

## Doppler ultrasonography principles and methods of evaluation of the reproductive tract in mares

Jair Camargo Ferreira<sup>1</sup>, Fernanda Saules Ignácio<sup>2</sup> & Cezinande de Meira<sup>2</sup>

### ABSTRACT

**Background:** Doppler ultrasonography is a non-invasive real time pulse-wave technique recently used for the transrectal study of the reproductive system hemodynamics in large animals. This technic is based in the Doppler Effect Principle that proposes the change in frequency of a wave for an observer (red blood cells) moving relative to the source of the respective wave (ultrasonic transducer). This method had showed to be effective and useful for the evaluation of the *in vivo* equine reproductive tract increasing the diagnostic, monitoring, and predictive capabilities of theriogenology in mares. However, an accurate and truthful ultrasonic exam requires the previous knowledge of the Doppler ultrasonography principles.

**Review:** In recent years, the capabilities of ultrasound flow imaging have increased enormously. The current Doppler ultrasound machines offer three methods of evaluation that may be used simultaneously (triplex mode). In B-mode ultrasound, a linear array of transducers simultaneously scans a plane through the tissue that can be viewed as a two-dimensional gray-scale image on screen. This mode is primarily used to identify anatomically a structure for its posterior evaluation using colored ultrasound modes (Color or Spectral modes). Colored ultrasound images of flow, whether Color or Spectral modes, are essentially obtained from measurements of moving red cells. In Color mode, velocity information is presented as a color coded overlay on top of a B-mode image, while Pulsed Wave Doppler provides a measure of the changing velocity throughout the cardiac cycle and the distribution of velocities in the sample volume represented by a spectral graphic. Color images conception varies according to the Doppler Frequency that is the difference between the frequency of received echoes by moving blood red cells and wave frequency transmitted by the transducer. To produce an adequate spectral graphic it is important determine the position and size of the simple gate. Furthermore, blood flow velocity measurement is influence by the intersection angle between ultrasonic pulses and the direction of moving blood-red cells (Doppler angle). Objectively colored ultrasound exam may be done on large arteries of the reproductive tract, as uterine and ovary arteries, or directly on the target tissue (follicle, for example). Mesovarium and mesometrium attachment arteries also can be used for spectral evaluation of the equine reproductive system. Subjectively analysis of the ovarian and uterine vascular perfusion must be done directly on the corpus luteum, follicular wall and uterus (endometrium and myometrium associated), respectively. Power-flow imaging has greater sensitivity to weak blood flow and independent of the Doppler angle, improving the evaluation of vessels with small diameters and slow blood flow.

**Conclusion:** Doppler ultrasonography principles, methods of evaluation and reproductive system anatomy have been described. This knowledge is essential for the competent equipment acquisition and precise collection and analysis of colored ultrasound images. Otherwise, the reporting of inconsistent and not reproducible findings may result in the discredit of Doppler technology ahead of the scientific veterinary community.

**Keywords:** Doppler ultrasound imaging, hemodynamics, blood-red cells, spectrum, color-flow, power-flow.

<sup>1</sup>Programa de Pós-graduação em Medicina Veterinária, FMVZ, Departamento de Reprodução Animal e Radiologia Veterinária, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, SP, Brasil. <sup>2</sup>Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Departamento de Reprodução Animal e Radiologia Veterinária, Botucatu, SP, Brasil. CORRESPONDÊNCIA: J.C. Ferreira [jaircvet@yahoo.com.br]. Departamento de Reprodução Animal e Radiologia Veterinária, Universidade Estadual Paulista Júlio de Mesquita Filho. CEP18608-210 Botucatu, SP, Brasil.

