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# Clinical application of Doppler ultrasound in the diagnosis of fertility problems in equines

*Aplicações da ultrassonografia Doppler no diagnóstico de problemas de fertilidade em equinos*

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***Abstract***

*The use of different ultrasound modalities (color, power and pulsed Doppler) in clinical research has enhanced knowledge of reproductive pathophysiology in equine, in addition to improving the clinical diagnosis of reproductive disorders in both the stallion and the mare. In the stallion, color Doppler ultrasound has improved the diagnostic potential of conventional ultrasound, improving the differential diagnosis of pathologies such as testicular torsion (decrease or absence of blood flow in the cord) and orchitis (increased blood flow in the cord). The function of the testicle is highly dependent on the blood flow it receives; therefore, any vascular alterations can affect both the quality and production of sperm. In recent years there have been a greater number of studies in which Doppler ultrasound is used as a tool in the diagnosis of testicular dysfunction and in the monitoring of medical and surgical treatments. In the mare, ultrasound technology has allowed numerous advances in basic research regarding the vascular hemodynamics of the ovary and uterus. Moreover, it has become an indispensable tool in reproductive practice. Among the clinical applications of this technique in mares are the evaluation of CL (corpus luteum) functionality, being a useful tool in the selection of recipients in embryo transfer programs or the use of power Doppler in the diagnosis of pregnancy as early as 7 days post-ovulation prior to flushing. Finally, the incorporation of color and pulsed Doppler ultrasound into the examination improves the diagnosis of mares with endometritis due to a pathological increase in uterine blood flow.*

***Keywords:*** *subfertility, power Doppler, color Doppler, pulse Doppler, stallion, mare, pregnancy diagnosis*

## Introduction

The use of ultrasound technology has allowed significant advances in equine reproduction research and has notably improved reproductive diagnosis in both stallions and mares. Ultrasound was first used in this species in the 80s, mainly for the reproductive evaluation of the mare (Ginther 2014). However, it has also gradually been introduced as part of the reproductive evaluation in stallions. B-mode ultrasound has contributed to knowledge of physiological aspects of the mare such as the characterization of follicular dynamics, the phenomenon of embryo mobility prior to implantation and the identification of signs prior to ovulation (endometrial edema, follicle deformity, serrated edge of the wall…) (Gastal, Gastal, and Ginther 2006b; Ginther 2014). In addition, it has enabled several important clinical findings such as the identification of endometrial cysts, hemorrhagic follicles, and accumulation of uterine fluid in endometritis, among others (Adams 1987). In the stallion, it has not only made it possible to diagnose certain pathologies such as hydrocele or inguinal hernia, but also to predict reproductive capacity by calculating the DSO (Daily Sperm Output) (Love CC et al. 1991; M. Pozor 2005).

The incorporation of Doppler ultrasound has led to new lines of research in this field. The study of testicular, uterine, and ovarian vascular hemodynamics in this species has added to knowledge of the pathophysiology of these organs. Doppler ultrasound has improved the diagnostic potential of conventional ultrasound, contributing to the identification of certain pathologies, and allows early detection of any pathology related to the perfusion of these organs. However, despite the great diagnostic potential of this technology, the lack of knowledge amongst practitioners about its possible applications

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in clinical diagnosis, means that this technique is not standardized, nor is it part of the basic reproductive examination.

The objective of this article is to provide an up-to-date review of recent advances and applications of Doppler ultrasound in reproductive diagnostics in both the stallion and the mare.

## Ultrasound modalities used in equine reproduction

Doppler ultrasound is based on the "Doppler effect", that is, on the difference between the frequency of the transmitted ultrasound waves and that of the echoes received by the ultrasound probe emitted by moving erythrocytes (Ginther and Utt 2004). Doppler effect was first described in 1842 by the Austrian physicist Christian Johann Doppler.

Most ultrasound machines currently have the different Doppler modalities incorporated and present an acceptable quality for the evaluation of both testicular and uterine vascularization. The three Doppler modalities used in equine reproductive medicine are color Doppler, power Doppler, and pulsed or spectral Doppler.

## Color Doppler

Color Doppler imaging has had a great impact on ultrasonography. This Doppler modality combines a B-mode two-dimensional image of tissue structures with a color representation of blood flow. It allows qualitative evaluation of the presence or absence of blood flow in an organ or area (Ginther and Utt 2004). Furthermore, the flow direction evaluated can be observed and the flow velocities can be subjectively assessed by coding the color intensity. It converts frequency changes into red (erythrocytes move towards the probe) and blue (they go in the opposite direction to the echoes). Brightness of the image is also taken into account; the brighter the image, the greater the frequency amplitude(Ginther and Utt 2004). Image analysis software such as Image J has been used in research work in order to semi-quantitatively evaluate the vascularization of different organs based on the area and intensity of pixels identified in the image. The extent of color can be estimated by percentage of a tissue with color signals or can be calculated using different software, where this calculation is made by assessing the number of colored pixels (Delorme et al. 1995).

