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Doppler Sonography of Umbilical Artery Throughout Pregnancy in Sheep

Sandeep Kumar[1]*, Ramesh Kumar Chandolia[1], Anand Kumar Pandey[1], Pradeep[1], Hitesh[1], Gyan Singh[1] and Gitesh Saini[2]

[1]Department of Veterinary Gynaecology and Obstetrics, COVS, LUVAS
[2] Department of Veterinary Gynaecology and Obstetrics, COVS, Rampura Phul, GADVASU

ABSTRACT

The Color Doppler study on 6 pregnant ewes was conducted twice a week from Days 20 to 60 post-mating, then at weekly intervals till the end of experiment by the transrectal and transabdominal ultrasonography. In order to evaluate the waveforms and velocity blood flow patterns, peak systolic velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI) and resistive index (RI), were measured. Data were nalysed by employing SPSS 21. The PSV and EDV increased from 38 to 139 days of gestation. The PI and RI of blood flow showed a non-significant decrease throughout gestation.

Key words: Sheep, Pregnancy, Doppler ultrasonography, Umbilical artery

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INTRODUCTION

Sheep have been frequently taken as biomedical model organisms in human obstetrics, especially related to foetal growth restriction and other pathological conditions. Pregnancy related veterinary studies in large domestic animal species are mainly concentrated on horses and cattle. The uterine and placental blood flow increases during pregnancy to supply the metabolic requirements of the growing foetus (Reynolds *et al.*, 2005). Therefore, tracking blood flow during pregnancy would enable us to obtain information about foetal development. Doppler ultrasonography (USG) is an imaging method that is helpful in monitoring the functional status of the foetal-placental unit and provides valuable information about the physiological and pathological differences in circulation between the mother and foetus (Fleischer *et al.*, 1994). Abnormal vascular findings in foetal and/or maternal compartments may be indicators of intrauterine growth restriction, foetal distress, or early pregnancy failure (Ozkaya *et al.*, 2007; Abdelheim *et al.*, 2013).

In particular, in sheep, the works about foeto-placental hemodynamic parameters (PSV, EDV, PI and RI) are related only to certain parameters pulsatility index (PI) and resistive index (RI) (Yilmaz *et al.*, 2015) or certain periods of pregnancy (Schoennagel *et al.*, 2014). But, the sequential

*Corresponding author.
E-mail address: sandeeppanihar48@gmail.com (Sandeep Kumar)
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scanning at short intervals throughout the pregnancy was lacking. So, aim of this study was to record the umbilical artery Doppler flowmetric indices in ewe, throughout the pregnancy, in order to contribute in increasing the knowledge on the vascular physiology in ovine pregnancy.

MATERIALS AND METHODS

The study was conducted on 6 pregnant Harnali ewes (2-2.5 years of age) maintained at sheep breeding farm, Dept.of AGB, LUVAS, Hisar during 2017-18. Following confirmation of pregnancy, sequential ultrasonographic examinations were performed from Day 20 and until lambing. The scanning was conducted twice a week from days 20 to 60 post-mating, then at weekly intervasl till the end of experiment. Examinations by the transrectal technique (days 20 to 70) and transabdominal ultrasonography (days 35 to 139) were performed using a Toshiba (Nemio XG) 3D machine with micro convex transrectal and convex transducer, 7.5 and 5.0 MHz frequency respectively.

Technique of Doppler Ultrasonography

For the Doppler ultrasonography, conventional two-dimensional ultrasound was performed from lower abdominal area around teats. Images of the urinary bladder as a large anechoic spherical structure, was taken as a landmark to view the genital tract in initial stages of image recording. Then the transducer was moved along lateral abdomen close to thighs over the udder region. Whenever there were clear images of the conceptus observed, the colour Doppler function of the machine was turned on. It was necessary to adjust the pulse repetition frequency, colour gain and the power in order to avoid aliasing. Transducer was moved in such a way that the Doppler angle made by the blood flow and direction of the ultrasound beam was kept minimum. The color Doppler function of the ultrasound device was used for detection and visualization of the uterine artery on the basis of red-blue color pattern and umbilical cord have a characteristic saw-tooth appearance of arterial flow in one direction and continuous umbilical venous blood flow in the other. Keeping the transducer constant, pulsed wave Doppler was started and gate size was adjusted such that sample volume was exactly in the centre of blood flow. Whenever there was clear waveform of the particular vessel, colour Doppler was switched off leaving the pulsed wave Doppler updating two-dimensional image such that error was minimum. The umbilical blood flow was recorded at every examination**.** The extent and direction of blood flow in the umbilical artery were indicated by colour signals (red or blue). In order to evaluate the waveform and velocity blood flow patterns, PSV, EDV, PI, and RI were measured. Recordings were obtained for at least three regularly consecutive arterial waveforms. Waves formed during foetal and maternal movements were disregarded. After getting waveform, images were frozen and track ball was rotated anticlock wise to review cine loop and desired waveform was saved in the machine itself for the offline analysis.

