Applications of Doppler ultrasonography in reproductive health and physiology of small ruminants

Aplicações da ultrassonografia Doppler na fisiologia e saúde reprodutiva de pequenos ruminantes

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Abstract

Major applications of Doppler ultrasonography for evaluating reproductive potential of healthy sheep and goats are discussed in this mini-review. Basic principles of Doppler imaging and examination techniques used are also briefly described. In ewes and does, Doppler ultrasonography has primarily been used in reproductive research involving superovulatory treatments and fetal development. In both species, color Doppler sonography has proven to be an accurate non-invasive method of detecting luteal structures and monitoring changes in luteal status/dynamics during the interovulatory period and pregnancy. Studies in rams have documented changes in testicular blood supply during the period encompassing the onset of puberty as well as seasonal fluctuations in velocimetric indices of testicular arteries and their relationships to spermatogenesis/semen quality, testicular volumes and circulating concentrations of reproductive hormones. Quantitative correlations between high-velocity antral follicular blood flow and superovulatory yields in ewes promise to provide a reliable non-invasive method to predict superovulatory outcomes. Umbilical cord hemodynamics are a useful marker of fetal development and health status. However, the relationships among velocimetric indices of major supplying arteries and ovarian/testicular or mammary gland function are still equivocal and require additional confirmatory studies of multiple influencing factors.

Keywords: sheep, goats, Doppler ultrasonography, puberty, pregnancy, superovulation, estrous cycle.

Introduction

Ultrasonography is one of the most commonly used techniques in animal diagnostics and research as it permits frequent, real-time, non-invasive, non-ionizing and non-destructive visualization of internal organs and tissues. In addition, the Doppler modality of ultrasound imaging allows for blood flow detection and quantification. Doppler ultrasonography has frequently been used in reproductive management of large animals, mainly cattle and horses. Application of Doppler ultrasonography in females of small ruminant species is significantly less widespread due mainly to difficulties associated with transrectal and transcutaneous detection and visualization of internal reproductive organs. Color-flow images are used to examine ovarian follicular blood flow, luteal vasculature and blood distribution in male genitalia. Spectral graphs are used to assess ovarian activity, blood flow in the uterus and fetus as well as spermatic cord hemodynamics. In addition, Doppler ultrasonography is an invaluable tool in the diagnosis of several pathological conditions in males and females including, but not limited to, persistent anovulatory follicles (ovarian cysts), spermatic cord traction or testicular/epididymal cancer. The main purpose of this mini-review is to highlight major functional correlates of blood flow indices obtained with the color and pulsed wave/spectral Doppler ultrasonography of reproductive organs in small ruminants. Basic principles of Doppler ultrasonography are discussed at the outset.

Physical principles of Doppler sonography

Blood flow can be displayed in color superimposed on a two-dimensional, grey-scale ultrasonographic image (color Doppler) or as a graph depicting pulsed Doppler-spectral analysis of blood velocity in a small area of a large blood vessel (spectral Doppler; Ginther, 2007). In both instances, the technology is based on Doppler-shift frequencies wherein the ultrasound beams reflected by moving erythrocytes vary as the cells move with different speed and also if they move toward or away from the ultrasound transducer. The angle at which ultrasound beams encounter the blood vessel or moving blood is called the Doppler angle or angle of insonation. The blood flow velocities and other indices computed during the Doppler scanning are a function of Doppler-shift frequencies and Doppler angle. When the direction of blood flow is towards the transducer, the echoes reflected by blood back to the transducer have a higher frequency than those emitted from the transducer and vice versa. A Doppler angle of 90° produces the weakest Doppler signals and an angle of 0°/180° is the most efficient angle for blood flow assessment. A Doppler angle of <60° is typically set and consecutive spectral waveforms/cardiac cycles are obtained; the Doppler gate is positioned inside the blood vessel and a spectral mode waveform is taken for calculating an array of velocimetric indices recorded during the systolic and diastolic stages.

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Doppler examination of the reproductive tract in non-pregnant ewes and goats

Apart from wound healing and certain pathological processes (e.g., cancer, edema), the vascular system of the adult is generally quiescent. The exceptions to this rule are ovarian structures and conceptuses in the gravid uterus. Intense angiogenesis and increased vascular permeability are markers of antral follicular growth and maturation, ovulation, luteogenesis, implantation and placentation. In the past, the most widely employed approach to study changes in angiogenesis was to use histological sections of the organs in which endothelial cells were stained with a specific angiogenic marker. Objective measurements of blood flow in capillary networks draining reproductive organs can be achieved non-invasively by color Doppler ultrasonography combined with computerized image analyses and arterial blood supply can be assessed by pulsed/spectral Doppler (Petridis et al., 2017).