This ultrasound modality is usually used to evaluate the vascularization of the CL, the follicle, and the uterus in the mare for diagnostic purposes (figure 1). In the stallion, this technique improves the diagnosis of pathologies such as varicocele and allows a differential diagnosis between testicular torsion (absence of flow in the cord) and inflammatory conditions such as orchitis or epididymitis (increased flow in the testicular cord)(Ortega-Ferrusola et al. 2014b)(figure 2). Color Doppler has also been used in the diagnosis of embryonic and fetal viability, as well as in the diagnosis of fetal sex after 120 days(Mebarki et al. 2019; Resende et al. 2014).

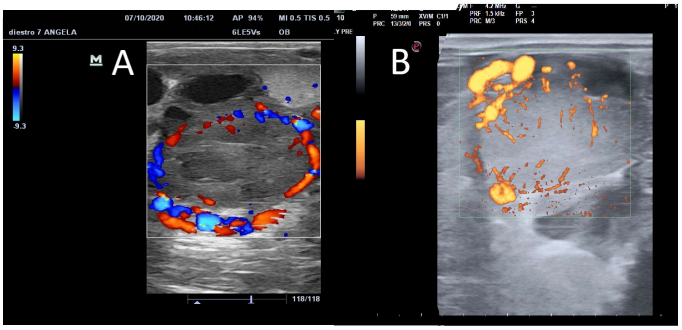


Figure 1. Images showing evaluation of a functional corpus luteum using color (A) and power (B) Doppler.

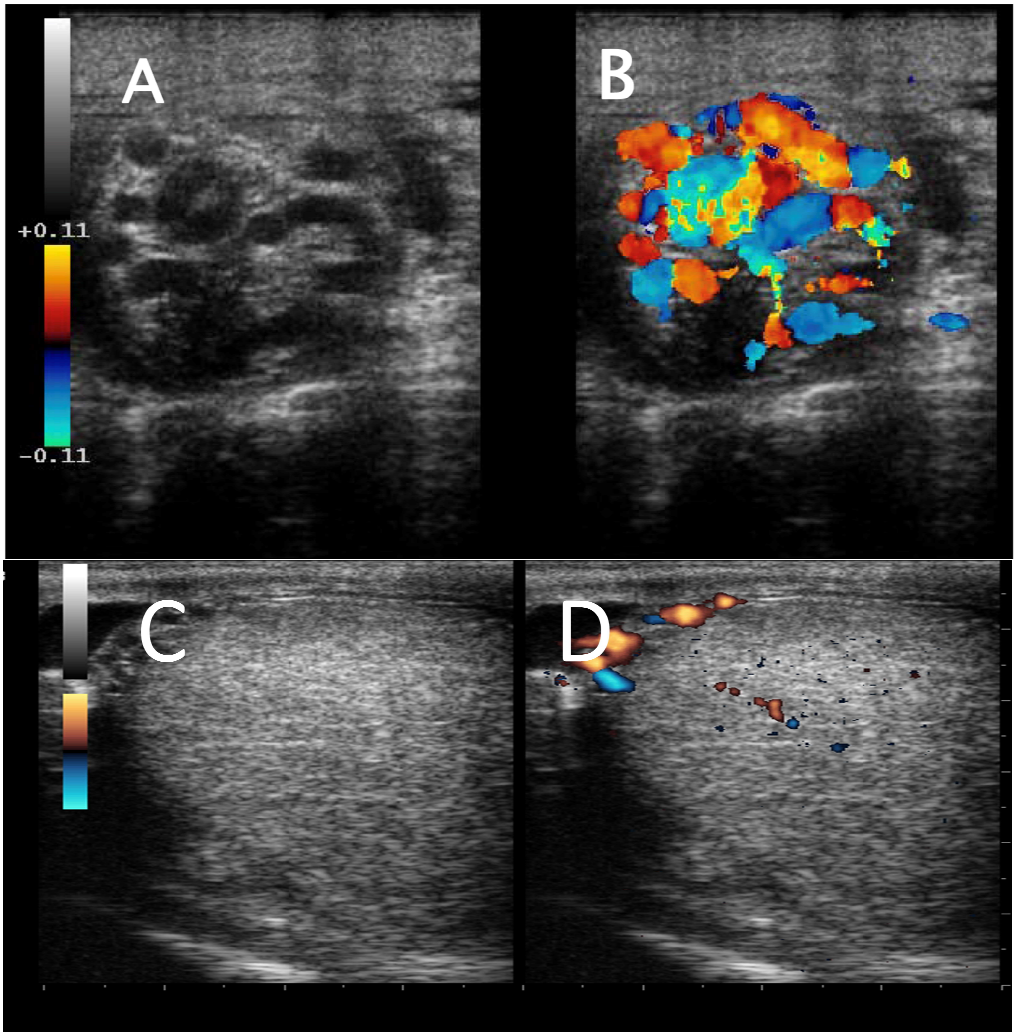


Figure 2. Comparison of B mode and colour Doppler ultrasound images of the supratesticular (A and B) and capsular (C and D) arteries in a stallion.

