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Doppler Ultrasound in the Reproduction of Mares

Camila Silva Costa Ferreira and Rita de Cássia Lima Morais

Abstract

Doppler ultrasonographic (US) is a method that provides real-time information on vascular architecture and hemodynamic aspects of blood vessels. It can determine the presence, direction, and speed of blood flow, being subdivided into the categories of color Doppler (color flow and power flow) and pulsed Doppler. The objective of this chapter was to compile data from several studies addressing the use of US Doppler correlated with pathophysiological phenomena of equine reproduction. Initially we decided to describe the technique, advantages, and disadvantages of each Doppler mode. Then the applicability of US Doppler in mares related to equine reproduction. Thus, within this chapter, you will find the form of use and descriptions of studies carried out on vascular perfusion of the follicular dynamics, the corpus luteum, the uterine segments, which we have divided into post-insemination evaluation, endometritis diagnosis and pregnancy diagnosis. So, we hope that this chapter will expand the knowledge about US Doppler and increase the number of veterinarians who will introduce the technique into their practical routine.

Keywords: equine, diagnosis, Doppler ultrasound, reproduction

1. Introduction

In ultrasonographic (US), images of the body are obtained from the reflection or scattering of a pulsed high frequency sound beam that is sent by a mobile transducer to examine the body [1]. Each time the sound beam encounters acoustic interfaces in its path, there are changes in the density or elasticity of the medium, where the fraction of sound energy is reflected or scattered. This can happen on the walls of an organ or even along a tissue with a heterogeneous structure. The retro-scattered wave (or "echo") is detected and processed by the device, which will assign a gray scale according to the amplitude of the signal returned. Therefore, an ultrasound image corresponds to a 2D map of the tissue's acoustic reflectivity. The body can also be investigated in Doppler mode to obtain flow information, widely applied in the analysis of the circulatory system [2].

In the 1980s, the renowned researcher Dr. O.J. Ginther stated that since the introduction of transrectal palpation, ultrasound diagnosis has been the most profound technological advance in the field of research and the reproductive clinic of large animals [3]. In the late 1990s, studies were started using US Doppler to determine physiological and pathological changes in the mare's reproductive tract [4]. Over the past decades, the use of US has reached great dimensions, not only in research centers, but also commercially in livestock activities, having made great improvements in the clinical diagnosis and reproductive efficiency of large animals [5].

In this context, the objective of this chapter was to compile data from several studies addressing the use of US Doppler correlated with pathophysiological phenomena of equine reproduction.

2. Doppler ultrasonographic

US Doppler is a method that provides real-time information on vascular architecture and hemodynamic aspects of blood vessels. It can determine the presence, direction and speed of blood flow, being subdivided into the categories of color Doppler (color flow and power flor) and pulsed Doppler [6].

In color flow Doppler US (UCF) is considered the classic examination of the color mode within US Doppler, which allows non-invasive assessment of the presence, direction, speed, and quality of blood flow. Two distinct colors are used, usually variations of red tones for positive flows and blue for negative flows representing the vascular blood perfusion of a structure, where the colored pixels indicate the direction of the blood cells in relation to the transducer [7–9].

In power flow Doppler US (UPF) is a variation of the UCF and has been used as the test of choice to evaluate the vascularization of the uterus and ovaries of mares and components of the testicular bag of the stallions [7, 9–15]. It is observed with this technique an increase of the sensitivity of display of the blood flow inside the tissue of 3 to 5 times, in comparison with UCF. The higher sensitivity allows the evaluation of vessels with a small diameter or slow flow that does not appear in a conventional color flow because of incompatible speed ranges and Doppler angles. [16]. Blood flow is generally characterized homogeneously by orange tones and the speed of flow is indicated in both modes by the intensity of the tones, the lighter the color the faster the flow and the darker the color the slower the flow [17]. In this way, the advantages of UPF are greater sensitivity to weak flow; effects of the Doppler angle on the Doppler frequency are ignored; and aliasing does not affect the display of colors [9].

