Haryana Vet. (December, 2022) **61(2),** 250-253

Research Article

ANALYSIS OF HEMODYNAMIC ALTERATION OF MIDDLE UTERINE ARTERY USING SPECTRAL DOPPLER ULTRASONOGRAPHY: A NOVEL METHOD FOR EARLY DIAGNOSIS OF PREGNANCY AND EMBRYONIC MORTALITY IN DAIRY CATTLE

MOHIT MAHAJAN*, MADHUMEET SINGH, AKSHAY SHARMA, AMIT SHARMA and PRAVESH KUMAR
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences,
Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India-176061

Received: 18.11.2021; Accepted: 06.01.2022

ABSTRACT

The current study was aimed to differentiate the state of pregnancy and embryonic mortality based on analysis of uterine blood flow. The present study was conducted on Jersey cows (N=22) with the objective to analyzethe comparative blood flow through the middle uterine artery on day 21 post insemination, in cows diagnosed subsequently pregnant (n=12), non-pregnant (n=6) and with embryonic mortality (n=4). Pulsatility, resistance and vascular perfusion indices (PI, RI and VPI), Time average mean velocity (TAMEAN), Time average maximum velocity (TAMAX), cross-sectional area of the artery and volume of blood flow (BFV-TAMEAN and BFV-TAMAX) were measured to study the spectral waveforms at 21 days post insemiantion. Non-pregnant cows had significantly higher (P<0.05) TAMAX for ipsi-lateral and contra-lateral middle uterine artery to corpus luteum as compared to pregnant and embryonic mortality diagnosed cows. Similarly, BFV-TAMAX was significantly higher (P<0.01) in ipsi-lateral as compared to the contra-lateral middle uterine artery of non-pregnant cows. Other parameters included had no significant difference (P>0.05) between ipsi-lateral and contra-lateral middle uterine artery on 21 days post insemination, although, PI, RI and VPI were non-significantly higher in pregnant and embryonic mortality diagnosed cows. In conclusion, spectral Doppler ultrasonography can be a useful tool for diagnosis of the physioloigical and pathological conditions of the uterus, *via* analysis of the uterine blood flow.

Keywords: Blood flow; Embryonic mortality, Estrous period, Jersey cows, Middle uterine artery, Spectral Doppler

How to cite: Mahajan, M., Singh, M., Sharma, A., Sharma, A. and Kumar, P. (2022). Analysis of hemodynamic alteration of middle uterine artery using spectral doppler ultrasonography: A novel method for early diagnosis of pregnancy and embryonic mortality in dairy cattle. *Haryana Vet.* **61(2)**: 250-253.

The success of the dairy industry depends on milk yield and calving rate. Efficiency in calving interval results in attaining one calf per year, for which early pregnancy diagnosis is pivotal. Early diagnosis of pregnancy and pathological conditions enables the farmers to identify, treat or rebreed them at the earliest opportunity, further decreasing the service rate and calving to conception interval (Stevenson, 2005).

Ultrasonography could be used for the evaluation of individual organs morphologically but does not provide information about its functional state, such as vascular perfusion to analyse the health of developing fetus (Melber and Ballas, 2021). The circulation of the bovine reproductive system in healthy and diseased animals were initially investigated using invasive procedures, Doppler ultrasonography is a non-invasive method for assessing blood flow to and within the reproductive organs during the inter-ovulatory interval and pregnancy (Bollwein *et al.*, 2004; Ginther, 2014). The assessment of corpus luteum and uterine blood flow can be done by studying the percentage of tissue with color Doppler signals and the blood flow indices in an individual middle uterine artery (Ginther *et al.*, 2007). The graphical representation of the blood flow is referred to as spectral Doppler, Doppler indices and flow parameters are derived from it (Sharma *et al.*, 2019). The present study was focused on deriving the comparative quantification of various blood flow indices at the end ofthe estrous period in pregnant, non-pregnantand cows with embryonic mortality.