**Power Doppler**

The increased flow sensitivity and improved vascular detail provided by power Doppler have been used to detect flow presence and characteristics in vessels that are poorly imaged with conventional color Doppler. Thus, this ultrasound modality is also a qualitative technique, but with greater sensitivity than color Doppler, allowing the detection of small diameter vessels with slower flow such as microvascularization of the endometrium, testicular parenchyma, and CL(Ginther and Utt 2004) (figure 1). This technique combines a two-dimensional image with staining in the gate area that reflects the number of erythrocytes in a vascular segment. Therefore, the signal strength at each point is related to the number of blood cells that are moving per unit of time. Unlike color Doppler, this modality is independent of the angle of insonation and does not provide information about the direction of blood flow, or speed. The computerized analysis of power Doppler images of organs such as the uterus, has allowed us, for example, to differentiate pregnant from non-pregnant mares 8 days after ovulation and prior to embryo recovery (Nieto-Olmedo et al. 2020). Initially, this software has been used for research purposes, allowing pixels of the irrigated areas to be counted, thus obtaining quantitative data from the examined area. However, they are now being developed for clinical purposes, as is the case with Ecotext (Humeco, Huesca, Spain), a computer program developed for the evaluation of testicular echotexture in various species, providing information on testicular functionality(Abecia et al. 2020).

## Pulsed Doppler

This is the only modality that allows objective quantification of blood flow in a given vessel, providing a series of flow velocity and peripheral vascular resistance parameters that allow early diagnosis of any vascular insult. Frequency changes are plotted on a graph as a function of time(Ortega-Ferrusola et al. 2014a). One of the aspects to consider with this ultrasound modality is that the ultrasound equipment must have the triplex ultrasound function, which allows movement of the image in B-mode, color Doppler mode and Pulsed Doppler mode simultaneously. This function allows a more precise evaluation of the blood flow of the vessel being evaluated, especially in organs as mobile as the testis or for the transrectal evaluation of flow in uterine and ovarian arteries.

The flow velocity parameters provided by the ultrasound equipment are the peak systolic velocity (PSV), the end diastolic velocity (EDV) and the time average mean velocity (TAMV). From these three velocities, most ultrasound machines automatically calculate Doppler indices. These indices are the resistance index or RI ([PSV - EDV] / PSV) and the pulsatility index or PI ([PSV - EDV] / MV)(Ortega-Ferrusola et al. 2014a). RI indicates the vascular resistance of blood flow distal to the measurement site, while PI quantifies the pulsatility of the cardiac wave or cycle. Evaluation of these parameters and indices provides information on the hemodynamics of the organ studied. In general terms, atrophy or degenerative conditions will be characterized by a decrease in both indices and Doppler velocities, whereas hyperemic conditions will present a decrease in Doppler indices and an increase in velocities. Finally, ischemic processes (testicular torsion, inguinal hernias) can be identified by an increase in Doppler indices and a decrease in velocities(Ortega-Ferrusola et al. 2014b).

The vascular hemodynamics of organs such as the testes can be influenced by numerous factors, both physiological (age of the animal, body size, seasonality, sexual activity, or the use of sedatives during the evaluation) and pathological (orchitis, testicular torsion, hydrocele...) (Ortega-Ferrusola et al. 2014b; Araujo and Ginther 2009). Thus, stallions may present with seasonal testicular hyperemia or that due to increased sexual activity or with inflammatory hyperemia due to, for example, orchitis.

## Clinical applications of Doppler ultrasonography in stallions

Ultrasound was first used in the 90s in the reproductive evaluation of the stallion, allowing not only diagnosis of some reproductive pathologies, but also the calculation of actual sperm production (DSOa) (M. Pozor 2005). One of the parameters most widely used in the diagnosis of testicular dysfunction in stallions is the calculation of reproductive efficiency (RE: (DSOa/DSOp) x 100)(Love CC et al. 1991). Normally, when the actual sperm production (DSOa: volume x concentration) is well below the predicted one, this confirms that the stallion suffers from testicular dysfunction (RE <50%). However, when this occurs, in most cases, it is usually too late for treatment to be effective(T. L. Blanchard et al. 2001). This has led to the use of other ultrasound modalities that allow an earlier diagnosis of subfertility, such as Doppler ultrasound. The testicle is a highly metabolic organ with a very low concentration of oxygen in the seminiferous tubules, so any changes in the vascular supply will affect the functionality of the testicle. For this reason, in recent years there have been a greater number of studies in which Doppler ultrasound is used as a tool both in the evaluation of the reproductive potential of the stallion, as well as in the diagnosis and monitoring of medical and surgical treatments.