## I. INTRODUCTION

## II. COLORED ULTRASONIC IMAGING

### 2.1 Spectral mode

### 2.2 Color-flow mode

## III. ARTERIAL SYSTEM OF THE REPRODUCTIVE TRACT

## IV. CONCLUSION

### I. INTRODUCTION

In large animals, Doppler ultrasonography is a non-invasive real time pulse-wave technique recently used for the transrectal study of the reproductive system hemodynamics. The introduction of this technology in current researches has allowed reevaluating previously conceptions considered definitive regarding the physiology of reproduction. This method had showed to be effective and useful for the evaluation of the *in vivo* equine reproductive tract increasing the diagnostic, monitoring, and predictive capabilities of theriogenology in mares [4].

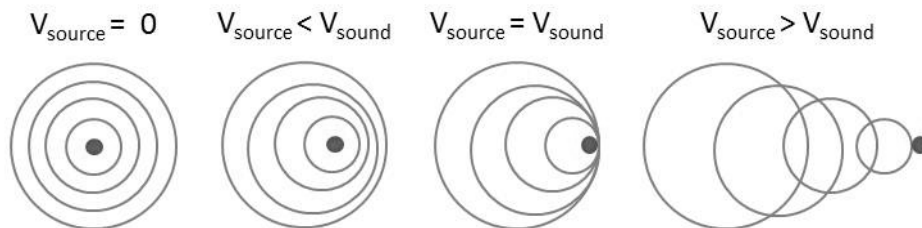
Color-Doppler ultrasonic imaging is based in the Doppler effect principle that proposes the change in frequency of a wave for an observer moving relative to the source of the respective wave (Figure

1). The frequency is constant when the wave source and observer are stationary. However, if there is a moving toward or away from each other, the returning echoes frequency increase and decrease, respectively [7]. At the moment, this principle is used for different applications as temperature, velocity and vibration measurements. In Doppler sonography, the wave source and stationary object are, respectively, the red-blood cells and the transducer.

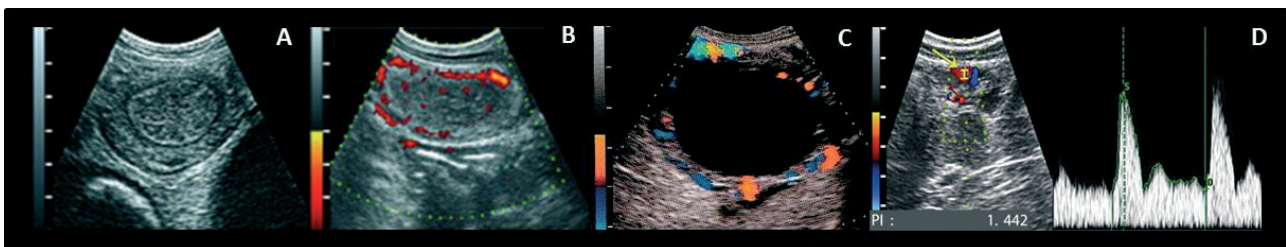
During an ultrasonic doppler exam is possible to perform a qualitatively and/or quantitatively evaluation of specific vessels and tissues. The direction and velocity of red-blood cells can be represented by different types and intensities of colors pixels or as a velocity spectral graphic. However, to select the most appropriated mode of exam first it is necessary to have a complete knowledge of the Doppler ultrasonography principles and methods of evaluation.

### II. COLORED ULTRASONIC IMAGING

There are two specifically approaches for colored ultrasonic imaging evaluation: Color-flow and Spectral modes (Figure 2). Color-flow mode uses Color-Doppler signals superimposes on a B-mode



**Figure 1.** Collection of images representing the Doppler Effect. Wave frequency emitted by a static object ( $V_{source} = 0$ ) relative to the observer is constant, concentric and equally spaced. When the pulse is emitted during relative motion between source and observer, the wave fronts are no longer concentric. Wave frequency of echoes increases and decreases, respectively, during approach and away movements ( $V_{source} < V_{sound}$ ). When the velocity of the object is similar to the sound speed ( $V_{source} = V_{sound}$ ), the crests of waves emitted overlap forming a single ridge that reaches the observer simultaneously with the source. Emitted wave reaches the observer after passing the sound source at supersonic speeds ( $V_{source} > V_{sound}$ ). Font: Personal file.



