default setting (i.e., the setting present after turning on) to ensure consistency of settings over the study period. To avoid teat deformation, the teat was immersed in a rectangular plastic container (130 mm $\times$ 60 mm $\times$ 60 mm), the edges of which were gently pressed against the adjacent skin around the base of the teat. The container was filled with approximately 400 mL of warm (30°C) 1.0% chlorhexidine lube (Chem Select Inc., Rancho Santa Margarita, CA). The 1.0% chlorhexidine solution was chosen as a standoff medium among 4 other items that were evaluated in early pre-trials based on ease of handling, image resolution, and motion artifacts [0.5% chlorhexidine solution (Vet One, Boise, ID); Udderdine110 (BouMatic, Madison, WI); Mineral Oil Light (AgriLabs LLC, St. Joseph, MO); and ultrasound gel (Medline Industries Inc., Northfield, IL)]. Ultrasound gel (Medline Industries Inc.) was used as a coupling medium between the ultrasound transducer and the plastic container. Two different sectional planes, sagittal and transverse (Supplemental Figures S1 and S2, respectively; <https://doi.org/10.3168/jds.2019-16670>), were obtained. Sagittal teat scans were taken from the caudal aspect of the teat with the transducer being held in a caudal-to-cranial direction. The longitudinal axis of the teat canal was used for orientation and centered in the ROI_{USG} with the distal end of the teat canal adjacent to the right boundary of the ROI_{USG} . Transverse teat scans were obtained from the right aspect of the teat with the transducer being held in a right-left direction. The cross section of the teat was centered in the ROI_{USG} . To facilitate manipulation of transducer and container, 2 wooden dowels were attached to both containers such that the transducer would fit tightly between the dowels, minimizing its mobility (Supplemental Figures S3 and S4; <https://doi.org/10.3168/jds.2019-16670>). This procedure aided in depiction of the longitudinal teat axis for sagittal

scans and alleviated scanning of the same position for transverse scans. When all structures and blood flow were clearly visible, an image was captured. All teat scans were stored on the integrated flash drive in JPEG format and transferred to a computer using a USB for image processing. Imaging was performed in duplicate by 2 operators (MW and IP) in the adjacent tiestall unit between milking sessions 1 and 2 on 3 d within a 2-wk period. Scanning of all 4 teats was performed sequentially in 4 rounds within each cow, as follows: (1) operator 1 obtained the first series of sagittal and transverse scans; (2) operator 2 obtained the first series of sagittal and transverse scans; (3) operator 1 obtained the second series of sagittal and transverse scans; (4) operator 2 obtained the second series of sagittal and transverse scans. This sequence ensured similar time intervals between duplicate scans within operators and scans of the same teat between operators.

After completion of trial 1, data were analyzed as outlined below. Because measures of precision yielded poor results, a second trial was conducted. Both operators reviewed the scanning technique, discussed possible sources of measurement error, and implemented the following changes. First, imaging was performed with a high-resolution ultrasound device (Logiq e Vet NextGEN, General Electric, Boston, MA) with a 4.2- to 13-MHz linear-array transducer (L4-12t-RS, General Electric). Second, sonographic video clips were obtained and stored in AVI format on the integrated flash drive. Third, only transverse sectional planes were obtained and were taken from the caudal aspect of the teat with the transducer being held in a caudal-to-cranial direction. Fourth, the sequence of scanning was modified such that each operator completed each series sequentially from each teat within a cow. This sequence minimized the time interval between repeated teat scans within operators.

In trial 2, another convenience sample of 8 cows that were in their first ($n = 7$) or fourth ($n = 1$) lactation and between 81 and 375 DIM was selected. The imaging modes used consistently throughout the trial were as follows: B-mode settings were frequency, 12 MHz; gain, 42; and depth, 3.5 cm. The power Doppler imaging (PDI) mode settings were frequency, 7.7 MHz; gain, 12; L/A (line density), 2/5; pulse repetition frequency, 0.6; wall filter, 109; and S/P (spatial filter), 2/16. The selection of the gain was based on Mattoon and Nyland (2015), who recommended increasing gain until artifactual speckles occur and then reducing it just to the point where they disappear. Consequently, multiple combinations of different settings of frequency, pulse repetition frequency, wall filter, and gain were tested until the highest image quality was achieved. Duplicate scans of all 4 teats were obtained between milking ses-

sions 1 and 2 in the adjacent tiestall unit by the same 2 operators as in trial 1 on 2 consecutive days.

