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Analyzing the correlation between uterine involution and blood flow in post-partum dairy cows

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ABSTRACT

The objective of this study was to assess the applicability of trans-rectal color Doppler ultrasonography for quantification of uterine blood flow in 30 healthy lactating Jersey cows on a weekly interval from days 0 to 56 after parturition (day 0). Significantly higher time average mean velocity (TAMEAN), time average maximum velocity (TAMAX) and blood flow volume (BFV-TAMEAN and BFV-TAMAX) in artery ipsilateral to previous gravid uterine horn (PGUH) was found as compared to contralateral middle uterine artery (MUA) on day 0 to 14 post-partum. Similarly, transverse diameter (D) of ipsilateral MUA was significantly higher as compared to contralateral MUA during first two weeks after parturition. Clinical uterine involution completed on day 32.47±0.85 post-partum as demonstrated by B-mode ultrasonography and a significant correlation (r= 0.31–0.44 from day 7–28) between size of PGUH and BFV-TAMAX was found. Also, significant correlation between transverse diameter of ipsilateral (r=0.56–0.80) and contralateral (r=0.51–0.79) MUA-D and BFV-TAMAX was found on different examination days. In conclusion, trans-rectal Doppler ultrasonography was a useful tool to investigate haemodynamic changes whereas PI, RI, Doppler pulse duration and systolic upstroke time were found unsuitable to indagate alterations in uterine perfusion during post-partum period.

Keywords: Dairy cows, Doppler, Post-partum period, Uterine blood flow, Uterine involution

Post-partum period is a very critical phase in the life of a cow during which uterine involution occurs (Frazer 2005). Delayed uterine involution has often been associated with compromised future fertility; therefore, understanding of this process is important for the successful management of dairy cows (Heppelmann *et al.* 2013, Sharma *et al.* 2018). Until recently, subjective evaluation via trans-rectal palpation has been commonly used (Krueger *et al.* 2009) but nowadays, B-mode ultrasonography has been in use due to its non-invasive nature (Sharma *et al.* 2017). In order to assess the physiological changes in reproductive tract, color Doppler ultrasonography have received great interest in the last several decades (Rawy *et al.* 2018). Therefore, research on hemodynamics of middle uterine artery (MUA) via triplex Doppler ultrasonography is of great utility as it has a positive impact on diagnostic and predictive abilities in large animal reproduction. This technique can be helpful in assessment of uterine blood flow during estrus, clinical endometritis, abortion and retention of placenta (Panarace *et al.* 2006a,b; Sharma *et al.* 2019a).

A higher level of estrogen in blood around parturition alters the velocity and volume of blood flow due to vasodilation (Krueger *et al.* 2009, Sharma *et al.* 2019b). Also, regeneration of new endometrium during post-partum period requires sufficient amount of blood supply (Sheldon and Dobson 2004), therefore, Doppler analysis allows a non-invasive investigation of blood flow of the entire reproductive tract. So, the present study envisages the indagation on uterine involution and various blood flow parameters during post-partum period in dairy cows.

MATERIALS AND METHODS

The study was conducted on thirty multiparous dairy cows (n=30) having body condition score ranging from 2.5 to 2.75 reared in a loose housing system under standard management conditions, fed a total mixed ration (once daily, *ad lib.*), mineral mixture (50 g daily) and had unrestricted access to water. Cows having normal parturition, i.e. no obstetrical complication at intra-partum stage and having received no treatment during pre-partumand post-partum period, were examined on a weekly interval, i.e. 4 h after parturition (day 0) upto day 56, for adjudging the uterine involution and uterine blood flow through middle uterine artery (MUA) by color Doppler trans-rectal ultrasonography. The MUA, a branch originating from internal iliac artery, is located cranial to the external iliac artery, can be found in the mesometrium as a movable arterial vessel and easily visualized by the color Doppler technique (Sharma *et al.* 2019b). For monitoring of uterine

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blood flow, Doppler examination of MUA ipsilateral and contralateral to uterine horn of previous pregnancy was performed using Mindray portable ultrasound device (Z5 VET) in pulsed-wave mode using a 5.7 MHz linear probe with a power of 50%, filter of 100 Hz and Doppler angle varying between 20º and 60º. The parameters displayed for each waveform by applying the automatic mode were pulsatility index (PI), resistance index (RI) and velocity of blood flow (TAMEAN, TAMAX). The MUA's transverse diameter (D) was calculated from the mean of three measurements of the diameter made from frozen two dimensional grey scale images just before Doppler measurements. Doppler pulse duration (DPD) and acceleration time/systolic upstroke time were also recorded as additional parameters to know their significance in assessment of uterine blood flow (Fig. 1a, 1b, 1c and 1d).