The evaluation of testicular vascularization can be carried out in three locations on the testicular artery: at the level of the cord (supratesticular artery), on the caudal pole of the testicle near the tail of the epididymis (capsular artery) or within the parenchyma (intratesticular artery). In a previous study by our group, we found that the capsular artery was the most reliable location to carry out spectral Doppler assessment. This artery has a straighter trajectory, unlike the supratesticular artery, which has a more contoured path. Furthermore, we were able to verify that blood flow parameters for this artery were most closely correlated with different parameters of sperm quality (Ortiz-Rodriguez et al. 2017). Measurement of flow in the intratesticular artery is feasible, but the position of these arteries and their small diameter meant that measurement of Doppler parameters was tedious and time-consuming.

## Diagnosis of testicular dysfunction

The diagnosis of testicular dysfunction in the stallion has traditionally been based on the evaluation of sperm quality and production, evaluation of levels of FSH, LH and estradiol in plasma, and

on the calculation of testicular volume and DSOp using B mode ultrasound (T. Blanchard et al. 2000; T. L. Blanchard et al. 2001; Love CC et al. 1991). However, most of these parameters are only affected in advanced stages of testicular degeneration(Oristaglio Turner 2007). In addition, they require samples to be sent to specialized laboratories or, as in the case of seminal evaluation, they require continuous monitoring of the animal at specific times (seasonal variations) for early detection of any decrease in sperm quality or production.

Doppler ultrasound provides the clinical practitioner with a simple and rapid diagnosis of any vascular insult that may ultimately affect the function of the testicle. Several studies in different species demonstrated that Doppler parameters are correlated with sperm quality and production parameters (Zelli et al. 2013; Gloria et al. 2018; M.A. Pozor et al. 2014; Biagiotti et al. 2002). Doppler indices have been found to be potential markers of testicular functionality. In humans, RI is a marker of testicular dysfunction widely used in reproductive medicine to identify patients with different causes of testicular dysfunction (Pinggera et al. 2008). In horses, the RI was also found to be increased in oligospermic stallions and in horses with pharmacologically induced testicular dysfunction (M.A. Pozor et al. 2014; Ortiz-Rodriguez et al. 2017), so it may be a promising method for the diagnosis of obstructive azoospermia (blockage of the ampulla) versus the non-obstructive type. In equines it has been found that the best Doppler parameters to predict sperm quality in stallions using ROC curves were: Doppler velocities (PSV, EDV and TAMV), the diameter of the capsular artery and TABF parameters (tissue perfusion parameters) (Ortiz-Rodriguez et al. 2017). In general terms, it was observed that oligospermic and asthenospermic stallions presented a decrease in Doppler velocities and an increase in Doppler indices. In the same study, reference values for Doppler parameters were established for the first time in equines. In addition, cut-off values were calculated to differentiate between fertile stallions and those with chronic testicular dysfunction. The diameter of the capsular artery was also a good predictor of subfertility, with diameters of this artery below 2.9 mm in subfertile stallions (oligospermic and asthenospermic). In this study, in addition to Doppler parameters, sperm quality parameters were evaluated by flow cytometry (viability, mitochondria and DNA fragmentation), DSO and sperm motility and velocity parameters using CASA systems. Important correlations were found between the Doppler parameters and the sperm quality parameters studied (Ortiz-Rodriguez et al. 2017). Thus, Doppler ultrasonography is a valuable diagnostic tool for use by clinical practitioners for the diagnosis of stallions with testicular dysfunction and could be a viable alternative to invasive procedures traditionally used for diagnosis of sub-fertility disorders.

**Monitoring of therapeutic outcomes following treatment**

Doppler ultrasound allows not only the diagnosis of pathological conditions but also the monitoring of medical treatments administered to patients. The functionality of the testicle is highly dependent on the blood flow it receives. Several studies have been carried out to see if certain drugs can improve testicular perfusion and therefore the quality of ejaculates. N-acetyl-cysteine (NAC) and pentoxifylline are drugs with antioxidant properties and also improve the microcirculation of tissues (HA Takhtfooladi et al. 2016). Pentoxifylline increases microcirculatory blood flow due to erythrocyte deformability, blood viscosity, platelet aggregation, and plasma fibrinogen concentrations(Ward and Clissold 1987). Several studies using the mouse model confirmed how treatment with pentoxifylline improved testicular vascularization in testes that had suffered torsion or some type of ischemia (Savaş et al. 2002; M. A. Takhtfooladi, Moayer, and Takhtfooladi 2015). Furthermore, it has been shown to improve seminal quality in patients with varicocele as well as in patients with asthenospermia and idiopathic oligozoospermia (Oliva, Dotta, and Multigner 2009; Schill 1982). In horses, oral administration of pentoxifylline over 2 months increased vascular perfusion of the testes, increasing the Total Arterial Blood Flow Rate (TABFR) and decreasing the Doppler indices in the stallions treated during and after treatment (Malgorzata A. Pozor et al. 2011). However, in this study sperm quality was not affected by treatment, possibly due to the short period of treatment administration (2 months), compared to similar studies in humans (3-6 months) (Marrama et al. 2009; Oliva, Dotta, and Multigner 2009). Another medical treatment that has been evaluated using Doppler ultrasound was the administration of a single dose of hCG (5000 IU), which improved testicular perfusion as early as 1 h after administration (Bollwein et al. 2008).