The extent of vascularization of the color US Doppler can be estimated subjectively through the percentage of pixels (colored signs) of a tissue or objectively by counting the colored pixels via software [10].

In spectral Doppler US mode, the artery blood flow wanted is found and then the cursor ("gate") is positioned in the lumen of the artery to evaluate the sample volume [18]. The volume evaluations of the samples are reflected in graphs, called spectrum, which represent the speed of the blood flow of the artery in question for several times within the cardiac cycle or of an individual arterial pulse [19]. The brightness of the spectral trace, represented in the gray scale, is also used to represent the amplitude of each frequency component, indicating the amount of blood cells that pass at a particular speed [6]. Changes in the evaluated vessel may suggest changes in the spectral tracing and this fact may be indicative of physiological, systemic changes or of some disease present at the site [20].

In the quantitative analysis of the spectral Doppler US tracing, most devices have automatic configuration to automatically calculate the average of the displacement frequency or speed. The maximum point reached in the spectrum is called the systolic peak velocity (PSV) and the minimum point in the wave morphology is the value of the final diastolic velocity (EDV). The medium flow can be calculated by multiplying the average speed by the vessel area [9, 20].

Hemodynamic indices, such as systole-diastole ratio, resistivity index (RI) and pulsatility index (PI), allow the comparison of flow during systole and diastole. RI and PI have a negative correlation with the vascular perfusion of the tissue irrigated

by the artery in question, that is, the lower the RI and PI, the greater the vascular perfusion in the tissue supplied by that vessel [10]. The changes in these indexes help in the identification of stenosis, thrombosis and changes in vascular resistivity and parenchyma dysfunctions, or in the characterization of disease malignancy [6].

3. Use in mares reproduction

3.1 Follicular dinamic

The UCF has the potential to predict the follicular state (ovulatory or anovulatory future) of the dominant follicles during the transition period. During the anovulatory transition season, vascular changes in the walls of the future anovulatory follicle and of a future dominant ovulatory follicle were studied from 25 mm in 7 days after the follicle was 30 mm. The blood flow area was smaller for anovulatory follicles of dominant size than for ovulatory follicles at the time when blood flow determinations started at 25 mm [21, 22]. There is a hypothesis for anovulation that involves hormones and follicular angiogenesis during the transition period [23]. In this regard, pre-ovulatory vascular changes were compared between the first and the next ovulation of the year in 40 pony mares for 6 days before ovulation [24]. Although the area of blood flow from the follicle increased the day of ovulation in both groups, the results demonstrated that follicle vascularization and LH peak were attenuated before the first ovulation of the year, with no indication that estradiol was involved in the differences between the first and the last ovulation.

Regarding the blood flow of the preovulatory follicle, recent studies have shown a daily increase in vascularization of the dominant follicle wall as it matures and approaches the day of ovulation [22, 24–26]. However, on the day of ovulation, a few hours before ovulation, an abrupt decrease in blood perfusion was detected in the wall of the preovulatory follicle [9, 25].

Vascular perfusion is related to the peripheral area of the follicle and its diameter [19]. Therefore, research shows that a higher pregnancy rate is associated with a greater blood flow in the preovulatory follicle (POF). Because a higher percentage of Doppler signals was observed in the follicular wall and a reduction in the Doppler indices of RI and PI in the ovarian vessels in mares that impregnated in comparison to mares that did not impregnate [27].

Another study demonstrated that larger, well-vascularized POF produce larger corpus luteum (CL), with more blood flow and a higher systemic concentration of progesterone (P4), which can lead to a better uterine environment for the establishment of pregnancy. In addition, the repetition of the POF diameter value in individuals during spring and autumn may be estimate the best breeding time during the transition period. The lower blood flow of CL observed during the last estrous cycle of the reproductive season is another important finding of the study, which may clarify the luteal insufficiency of the transition period [28].