*Corresponding author: mohitmahajan108@gmail.com

MATERIALS AND METHODS

The study was conducted on Jersey cows (n=22) housed in concrete dry sheds, fed green fodder and dry fodder/hay, with concentrate and mineral supplementation. Cows had free access to clean drinking water. Cows were healthy with no apparent clinical signs of any disease or deficiency. Trans-rectal ultrasonography (Mindray Z5 VET) of the middle uterine artery (MUA; Fig. 1) was done for diagnosing the state of pregnancy, non-pregnancy and embryonic mortality in brightness mode (B-mode), color Doppler mode and spectral mode on Day 21 post insemination.

The middle uterine artery (MUA), a branch originating from the 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.*, 2019). Doppler examination was performed in pulsed -wave mode using a 5.7 MHz linear probe with a power of 50%, filter of 100 Hz and Doppler angle of 60º. All examinations were conducted by the same operator and lasted for 15-20 minutes. To prevent any influences on uterine blood flow by trans-rectal manipulation, the uterus was not palpated before and during the blood flow measurements (Fig. 2).

Examination of both middle uterine arteries (ipsilateral [i] and contra-lateral [c] to corpus luteum) was done for evaluation of the uterine blood flow parameters and Doppler indices i.e. Pulsatility index (PI), Resistance index (RI), Vascular perfusion index (VPI), Time average mean velocity (TAMEAN), Time average maximum velocity (TAMAX), Blood flow volume- Time average mean velocity (BFV-TAMEAN), Blood flow volume-Time average maximum velocity (BFV-TAMAX), cross-sectional area of middle uterine artery (Fig. 2) and systole:diastole ratio in subsequently pregnant, cows with embryonic mortality and non-pregnant cows (Fig. 3a,b,c).

Blood flow volume in mL/min was calculated using the equation (Varughese *et al.*, 2013): Blood flow volume-TAMEAN=TAMEAN×π×(D×0.1/2)^2×60

Blood flow volume-TAMAX=TAMAX×π×(D×0.1/2)^2 ×60

Numeric data for all the parameters are expressed as mean±SD and statistical analysis was carried out using Student's t-Test and one-way ANOVA (Statistical Analysis Software® 9.2 TS Level version 2M2 for windows). All the experiments have been carried after the approval of the ethical committee of the institute and the principles under the declaration of Helsinki were also taken into consideration.

RESULTS AND DISCUSSION

In the present study, the mean PI and RI were found to be non-significantly higher (P>0.05) in [i] as compared to [c] MUA in subsequently pregnant and embryonic mortality' diagnosed cows. Albeit, non-significantly lower (P>0.05) PI and RI were recorded in MUAs of non-pregnant cows as compared to pregnant and embryonic mortality diagnosed cows.In contrary to findings mentioned above, the VPI was found to be non-significantly lower (P>0.05) in [i] and [c] MUA of pregnant and embryonic mortality' diagnosed cows as compared to non-pregnant cows (Table 1).

No significant difference (P>0.05) between [i] and [c] MUA in terms of TAMEAN was observed in pregnant, embryonic mortality and non-pregnant cows. However, TAMAX was significantly higher (P<0.05) between [i] and [c] MUA of non-pregnant cows as compared to pregnant and embryonic mortality diagnosed cows. Similarly, BFV-TAMAX was significantly higher (P<0.01) in [i] as compared to [c] MUA of non-pregnant cows.The cross-sectional area of the MUA was found to be non-significantly higher (P>0.05) in the [i] as compared to [c] MUA in pregnant, non-pregnant and cows with embryonic mortality (Table 1).

With the development of the foeto-maternal placental unit in pregnant cows during the phase of maternal recognition of pregnancy (Jaffe, 1995), the pulsatility index of the middle uterine artery is adapted. The side of pregnancy and formation of extra-embryonic membranes (Bollwein *et al.*, 2004) is represented by a higher PI, which is in accordance with the findings of the present study.Similarly, Silva *et al.* (2009) and Varughese *et al.* (2013) reported that higher PI in pregnant as compared to non-pregnant cows during early gestation. In cows with embryonic mortality,a higher PI compared to non-pregnant cows, indicated placental insufficiency, intrauterine growth retardation, or gestational hypertension (Varughese *et al.*, 2013).