Perfusion intensity measurements from single images (trial 1) and video images (trial 2) were performed by 2 trained investigators (trial 1, MW; trial 2, SS) with a commercially available software program (PixelFlux, Chameleon Software, Münster, Germany) according to Scholbach et al. (2012). Supplemental File S1 (<https://doi.org/10.3168/jds.2019-16670>) outlines a detailed description of measurements.

Statistical analyses were performed using Excel (2016, Microsoft Corp., Redmond, WA) and R Statistical Software (R Core Team, 2018). Interoperator reproducibility was assessed using only the first data set (no duplicate scans) by calculating mean coefficients of variation [**CV** (95% CI)], Pearson correlation coefficients [**ICC** (95% CI)] according to Shrout and Fleiss (1979) using the “psych” package (Revelle, 2016), and concordance correlation coefficients [**CCC** (95% CI)] according to Lin (1989) for nonrepeated measurements as described by Carrasco et al. (2013) using the “ccrm” package (Carrasco and Martinez, 2015). Intraoperator repeatability of duplicate scans was evaluated by calculating mean CV (95% CI), Pearson correlation coefficients, ICC (95% CI) with the “psych” package (Revelle, 2016), and CCC (95% CI) for nonlongitudinal repeated measurements, as described by Carrasco et al. (2013) with the “ccrm” package (Carrasco and Martinez, 2015). Pearson correlation coefficients were classified as negligible (0.00 to 0.09), weak (0.10 to 0.39), moderate (0.40 to 0.69), strong (0.70 to 0.89), or very strong (0.90 to 1.00) according to Schober et al. (2018). Results of ICC and CCC were classified as indicating poor (<0.40), fair (0.40 to 0.59), good (0.60 to 0.74), or excellent (0.75 to 1.00) agreement, according to Cicchetti (1994). General linear mixed models were generated to determine differences between operators (interoperator reproducibility) and duplicate scans (intraoperator repeatability) with the “nlme” package (Pinheiro et al., 2016) for trials 1 and 2. To account for dependence of images (trial 1) or videos (trial 2) among different operators, respectively, image or video was included as random effect. The covariance of repeated (duplicate scans) measurements was modeled with the first-order autoregressive covariance structure. Operator and duplicate scans were included as fixed effects. Least squares means (**LSM**) and 95% CI were generated using the “lsmeans” package (Lenth, 2016). Significance was declared at $P < 0.05$.

A total of 384 teat scans [trial 1, $n = 256$ (sagittal plane, $n = 128$; transverse plane, $n = 128$); trial 2, $n = 128$ (transverse plane)] from 16 cows were obtained. Overall (trials 1 and 2) mean $\pm$ SD perfusion intensity was $0.41 \pm 0.41\%$, ranging from 0.004 to 2.48%

[trial 1: sagittal, $0.49 \pm 0.39\%$ (range: 0.03 to 2.09%); transverse, 0.62 ± 0.45 (range: 0.13 to 2.48%); trial 2: $0.13 \pm 0.16\%$ (range: 0.004 to 0.75%)]. Table 1 provides results for assessment of interoperator reproducibility and intraoperator repeatability in trials 1 and 2. In trial 1, measurements between operators 1 and 2 were weakly correlated for both sectional planes ($r \leq 0.27$, $P \geq 0.13$). Interoperator ICC and CCC indicated poor agreement (ICC ≤ 0.26 , CCC ≤ 0.26). Least squares means from general linear mixed model yielded no differences between operators ($P \geq 0.35$). Interoperator CV were 48.8% (95% CI: 35.4 to 62.2) and 40.6% (95% CI: 31.1 to 50.0) for measurements of the sagittal and transverse planes, respectively. Duplicate measurements within operators were weakly correlated for both planes ($r \leq 0.21$, $P \geq 0.16$). Intraoperator ICC and CCC demonstrated poor agreement between duplicate measurements within operators (ICC ≤ 0.19 , CCC ≤ 0.19). General linear mixed models demonstrated no differences between duplicate measurements ($P \geq 0.053$). Compared with trial 1, interoperator correlation coefficient, ICC, and CCC increased; however, correlation of measurements between operators was still weak ($r = 0.36$, $P = 0.04$) and interoperator ICC and CCC yielded poor agreement (ICC = 0.35, CCC = 0.34). Interoperator CV was 65.8% (95% CI: 50.8 to 80.8). Conversely, duplicate measurements within operators were moderately correlated ($r = 0.44$, $P = 0.0003$), and intraoperator ICC and CCC showed fair agreement (ICC = 0.44, CCC = 0.57). Analysis for each operator separately indicated a strong correlation between duplicate measurements (operator 1: $r = 0.71$, $P < 0.0001$; operator 2: $r = 0.72$, $P < 0.0001$) and good agreement (operator 1: ICC = 0.69, CCC = 0.68; operator 2: ICC = 0.68, CCC = 0.69). Intraoperator CV over both operators ranged from 50.1 to 53.1%. A general linear mixed model showed no differences between operators ($P = 0.36$) or duplicate measurements within operators ($P = 0.61$).