**Figure 2.** Ultrasonic images collection of female reproductive tract in B, Power, Color and Spectral modes (A, B, C and D, respectively). (A and B) Cross section image, using B-mode and Power-flow Doppler, of a non-pregnant uterine horn in diestrus. (C) Color Doppler evaluation of a pre-ovulatory follicle. (B and C) Presence of colored pixels within uterine tissue (endometrium and myometrium) and follicular wall indicate the red blood cells displacement and have been used to estimate the tissue vascularity. (D) Spectral graph consisting of maximum speeds values of blood flow in mesometrial artery. Source: Personal file.

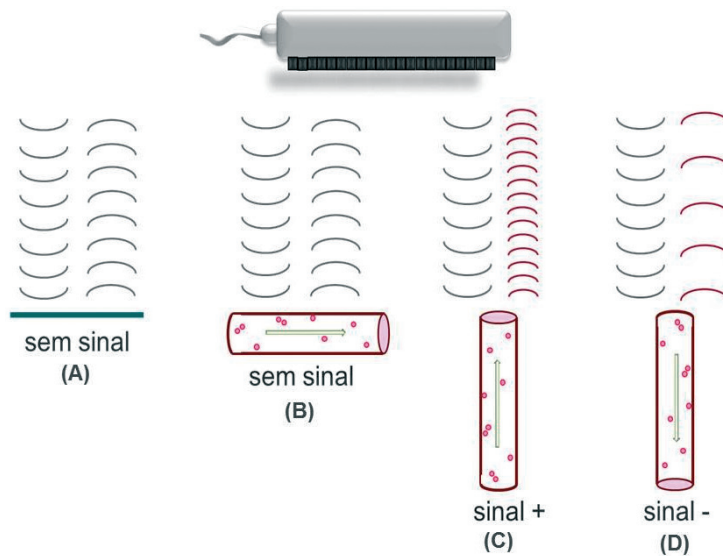
image of a structure to estimate its vascularity, while pulsed-Doppler spectral analysis of blood velocities of a specific artery is done on Spectral mode. Both methods are based on Doppler-shift frequencies, also called Doppler frequency [7].

Doppler frequency is the difference between the frequency of received echoes and frequency transmitted by the transducer (Figure 3). The transducer transmitted frequency is constant while the frequency of the returning echoes varies according to the Doppler Effect principle. Therefore, when the wave source (blood-red cells) is stationary or moving parallel to the transducer there is no difference between transmitted and returning frequencies and colored Doppler signals are not detected. If the blood flow moves toward to the transducer, the returning frequency is greater than the transmitted frequency resulting in a positive Doppler frequency. A negative Doppler signal is produced when the returning frequency is lower than the transmitted frequency or, in other words, when the blood-red cells moves away to the transducer [11].