Transrectal Doppler ultrasonography of the reproductive tract in ewes and goats can be accomplished using a 6.0-8.0–MHz probes in conscious animals restrained in a standing positon or dorsal recumbency. Transabdominal technique has successfully been used to monitor blood flow in the uterine artery of standing ewes (Ioannidi et al., 2017). If the diagnostic system/ultrasound scanner is equipped with a dual screen mode, Doppler readings can be obtained simultaneously to B-mode imaging; initial detection and localization of internal organs of interest is sometimes easier with the B-mode scanning. Evaluation of blood flow in the ovarian structures can also be performed with power Doppler. Power Doppler sonography is a new scanning modality that displays in color the strength of the Doppler signal rather than the speed and direction of blood flow. Therefore, it is approximately three times more sensitive than conventional color Doppler in detecting blood flow and hence is particularly useful for small blood vessels and those with low-velocity flow (El-Sherry et al., 2013). Still images or recordings obtained during power/color Doppler examinations can be stored for analyses with various image analytical programs at a later date. The vascularized (colored) area of an ultrasonogram may be expressed as a proportion or percentage of the total cross-sectional area of the organ or tissue, and pixels corresponding to various blood flow velocities can be enumerated (El-Sherry et al., 2013; Oliveira et al., 2014; Figueira et al., 2015).

Color Doppler ultrasonography has the makings of a practical tool to assess luteal function in sheep. The luteal vascularization area is positively correlated with plasma progesterone (P_4) concentrations of during corpus luteum (CL) formation and regression (Figueira et al., 2015). Therefore, color Doppler imaging can be used to evaluate the health status of CL at specific stages of the interovulatory interval and during early pregnancy in sheep and goats.

Ovarian blood supply during the superovulatory treatment

Quantitative relationships between antral follicular vasculature determined by color Doppler sonography and superovulatory yields in Santa Inês ewes were studied by Oliveira et al. (2014, 2017). There was a positive correlation between the quantitative estimates of follicular blood flow (color Doppler area (DA) and color Doppler area percentage (DA/TAx100%) computed for each ovary; TA=total cross-sectional area of the ovary) on the final day of the 4-day superovulatory treatment and the number/percentage of unfertilized eggs retrieved from superovulated donor ewes. Moreover, the number of “high-velocity pixels” (HPVs; 0.055-0.11 m/s) on Day 1 correlated directly with the number of corpora lutea (CL) and of transferable embryos. Correlations were also recorded between the number of HVPs on Day 3 and the embryo recovery rate, viability rate as well as percentage of degenerated embryos. The percentage of HVPs relative to the total area of ovarian cross section was correlated with the number of CL and of viable embryos on Day 1 and with the recovery rate on Days 0 and 3. It was concluded that sonographic assessment of high-velocity antral follicular blood flow was a useful method to predict the outcome of the superovulatory treatment in ewes.

B-mode or color Doppler ultrasonography on the day of embryo recovery and those observed with provides a means to accurately enumerate luteal structures in superovulated ewes (Oliveira et al., 2018a; Pinto et al., 2018). The use of color Doppler imaging was associated with increased accuracy of CL detection. compared with grey scale ultrasonography (82.3% vs 73.6%; Fonseca et al., 2019). Even though Pinto et al. (2018) showed that sensitivity of the color Doppler sonography decreased when more than four CL were present, those results support the notion that color Doppler can effectively be used for estimating ovarian responses in superovulated ewes and does. Noteworthy, color Doppler imaging permits the detection of both normal CL and luteinized unovulated follicles. Although the color Doppler technique allows for monitoring the hemodynamic changes in ovarian structures, it was not possible to visually identify vascular differences between normal and prematurely regressing CL (Oliveira et al., 2018a). The latter warrants further studies of the computerized analyses of color Doppler images to non-invasively distinguish different types of luteal structures.

Recently, a study has been undertaken to examine if blood flow indices of ovarian arteries determined during the superovulatory treatment were correlated with antral follicular development, ovarian responses and embryo yields in Santa Inés ewes pre-treated with progesterone/estradiol benzoate to synchronize follicular wave emergence prior to superovulatory regimen (Oliveira et al., 2018b). It was hypothesized that since superovulation propels the growth and metabolic activity of multiple antral follicles, the ovaries of animals undergoing hormonal ovarian superstimulation would require a significantly greater blood supply. The mean number of large (>4.5 mm in diameter) antral follicles was greater on Days 3 and 4 compared with Day 2 and was greater on Day 3 compared

with Day 1 of the superovulatory treatment. Mean peak systolic velocity (SVp) values increased from Day 1 to Day 4 by 38%, and mean velocity (Vm) as well as end-diastolic velocity (EDV) rose significantly from Day 2 to Day 4 of the superovulatory regimen (by 57% and 75%, respectively). However, there was a lack of consistency between the left and right ovary/uterine horn in the velocimetric correlates of antral follicle numbers and superovulatory responses. Therefore, the usefulness of spectral Doppler indices of ovarian arteries to consistently and accurately predict superovulatory yields in sheep remains highly equivocal.