Statistical Analyses

Data were analysed using SPSS 21 and are expressed as the mean ± standard error of mean (S.E.M). Simple one-way ANOVA was used to study the effect of age on different cord Doppler flow metric parameters and Duncan's multiple range test was used to differentiate between significant means. Differences with values of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Serial ultrasonographic examinations were conducted twice a week from Days 20 to 60, and weekly between 60 and 139 days post-mating. Mean duration of pregnancy was 147 days (range: 146–150 days). All lambing occurred normally without assistance.

Doppler ultrasonography is a diagnostic image technique that allows for obtaining values for several hemodynamic variables of blood vessels, frequently utilized in human and animal obstetrics for the evidence of physiological changes in uterine hemodynamic profile. Doppler examinations are used commonly to monitor high risk pregnancy in human medicine (Dubiel *et al*., 2003) and canine (Simon *et al.,* 2022). Various foetal blood flow waveform evaluations have been performed to judge the foetal wellbeing, normal and abnormal placental circulation, intrauterine growth restriction of foetus and various foetal abnormalities during antenatal development. In veterinary medicine, there are only few reports that have examined the blood flow pattern in the umbilical cord in sheep (Lemley *et al*., 2012), goat (Kumar, 2014) and bitch (Di-salvo *et al*., 2006, Scotti *et al.*, 2008 and Simon *et al.,* 2022).

In the present study, umbilical artery was detected first time during study on day 38 of pregnancy and flow velocity patterns of umbilical vein and artery were recorded. The umbilical artery was the first foetal vessel to be evaluated by Doppler velocimetry in human medicine. In the present study, flow velocity waveforms from the umbilical cord have a characteristic saw-tooth

appearance of arterial blood flow in one direction and umbilical venous blood flow on the other side of baseline as continuous wave (Fig. 1). The umbilical vein blood flow pattern was almost flat with slightly wavy margin during all weeks of gestation without any abnormality with foetal outcome. These observations are in accordance with previous studies inwomen (Dubiel *et al*., 2003), in bitch (Di-salvo *et al*., 2006), in queen (Scotti *et al*., 2008) and in goat (Serin *et al*., 2010 and Kumar, 2014).

Fig. 1. Colour Doppler image showing pattern of blood flow in umbilical artery and umbilical vein which is present above and below the baseline respectively in spectral mode.

The umbilical cord waveforms were identified by the simultaneous presence of arterial and venous flow. This was in agreement with Kumar (2014). The umbilical artery blood flow was characterized only by the systolic waveform until the Day 59 of pregnancy and diastolic component was observed after Day 62nd of pregnancy. There is no similar study in this area. However, Serin *et al.* (2010) detected umbilical artery systolic waveform in goat from Days 40 to 85 of gestation and diastolic waveforms were observed after Day 85 of gestation, whereas Kumar (2014) observed the systolic waveform only until 69th day of pregnancy in umbilical blood flow. This difference may be due to species differences.

The peak systolic velocity (PSV) increased from Day 38 (45.02 ± 1.43 cm/sec.) to Day 139 (92.5 ± 1.74cm/sec.) of gestation with a significant ($p<0.05$) increase onDay 104 (82.48 ± 1.77 cm/sec.) of gestation. However, the significant increase was also observed on alternate week except 10 to 13 weeks and 18 to 20 weeks of gestation (Fig. 2). The end diastolic velocity (EDV) increased non-significantly ($p<0.05$) at every week of gestation and value increased from Day 38 (9.21 ± 2.56cm/sec.) to Day 139 (53.86 ± 5.49cm/sec.) of gestation (Fig. 2).