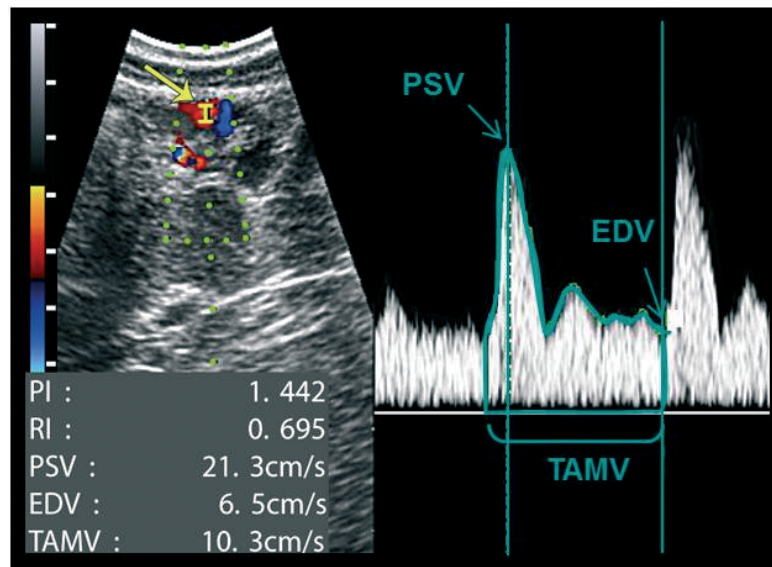
### 1.1 Spectral mode

In the spectral mode, blood flow velocity variations are represented as a graphic wave form called spectrum (Figure 4). The spectrum provides maximum velocities values as Peak systolic (PSV), End diastolic (EDV) and Time-average maximum (TAMV) velocities. PSV is the maximum point along the length of the spectrum, while EDV is the ending point of the cardiac cycle. TAMV is the maximum velocity values average. Additionally, the position of the spectrum in relation to the baseline of the monitor indicates the blood flow direction. By convection, wave forms above and under the baseline indicate, respectively, blood-red cells moving toward and away from the transducer [4].

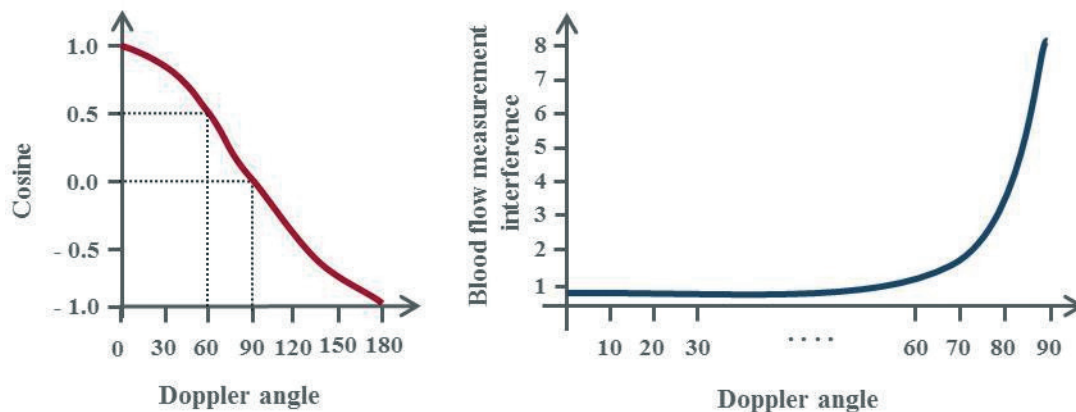
To measure blood velocities is necessary to determine the Doppler angle. Doppler angle or angle of insonation is the angle of intersection of the ultrasonic pulse with the direction of moving blood-red cells (Figure 5). To produce an accurate spectrum is required a doppler angle of 30° to 60°. However, determine the angle of insonation from uterine



**Figure 3.** Illustration of frequency echoes changes during a colored Doppler ultrasonic exam. Doppler frequency varies according to erythrocytes movement in relation to the transducer. (A and B) Frequency of echoes sent and received is similar in cases of stationary sources or perpendicular motion, resulting in no production of colored pixels. (C) During an approach movement, the received frequency is greater than the emitted, generating positive Doppler signals. (D) Received frequency is lower than emitted frequency when red cells move away from the transducer, producing negative Doppler signals. Fontt: Personal file.



**Figure 4.** Spectral Doppler evaluation of mesometrial attachment artery. The gate (yellow) is placed on an artery of the mesometrial attachment (red) to capture velocities data of uterine blood flow and, posteriorly, produce a spectral graph. Peak systolic (PSV) and end diastolic (EDV) velocities are, respectively, the maximum and ending blood flow velocities of a cardiac cycle. TAMV is the average of an espectral maximum blood flow velocities. Doppler device automatically calculates Pulsatility and Resistance indices (RI and PI, respectively). Spectral graph located above the baseline indicates erythrocytes moving toward to the transducer. Font: Personal file.