Blood flow volume in mL/min was calculated using the equation (Bollwein *et al.* 2000)

$$\text{Blood flow volume-TAMEAN} = \text{TAMEAN} \times \pi \times (\text{D} \times 0.1/2)^2 \times 60$$

$$\text{Blood flow volume-TAMAX} = \text{TAMAX} \times \pi \times (\text{D} \times 0.1/2)^2 \times 60$$

The diameter of both the uterine horns, i.e. previous gravid uterine horn (PGUH) and previous non-gravid uterine horn (PNGUH) was assessed from day 7 to 42 via B-mode ultrasonography (5–7.5 MHz) and mean of both the uterine horns was calculated for carrying out investigation of uterine involution (Fig. 2a). Uterine involution was considered complete when both gravid and non-gravid uterine horns were nearly in symmetrical measure (Fig. 2b) and no further change took place between two consecutive examinations in diameter of uterine horns (Abdel-Khalek *et al.* 2013).

Numeric data for all the parameters are expressed as mean±SD and statistical analysis was carried out using Student's t-Test. Pearson's correlation coefficient was calculated to ascertain the correlation between blood flow parameters and size of previous gravid uterine horn up to completion of uterine involution. All the experiments were carried out after the approval of ethical committee of the institute.

RESULTS AND DISCUSSION

In cattle reproduction, visualization of physiological and pathological processes of the reproductive system can be

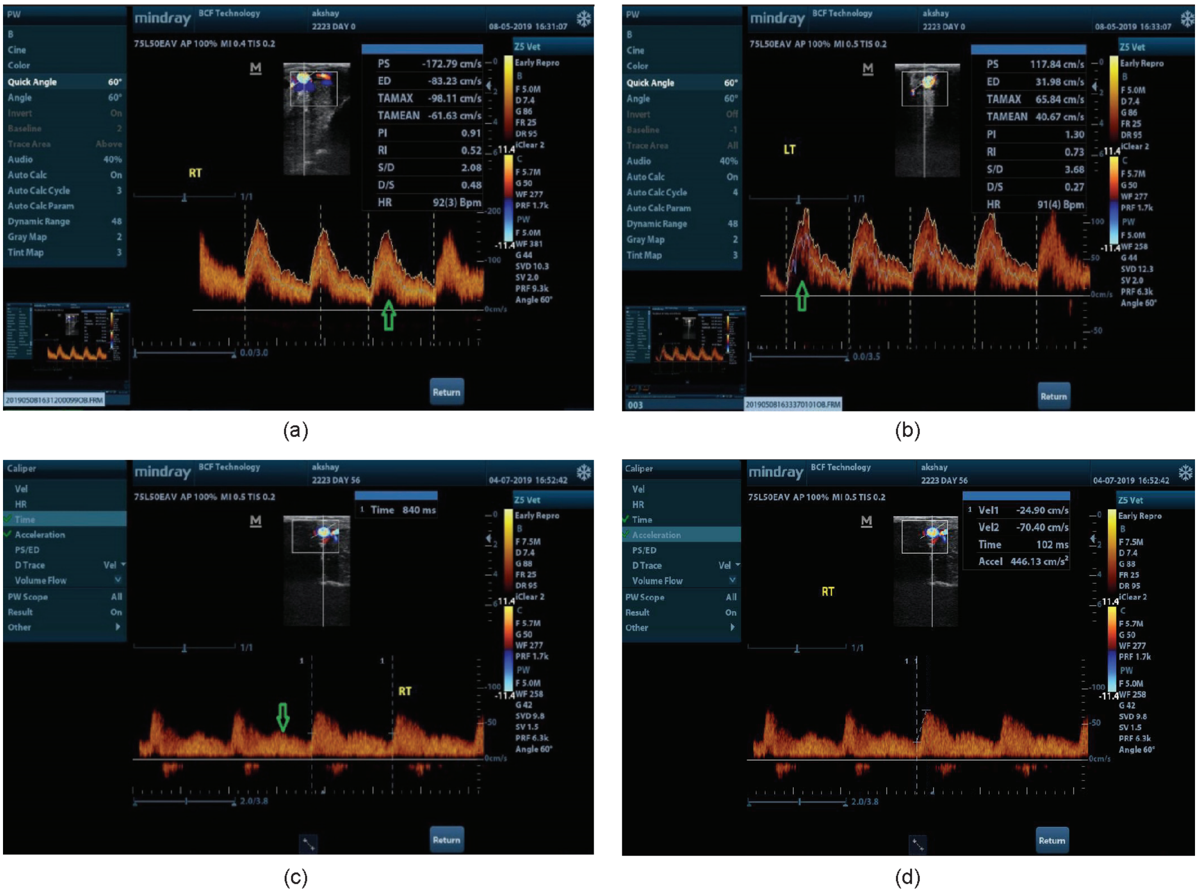
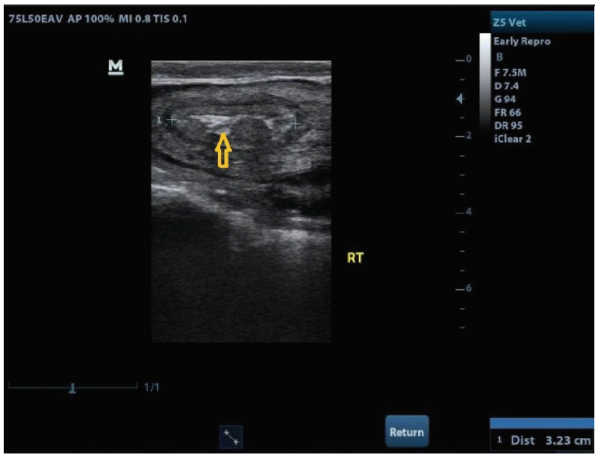