The morphology and vascularization of anovulatory hemorrhagic follicles (HAF) in mares and the endocrinology immediately prior to the formation of HAF were studied in control and HAF groups [29]. The day of ovulation and the first day of HAF formation, as indicated by the turbidity of the follicular fluid, were defined as Day 0. The frequency of discrete ultrasound indicators in imminent ovulation gray scale and the diameter of the follicle on Day −1 did not differed between the groups with future ovulation and HAF. However, the circumference of the follicle wall of future HAF had more signs of color Doppler than in control mares [25].

3.2 Corpus luteum

The UCF can be effective in identifying mares that fail or regress CL, before any decrease in P4 circulation, decrease in CL area, echogenicity changes in B-mode image or when uterine/cervical tone becomes apparent [30].

A daily assessment of CL between the day of ovulation and the eighth day after ovulation was performed by Romano et al. [15]. The authors observed that the CL area was weakly correlated with luteal vascular perfusion and plasma P4 concentrations. However, a positive correlation was observed between luteal vascular perfusion and plasma P4 concentrations. Furthermore, the number of colored pixels and the total pixel intensity were positively correlated with vascular perfusion and the plasma P4 concentration.

From a daily analysis, via UPF of the CL, a transient increase in the total luteal area was observed during the first days after ovulation, demonstrating that old mares had a greater luteal area than young mares between D2 and D8 and in D18-D19 ($p < 0.05$). However, old mares have a late increase in luteal vascularization during the first gestational days ($p < 0.05$). However, the CL of young and old mares showed similar and constantly high vascularization from D14. It has also been observed that the progressive increase in plasma P4 concentrations observed up to D8 was followed by a gradual decrease until to intermediate levels of P4. Thus, concluding that the newly formed CL of old mares underwent a compensatory structural remodeling to guarantee the local blood supply and the continuous output of P4 during early pregnancy to maintain it without suffering from the age effect [11].

To check if there could be a difference between the vascularization of the CL between the possible days for embryo transfer in the recipient mares, it was observed that the CL classified as adequate for the embryo transfer procedure by US mode B were also those classified with the better vascularization by UPF maintaining the pattern of proportional growth of the size of the CL with the concentration of P4 [31].

De Vasconcelos Azevedo et al. [32] evaluated the vascularization and function of CL of recipient mares of the Mangalarga Marchador embryo at the time of embryo transfer through UCF. As a result, they noticed that the mares that were pregnant showed a correlation with the increase in the vascularization of the CL, as well subjective methods as objective methods, and the plasma concentration of P4.

3.3 Uterine evaluation

The hemodynamic evaluation of the uterus can be done by spectral data collected from the uterine arteries and their ramifications [33], or by subjective or objective assessments of the blood flow of the endometrium that provide data regarding local and specific changes in the evaluated area [8, 34].

The first investigations relating US Doppler to equine uterine physiology were carried out by Stolla and Bollwein [35], Mayer et al. [36] and Bollwein et al. [33], who obtained a cyclic pattern in uterine blood flow. Bollwein et al. [33] performed the measurement of the RI of the uterine artery on different days of the equine estrous cycle. They are verifying that there was no difference between the values of the left and right arteries. Not showing a correlation of the values found in the flow with the presence or absence of follicles or luteum corpus ipsilateral to the evaluated horn, indicating that the circulation is distributed equally to the two horns of the uterus. The authors also found that the average RI for all evaluated days was higher when observed in multiparous mares than when observed in nulliparous mares. This work also found significantly higher RI values on days 0 and 10 of the

estrous cycle compared to days 5, 15 and 20 (considering D0 the day of ovulation). The authors correlated the lower RI value observed on day 5 to the possibility of an increase in blood supply to the uterus in this initial luteal phase due to the moment of embryo entry into the uterus.