Higher values of resistance index (RI) indicate vascular impedance in a blood vessel. An increased uterine blood flow in response to neo-vascularization due to formation of placental complex, development of vascular bed for fetal circulation and constricted uterine arteries in response to progesterone dominance leads to higher RI in pregnant as compared to non-pregnant cows (Silva *et al.*, 2009; Gupta *et al.*, 2009; Melber and Ballas, 2021). Similar findings have been reported in pregnant cows (Panarace *et al.*, 2006; Abdelnaby, 2020), buffaloes (Varughese *et al.*, 2013) and mares (Silva *et al.*, 2009). In comparison to pregnant cows, the vaso-dilatory effect of estrogen during estrus (Herzog and Bollwein, 2007) in non-pregnant cows leads to decreased resistance index (Rawy *et al.*, 2018; Sharma *et al.*, 2019).

Vascular Perfusion Index (VPI) describes the extent of tissue perfusion carried out by a blood vessel in an organ. Under the influence of increased gonadotrophin secretion, ovarian activity, follicular growth and accompanied increased estrogen secretion at the end of the estrous period (Sharma *et al.*, 2019), an increased blood flow in MUA and higher tissue perfusion was observed in non-pregnant cows which is in agreement with our findings (Honnens *et al.*, 2008; Rawy *et al.*, 2018). However, low perfusion index in pregnant cows is evident,with the formation of the foeto-maternal placental unit, growth of new vessels and remodeling of existing vasculature during early gestationunder the influence of luteal progesterone (Silva and Ginther, 2010; Pinaffi *et al.*, 2017). Increased tissue perfusion in cows with embryonic mortality occurs due to loss of embryo and re-initiation of cyclicity (Pinaffi *et al.*, 2017). In a study, Singh *et al.* (2018) observed that resistance index and pulsatility index of middle uterine artery was invariably similar (p>0.05) from day 30 of gestation onward. However, these indices exhibited a decreasing (p>0.05) trend till nine-month of gestation in fetal umbilicus.

In non-pregnant cows, elevated levels of estrogen have a vaso-dilatory effect on blood vessels (Rupnow *et al.*, 2001) which consequently raises the velocity and volume of blood flow through MUA as compared to pregnant cows (Kim-Engloff *et al.*, 2016). However, higher blood flow volume in pregnant cows as compared to

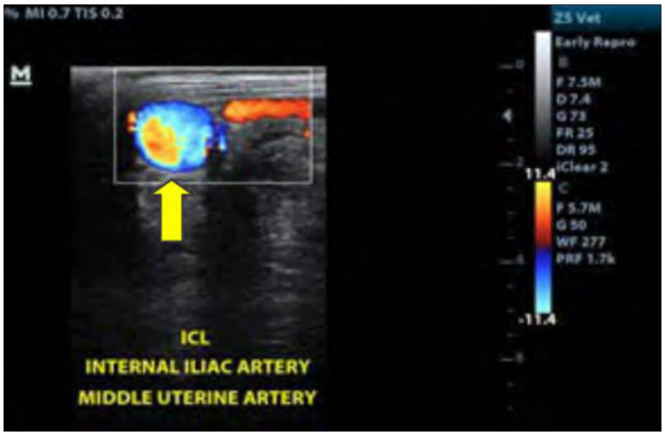

Fig. 1. Middle uterine artery (left- yellow arrow) and middle uterine vein (right)