Fig. 1. Doppler sonogram images illustrating waveforms during post-partum period. (a) PGUH ipsilateral MUA waveform of healthy cow on day 0 (4 h after parturition) characterized by high TAMAX and obliteration of spectral window suggestive of turbulent flow (green arrow). (b) PGUH contralateral MUA waveform of healthy cow on day 0 (4 h after parturition) characterized by low TAMAX and less obliterated spectral window (green arrow). (c) Calculation of DPD and prolonged diastolic phase (green arrow) indicative of normal blood flow on day 56 post-partum. (d) Calculation of systolic upstroke time.

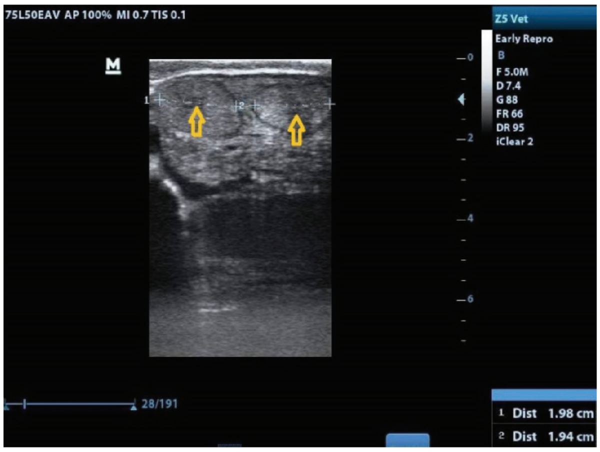
Table 1. Haemodynamic parameters of middle uterine arteries in healthy cows during post-partum period expressed as mean±SD.