Subsequent studies by Bollwein et al. [37], confirmed these statements, in addition to noting that the lowest uterine PI values were record during the initial diestrus, in a stage, when they found a peak in uterine tonus in mares. They speculated, therefore, that the tone and contractility of the uterus do not seem to be regulated by the uterine blood supply in cyclic mares.

Ferreira et al. [31] evaluated the flow of the uterine artery of recipient's mares on the day of embryo transfer. It was observed that in animals where the RI of the dorsal branches of the uterine arteries close to 1.0 are proportional to mares with greater vascularization of the corpus luteum and a high plasma P4 concentration. Being able to use these indices to select mares with greater aptitude for the development of the embryo when comparing two similar mares clinically by conventional ultrasound exams.

Other studies correlating the uterine flow of normal mares with subfertility characterized by biopsy endometrial were performed by Stolla and Bollwein [35] and Blaich et al. [38]. The authors show that in all mares there was an increase in vascular resistance in the pre-ovulatory phase and in 8 mares the peak occurred 8 days after ovulation. The blood flow impedance decreased from D1 after ovulation until it reached its lowest level during a luteal phase in all mares. They also observed that there was a gradual increase in vascular resistance in the initial follicular phase and that cyclical changes occurred in all mares with varying amplitudes. As they compared the flows according to the endometrial classifications, they observed that, for all mares with histological category IIb and III, there was an increase in the PI and average RI throughout the estrous cycle, when compared with the mares in the group that had normal fertility.

3.3.1 Uterine evaluation after artificial insemination

Although adequate blood flow is essential for the normal functioning of the reproductive system [9], there are few in vivo studies that describe the uterine hemodynamics of non-pregnant mares [34, 39].

Changes in blood flow velocity verified by spectral Doppler US of the dorsal uterine arteries after reproduction, suggest that there is an increase in endometrial blood flow during semen transport and uterine clearance [40]. However, currently, the evaluation of the Doppler indices of the mesometrial arteries and the vascular perfusion of the uterine tissue during the pre- and post-reproductive periods and in mares with endometritis have been little researched.

Bollwein et al. [41] also evaluated, during three estrous cycles, the vascular perfusion of the uterine artery using the mean maximum velocity spectral Doppler index (MVM). The authors related the quantification of flow to the effect of the infusion of semen extender, seminal plasma, or pure semen. In response to the dilution infusion, no effect on uterine blood flow was observed. Controversial results have been reported after one hour of infusion of seminal plasma or pure semen, where an increase in VMM values was observed in both uterine arteries. Therefore, the authors concluded that the increase in endometrial perfusion in these groups may be associated with inflammation and vasodilator components present in the seminal plasma.

Ferreira et al. [8] observed a transient increase in uterine vascular perfusion without mesometrial changes in the PI during the first 8 hours after artificial insemination. However, in the research by Ferreira et al. [42, 43], where PI Doppler

measurements of the mesometrial arteries and UPF of the organ in relation to the semen effect were used, changes in the blood flow velocity of the uterine arteries were observed only in the first hour after the infusion of crude semen.

Ferreira et al. [42, 43] also evaluated uterine vascular perfusion before and after artificial insemination correlating with the age of the mares and the presence/absence of endometrial degenerative processes. There were no differences in perfusion between the horns, however, they showed in the organ, an early and transient increase in the blood flow of the uterus in response to artificial insemination in all mares. However, the increase in mesometrial arterial resistance was strongly associated with severe endometrial degenerative changes after AI, regardless of age.