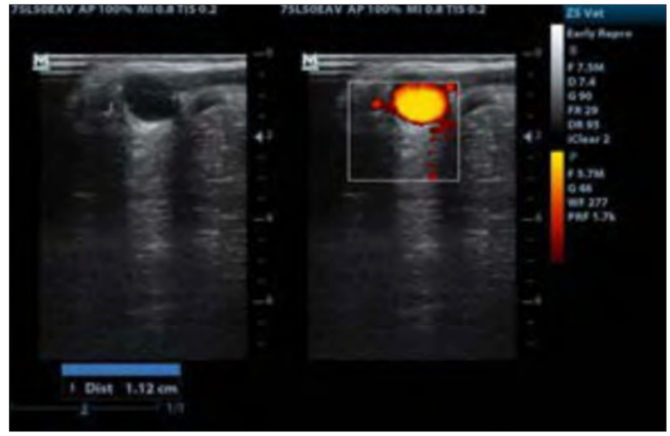

Fig. 2. Cross sectional image of middle uterine artery

Fig. 3. Spectral Doppler image of middle uterine artery (i) in pregnant cows (a), cows with embryonic mortality (b), non-pregnant (c)

Table 1. Spectral Doppler sonography of middle uterine artery (MUA) in cows diagnosed pregnant, non-pregnant and with embryonic mortality on Day 21 post insemination

Parameters	Cows	MUA ipsi-lateral to CL (i)	MUA contra-lateral to CL (c)
Pulsatility index	Pregnant (n=12)	1.14±0.28 (0.18-3.6)	0.66±0.10 (0.21-1.34)
	Embryonic mortality (n=4)	1.16±0.34 (0.52-1.71)	0.54±0.17 (0.23-0.82)
	Non-pregnant (n=6)	0.43±0.17 (0.15-1.22)	0.35±0.07 (0.11-0.54)
Resistance index	Pregnant (n=12)	0.51±0.07 (0.14-0.99)	0.41±0.05 (0.19-0.71)
	Embryonic mortality (n=4)	0.46±0.05 (0.29-0.54)	0.29±0.07 (0.15-0.41)
	Non-pregnant (n=6)	0.19±0.02 (0.11-0.29)	0.24±0.04 (0.10-0.37)
Vascular perfusion index	Pregnant (n=12)	0.49±0.07 (0.01-0.86)	0.59±0.05 (0.29-0.81)
	Embryonic mortality (n=4)	0.54±0.05 (0.46-0.71)	0.71±0.07 (0.59-0.85)
	Non-pregnant (n=6)	0.81±0.02 (0.71-0.89)	0.76±0.05 (0.63-0.90)
Time average mean velocity (cm/sec)	Pregnant (n=12)	6.89±0.79 (2.16-11.09)	6.93±0.96 (3.64-11.86)
	Embryonic mortality (n=4)	4.96±1.47 (2.16-8.54)	5.87±1.49 (3.12-8.24)
	Non-pregnant (n=6)	7.44±1.32 (2.95-12.8)	6.54±1.09 (4.8-10.51)
Time average maximum velocity (cm/sec)	Pregnant (n=12)	13.57±1.01(7.07-19.44)	15.23±1.45 (8.69-27.01)
	Embryonic mortality (n=4)	10.75±1.58 (7.07-14.19)	12.39±1.11 (8.25-15.24)
	Non-pregnant (n=6)	17.57±2.10^{x}(13.82-25.56)	11.36±1.23^{y} (8.05-14.58)
Blood flow volume-TAMEAN (ml/min)	Pregnant (n=12)	365.89±45.06 (120.73-592.10)	252.19±31.43 (124.29-439.55)
	Embryonic mortality (n=4)	301.52±112.78 (84.24-618.47)	209.58±74.11 (98.81-350.26)
	Non-pregnant (n=6)	405.17±62.07 (186.96-555.61)	302.72±61.74 (137.24-463.47)
Blood flow volume- TAMAX (ml/min)	Pregnant (n=12)	715.02±53.49 (538.25-1067.55)	568.23±63.66 (260.95-958.32)
	Embryonic mortality (n=4)	609.85±109.81 (364.68-898.74)	433.61±113.53 (261.27-647.81)
	Non-pregnant (n=6)	1003.93±157.48^{a} (483.88-1619.94)	515.04±76.69^{b} (289.31-764.52)
Cross-sectional area (cm^2)	Pregnant (n=12)	0.92±0.07 (0.61-1.41)	0.72±0.10 (0.28-1.33)
	Embryonic mortality (n=4)	0.98±0.19 (0.65-1.41)	0.57±0.07 (0.48-0.71)
	Non-pregnant (n=6)	0.96±0.12 (0.55-1.31)	0.85±0.16 (0.44-1.58)
Systole/diastole ratio	Pregnant (n=12)	2.52±0.45 (1.17-6.28)	1.87±0.21 (1.23-3.5)
	Embryonic mortality (n=4)	1.59±0.24 (1.00-2.08)	1.36±0.06 (1.25-1.47)
	Non-pregnant (n=6)	1.27±0.04 (1.13-1.42)	1.34±0.08 (1.11-1.58)