Days post-partum	Side of MUA in relation to uterine horn	Parameter								
		PI	RI	TAMEAN	TAMAX	Diameter of MUA	BFV-TAMEAN	BFV-TAMAX	DPD	Systolic upstroke time
Day 0	Ipsilateral	0.72±0.09	0.43±0.09	49.35±4.16[a]	88.77±5.87[a]	1.10±0.03[a]	3378.56±581.01[a]	5082.37±392.08[a]	645.69±19.27	147.54±10.11
	Contralateral	0.87±0.08	0.49±0.04	33.15±2.70[b]	63.63±4.48[b]	0.97±0.03[b]	1493.99±183.43[b]	2880.18±290.79[b]	647.54±19.69	180.62±13.62
Day 7	Ipsilateral	0.80±0.06	0.49±0.03	41.64±2.87[a]	74.43±4.49[a]	1.03±0.03[a]	2087.286±184.57[a]	3698.80±284.19[a]	712.15±16.17	163.84±12.16
	Contralateral	0.87±0.07	0.51±0.04	29.05±1.98[b]	56.08±3.13[b]	0.95±0.02[b]	1228.93±103.37[b]	2363.76±176.94[b]	710.77±16.34	180±22.20
Day 14	Ipsilateral	0.85±0.08	0.48±0.04	34.93±1.58[a]	63.29±2.73[a]	0.98±0.02[a]	1607.19±116.15[a]	2875.85±168.00[a]	721.63±24.29	153.5±7.37
	Contralateral	0.81±0.08	0.48±0.04	28.39±1.93[b]	54.52±2.86[b]	0.88±0.02[b]	1029.17±56.57[b]	1999.96±103.49[b]	730.5±26.69	184.13±14.12
Day 21	Ipsilateral	1.02±0.07	0.57±0.03	28.52±1.99[x]	53.65±2.84[x]	0.94±0.02[x]	1263.69±148.30[x]	2341.62±220.62[x]	747±27.82	142.29±10.25
	Contralateral	0.98±0.07	0.54±0.04	23.55±1.05[y]	46.22±2.01[y]	0.92±0.02[y]	931.69±68.68[y]	1812.46±127.39[y]	723.29±21.61	138±10.95
Day 28	Ipsilateral	1.03±0.10	0.56±0.04	26.99±1.59	51.81±2.58	0.93±0.02	1100.30±87.67	2129.57±162.22	708.35±22.85	159.53±14.47
	Contralateral	0.92±0.09	0.51±0.04	24.65±1.10	48.65±1.71	0.88±0.02	900.75±61.43	1776.14±103.92	716.47±23.92	166.70±14.30

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



(a)



(b)

Fig. 2. B-mode sonogram images illustrating uterine involution. (a) Diameter of previous gravid uterine horn (PGUH) on day 14 post-partum and lochia inside uterine lumen (yellow arrow). (b) Completion of uterine involution characterized by nearly symmetrical uterine horns on day 28 post-partum and inconspicuous uterine lumen suggestive of no inflammatory process.

achieved by color Doppler technique (Ginther and Utt 2004), especially during post-partum period where minimum invasion is required (Heppelmann *et al.* 2013). Uterine involution is characterized by shrinkage of tubular genital tract following decrease in length of muscle fibres after parturition in response to strong myometrial contractions under influence of 15-keto-13,14-dihydro-$PGF_2\alpha$ (Sheldon and Dobson 2004, Sheldon *et al.* 2006). Uterine involution happens to be an important event during post-partum period and completed at day 32.43±0.85 (Mean±SD) in all the cows under study. The percent reduction in size of PGUH and PNGUH was 25 and 24% between day 7–14, 33 and 26% between day 14–21, 17 and 6% between day 21–28 and 9 and 4% between day 28–35, respectively. Similarly, percent reduction in blood flow volume (BFV-TAMAX) of PGUH and PNGUH was 27 and 18% between day 0–7, 22 and 15% between day 7–14, 19 and 9% between day 14–21, 9 and 2% between day 21–28 and 12 and 6% between day 28–35, respectively

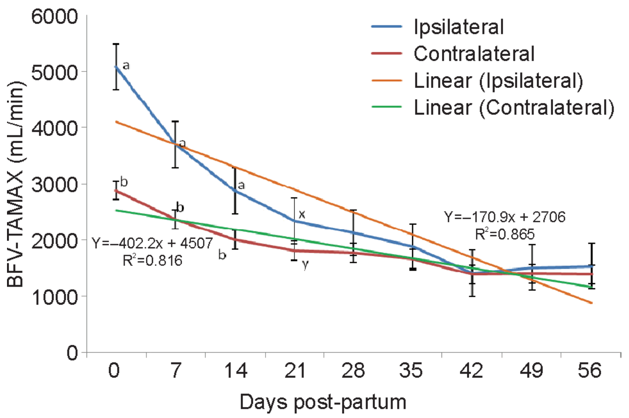


Fig. 3. Mean±SD of the blood flow volume-TAMAX (BFV-TAMAX) of the ipsilateral (PGUH) and contralateral (PNGUH) middle uterine artery between day 0 (4 h after parturition) and day 56 post-partum in 30 healthy cows. The orange and green lines are the trend lines (along with regression equation and R^2 value) for ipsilateral and contralateral middle uterine artery, respectively. Means with different letters on day 0, 7, 14 (P<0.01) and 21 (P<0.05) are significantly different.