In horses, the AI procedure can be performed by depositing the semen in the body of the uterus or at the apex of the uterus horn to use a reduced inseminating dose. In order to assess the uterine inflammatory response to the different semen deposition sites, Araújo [44] analyzed the uterine perfusion by means of spectral Doppler US of the dorsal uterine arteries and with subjective UCF in the uterus. The values of RI and PI identified after AI were similar on the contralateral and ipsilateral sides of the mesometrial and dorsal uterine arteries. Regarding the evaluation time, a difference was observed where, both the RI and the PI, were lower in the moment before ovulation and AI and higher values were recorded in 24 h after. As for the analysis of organ perfusion by score, this did not show any difference within each experimental group regarding the evaluation time. Regarding the place of insemination, the color scores showed significant differences between the two experimental groups, with a predominance of scores 1.0 and 2.0 in the group of mares inseminated at the apex of the uterine horn, and the score 3.0 in the group inseminated in the body of the uterus.

3.3.2 Endometritis' diagnosis

A research to measure uterine blood perfusion using UCF (subjective and objective) was carried out by Sá et al. [45] to investigate changes in vascularization of uterine segments of mares after intrauterine inoculation of E. coli. The authors found significant differences in the blood flow evaluated before inoculation of the bacteria (M0) and 24 h after inoculation (M1), where blood perfusion in M1 showed almost twice the M0 in the three uterine segments evaluated, but no significant differences were found in evaluations carried out between the follow-ups. In four animals evaluated, endometrial cytology and mode B ultrasound examinations were not sufficient to detect uterine infection, however, through UCF, it was possible to verify a significant increase in uterine vascularization in these mares compared to the values before inoculation.

In a second experiment carried out by Sá et al. [45], the evaluation by the UCF was performed 24 h after the intrauterine infusion of E. coli. Half of the mares with uterus inoculated by bacteria were subjected to a treatment consisting of a uterine lavage using 50 mL of the Fitoclean® phytotherapy solution (Organnact Saúde Animal, Brazil) diluted in 950 mL of Ringer with Lactate and subsequent intrauterine infusion with 40 mL phytotherapy solution Fitoclean® diluted in 60 mL of Ringer with Lactate. In the control group, an intrauterine wash was performed with Ringer's serum with pure lactate, with subsequent infusion of 100 mL of the same substance. UCF was performed before treatment (A), 24 h (B) and 48 h (C) after treatment. The authors reported that uterine perfusion was greater at time A than at time B and C, but the decrease in uterine bacterial load was not verified. The authors assume that the decrease in vascularization in the post-treatment groups can be attributed to the vasoconstriction caused by the reaction of components with

anti-inflammatory properties present in the product, which may have caused the decrease in local perfusion even with the presence of microorganisms.

A third research was carried out in the study by Sá et al. [45]. The mares in which pathogenic agents were identified in the samples collected 10 days after the end of the second mentioned experiment, were subjected to antibiotic therapy by intrauterine infusion of 100 mL Gentamicin (Gentrin® Uterine Infusion, Ourofino Saúde Animal, São Paulo, Brazil) for three days, according to the sensitivity shown in the antibiogram. UCF was performed seven days after treatment, comparing the tests performed before E. coli pre-inoculation, post-treatment with Fitoclean® and one week after antibiotic therapy (M0, M1 and M2, respectively). The authors observed a reduction in vascularization in the group treated with Fitoclean® after antibiotic therapy, however, blood perfusion in this group was still greater than M0, even with antibiotics being able to eliminate the bacteria present. Based on the results obtained in this study, it was possible to identify acute endometritis through the UCF, but the vascular perfusion identified did not correlate with the uterine laboratory tests performed.

Abdelnaby et al. [46] performed spectral Doppler ultrasonography of the dorsal uterine arteries and UCF of the uterus of mares with and without endometritis, correlating the data with the impact of the pathology on the oxidative and hormonal state. The results revealed a significant increase in the metabolites of estradiol, malondialdehyde and nitric oxide associated with a significant decrease in progesterone and total antioxidant capacity in the endometritis group. The uterine blood flow analyzed by the UCF showed a significant increase in the endometritis group, while the spectral mode showed a significant increase in the PSV and TAMV indices and in the blood flow area rate accompanied by a significant decrease in the PI and RI Doppler indices. In addition, the elevated uterine blood perfusion was correlated with the accumulation of fluid inside the uterus, with a marked difference between the uterine horns in relation to the size of the UCF staining area, which may be due to the marked increase in fluid accumulation in the right in endometritis group.