Doppler ultrasound has also allowed monitoring of surgical techniques. Acquired inguinal herniation is a very common condition in stallions, usually leading to unilateral or bilateral castration to

prevent future recurrence. Recently, several surgical techniques such as the standing laparoscopic peritoneal flap hernioplasty (SLPFH) have been developed to avoid herniation recurrence and preserve the breeding activity of high economic value stallions (Gracia-Calvo, Ortega Ferrusola, and Ezquerra 2014). Our research group carried out a study evaluating whether the preventive closure of the inguinal rings using standing laparoscopic peritoneal flap hernioplasty (SLPFH) compromised the testicular vascularization and the seminal quality of these horses. In this study pulsed Doppler ultrasound and conventional seminal evaluation techniques were performed in order to check whether reproductive function of stallions was preserved after this intervention (Gracia-Calvo et al. 2015; 2014). Sperm production and sperm quality parameters of stallions on which surgery was performed were not affected during the year after surgery. However, curiously, it was observed that all the stallions presented a decrease in Doppler indices and an increase in Doppler velocities at 12 months after surgery, corresponding with a hyperemic, non-ischemic process as expected. This hyperemia could be due to an increase in the sexual activity of the stallions because these stallions had been never used as breeding males before or otherwise, the SLPFH may have been compressing the spermatic cord at the level of the internal inguinal ring, causing a slight external stenosis of the testicular artery. This would trigger a compensatory hyperemia to support the deficit of blood flow supplying the testis (Gracia-Calvo et al. 2015).

## Early diagnosis of acute subfertility problems

Doppler ultrasound is a good tool for early diagnosis of testicular dysfunction. In a preliminary study by our research group in which we induced testicular dysfunction through anti-GnRH immunization, we were able to verify that immunocastration with Improvac® reduced the blood flow to the testis before the sperm quality and production were affected. Testicular vascularization (TABF) significantly decreased one month after the first Improvac® vaccine. However, all the quality and production parameters evaluated significantly decreased two months after the first vaccine. Once again, highlighting the importance of adequate irrigation for correct functioning of the testicle.

## Clinical applications of Doppler ultrasonography in mares

B-mode ultrasound has become an indispensable tool in the reproductive examination of the mare. In contrast, the use of Doppler ultrasound by clinical practitioners is not as widespread. The first published work on Doppler ultrasound in mares was in 1998 by Dr Bollwein's group (H Bollwein et al. 1998). Subsequently, numerous studies have been carried out on the use of Doppler ultrasound in the mare.

## The use of Doppler ultrasound for evaluation of the CL and follicle

One of the most widespread applications of color Doppler ultrasound in reproductive practice is assessment of the functionality of the corpora lutea. Several studies have shown that the area of luteal blood vessels correlates well with circulating progesterone [$P_4$] concentrations in estrous cycling mares (H. Bollwein et al. 2002). Moreover, luteal blood flow is positively correlated with circulating [$P_4$] in the non-pregnant mare, where it is a more reliable predictor of CL function than CL size, particularly during luteal regression (Ginther, Gastal, Gastal, Utt, et al. 2007; H. Bollwein et al. 2002).

Numerous studies have been carried out aimed at learning more about follicular dynamics in the mare. Among the most important findings are the greater vascularization that dominant follicles present compared to the subordinate ones during the follicular selection process. This greater vascularization occurs before changes in the follicular diameter are detected (Acosta et al. 2004); a decrease in the percentage of follicle circumference with signs of blood flow in the follicle wall occurs 4 hours before ovulation (Gastal, Gastal, and Ginther 2006a). Serration of the granulosa opposite the future site of ovulation results from blood vessels beneath the granulosa (Ginther, Gastal, and Gastal 2007) or the differences in the vascularization pattern between preovulatory follicles and hemorrhagic follicles (Ginther, Gastal, Gastal and Beg, 2007).

## The use of Doppler ultrasound in embryo transfer programs

All advances in Doppler ultrasound have promoted its use and paved the way for new

applications of this technique in embryo transfer programs (Pugliesi et al. 2018). The high precision and sensitivity of this technique in the evaluation of CL function has led to new applications such as the selection of recipient mares at the time of embryo transfer based on luteal blood flow (Brogan et al., 2016; Ferreira et al., 2020a). A recent study in which both color and pulsed Doppler ultrasound were used for the selection of recipients, found that mares with a RI in their uterine arteries close to 1.0 correlated with mares with high CL vascularization and elevated P4 concentrations (Ferreira et al. 2020b). In addition, it has been proven that CL area, area of blood flow, or [$P_4$] are predictive of pregnancy (Brogan et al. 2016).