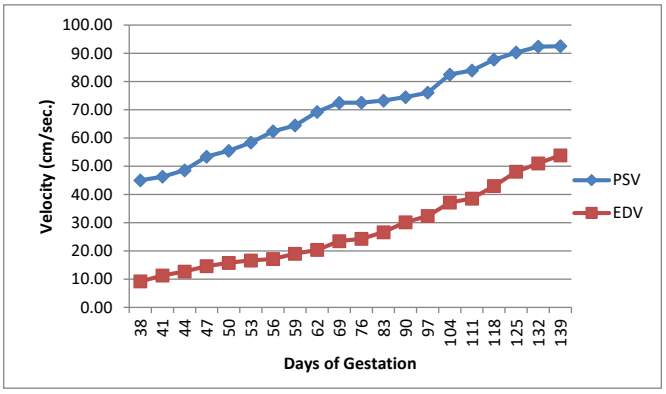

Fig. 2. Line diagram showing ultrasonographic changes in PSV and EDVof umbilical artery in relation to gestational age in days

In the current study, peak systolic velocity (PSV) and end diastolic velocity (EDV) increased significantly and non-significantly, respectively from Days 38 to 139 of gestation. Parallel data is not available in the ewe. However, this was in agreement with Di-salvo *et al*., (2009) in the bitch. Similarly, Scotti *et al.* (2008) and Polisca *et al.* (2010) observed the same pattern of waveforms in queen and rabbit, respectively. However, Kumar (2014) observed increasing pattern only in PSV in goat.

The resistive index (RI) showed a non-significant ($p<0.05$) decrease throughout the gestation from Day 38 (0.8 ± 0.05) to Day 139 (0.42 ± 0.05) of gestation (Fig. 3). The similar trend was also observed in the case of pulsatile index (PI) (Fig.3). The value of PI (Mean ±SE) decreased non-significantly from Day 38 (3.98 ± 0.35to Day 139 (0.7 ± 0.13) of gestation.

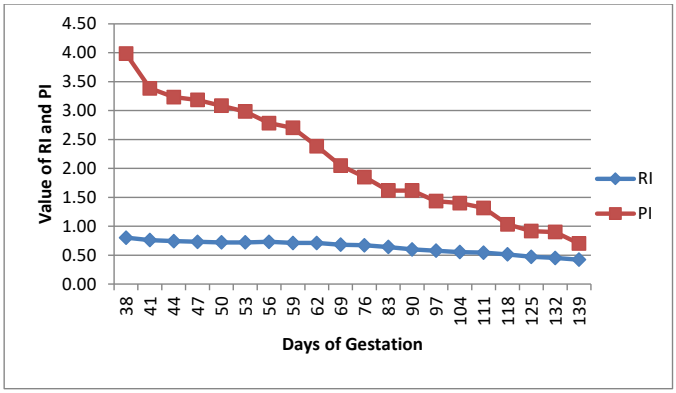

Fig. 3. Line diagram showing ultrasonographic changes in PI and RI of umbilical artery in relation to gestational age in days

The PI and RI values decreased almost non-significantly from Days 38 to 139 of gestation. This was in agreement with Di-salvo *et al.* (2009) in bitch, Scotti *et al.* (2008) in queen and Polisca *et al.* (2010) in rabbit. However, Kumar (2014) observed that PI increased from Days 39 to 60 of gestation and then decreased up to 90 days of gestation in goats. Similar findings were reported in

goat by Serin *et al.* (2010).Whereas the RI values decreased non-significantly throughout gestation according to Kumar (2014).

Physiologically the uterine and umbilical blood flow increases continuously during pregnancy to meet the increases in foetal nutrient demand and placental growth (Meschia 1983; Reynolds *et al.* 1986), which was also true in the present study. In women, increased blood flow and velocity are supposed to be due to physiological changes in maternal spiral arteries in addition to altered blood viscosity and peripheral resistance (Lees *et al.,* 1967). An increased blood viscosity has been recognized in pregnant sheep which were analysed at a gestational age of 120-130 days (Stow *et al.,* 2003). Surplus, hormonal secretion (oestrogen and/or progesterone), which is necessary for pregnancy maintenance, has been mentioned as a regulatory factor (Rosenfeld *et al.,* 1974; Ford *et al.,* 1979; Caton and Wilcox, 1980; Ford, 1982; Reynolds *et al.,* 1984; Magness and Rosenfeld, 1989; Magness*et al.* 1993). In a study by Elmetwally and Meinecke-tillmann (2012) in sheep, these impedance indices (PI, RI) decreased during pregnancy until parturition. Furthermore, the results clearly showed a significantly negative correlation between these impedance indices and blood flow volume in sheep. This indicates an inverse relationship between uterine BFV and blood flow impedance indices. Similar results werealso reported by Scotti*et al.* (2008) in queen. The decrease in resistance maintains the increased uterine blood flow volume during later stages of normal pregnancy so as to meet the foetal demands for different nutrients as well as oxygen.

CONCLUSIONS

Color Doppler evaluation of the blood flow of umblical artery during different stages of pregnancy were useful to correlate development of foetus in uterus. These indices can be helpful to assess normal development of foetus.

CONFLICT OF INTEREST

The authors declare bo conflict of interest among them-selves.

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