Morais [13] evaluated, through UPF, the intensity of colored pixels (IPC) of the uterine segments (body and horns) during estrus before and after uterine treatment with DMSO. From the evaluations it was noted that mares with negative cytology (despite positive culture test) had less blood flow than mares with positive cytology. IPC was reduced in mares that became pregnant. In the mares that remained empty, the CPI remained high. Thus, UPF can be used as an auxiliary diagnostic method in some cases of equine endometritis. In pregnant mares, blood flow in the endometrium and RI decreased. Empty mares blood flow in the endometrium and RI remained high or increased.

3.4 Doppler ultrasonography in pregnancy diagnosis

Changes in the hemodynamics of the equine reproductive tract during early and late pregnancy have already been described several times in the literature. Bollwein et al. [40] to compare the vascularization of the right and left uterine horns of cyclical mares and in initial pregnancies, observed that on days 11 and 15 to 29 of gestation, mean values of blood flow velocity were higher and RI lower in both horns uterine of pregnant mares compared to empty mares.

Subsequently, Bollwein et al. [47] investigated the blood flow in both uterine arteries (ipsilateral and contralateral to the fetus) every four weeks, from the second week until the moment of delivery. A highly significant regression was observed in the RI averages according to the week of gestation, reaching, at the end of the evaluations, values lower than half of those initially recorded (0.89 ± 0:01 to

0.39 ± 0:03). The volume of blood flow in the ipsilateral and contralateral uterine horns increased significantly according to the week from the middle of gestation.

In other research, it was shown that the transient changes in vascular perfusion accompany the mobility and the fixation of the embryonic vesicle [34]. Pregnant and non-pregnant mares have similar and low endometrial vascularity in the first eight days after ovulation. However, from D11 there was a gradual increase in the volume of blood flow in both uterine horns during the embryonic mobility phase and a higher speed in blood flow in the uterine horn of fixation of the conceptus, in relation to the opposite side.

Silva and Ginther [29] observed, through UCF, an early vascular indicator of the future position of the embryo itself, which consisted of a colored point in the image of the endometrium close to the wall of the embryonic pole. The early indicator was detected in each mare 0.5 ± 0.1 days after fixation and 2.5 ± 0.2 days before the first visible embryo detection. The author also reports that by US Doppler we can monitor the inadequate early orientation of this embryo. Studies have shown that this type of problem is correlated with a flabby uterus and a defective embryonic dorsal invasion of the endometrium. However, the asymmetric increase in the allantoic sac can spontaneously correct the disorientation, so that the orientation for the formation of the umbilical cord is in a normal position around 12 o'clock.

Using spectral-mode US Doppler, Chen and Stolla [48] developed the uterine index (UI) to predict embryonic death in mares. The calculation used by the authors considers the following formula: UI = (RI-p - RI-np) x10, where RI-p is the RI of the uterine artery on the side of the pregnant uterine horn and RI-np is the RI of the artery uterine horn of the non-pregnant uterine horn. According to the authors, UI less than five is indicative of embryonic death evident in the next 24 hours, while mares with UI more than 10 did not present any apparent hemodynamic disorder.

Ferreira et al. [12] observed that the uterine doppler indices (RI and PI) of pregnant mares decreased progressively. Unlike other studies, this research also observed, through measurements of mesometrial RI and PI, an increase in vascular perfusion between D3 and D6 post-OV. This study was one of the triggers that helped future inquiries regarding the use of the technique as an early pregnancy diagnosis and determination of the ideal moment for embryo collection, especially when aimed at cryopreservation.

