Doppler Sonography for Evaluation of Haemodynamic Changes of Uterine Arteries and Umbilicus during Different Months of Gestation in Dairy Cows

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Abstract. *The present study was conducted with an objective of evaluating the haemodynamic parameters for the middle uterine artery and the umbilicus and their inter-relationship in pregnant dairy cows (N = 12). Trans-rectal Doppler ultrasonography in dairy cows was carried out at an interval of 14 days beginning from day 14 to 238 of gestation. Pearson correlation coefficients were calculated using the CORR matrix in NCSS 2020. Pulsatility and resistance indices (PI and RI), time averaged maximum velocity (TAMAX), time averaged mean velocity (TAMEAN), diameter of the artery, maximum and mean volume of blood flow (BFV-TAMAX and BFV-TAMEAN), diameter, Doppler pulse duration (DPD) and systolic upstroke/acceleration time (AT) of middle uterine arteries and the umbilicus were measured to study the uterine and umbilical blood flow during different stages of gestation. Results revealed that haemodynamic indices, i.e., PI, RI, time averaged maximum and mean velocities, blood flow volume (mean and maximum) to the uterus and diameter of middle uterine artery, were significantly different ($p < 0.01$ and $p < 0.05$) between the middle uterine artery ipsilateral and contralateral to the gravid horn during the first 8 months of gestation. Pearson correlation analysis showed that a significant positive and negative correlation in the ipsilateral ($r = -0.6542$–0.9188; $p < 0.01$ and $p < 0.05$) and contralateral ($r = -0.4682$–0.9363; $p<0.01$ and $p<0.05$) middle uterine artery, respectively, during the first 8 months of gestation was present. Also, there was a linear change in the haemodynamic indices of the foetal umbilicus during the first 6 months of gestation along with a significant positive and negative correlation ($r = -0.5793$–0.9520; $p < 0.01$ and $p < 0.05$) between haemodynamic indices. In conclusion, the significant changes in haemodynamic changes of the middle uterine artery and the umbilicus occur during mid to late gestation in dairy cows.*

Introduction

With respect to normal physiology, alterations in uterine perfusion reflect structural and functional changes in the endometrium and, thus, may be an indirect measure for embryo-maternal communication during the establishment of pregnancy (Hassan et al., 2020). Modern diagnostic modalities including transrectal spectral Doppler ultrasonography allow quantification of the uterine blood flow both in cyclic, pregnant and pathological conditions and also help in supplementing the already established protocols of pregnancy and parturition (Panarace et al., 2006; Sharma et al., 2019). A characteristic pattern has been observed in the uterine vascularity throughout the estrous cycle concurrent with the serum progesterone and estradiol concentrations (Bollwein et al., 2016) whereas pregnant cows show a marked increase in the uterine blood flow beginning from week 3 in the gravid horn in contrast to the non-gravid horn (Silva and Ginther, 2010). Both pulsatility index (PI) and resistivity index (RI) serve as the most useful indicators in measuring the resistance offered to the blood within the vessels due to lying down of the microvasculature distal to the site of measurement, and also remain independent of the Doppler angle and the diameter of blood vessels (Maulik, 1993). Similarly, Doppler assessment of the umbilical arterial blood flow can be used as a marker of placental insufficiency (Scotti et al., 2008), as a close relationship exists between birth weight of the foetus, placental size, uterine and umbilical perfusion (Reynolds and Redmer, 1995). The umbilical arterial waveform has a characteristic saw-tooth appearance with the only systolic component while the umbilical venous waveform remains flat (Kumar et al., 2015). A consistent decline in resistance values of the umbilical blood flow with an increase in uterine irrigation has been observed throughout the gestational period (Serin et al., 2010). Hence, an effort was made to evaluate the sequential changes in haemodynamic indices of the middle uterine artery and the foetal umbilicus throughout gestation in dairy cattle.