**Figure 5.** Cosine values of the Doppler angle ( $\cos\theta$ ) and relationship between insonation angle and accuracy of measurement of blood flow velocities. Determination of blood flow velocity varies with the Doppler frequency (DF).  $DF = 2 \cdot v \cdot \cos\theta / c$ . While the movement of erythrocytes in relation to the transducer is perpendicular ( $90^\circ$ ),  $\cos\theta$  is zero and the device do not produce Doppler signals. Value of  $\cos\theta$  is close to 1 when  $0^\circ < \text{Angle Doppler} < 60^\circ$ , and do not influence blood flow velocity measurement. Font: Personal File.

(mesometrial, endometrial and myometrial) and ovarian (mesovarium, luteal and follicular) arteries is difficult considering their tortuous anatomy. An alternative used in early studies [2,3,9,10] is to calculate the Doppler index. They are good indicators of vascular perfusion of reproductive organs and are independent of the doppler angle [7]. Resistance and Pulsatility indeces (RI and PI, respectively) are calculated using the ratio of the three velocities measurements (PSV, EDV and TAMV) and both indices

have a negatively correlation with the vascular perfusion of the tissue downstream from the sample gate [4].

For providing precisely values of blood velocities, spectrum mode is an objectively method for the evaluation of normal and pathologic blood flow disturbance at reproductive system. However, even showing a great potential for research and clinical purposes it is avoided by equine farm veterinaries in consequence of the difficulty and time



consuming to collect data with precision [4]. To produce a trustful spectral graphic is crucial to place the sample gate Doppler into an artery. Usually, this may be problematic considering the vessels dimensions and the animal and visceral mobility. It is recommended to perform the spectral-doppler exam in a closed dark room with temperature control to minimize animal movement and breathless. Evaluations of animals without feeding and exercising also can be helpful for avoid excessive visceral mobility. Additionally, according to Araujo & Ginther [1] sedation of pony mares do not affect the hemodynamics of the reproductive organs and are beneficial for the evaluation of the corpus luteum and endometrium.

### 1.2 Color-flow mode

Color-flow Doppler provides an immediately qualitative evaluation of the blood flow of different organs and tissues. The vascularity of a structure is estimated subjectively considering the extension of a tissue with colored pixels during a continuous real-time exam. Different than Spectral mode, Color-flow mode is a simple, rapid and functional method of evaluation, which has been applied for farm practical purposes [4].

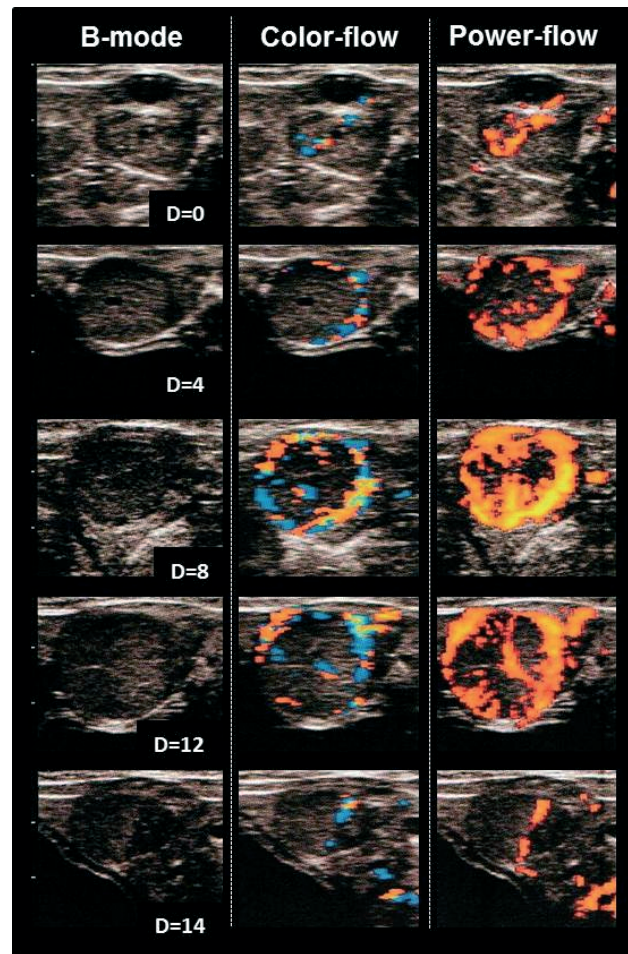
The current Doppler equipment offer two different modes of color-flow imaging: Color-flow and Power-flow modes. The classic color-flow mode use two distinct colors, usually variations of red and blue colors, to represent the vascular blood perfusion of a structure. Also, colored pixels indicate the blood-red cells direction in relation to the transducer. By convection, red colored spots indicate blood flow moving toward to the transducer, while blue colored spots represent blood-red cells moving away from the probe. Additionally, the intensity of the colored pixels suggests the velocity of blood flow ranging from dark to bright tonalities for slower and faster velocities, respectively.