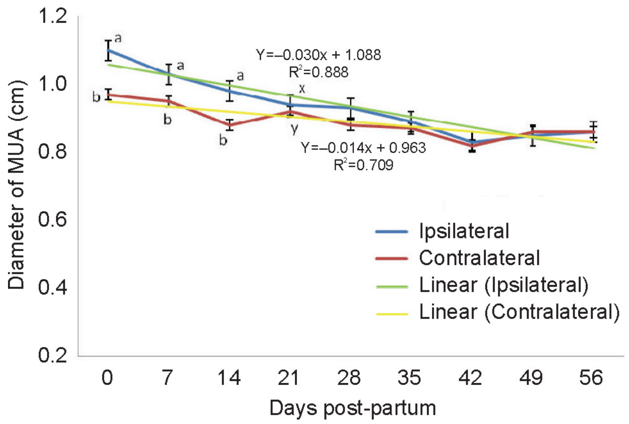


Fig. 4. Mean±SD of the diameter of MUA (cm) of the ipsilateral (PGUH) and contralateral (PNGUH) middle uterine artery between day 0 (4 h after parturition) and day 56 post-partum in 30 healthy cows. The green and yellow lines are the trend lines (along with regression equation and R^2 value) for ipsilateral and contralateral middle uterine artery, respectively. Means with different letters on day 0, 14 (P<0.01) and 7 (P<0.05) are significantly different**.**

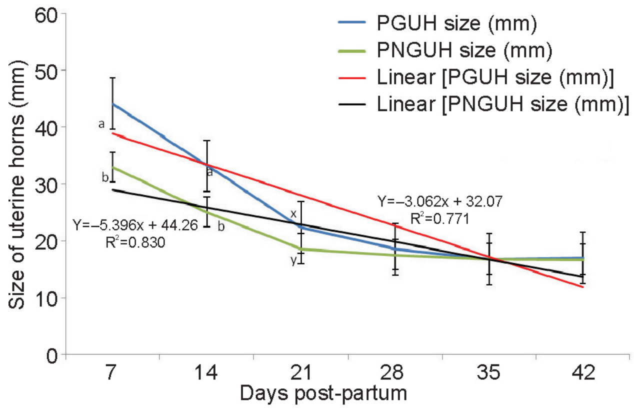


Fig. 5. Mean±SD of the size of uterine horns (mm) from day 7 to 42 post-partum in 30 healthy cows. Means with different letters on day 0, 14 (P<0.01) and 7 (P<0.05) are significantly different. The green and yellow lines are the trend lines (along with regression equation and R^2 value) for ipsilateral and contralateral middle uterine artery, respectively.

(Fig. 5). A nearly similar percent decrease in diameter of uterine horns was reported during puerperal phase (Heppelmann *et al.* 2013) as found in our study.

Transverse diameters of both the uterine horns were found symmetrical between 4–5 weeks after parturition which is similar to our findings (Hajurka and Zemljic 2006, Sharma *et al.* 2018). Transverse diameter of MUA was significantly higher (P<0.01) in side ipsilateral to PGUH as compared to PNGUH upto 2 weeks post-partum. Increased diameter of uterine artery plays a pivotal role in supplying the adequate amount of blood to the uterus for post-partum uterine changes such as uterine involution, regeneration of endometrium and ovarian rebound. Significant decrease in transverse diameter of MUA following subsequent observations after parturition has been reported (Krueger *et al.* 2009, Heppelmann *et al.* 2013).

The Time average mean velocity (TAMEAN) and Time average maximum velocity (TAMAX) was significantly higher (P<0.01) in PGUH as compared to PNGUH on day 0, 7, 14 post-partum. Significantly higher (P<0.01) blood flow through MUA ipsilateral to PGUH, i.e. BFV-TAMEAN and BFV-TAMAX, was indicative of higher uterine perfusion in comparison to PNGUH on day 0, 7, 14 post-partum (Fig. 3). In response to increased estrogen concentration around parturition, acceleration of the blood flow to the uterus also increases owing to various haemodynamic variables (TAMEAN, TAMAX, BFV-TAMEAN, BFV-TAMAX and diameter of the uterine arteries), albeit, other indices (PI and RI) responsible for slowing the rate of uterine perfusion decrease simultaneously (Bollwein *et al.* 2000, Rawy *et al.* 2018).