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Materials and methods

Animals

Twelve pregnant Jersey crossbred multiparous cows (parity = 3; N = 12) reared in a loose housing system under standard management conditions, fed a total mixed ration, once daily *ad libidum*, with unrestricted access to water (32.6°N, 76.3°E, altitude 1290.8 m) were enrolled for the research after normal parturition. The cows were milked twice daily (04:00 and 15:00 h). All the experiments were carried after the approval of the ethics committee of the institute.

Trans-rectal Doppler sonography of the middle uterine artery for assessment of uterine perfusion

The middle uterine artery (MUA) originates from the internal iliac artery, can be found in the mesometrium as a movable arterial vessel, and is located cranial to the external iliac artery (Sharma et al., 2019). For monitoring of the uterine blood flow, Doppler examination of the MUA ipsilateral and contralateral to the pregnant uterine horn was performed from day 14 to 238 of gestation (Fig. 1, a–d) at an interval of 14 days using a linear probe of portable Mindray Z5 ultrasound machine at a frequency of 7.5 MHz, with a filter of 100 Hz and the Doppler angle varying between 30° and 60°. Visualization of the umbilicus begins after day 25 in cattle, and the umbilical pulsation was measured on the free fluctuating portion of the umbilical cord, between the conceptus and the gestational sac at an insonation angle of 60° in a spectral Doppler mode from day 28 to 182 of gestation (Fig. 2 a, b). The parameters displayed for each waveform by applying the automatic mode were pulsatility index (PI), resistivity index (RI), time averaged mean velocity (TAMEAN), time averaged maximum velocity (TAMAX), mean blood flow volume (BFV-TAMEAN) and maximum blood flow volume (BFV-TAMAX). The MUA's transverse diameter (D-MUA) was calculated from the mean of three measurements of the diameter made from frozen two-dimensional grey scale images just before Doppler measurements. Similarly, the width/diameter of the umbilical