Recently, Power-flow imaging has been used to evaluate uterine and ovarian vascularity of mares and cattle [2,3,6,10]. In power-Doppler mode, the blood flow movement is graduated using a single color and the color pixels intensity vary according to the power of the Doppler signals (number of blood-red cells moving at a specific velocity). Power mode Doppler has advantages over conventional color-flow imaging for the evaluation of reproductive system hemodynamics in mares. According to Ginther [4], power-flow imaging has greater sensitivity to weak

blood flow and is independent of the doppler angle. Also, its color display is not affected by aliasing and reduced blooming artifacts are observed when compared to the classic color-mode Doppler (Figure 6). Therefore, power mode Doppler could improve the evaluation of vessels with small diameters and slow blood flow, as are present in the uterus (mesometrium attachment, endometrium and myometrium) and ovaries (mesovarium, corpus luteum and follicular wall) of mares [2,3].

### III. ARTERIAL SYSTEM OF THE REPRODUCTIVE TRACT

The use of color and power Doppler ultrasonography has become one of the best available



**Figure 6.** Selection of conventional and colored (Color and Power-flow) ultrasonic images of an equine corpus luteum. Sonograms within a same line were obtained simultaneously. Day of ovulation was considered day zero (D=0) of the estrous cycle. Number and intensity of colored pixels present inside and around the CL indicate luteal vascular perfusion. At a same time, extension of luteal tissue with color pixels using color-flow mode is lower than that displayed by Power-flow evaluation, showing the higher sensitivity of the second mode to weak and slow blood flows, as those present in the reproductive tract of mares. Font: Adapted from Ginther [4].

and reliable techniques for the diagnoses and studies of reproductive hemodynamics [2,4]. Considering the presence of a substantial number of small vessels with slow blood flow that do not appear on a conventional color-flow image, it is suggested the use of power-flow mode for the vascular perfusion evaluation of follicles, corpus luteum and uterus [2, 6, 8].

The uterine vascular perfusion evaluation, using color-flow imaging, must be done considering all uterine layers [2,3]. The vascularity of the uterus is estimated subjectively using the percentage of uterine tissue with color signals Doppler during a real-time cross-sectioning exam of the uterus in a continuous span of 1 minute. Multiple cross sections must be viewed, as consequence of animal and uterine movements [9].

The mesometrium attachment is an important reference point in equine transrectal Doppler ultrasound for indicating the area of entry of the blood vessels into the uterus [4]. According to Silva *et al.* [9], color Doppler signals within the endometrium are commonly inadequate for the production of spectral waveforms. However, early studies have reported the successfully use of mesometrial arteries as an alternative assessment for the spectral evaluation of uterine blood flow velocities of pregnant and non-pregnant mares [3,9,10].