Another application of Doppler ultrasound in embryo transfer programs has been the diagnosis of pregnancy on day 7 or 8 prior to collection of the donor embryo. Flushing for embryo recovery from the donor mare's uterus is normally performed without a previous pregnancy diagnosis since early pregnancy diagnosis techniques in mares (before 10 days) did not exist until now. Previous research has revealed that migration of the embryo could have a local effect on endometrial vascularization, stimulating uterine blood flow (Heinrich Bollwein, Mayer, and Stolla 2003). Using power Doppler ultrasound and subsequently analyzing the images with Image J v1.48 software (computer analysis of Doppler images), significant differences were detected in uterine blood flow between pregnant and non-pregnant mares. Vascular perfusion of the endometrium was analyzed using spot meter techniques, measuring mean pixel intensity and area of blood flow (figure 3). Mares with positive flushings presented a higher uterine blood flow area (one embryo: 54.01 ± 2.27 mm2 or two embryos: 61.01 ± 6.73 mm2) compared to barren mares (21.77 ± 2.22 mm2) ($p < 0.05$). However, significant differences in vascular perfusion were not detected between single or twin pregnancies. Blood flow is a good predictor for differentiation between pregnant and non-pregnant mares with an AU C: 0.869; $p < 0.001$ and an optimal cut-off value of 37.21 mm.

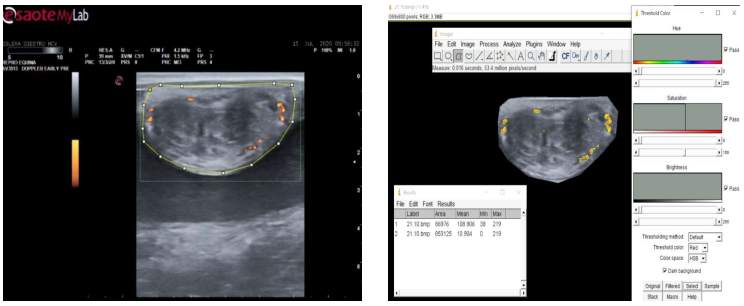

Figure 3. Computer analysis of power Doppler images of a uterine horn, used to differentiate pregnant from non-pregnant ares 8 days post-ovulation. Transverse echographic sections of the uterine horn are *first obtained using power Doppler. The uterine horn area is subsequently defined and Image J software is used to calculate mean pixel intensity and area of blood flow employing spot meter techniques.*

**The use of Doppler ultrasound in the diagnosis of endometritis**

Endometritis is one of the most frequent pathologies in mares and difficult to diagnose in many cases (Christoffersen and Troedsson 2017). Therefore, an increased understanding of the mechanisms involved in the disease progression is necessary to improve the treatment and management of these mares. In a recent study, color and pulsed Doppler ultrasound was incorporated into the diagnostic work up in mares with endometritis (Abdelnaby et al. 2020)

Uterine blood flow analyzed using color mode showed a significant increase in the endometritis group, while the spectral mode showed a significant increase in Doppler velocities and blood flow rate accompanied by a significant decrease in Doppler indices. The mares with endometritis had pathological hyperemia in the uterus. Therefore, the incorporation of Doppler ultrasound into standard diagnostics could improve diagnosis of endometritis in mares because it showed changes in uterine vascularization.

In a previous study by our group, we observed that older barren mares showed higher endometrial vascularity (35.06 ± 2.56 mm2) than younger ones (17.21 ± 1.26 mm2) and middle aged non-pregnant mares (23.84 ± 1.50 mm2) ($p < 0.05$). However, older (12-18 years old) and middle-aged pregnant mares (5-11 years old) had increased uterine vascularization compared to young pregnant mares (2-5 years old) ($p < 0.001$) but there was no difference between these two groups. This increased irrigation

in barren geriatric mares may be a consequence of a subclinical endometritis due to repeated flushing for embryo recovery(Nieto-Olmedo et al. 2020).

## The use of Doppler ultrasound for monitoring treatment

Doppler ultrasound has also been used as a tool to monitor medical treatment in mares. Several studies have evaluated treatments aimed at improving uterine and ovarian perfusion in mares such as supplementation with L-arginine and administration of pentoxifylline(Bailey et al. 2012; Kelley et al. 2014; Mesa et al. 2015).

## Conclusions

The use of the different ultrasound modalities in clinical research has enabled a significant amount of knowledge to be gained regarding reproductive pathophysiology in equines, in addition to improving the diagnosis of reproductive pathologies. The incorporation of pulsed Doppler ultrasound into the reproductive evaluation of the stallion has enabled early identification of stallions with testicular dysfunction, thus allowing administration of timely treatment and subsequent improvements of the fertility prognosis for these animals. In addition, this technique has been used in the monitoring of patients undergoing medical and surgical treatments, thus verifying their efficacy.