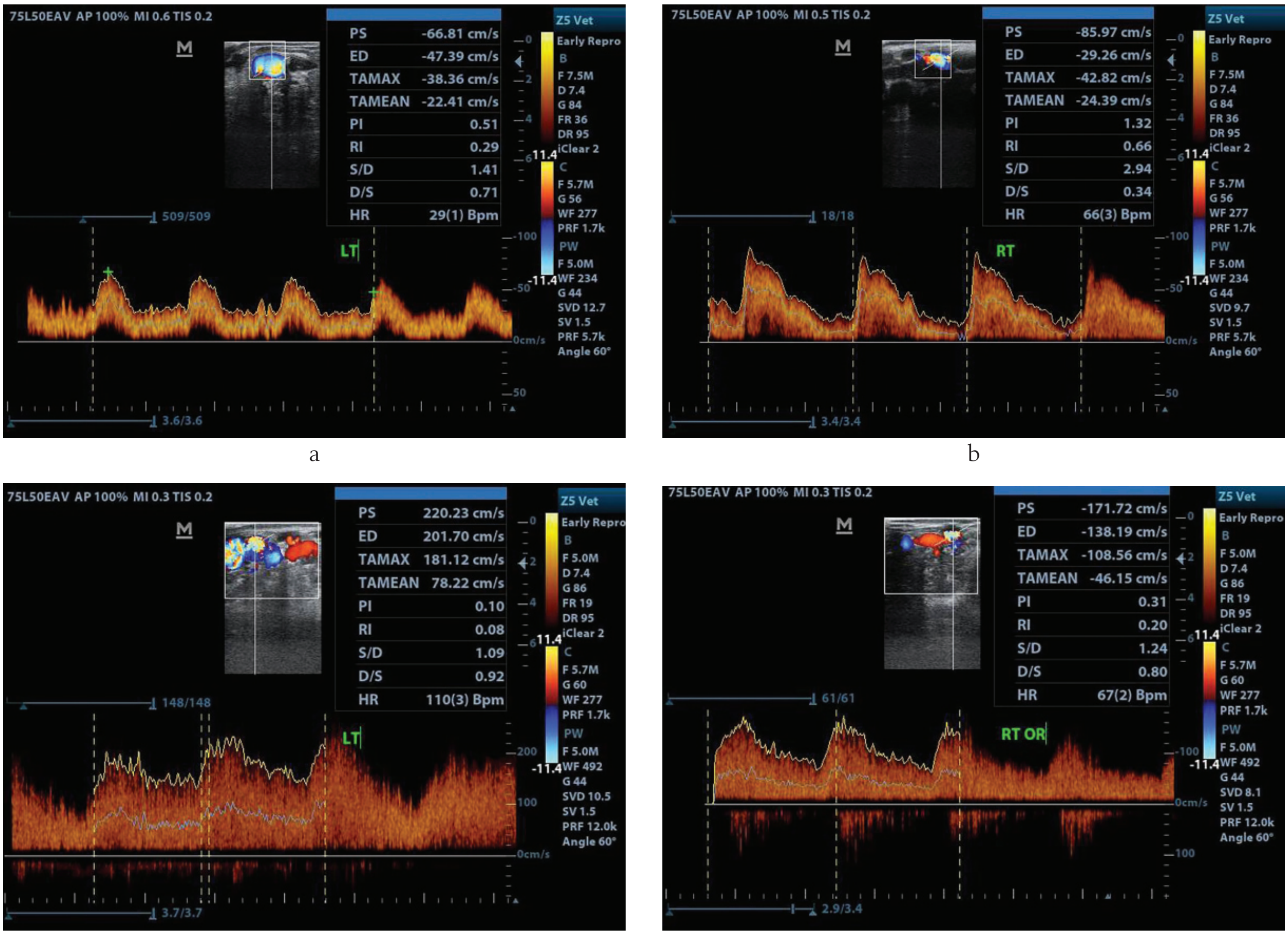

Fig. 1. Ultrasonographic imaging of middle uterine artery.
(a) day 42 of gestation (ipsilateral); (b) day 42 of gestation (contralateral);
(c) day 210 of gestation (ipsilateral); (d) day 210 of gestation (contralateral).

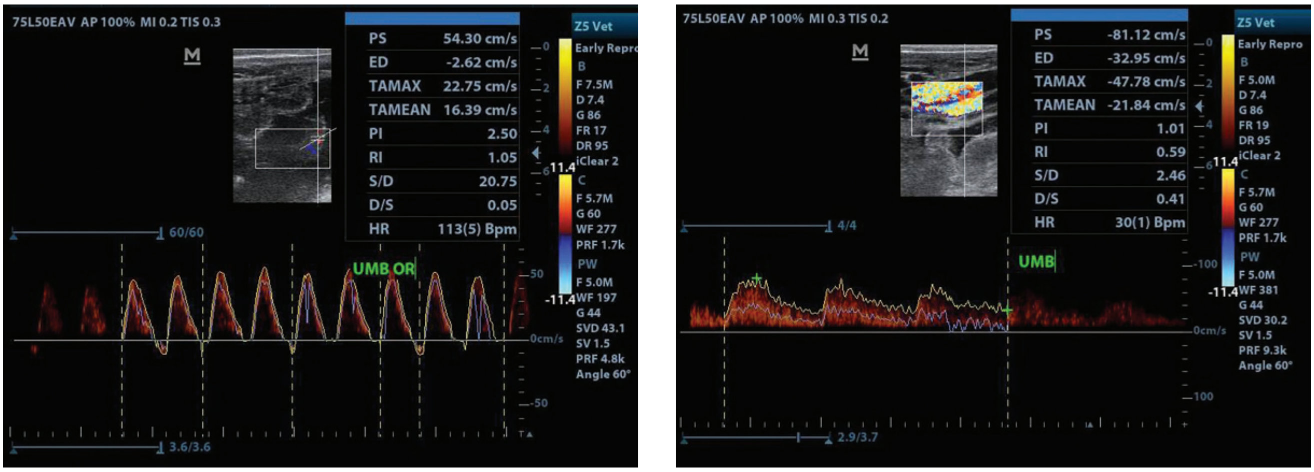

Fig. 2. Sonographic visualization of umbilicus.
(a) day 98 of gestation (characteristic saw-tooth pattern);
(b) day 196 of gestation (Flattening of umbilical waveform).