In this study, we showed that repeatable measurements of blood flow of cows' teats can be obtained using power Doppler USG. We believe that the remarkable improvement in intraoperator repeatability of transverse teat scans can be attributed mostly to changes implemented after trial 1 (i.e., ultrasound device, transverse plane only, scanning sequence, and video clips vs. images). To a smaller extent, differences could have been due to an increase in training of both operators over time and the discrepancy between investigators performing the perfusion intensity measurements. Using a high-resolution ultrasound device in trial 2, we were able to readily visualize blood flow of teats, whereas both operators had difficulties detecting blood flow in trial 1. Depiction of frequency and gain using

Table 1. Mean coefficients of variation (CV; 95% CI), Pearson correlation coefficients (r), intraclass correlation coefficients (ICC; 95% CI), and concordance correlation coefficients (CCC; 95% CI) for assessment of interoperator reproducibility and intraoperator repeatability, and LSM (95% CI) for each operator and duplicate scans, respectively, derived from mixed-effects ANOVA for perfusion intensity measurements (%) in teats of dairy cows using power Doppler ultrasonography

Item	Trial 1			Trial 2 (transverse only)		
	Interoperator reproducibility		Intraoperator repeatability	Interoperator reproducibility	Intraoperator repeatability	
	Sagittal	Transverse				
CV (95% CI) ¹	48.8 (35.4; 62.2)	40.6 (31.1; 50.0)	47.5 (38.2; 56.8)	65.8 (50.8; 80.8)	51.6 (42.3; 60.9)	
OP1			47.4 (34.0; 60.7)		50.1 (35.8; 64.3)	
OP2			47.6 (33.8; 61.3)		53.1 (40.4; 65.8)	
r (P-value) ²	0.19 (0.30)	0.27 (0.13)	0.17 (0.19)	0.36 (0.04)	0.44 (0.0003)	
OP1			0.03 (0.86)		0.71 (<0.0001)	
OP2			0.17 (0.34)		0.72 (<0.0001)	
ICC (95% CI) ^{1,3}	0.15 (−0.21; 0.48)	0.26 (−0.10; 0.56)	0.15 (−0.10; 0.38)	0.35 (0.002; 0.62)	0.44 (0.22; 0.62)	
OP1			0.03 (−0.32; 0.37)		0.69 (0.45; 0.84)	
OP2			0.15 (−0.15; 0.45)		0.70 (0.47; 0.84)	
CCC (95% CI) ^{1,3}	0.15 (−0.08; 0.36)	0.26 (0.08; 0.41)	0.13 (−0.07; 0.31)	0.34 (0.01; 0.61)	0.57 (0.38; 0.71)	
OP1			0.03 (−0.23; 0.28)		0.68 (0.45; 0.83)	
OP2			0.15 (−0.06; 0.35)		0.69 (0.46; 0.84)	
ANOVA ⁴						
OP1	0.51 (0.41; 0.61)	0.59 (0.47; 0.71)		0.12 (0.06; 0.18)		
OP2	0.47 (0.36; 0.57)	0.66 (0.54; 0.78)		0.14 (0.08; 0.19)		
P-value	0.52	0.35	0.55 (0.45; 0.65)	0.36	0.12 (0.07; 0.18)	
DUP1			0.42 (0.32; 0.52)		0.13 (0.08; 0.19)	
DUP2			0.05		0.61	
P-value					0.61	