In the mare, the use of color Doppler and power Doppler ultrasound allow a quick and reliable evaluation of the functionality of the CL, proving to be a useful tool in the selection of recipients in embryo transfer programs. Power Doppler ultrasound allows a pregnancy diagnosis to be made as early as 7 or 8 days after ovulation. Finally, mares with endometritis experience a pathological increase in uterine blood flow that can be diagnosed by color and pulsed Doppler ultrasound.

## References

**Abdelnaby, Elshymaa A., Ibrahim A. Emam, Noha Y. Salem, Eman S. Ramadan, Marwa S. Khattab, Haithem A. Farghali, and Naglaa A.** Abd El Kader. 2020. "Uterine Hemodynamic Patterns, Oxidative Stress, and Chromoendoscopy in Mares with Endometritis." Theriogenology 158 (December). https://doi.org/10.1016/j.theriogenology.2020.09.012.

**Abecia, J.A., M. Carvajal-Serna, A. Casao, C. Palacios, L. Pulinas, M. Keller, P. Chemineau, and J.A. Delgadillo.** 2020. "The Continuous Presence of Ewes in Estrus in Spring Influences Testicular Volume, Testicular Echogenicity and Testosterone Concentration, but Not LH Pulsatility in Rams." Animal 14 (12). https://doi.org/10.1017/S1751731120001330.

Acosta**, T.J., E.L. Gastal, M.O. Gastal, M.A. Beg, and O.J. Ginther.** 2004. "Differential Blood Flow Changes Between the Future Dominant and Subordinate Follicles Precede Diameter Changes During Follicle Selection in Mares1." Biology of Reproduction 71 (2). https://doi.org/10.1095/biolreprod.104.027896.

**Adams, GP, Kastelic, JP, Bergfelt, DR, Ginther OJ.** 1987. "Effect of Uterine Inflammation and Ultrasonically-Detected Uterine Pathology on Fertility in the Mare ." J Reprod Fertil Suppl 35: 445–54.

**Araujo, Reno R., and O. J. Ginther.** 2009. "Vascular Perfusion of Reproductive Organs in Pony Mares and Heifers during Sedation with Detomidine or Xylazine." American Journal of Veterinary Research 70 (1). https://doi.org/10.2460/ajvr.70.1.141.

**Bailey, C.S., R.B. Sper, J.L. Schewmaker, C.N. Buchanan, T.M. Beachler, M.A. Pozor, and M.D. Whitacre.** 2012. "Uterine Artery Blood Flow Remains Unchanged in Pregnant Mares in Response to Short-Term Administration of Pentoxifylline." Theriogenology 77 (2). https://doi.org/10.1016/j.theriogenology.2011.08.018.

**Biagiotti, G., G. Cavallini, F. Modenini, G. Vitali, and L. Gianaroli.** 2002. "Spermatogenesis and Spectral Echo-Colour Doppler Traces from the Main Testicular Artery." BJU International 90 (9). https://doi.org/10.1046/j.1464-410X.2002.03033.x.

**Blanchard, T, D Varner, L Johnson, J Roser, J Hill, and C Miller.** 2000. "Testicular and Hormonal Changes in Stallions with Thermally Induced Testicular Degeneration." J Reprod Fertil Suppl 56: 51–59.

**Blanchard, T.L., L. Johnson, D.D. Varner, S.L. Rigby, S.P. Brinsko, C.C. Love, and C. Miller**. 2001. "Low Daily Sperm Output Per Ml of Testis as a Diagnostic Criteria for Testicular Degeneration in Stallions." Journal of Equine Veterinary Science 21 (1). https://doi.org/10.1016/S0737-0806(01)70228-6.

**Bollwein, H, J Maierl, R Mayer, and R Stolla.** 1998. "Transrectal Color Doppler Sonography of the in Cyclic Mares." Theriogenology 49 (8). https://doi.org/10.1016/S0093-691X(98)00094-6.

**Bollwein, H., R. Mayer, F. Weber, and R. Stolla.** 2002. "Luteal Blood Flow during the Estrous Cycle in Mares." Theriogenology 57 (8). https://doi.org/10.1016/S0093-691X(02)00705-7.

**Bollwein, Heinrich, Rainer Mayer, and Rudolf Stolla.** 2003. "Transrectal Doppler Sonography of Uterine Blood Flow during Early Pregnancy in Mares." Theriogenology 60 (4). https://doi.org/10.1016/S0093-691X(03)00080-3.

**Bollwein, Heinrich, Johann Joachim Schulze, Akio Miyamoto, and Harald Sieme.** 2008. "Testicular Blood Flow and Plasma Concentrations of Testosterone and Total Estrogen in the Stallion after the Administration of Human Chorionic Gonadotropin." Journal of Reproduction and Development 54 (5). https://doi.org/10.1262/jrd.20014.