Values with different superscripts (a, b) within a row for a particular parameter differ significantly (P<0.01) and (x, y) differ significantly (P<0.05) cows with embryonic mortality can be attributed to coping up with the nutritional and metabolic demands of the developing embryo which is in unison with our findings (Bollwein *et al.*, 2002; Panarace *et al.*, 2006; Satterfield *et al.*, 2010; Varughese *et al.*, 2013). With the collapse of the placental unit and the absence of neovascularization in cows with embryonic mortality, a reduction in blood flow volume occurs.

The higher cross-sectional area of the ipsilateral as compared to contra-lateral MUA in non-pregnant and embryonic mortality diagnosed cows has been attributed to the vaso-dilatory effect of estrogen during re-initiation of cyclicity (Bollwein *et al.*, 2002; Sharma *et al.*, 2019) and activation of uterine clearance mechanism and greater perfusion for laying new endometrial layer following late embryonic mortality, respectively. As far as higher systole diastole ratio is concerned,the expansion of embryonic membranes, the formation of placental bed and subsequent, greater impedance in blood vessels can contribute towards these findings in pregnant cows.

CONCLUSION

The analysis of spectral Doppler indices PI, RI, VPI, TAMEAN, TAMAX, BFV-TAMEAN, BFV-TAMAX and the cross-sectional area of the middle uterine artery gives a non-invasive, non-manipulative and accurate determination of the functional reproductive status in dairy cows. It can therefore be used for the identification of pregnancy, embryonic mortality or re-breeding cows as early as 21 days post insemination. Therefore spectral Doppler analysis of the middle uterine artery has the potential for changing the dynamics of the economy in large-scale dairy farms.

REFERENCES

Abdelnaby, E.A. (2020). Hemodynamic changes in arterial flow velocities throughout the first six months of pregnancy in buffalo heifers by Doppler ultrasonography. *Asian Pacific J. Reprod.* **9(4)**: 204.

Bollwein, H., Baumgartner, U. and Stolla, R. (2002). Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenol.* **57(8)**: 2053-2061.

Bollwein, H., Weber, F., Woschee, I. and Stolla, R. (2004). Transrectal Doppler sonography of uterine and umbilical blood flow during pregnancy in mares. *Theriogenol.* **61(2-3)**: 499-509.

Ginther, O.J. (2014). How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. *Theriogenol.* **81(1)**: 112-125.

Ginther, O.J., Silva, L.A., Araujo, R.R. and Beg, M.A. (2007). Temporal associations among pulses of 13, 14- dihydro-15-keto-PGF2 alpha, luteal blood flow, and luteolysis in cattle. *Biol. Reprod.* **76(3)**: 506-513.

Gupta, U., Qureshi, A. and Samal, S. (2009). Doppler velocimetry in normal and hypertensive pregnancy. *Inter. J. Gynae. Obst.* **11(2)**: 1528-8439.

Herzog, K. and Bollwein, H. (2007). Application of Doppler ultrasonography in cattle reproduction. *Reprod. Domest. Anim.* **42(2)**: 51-58.