cord (D) was obtained from the images showing a straight longitudinal section of the umbilical cord and measured from the outer sides of the umbilical cord. In order to assess the significance of other haemodynamic parameters, Doppler pulse duration (DPD) and acceleration time / systolic upstroke time were also recorded.

Blood flow volume in mL/min was calculated using the equation (Varughese et al., 2013):

$$\text{Blood flow volume-TAMEAN} = \text{TAMEAN} \times \pi \times (D\times0.1/2)^2 \times 60$$

$$\text{Blood flow volume-TAMAX} = \text{TAMAX} \times \pi \times (D\times0.1/2)^2 \times 60$$

Statistical analysis

Numeric data for all the parameters were expressed as mean ± SE and statistically analyzed using repeated measures ANOVA and Pearson matrix correlation analysis with NCSS 2020, USA (Version 20.0.1).

Results

The PI and RI between ipsilateral and contralateral middle uterine arteries (MUA) differed significantly on days 140 ($p < 0.01$), 154 and 168 ($p < 0.05$), respectively. Similarly, the TAMAX and TAMEAN values of the ipsilateral MUA differed significantly throughout gestation on various days of examination with the significance level varying from $p < 0.01$ to $p < 0.05$. Also, the diameter (D-MUA) of the ipsilateral MUA showed a significant increase ($p < 0.01$ and $p < 0.05$) in the values when compared with the contralateral MUA throughout gestation. The volume of the blood flow evaluated through BFV-TAMAX and BFV-TAMEAN values differed significantly ($p < 0.01$ and $p < 0.05$) between ipsilateral and contralateral MUAs until the end of the period of examination (Table 1). Also, the mean blood flow values witnessed a 5.36 times increase throughout gestation in the ipsilateral MUA while the contralateral MUA showed a rise by a factor of 2.63.

The PI values were found to increase intermittently throughout gestation while the RI values showed an intermittent decline during the first trimester followed by a decrease in a linear fashion during the second trimester while studying the umbilical haemodynamics. Also, TAMAX and TAMEAN values showed a characteristic linear surge throughout the first and the second trimester (Table 2). Similarly, the umbilical diameter increased linearly during the period of examination with a 6.78-fold increase as compared with initial values on day 182 of gestation. The blood flow volume through the umbilicus also increased during the first and the second trimester with a dramatic increase by 2.1 times at the onset of the second trimester.

In correlation analysis of MUA haemodynamic parameters, PI and RI shared a significant positive correlation ($p < 0.01$) whereas other parameters such as TAMAX, TAMEAN, BFV-TAMAX and BFV-TAMEAN had a significantly negative correlation with PI and RI ($p < 0.01$) for ipsilateral and contralateral MUAs throughout gestation. Also, parameters for velocity and volume of the blood flow, i.e., TAMAX, TAMEAN and BFV-TAMAX and BFV-TAMEAN and D-MUA, were found to be positively correlated with each other ($p < 0.01$) for ipsilateral and contralateral MUAs throughout the period of examination (Table 3).

In a correlation analysis of umbilical haemodynamic parameters, PI and RI shared a significant positive correlation ($p < 0.05$) but were negatively correlated with TAMAX, TAMEAN, BFV-TAMAX, BFV-TAMEAN ($p < 0.01$) and diameter of the umbilicus ($p < 0.01$ and $p < 0.05$, respectively). The diameter of the umbilicus also had a significant positive correlation ($p < 0.01$) with parameters for velocity and volume of the blood flow during the first 6 months of gestation (Table 4).