¹The mean (95% CI) CV, ICC, and CCC were calculated for both operators together and for each operator (OP1 and OP2) separately.
²The classification system for Pearson correlation coefficients (r) was according to Schober et al. (2018): negligible, 0.00 to 0.09; weak, 0.10 to 0.39; moderate, 0.40 to 0.69; strong, 0.70 to 0.89; and very strong, 0.90 to 1.00.
³The classification system for ICC and CCC was according to Cicchetti (1994): poor, <0.40; fair, 0.40 to 0.59; good, 0.60 to 0.74; excellent, 0.75 to 1.00.
⁴Mixed-effects ANOVA including the fixed effects of operator (OP) and duplicate (DUP; DUP1, first scan; DUP2, second scan) measurements. LSM (95% CI) and P-value for the fixed effect of operator and duplicate measurement, respectively, are presented.

the Logiq e Vet NextGEN in trial 2 increased the range of settings and improved the ability to detect low blood flow. Further, by omitting sagittal teat scans, both operators could exclusively focus on one scanning technique. Both factors improved the overall quality of teat scans. By modifying the sequence of scanning such that repeated scans within operator were measured directly one after another, we reduced the variability in blood flow that could have arisen over the course of a naturally occurring muscular contraction between repeated scans (Hamann and Mein, 1990). Because changes in blood flow may have completely impeded the ability to obtain repeatable measurements for reasons other than measurement error, we decided to minimize the time interval between 2 repeated teat scans within operator in trial 2. We acknowledge that this may have led to information bias affecting intraoperator repeatability, which may help explain the contrary results in interoperator reproducibility and intraoperator repeatability of transverse teat scans between trials 1 and 2. Analyzing video clips (i.e., a sequence of images) in trial 2 rather than 1 single image eliminated the “snapshot effect” and reduced the effect of images with extreme values by calculating the average from a sequence of video images. Results of Pearson correlation coefficients, ICC, and CCC were consistent, leading to similar conclusions for interoperator reproducibility and intraoperator repeatability in trials 1 and 2, respectively. In contrast, LSM from general linear mixed models failed to detect poor agreement of reproduced and repeated measurements in trial 1 and reproduced measurements in trial 2. This was most likely caused by a large residual error due to very scattered data, as outlined by Lin (1989). Coefficients of variation indicated large dispersion of both, measurements between operators and duplicate measurements within operators with greater dispersion being present in trial 2, suggesting the need to further decrease measurement error. Measures to further improve precision include modification of the standoff (i.e., container or standoff medium), training of operators, and further standardization of image analysis (e.g., automatized determination of the region of interest subjected to computational analysis with PixelFlux). Recently, we showed that recovery of teat tissue alterations that result from machine milking can last several hours or exceed the 8-h milking interval in cows milked 3 times a day (Melvin et al., 2019). In the current study, we performed teat scanning between 3 and 6 h after milking, which likely resulted in scanning of teats in a state of remodeling. This may have hampered the ability to obtain reproducible and repeatable measurements and may partially explain the relative low values in perfusion intensity. Our ultimate goal is to assess changes in blood flow that occur as a

result of machine milking. The ability to detect variability attributed to the effect of forces created by the milking machine will ultimately depend on its magnitude relative to measurement error, as well as the sample size. Dynamics in blood flow that are caused by naturally occurring muscular contractions or manipulations will have to be considered in such studies. A possible sampling protocol that may help decipher these dynamics is to assess blood flow (1) before premilking udder preparation, (2) after completion of premilking udder preparation, immediately before attachment of the milking unit, and (3) after machine milking, in accordance to the protocol described by Gleeson et al. (2002) for assessment of teat dimensions using B-mode USG. We conclude that repeatable measurements of blood perfusion intensity of cows' teats can be obtained using power Doppler USG. Thus, power Doppler USG may be an acceptable diagnostic technique to assess changes in blood circulation that result from machine milking in teats of dairy cows, given that measurements are obtained by 1 operator. However, future research is necessary to test this hypothesis.

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