In unison with our findings, a significant (linear; P<0.01) decrease in TAMAX and blood flow volume to the uterus has been reported by various researchers between days 0 to 28 post-partum which can be attributed to decrease in diameter of MUA (Ford *et al.* 1982, Guilbault *et al.* 1984, Krueger *et al.* 2009). However, the difference in velocity and volume reduced significantly (P<0.05) on day 21 post-partum as uterine involution approaches (Fig. 4). To establish new parameters for assessing the uterine blood flow, DPD and systolic upstroke time were recorded but there was no significant difference found (P>0.05) between PGUH and PNGUH till last day of examination, i.e. day 56 (Table 1). Envisaging the study of new parameters, DPD and systolic upstroke time have been considered as the important haemodynamic parameters for investigating the uterine changes during endometritis (Sharma *et al.* 2019b) but found non-significant (P>0.05) during any stage after parturition.

With increase in post-partum days, blood flow to uterus started decreasing after completion of regeneration of intercaruncular epithelial lining of endometrium. Albeit, no significant difference (P>0.05) between PI and RI of PGUH and PNGUH at any post-partum stage was recorded. On the other hand, PI and RI, despite showing an initial increase for first few weeks post-partum, did not change significantly which is in disagreement with others findings (Krueger *et al.* 2009). Therefore, PI which is mainly considered as a main variable for quantification of uterine perfusion in women (Tekay and Jouppila 1994) does not hold much significance in cows during post-partum period.

Correlation analysis justified a strong association between two or more variables included in a study. Correlation analysis was done for blood flow parameters of the mean values of previous gravid and non-gravid uterine horns separately. The PI was positively correlated with RI at every stage of examination for PGUH and PNGUH, respectively (r=0.96, 0.96 on day 0; r=0.97, 0.94 on day 7; r=0.93, 0.95 on day 21, 28, 49; r=0.95, 0.91 on day 35; r=0.96, 0.93 on day 42; r=0.95, 0.98 on day 56; P<0.0001). High correlation coefficient between PI and RI throughout pregnancy (Varughese *et al.* 2013) and endometritis (Sharma *et al.* 2019b) has been reported which is in agreement with our findings.

In present study, transverse diameter of middle uterine artery (D) was significantly correlated with BFV-TAMAX for PGUH (r= 0.72, P<0.0001 on day 0; r=0.64, P<0.05 on day 7; r=0.75, P<0.05 on day 14; r=0.80, P<0.05 on day 21; r=0.66, P<0.05 on day 28; r=0.79, P<0.05 on day 35; r=0.75, P<0.05 on day 42; r=0.56, P<0.05 on day 49; r=0.64, P<0.05 on day 56) and PNGUH (r=0.64, P<0.01 on day 0; r=0.52, P<0.05 on day 7; r=0.51, P<0.05 on day 14; r=0.79, P<0.05 on day 21; r=0.71, P<0.01 on day 28; r=0.79, P<0.05 on day 35; r=0.68, P<0.01 on day 42; r= 0.55, P<0.01 on day 49; r=0.91, P<0.05 on day 56). Strong significant correlation (r=0.87; P<0.01) between diameter of ipsilateral MUA and BFV-TAMAX have been reported (Heppelmann *et al.* 2013) which is not much akin to our findings on the day of parturition. Similarly, a decrease in correlation was evident with increase in day post-partum (Krueger *et al.* 2009). Other parameters were found to be having no significant correlation with diameter of MUA. However, size of PGUH and BFV-TAMAX were holding a significant correlation (P<0.05) upto day 28 of examination (r=0.32, day 7; r=0.44, day 14; r=0.31, day 21; r=0.41, day 28) but no such correlation between size of PNGUH and blood flow parameters was found. In concurrence with our findings, increased perfusion of uterus helps in rapid involution as evident in our study where strong correlation between size of uterine horns and BFV-TAMAX was found upto completion of uterine involution. Therefore, it is justified that higher perfusion of uterus during early post-partum period helps in rapid uterine involution and achieving desirable future fertility.

Based on the scope of present study, characteristic changes in uterus and its perfusion could be easily evaluated by trans-rectal triplex Doppler sonography. Diameter of middle uterine artery and blood flow volume-time average maximum velocity had an important role in completing the uterine changes whereas pulsatility and resistance indices did not hold any significance related to uterine perfusion during post-partum period. New parameters, i.e. Doppler pulse duration and systolic upstroke time, were not reliable to be established as important haemodynamic variables for studying the status of uterine perfusion in post-partum healthy cows.

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