Table 1. Uterine perfusion adjudged on Doppler indices of middle uterine arteries during the first 8 months of gestation in dairy cows (N = 12) (Mean ± SE)

Day of gestation	MUA side ipsilateral to gravid horn	Pulsatility index	Resistivity index	Time averaged maximum velocity (cm/sec)	Time averaged mean velocity (cm/sec)	D-MUA (mm)	BFV-TAMAX (mL/min)	BFV-TAMEAN (mL/min)
Day 28	Ipsilateral	1.05±0.16	0.56±0.08	40.75±4.15	21.99±3.37	10.90±0.06[a]	2362.95±359.48	1302.91±235.02
	Contralateral	1.10±0.13	0.58±0.07	39.47±3.98	22.37±2.62	10.50±0.04[b]	2121.72±334.60	1197.24±204.44
Day 42	Ipsilateral	1.02±0.10	0.55±0.08	40.16±2.28	22.36±1.64	11.60±0.04[a]	2535.36±177.23[x]	1375.88±89.15
	Contralateral	1.15±0.14	0.58±0.07	39.10±2.44	21.87±1.72	10.30±0.05[b]	1954.14±162.58[y]	1099.94±110.66
Day 56	Ipsilateral	1.19±0.13	0.59±0.04	46.88±6.66	26.86±4.14	11.50±0.04[a]	2998.50±450.66[x]	1669.12±233.75
	Contralateral	1.14±0.15	0.57±0.07	34.78±3.20	20.77±2.40	10.30±0.07[b]	1876.32±305.83[y]	1134.27±216.67
Day 70	Ipsilateral	1.01±0.17	0.52±0.07	43.02±4.33	25.26±2.45[a]	12.20±0.07[a]	3101.38±416.50[x]	1823.36±251.78[x]
	Contralateral	1.31±0.10	0.65±0.04	35.47±2.31	19.28±1.31[b]	10.60±0.05[b]	1954.85±235.73[y]	1048.48±114.20[y]
Day 84	Ipsilateral	1.29±0.11	0.60±0.05	36.48±1.84	19.59±1.11[y]	12.90±0.05[a]	2889.43±256.84	1540.11±131.10
	Contralateral	1.30±0.17	0.63±0.05	43.96±3.78	26.27±3.02[x]	11.10±0.03[b]	2627.60±23.43	1574.87±231.75
Day 98	Ipsilateral	1.20±0.14	0.68±0.08	37.70±4.37	21.12±1.75	12.60±0.05[a]	2952.54±256.84	1540.11±131.10
	Contralateral	1.17±0.11	0.60±0.06	40.01±5.84	22.45±4.34	10.80±0.04[b]	2255.35±364.15	1263.41±254.53
Day 112	Ipsilateral	0.98±0.15	0.51±0.07	40.26±3.51	22.30±2.68	13.30±0.05[a]	3457.35±469.48	1902.81±299.12
	Contralateral	1.12±0.21	0.53±0.09	44.74±3.47	26.75±1.98	11.20±0.03[b]	2660.00±290.78	1551.90±103.04
Day 126	Ipsilateral	1.03±0.24	0.53±0.11	52.78±6.46	28.55±3.91	13.90±0.06[a]	4960.39±623.68[a]	2601.25±345.60[x]
