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-partum and 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...
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 20° and 60°. 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)

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

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

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
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-PGF₂α (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. 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.](image)

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 | Diameter of MUA | TAMAX | TAMAX/MEAN | BFV-TAMAX | BFV-TAMAX/MEAN | DPD | Systolic upstroke time |
|------------------|------------|----------------|-------|------------|-----------|----------------|------|-----------------------|
| Day 0            | Ipsilateral | 0.72±0.09      | 4.35±1.69 | 88.72±5.87 | 0.43±0.09 | 49.35±4.16 | a   | 3378.56±581.01a       |
|                  | Contralateral | 0.87±0.08      | 4.96±2.67 | 63.63±4.68 | 0.48±0.04 | 33.15±2.96 | a   | 88.77±5.87a           |
| Day 7            | Ipsilateral | 0.80±0.06      | 4.91±2.37 | 64.64±2.97 | 0.46±0.03 | 41.51±2.79 | a   | 2087.29±184.57a       |
|                  | Contralateral | 0.87±0.07      | 4.86±2.57 | 74.43±4.89 | 0.48±0.04 | 34.93±1.87 | a   | 1287±103.74a          |
| Day 14           | Ipsilateral | 1.02±0.08      | 5.34±1.93 | 63.29±2.73 | 0.51±0.01 | 29.05±1.98 | a   | 1207.37±106.15a       |
|                  | Contralateral | 0.81±0.08      | 5.48±1.04 | 63.29±4.18 | 0.48±0.01 | 28.59±1.03 | a   | 1029±74±56.57a        |
| Day 21           | Ipsilateral | 1.02±0.07      | 5.37±1.80 | 54.52±2.86 | 0.57±0.01 | 28.52±1.99 | a   | 2341±380±148.30a      |
|                  | Contralateral | 0.98±0.07      | 5.40±1.04 | 53.65±2.28 | 0.54±0.01 | 28.54±1.99 | a   | 1812±62±127.40a       |
| Day 28           | Ipsilateral | 1.03±0.08      | 5.50±1.04 | 51.82±2.15 | 0.56±0.01 | 26.99±1.59 | a   | 2129±57±162.22        |
|                  | Contralateral | 0.92±0.09      | 5.48±1.04 | 48.65±1.71 | 0.51±0.01 | 24.61±1.10 | a   | 716±47±23.92          |

Values with different superscripts within the same column for the same day are significantly different (P<0.05). *Values with different superscripts within the same column for the same day are significantly different (P<0.01).
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 inter-
caruncular 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 have 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 PNUGH 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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