Ousey et al. [49] evaluated the blood flow of the uterine artery and other Doppler indices during pregnancy to compare placental and fetal development in young and elderly mares. However, no difference was found in the evaluated indexes. Thus, the authors stated that the similarity in the Doppler indices between the groups of elderly and young mares reflects the absence of severe pathological changes in the endometrial vascularization and glandular tissue found in the elderly mares used. However, employing other types of blood perfusion measurements, it has already been observed that mares with diffuse endometrial degeneration had reduced uterine vascular perfusion when compared to pregnant mares with unchanged endometrium. The hypothesis was then raised that severe angiosis can reduce the capacity of the vessels to adapt to the varied demands of uterine circulation [50]. In addition, endometrial pathological changes have been strongly associated with degeneration of uterine vessels in mares [51].

Ferreira et al. [42], used in their research, in addition to the analysis of uterine perfusion with UPF, a spectral analysis of the insertion of uterine arteries in the mesometrium, which is also reported to be an efficient method for the objective examination of uterine blood flow during the first 20 days gestation. With this methodology, the authors detected early and transient increases in uterine blood flow in pregnant mares, regardless of age and presence of endometrial changes. The increase in uterine blood flow in the initial pregnancy described by this and other

authors, may be caused by the effects of vasoactive factors sought by pre-implanted embryos, as described in horses [34].

Another analysis carried out in the study by Ferreira et al. [42], was an observation of the negative effect of age and endometrial degeneration without uterine blood flow from mares during early pregnancy. The authors associated this apparent inability of the aged uterus to respond to vasoactive factors derived from the embryo with the progressive changes in the architecture of the uterine vascular network observed in older mares. In addition, it has already been added that age-related degenerative changes in the endometrium can affect the development of placental microcotyledons and their associated blood flow [49].

With UCF it is also possible to determine fetal equine sex between 90 and 180 days of gestation by observing fetal gonads [52]. This assessment was later confirmed by Pricking et al. [53], who obtained high rates of sex determination accuracy when associated with Us mode B, color Doppler and 3D transabdominal tomography ultrasound techniques. In addition, the application of this new 3D imaging ultrasound technology has enabled the diagnosis of gender in 18 cases in which B-mode and Doppler ultrasonography have shown dubious results.

In relation to the gestation of hybrids, pregnant donkey showed greater uterine vascularization in the uterine horn contralateral to that of embryonic fixation. And that older mares showed less blood flow in the uterine artery, as they had higher PI and RI [54]. Another difference observed was comparing the PI of the fetal umbilical cord artery, which showed a statistical difference in pregnant with donkey semen when compared to pregnant with stallion semen [55].

Nieto-Olmedo et al. [14] detected differences in uterine vascular perfusion between pregnant and non-pregnant mares early between days 7 and 8 post-ovulation, demonstrating that the UPF associated with computerized analyzes is an effective method for the early diagnosis of pregnancy. The authors observed that the area of vascular perfusion of the uterus (mm^2) and pixel intensity increases in pregnant mares compared to mares without embryonic recovery. The technique can be used in routine clinical practice to maximize the embryo recovery rates of donor mares and to predict the diagnosis of pregnancy before embryo collection.

Relationship of uterine vascular perfusion associated with involution of the postpartum uterus was mentioned in the study by Lemes et al. [56] using the UCF mode of uterine segments. The authors observed that vascular perfusion increased in endometrial and mesometrial tissues in the first 2 to 4 days after delivery, followed by a progressive decrease until the second week postpartum. The profile of vascular perfusion in the uterus described after the first postpartum ovulation is similar to that observed during the estrous cycles and initial gestation observed previously, indicating a rapid return of the uterus to the pre-gestational uterine characteristics in the mares. In such cases, it has been reported that the rapid reduction in uterine diameter, absence of intrauterine fluid and vascular decrease in the layers of the uterus, may indicate a favorable uterine environment for the development of the embryo in the foal's heat.

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