	Contralateral	1.36±0.20	0.68±0.07	44.65±4.31	25.58±2.61	11.30±0.04[b]	2707.56±295.33[b]	1544.88±176.73[y]
Day 140	Ipsilateral	0.74±0.10[b]	0.38±0.09[b]	49.40±6.26	24.64±2.37	14.00±0.04[a]	4454.98±540.92[a]	2255.42±270.36[x]
	Contralateral	1.46±0.12[a]	0.70±0.04[a]	43.29±2.98	25.80±1.27	11.40±0.05[b]	2672.62±304.63[b]	1588.35±157.90[y]
Day 154	Ipsilateral	0.66±0.14[y]	0.40±0.07[y]	80.75±6.67[a]	39.41±4.73[x]	13.20±0.04[a]	6543.55±555.02[a]	3197.58±358.87[a]
	Contralateral	1.18±0.15[x]	0.63±0.06[x]	50.11±5.77[b]	26.22±2.37[y]	11.10±0.05[b]	2876.90±318.56[b]	1554.91±214.03[b]
Day 168	Ipsilateral	0.69±0.17[y]	0.40±0.08[y]	75.03±5.86[x]	38.10±3.72	14.20±0.03[a]	7205.89±665.56[a]	3657.71±392.34[a]
	Contralateral	1.25±0.16[x]	0.65±0.06[x]	51.94±6.40[y]	29.16±3.31	11.30±0.03[b]	3154.16±427.14[b]	1759.73±204.48[b]
Day 182	Ipsilateral	0.61±0.11	0.37±0.06	103.24±11.34[a]	51.41±7.75	14.30±0.06[a]	9608.84±963.44[a]	4821.50±760.94[a]
	Contralateral	0.58±0.14	0.28±0.08	58.67±7.29[b]	35.22±4.93	11.10±0.05[b]	3480.75±492.49[b]	2053.20±326.87[b]
Day 196	Ipsilateral	0.41±0.16	0.27±0.09	120.50±14.32[a]	58.26±9.04	15.10±0.10[a]	12383.87±806.39[a]	5880.47±506.71[a]
	Contralateral	0.75±0.15	0.43±0.07	69.14±10.30[b]	38.09±7.19	11.40±0.07[b]	3928.24±238.19[b]	2159.13±269.55[b]
Day 210	Ipsilateral	0.50±0.12[y]	0.34±0.07	133.61±15.53[a]	64.07±10.14[x]	14.80±0.14[a]	12816.28±1032.68[a]	6071.79±714.23[a]
	Contralateral	0.92±0.16[x]	0.51±0.07	71.51±12.11[b]	33.33±4.41[y]	11.40±0.07[b]	4087.19±400.45[b]	1840.12±295.88[b]
Day 224	Ipsilateral	0.47±0.10	0.33±0.06	145.09±17.40[a]	72.00±7.93	13.80±0.11[a]	12538.68±1321.36[a]	6239.04±639.51[a]
	Contralateral	1.02±0.28	0.53±0.13	73.20±14.98[b]	43.01±10.64	11.50±0.10[b]	4217.33±499.16[b]	2419.06±255.73[b]
Day 238	Ipsilateral	0.38±0.08	0.27±0.05	139.08±16.15	71.83±11.80	13.80±0.09[a]	12671.27±2038.24[a]	6313.45±1004.75[a]
	Contralateral	0.40±0.12	0.28±0.08	110.02±26.30	57.85±14.73	10.90±0.08[b]	5586.92±898.45[b]	2929.45±543.24[b]