Luteal vascularity is estimated while viewing, in a slow and continuous motion, of the entire CL real-time scan [6]. Color spots at the periphery and within the CL must be included in the luteal vascular perfusion estimation. Spectral mode from CL is considered too time consuming and, usually, it is not

used for objectively evaluation [5]. An alternative to validate the subjectively exam of CL is determining the number of colored pixels in a captured selected image representing the largest cross-section of the CL with distinctive colored areas, as described previously by Ginther & Utt [7].

Vascular blood perfusion of follicles is estimated considering the colored Doppler signals from the vessels of the follicular wall [8]. The entire follicle must be scanned several times, in a slow and continuous motion. However, the complete real-time exam of preovulatory follicles is, commonly, unable due to their dimensions. In these cases, the operator must scan parts of the follicle and use their average to estimate the vascularity. Similar to the reported for CL, the spectral mode is not recommended for objectively evaluation of follicular hemodynamics been suggested the validation of the subjectively exam using the colored pixels count.

#### IV. CONCLUSION

The advent of the Doppler ultrasonography had enabled to re-evaluate reproductive physiologic conceptions before considering them absolutes. However, the complete knowledge of Doppler principles, methods of evaluation and arterial anatomy of the reproductive system are essential to execute an exam with accuracy and excellence. Special care must be taken in order to enhance studies in this area. Otherwise, inconsistent and not reproducible findings may result in the discredit of Doppler technology ahead of the society and scientific veterinary community.

#### REFERENCES

- 1 Araujo R.R. & Ginther O.J. 2009. Vascular perfusion of reproductive organs in pony mares and heifers during sedation with detomidine or xylazine. *American Journal of Veterinary Research*. 70(1): 141-148.
- 2 Ferreira J.C., Gastal E.L. & Ginther O.J. 2008. Uterine blood flow and perfusion in mares with uterine cysts: effect of the size of the cystic area and age. *Reproduction*. 135(4): 541-50.
- 3 Ferreira J.C., Ignácio F.S. & Meira C. 2010. Uterine vascular perfusion and spectral-Doppler measurements during early gestation in mares: New concepts of evaluation. *Animal Reproduction Science*. 121(S): 281-283.
- 4 Ginther O.J. 2007. Ultrasonic Imaging and Animal Reproduction: Color-Doppler Ultrasonography, Ginther O.J. (ed). Cross Plains: Equiservices Publishing, 258p.
- 5 Ginther O.J., Gastal E.L., Gastal M.O., Utt M.D. & Beg M.A. 2007. Luteal blood flow and progesterone production in mares. *Animal Reproduction Science*. 99(1-2): 213-220.

- 6 Ginther O.J., Rodrigues B.L., Ferreira J.C., Araujo R.R. & Beg M.A. 2008.** Characterization of pulses of 13,14-dihydro-15-keto-PGF<sub>2</sub>alpha (PGFM) and relationships between PGFM pulses and luteal blood flow before, during, and after luteolysis in mares. *Reproduction, Fertility and Development*. 20(6): 684-693.
- 7 Ginther O.J. & Utt M.D. 2004.** Doppler Ultrasound in Equine Reproduction: Principles, Techniques, and Potential. *Journal of Equine Veterinary Science*. 24: 516-526.
- 8 Siddiqui M.A., Ferreira J.C., Gastal E.L., Beg, M.A., Cooper D.A. & Ginther O.J. 2010.** Temporal relationships of the LH surge and ovulation to echotexture and power Doppler signals of blood flow in the wall of the preovulatory follicle in heifers. *Reproduction, Fertility and Development*. 22 (7): 1110-1117.
- 9 Silva L.A., Gastal E.L., Beg M.A. & Ginther O.J. 2005.** Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. *Biology of Reproduction*. 72(3): 755-761.
- 10 Silva L.A., Ginther O.J. 2006.** An early endometrial vascular indicator of completed orientation of the embryo and the role of dorsal endometrial encroachment in mares. *Biology of Reproduction*. 74(2): 337-343.
- 11 Zagzebski J.A. 2005.** Physics and instrumentation in Doppler and B-Mode ultrasonography. Philadelphia: Elsevier Saunders, 752p.