Doppler examinations in pregnant ewes and goats

The uterine blood flow volume (BFV) in sheep and goats increased approximately 60 fold until the end of pregnancy, with a rapid increase before and a slow increase after gestational week 18 (gw18; Elmetwally et al., 2016). Time averaged maximum velocity in the uterine artery (UtA) increased throughout pregnancy in sheep and goats whereas time averaged mean velocity decreased at gw18/20 in both species. Furthermore, there was an effect of the stage of pregnancy on UtA resistance index (RI) and pulsatility index (PI); both indices decreased until the end of gestation.

During the second part of the study, different velocimetric indices of the uterine artery were determined in animals allocated to two groups, namely “more reactive/anxious” (MR) and “less reactive /anxious” (LR) sheep and goats on the basis of repeated Arena tests. In sheep, UtA-PI and Ut-RI were significantly higher in MR as compared to LR ewes especially at gwk 6, 10 and 12. In pregnant goats, UtA-RI at gwk 6, 16, 18 and 20 and UtA-PI at gwk 4, 8, 18 and 20 were significantly influenced by reactivity/anxiety of the dam. Umbilical artery (UMA) RI was higher in MR than LR animals at gwk 14 and 20. It was concluded that although the results of the two parts of the study are supportive of the clinical applicability of Doppler ultrasound as a tool to evaluate the uterine and the umbilical blood flow throughout the pregnancy in small, fetal and uterine changes in blood supply are strongly influenced by the animals’ temperament. Velocimetric indices of the umbilical artery may provide information about the development of the fetoplacental capillary bed; assessment of blood flow can be an early indication of potential fetal growth restriction or placental insufficiency (Serin et al., 2010). In fact, a vast majority of the work aimed to establish normal clinical reference ranges for pregnant women had been performed in an ovine model.

Luteal dynamics in pregnant and non-pregnant Saanen goats were studied using B-mode and color Doppler ultrasonography (Balaro et al., 2017). The assessment of color Doppler images was more reliable in predicting the luteal functionality than CL morphometrics; the number of colored pixels accurately predicted circulating P_4 concentrations >1.0 ng/mL. In the following study, it was shown that color Doppler and B-mode ultrasonographic CL detection on Days 21 and 23 of gestation in goats had similar sensitivity, specificity and accuracy (Consentino et al, 2018).

Doppler examination of the udder

Velocimetric evaluation of the external pudendal artery (PA), the main artery of the udder, can be performed using a 5.0-8.0−MHz transducer at a 60-mm scanning depth in restrained sheep and goats examined in a standing position (Petridis et al., 2017). Blood flow into the mammary gland increases progressively with lactogenesis and declines during the mammary gland involution. However, there is a paucity of information on the relationships between PA blood flow parameters and milk yields.

Doppler examination of the male genital system

Spectral Doppler scans of testicular arteries (TA) were performed in peri- and post-pubertal Dorper rams using a 7.5-MHz probe (Camela et al., 2017). There were no significant shifts in any TA velocimetric charactericts at the time of puberty but ejaculate volume was negatively correlated with PI of testicular arteries. Most recently, Hedia et al. (2019) have documented changes in velocimetric indices of TA in fat-tailed rams over a 12-month period, and examined them for temporal associations with testicular volume, plasma steroid concentrations and semen characteristics. Mean values for resistive index (RI) were lowest between September and March, increased significantly in April and reached maximum vlues in June. The average pulstaily index (PI) values in TA of rams increased significantly in March ad then reached a peak in the month of July. The authors concluded that significant increases in testicular blood flow volume (i.e., a decline in both RI and PI) during the breeding season were due mainly to the marked rise in testicular volume and plasma estradiol-17β concentrations recorded during that period. In addition, both the RI and PI values were strongly and negatively correlated with sperm cellconcentration and progressive motility.

Concluding remarks

Doppler ultrasonography in combination with B-mode ultrasound imaging is as extremely useful clinical tool. With the inevitable advancements in imaging technology and ultrasonographic equipment, it will become a widely used non-invasive diagnostic method in small ruminant reproduction. However, its application and benefits in the field of reproductive research progresses rather slowly compared with those of real-time, grey-scale ultrasonography and computer-assisted image analysis. At present, power and color Doppler modalities employed to study ovarian blood perfusion and umbilical cord velocimetric parameters appear to offer the greatest value, and can be used to monitor and predict structural and functional changes in ovarian structures and fetoplacental units, respectively.

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