[a,b]Values with different superscripts within the same column for the same parameter and day are significantly different ($p < 0.01$).
[x,y]Values with different superscripts within the same column for the same parameter and day are significantly different ($p < 0.05$).

Discussion

Adaptations of the uterine artery in response to foetal demands reflect in the haemodynamic changes such as volume and velocity of the blood flow to the uterus (Hassan et al., 2020). The beginning of pregnancy is marked by relatively higher resistivity with high peak systolic velocity, low diastolic velocity and presence of the notch signal (Panarace et al., 2006), which is similar to the findings of the present study (Bollwein et al., 2000). However, with advancement of pregnancy, significant reduction in RI and PI coincides with vasculature development in the distal tissues and vascular remodelling linked with vascular endothelium (Gibbons and Dzau, 1994).

In concurrence with our study, the entire gestation period was marked by an increase in TAMAX and BFV-TAMAX values in both the middle uterine arteries, with a steep increase by 3 times of the initial values in the ipsilateral artery by the end of pregnancy (Nishida et al., 2006). Similarly, Varughese et al. (2013) reported an increase in TAMAX values

Table 2. Haemodynamic indices of the umbilicus following Doppler ultrasonography during the first 6 months of gestation in dairy cows (N = 12) (Mean ± SE)

Days of gestation	Pulsatility index	Resistivity index	Time averaged maximum velocity (cm/s)	Time averaged mean velocity (cm/s)	Diameter (mm)	BFV-TAMAX (mL/min)	BFV-TAMEAN (mL/min)
Day 28	2.04 ± 0.59	0.75 ± 0.05	7.02 ± 0.48	3.82 ± 1.06	2.30 ± 0.03	16.98 ± 5.73	8.64 ± 0.55
Day 42	3.10 ± 0.35	0.91 ± 0.06	10.07 ± 1.33	4.71 ± 0.64	4.20 ± 0.07	21.21 ± 3.08	37.21 ± 13.27
Day 56	3.52 ± 0.30	1.04 ± 0.02	12.61 ± 2.49	5.28 ± 0.27	6.70 ± 0.07	282.30 ± 71.06	119.77 ± 26.68
Day 70	2.79 ± 0.43	2.09 ± 0.86	14.19 ± 1.62	7.34 ± 1.22	8.00 ± 0.07	483.08 ± 118.56	244.33 ± 65.59
Day 84	2.41 ± 0.26	0.91 ± 0.06	20.17 ± 2.24	10.64 ± 1.28	10.40 ± 0.08	876.60 ± 259.67	455.37 ± 123.29
Day 98	2.21 ± 0.24	0.94 ± 0.09	20.68 ± 2.95	11.01 ± 1.77	12.70 ± 0.11	1833.58 ± 344.65	957.94 ± 183.74
Day 112	2.43 ± 0.36	0.95 ± 0.06	25.11 ± 3.00	13.86 ± 2.02	14.20 ± 0.09	2579.45 ± 275.10	1377.12 ± 91.85
Day 126	1.55 ± 0.38	0.76 ± 0.08	27.82 ± 5.38	15.43 ± 3.54	15.90 ± 0.12	3184.99 ± 848.30	1955.43 ± 377.33
Day 140	1.27 ± 0.29	0.69 ± 0.10	29.52 ± 3.15	16.23 ± 4.03	14.95 ± 0.08	2523.38 ± 222.14	1802.81 ± 465.90
Day 154	1.94 ± 0.89	0.67 ± 0.10	30.32 ± 7.14	16.98 ± 5.73	14.68 ± 0.07	3368.35 ± 1023.27	1909.68 ± 621.08
Day 168	0.56 ± 0.02	0.40 ± 0.04	35.77 ± 2.57	21.21 ± 3.08	16.60 ± 0.24	3590.00 ± 862.26	2090.02 ± 453.14
Day 182	0.50 ± 0.13	0.36 ± 0.08	31.94 ± 3.51	20.86 ± 2.93	15.60 ± 0.17	3547.62 ± 479.21	2446.77 ± 434.51

Table 3. Pearson correlation matrix for various haemodynamic indices of middle uterine arteries (MUA, ipsilateral and contralateral) during the first 8 months of gestation in dairy cows (N = 12)