Honnens, A., Voss, C., Herzog, K., Niemann, H., Rath, D. and Bollwein, H. (2008). Uterine blood flow during the first 3 weeks of pregnancy in dairy cows. *Theriogenol.* **70(7)**: 1048-1056.

Jaffe, R. (1995). Colour Doppler imaging in the evaluation of normal and abnormal early uteroplacental circulation and gestational outcomes. *Theriogenol.* **43(1)**: 121-127.

Kim-Egloff, C., Hassig, M., Bruckmaier, R. and Bleul, U. (2016). Doppler sonographic examination of uterine and placental perfusion in cows in the last month of gestation and effects of epidural anesthesia and isoxsuprine. *Theriogenol.* **85(5)**: 986-998.

Melber, D.J. and Ballas, J. (2021). Clinical applications for Doppler ultrasonography in obstetrics. *Current Radiol. Repor.* **9(2)**: 1-10.

Panarace, M., Jauregui, G., Garnil, C., Segovia, A., Gutierre, J., Lagioia, J.J., Marfil, M., Rodriguez, E. and Medina, M. (2006). Color Doppler evaluation of umbilical blood flow during pregnancy in cows. *Reprod. Fertil. Develop.* **19(1)**: 323-33.

Pinaffi, F.L.V., Araujo, E.R., Silva, L.A. and Ginther, O.J. (2017). Color -Doppler signals of blood flow in the corpus luteum and vascular perfusion index for ovarian and uterine arteries during expansion of the allantochorion in *Bos taurus* heifers. *Theriogenol*. **102**: 35-43.

Rawy, M., Mido, S., Ali, H.E.S., Derar, D.E., Meghaded, G.E., Kitahara, G. and Osawa, T. (2018). Effect of exogenous estradiol benzoate on uterine blood flow in postpartum dairy cows. *Anim. Reprod. Sci.* **192**: 136-145.

Rupnow, H.L., Phernetton, T.M., Shaw, C.E., Modrick, M.L., Bird, I.M. and Magness, R.R. (2001). Endothelial vasodilator production by uterine and systemic arteries-estrogen and progesterone effects on eNOS. *American J. Physiol.* **280(4)**: 1699-1705.

Satterfield, M.C., Bazer, F.W., Spencer, T.E. and Wu, G. (2010). Sildenafil citrate treatment enhances amino acid availability in the conceptus and fetal growth in an ovine model of intrauterine growth restriction. *J. Nutrit.* **140(2)**: 251-258.

Sharma, A., Singh, M., Kumar, P., Sharma, I., and Rana, A. (2019). Mid-estrus uterine blood flow in endometritic and non-endometritic dairy cows using transrectal Doppler ultrasonography. *Biolog. Rhythm Res.* https://doi.org/10.1080/09291016.2019.1613792.

Silva, L.A. and Ginther, O.J. (2010). Local effect of the conceptus on uterine vascular perfusion during early pregnancy in heifers. *Reprod.* **139(2)**: 453-463.

Silva-del-Rio, N., Colloton, I.D. and Fricke P.M. (2009). Factors affecting pregnancy loss for single and twin pregnancies in a high-producing dairy herd. *Theriogenol.* **71(9)**: 1462-1471.

Singh,G., Chandolia, R.K., Dutt, R., Saini, A. and Malik, R.K. (2018). Characteristics of middle uterine artery and umbilical blood flow in pregnant Murrah buffalo. *Indian J. Anim. Reprod.* **39(1)**: 11-14.

Stevenson, J.S. (2005). Breeding strategies to optimize reproductive efficiency in dairy herds. *Vet. Clin. North Am. Food Anim. Pract.* **21(2)**: 349-365.

Varughese, E.E., Brar, B.S. and Dhindsa, S.S. (2013). Uterine blood flow during various stages of pregnancy in dairy buffaloes using transrectal Doppler ultrasonography. *Anim. Reprod. Sci.* **140(1-2)**: 34-39.