Variables	Pulsatility index	Resistivity index	TAMAX	TAMEAN	D-MUA	BFV-TAMAX	BFV-TAMEAN
Ipsilateral MUA							
Pulsatility index	1.0000						
Resistivity index	0.8796*	1.0000					
TAMAX	–0.5793*	–0.4949*	1.0000				
TAMEAN	–0.5512*	–0.4808*	0.9520*	1.0000			
D-MUA	–0.0571	–0.0249	0.1753**	0.1179	1.0000		
BFV-TAMAX	–0.4865*	–0.3972*	0.8521*	0.7792*	0.5916*	1.0000	
BFV-TAMEAN	–0.4554*	–0.3821*	0.7775*	0.7721*	0.5335*	0.8922*	1.0000
Contralateral MUA							
Pulsatility index	1.0000						
Resistivity index	0.9363*	1.0000					
TAMAX	–0.4682*	–0.4192*	1.0000				
TAMEAN	–0.4103*	–0.3789*	0.9068*	1.0000			
D-MUA	0.1830**	0.2323*	–0.1371	–0.1201	1.0000		
BFV-TAMAX	–0.3609*	–0.2867*	0.7911*	0.7147*	0.4410*	1.0000	
BFV-TAMEAN	–0.2524*	–0.2032**	0.5577*	0.5601*	0.3397*	0.7164*	1.0000

*p < 0.01. **p < 0.05.

Table 4. Pearson correlation matrix for various haemodynamic indices of umbilicus during first 6 months of gestation in dairy cows (N = 12)

Variables	PI	RI	TAMAX	TAMEAN	D	BFV-TAMAX	BFV-TAMEAN
PI	1.0000						
RI	0.2572**	1.0000					
TAMAX	–0.4914*	–0.3328*	1.0000				
TAMEAN	–0.4651*	–0.3444*	0.9188*	1.0000			
D	–0.4795*	–0.2960**	0.6617*	0.6366*	1.0000		
BFV-TAMAX	–0.5115*	–0.4364*	0.7021*	0.6730*	0.7231*	1.0000	
BFV-TAMEAN	–0.6542*	–0.3800*	0.6452*	0.6631*	0.7170*	0.6581*	1.0000

*p <0 .01. **p < 0.05.

beginning from the first to the second trimester with a 1.3–1.8 times surge in the values throughout the last trimester in the ipsilateral artery; however, the contralateral artery showed a steady increase by 1.1–1.2 times. Not much akin to the findings of the present study, Herzog et al. (2011) reported a 28% and 36% higher BFV during 21st and 39th week of gestation.

For umbilical haemodynamics, a gradual fall in RI and PI values has been observed in the umbilical artery along with the advancement of gestation in buffaloes, which is similar to our study (Singh et al., 2018). In another study, foetal umbilical indices of RI and PI have been reported to decline by 26 weeks of gestation after which no change was observed (Panarace et al., 2006). However, very little to no changes have been observed in RI and PI values of the umbilicus throughout the gestation period in Murrah buffaloes using trans-abdominal ultrasonography, which is not akin to the findings of the present study (Singh et al., 2017).

During correlation analysis, RI of the ipsilateral and contralateral artery has been reported to be positively correlated with PI and negatively correlated with TAMAX, and blood flow volume to the uterus throughout gestation in cows (Bollwein et al., 2002) and buffaloes (Varughese et al., 2013; Abdelnaby, 2020), which is similar to our study. On the contrary, RI values have been found to be positively correlated with velocity and volume of the blood flow to the uterus and negatively correlated with other parameters (Panarace et al., 2006). In further concurrence with the present study, the diameter of both ipsilateral and contralateral arteries had a high positive correlation with the blood flow volume and a negative correlation with PI and RI beginning from month 1 to 8 of gestation (Hassan et al., 2020). Also, the umbilical diameter has been found to increase along with gestational age when measured between days 73 and 190 of gestation (Hunnam et al., 2009), which is in agreement with our findings.

In conclusion, haemodynamic indices of middle uterine arteries (MUA), i.e., pulsatility and resistivity index, time averaged mean and maximum velocities, blood flow volume (mean and maximum) to the uterus and diameter, were significantly higher in the ipsilateral MUA after day 140 of gestation. With a scope for future research, circulatory adaptations of the foeto-maternal unit during the course of gestation can be carefully monitored by regular examination of haemodynamic parameters to study the physiological and anatomical changes during normal and complicated pregnancy.

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