**Brogan, P.T., H. Henning, T.A.E. Stout, and M. de Ruijter-Villani.** 2016. "Relationship between Colour Flow Doppler Sonographic Assessment of Corpus Luteum Activity and Progesterone Concentrations in Mares after Embryo Transfer." Animal Reproduction Science 166 (March). https://doi.org/10.1016/j.anireprosci.2015.12.010.

**Christoffersen, M, and MHT Troedsson.** 2017. "Inflammation and Fertility in the Mare." Reproduction in Domestic Animals 52 (August). https://doi.org/10.1111/rda.13013.

**Delorme, Stefan, Gerald Weisser, Ivan Zuna, Martin Fein, Adolf Lorenz, and Gerhard van Kaick.** 1995. "Quantitative Characterization of Color Doppler Images: Reproducibility, Accuracy, and Limitations." Journal of Clinical Ultrasound 23 (9). https://doi.org/10.1002/jcu.1870230906.

**Ferreira, Camila Silva Costa, Rita de Cássia Lima Morais, Amanda Bricio Pereira Andrade, Mário Felipe A. Balaro, José Antônio Silva Ribas, Gustavo Mendes Gomes, and Aline Emerim Pinna.** 2020a. "Spectral Doppler Ultrasound in Selecting an Equine Embryo Receiver." Reproduction in Domestic Animals 55 (6). https://doi.org/10.1111/rda.13676.

**Gastal, E L, M O Gastal, and O J Ginther.** 2006a. "Relationships of Changes in B-Mode Echotexture and Colour-Doppler Signals in the Wall of the Preovulatory Follicle to Changes in Systemic Oestradiol Concentrations and the Effects of Human Chorionic Gonadotrophin in Mares." Reproduction 131 (4). https://doi.org/10.1530/rep.1.01011.

**Gastal, E.L., M.O. Gastal, and O.J. Ginther**. 2006b. "Serrated Granulosa and Other Discrete Ultrasound Indicators of Impending Ovulation in Mares." Journal of Equine Veterinary Science 26 (2). https://doi.org/10.1016/j.jevs.2005.12.005.

**Ginther, O.J.** 2014. "How Ultrasound Technologies Have Expanded and Revolutionized Research in Reproduction in Large Animals." Theriogenology 81 (1). https://doi.org/10.1016/j.theriogenology.2013.09.007.

**Ginther, O.J., E.L. Gastal, and M.O. Gastal**. 2007. "Spatial Relationships between Serrated Granulosa and Vascularity of the Preovulatory Follicle and Developing Corpus Luteum." Journal of Equine Veterinary Science 27 (1). https://doi.org/10.1016/j.jevs.2006.11.012.

**Ginther, O.J., E.L. Gastal, M.O. Gastal, and M.A. Beg**. 2007. "Incidence, Endocrinology, Vascularity, and Morphology of Hemorrhagic Anovulatory Follicles in Mares." Journal of Equine Veterinary Science 27 (3). https://doi.org/10.1016/j.jevs.2007.01.009.

**Ginther, O.J., E.L. Gastal, M.O. Gastal, M.D. Utt, and M.A. Beg**. 2007. "Luteal Blood Flow and Progesterone Production in Mares." Animal Reproduction Science 99 (1–2). https://doi.org/10.1016/j.anireprosci.2006.05.018.

**Ginther, O.J., and Matthew D. Utt.** 2004. "Doppler Ultrasound in Equine Reproduction: Principles, Techniques, and Potential." Journal of Equine Veterinary Science 24 (12). https://doi.org/10.1016/j.jevs.2004.11.005.

**Gloria, Alessia, Augusto Carluccio, Laura Wegher, Domenico Robbe, Claudio Valorz, and Alberto Contri.** 2018. "Pulse Wave Doppler Ultrasound of Testicular Arteries and Their Relationship with Semen Characteristics in Healthy Bulls." Journal of Animal Science and Biotechnology 9 (1). https://doi.org/10.1186/s40104-017-0229-6.

**Gracia-Calvo, L.A., J. Duque, C. Balao da Silva, J. Ezquerra, and C. Ortega-Ferrusola.** 2015. "Testicular Perfusion after Standing Laparoscopic Peritoneal Flap Hernioplasty in Stallions." Theriogenology 84 (5). https://doi.org/10.1016/j.theriogenology.2015.05.014.

**Gracia-Calvo, L.A., L.J. Ezquerra, M. Martín-Cuervo, M.E. Durán, H. Tapio, J.M. Gallardo, F.J. Peña, and C. Ortega-Ferrusola.** 2014. "Standing Laparoscopic Peritoneal Flap Hernioplasty of the Vaginal Rings Does Not Modify the Sperm Production and Motility Characteristics in Intact Male

Horses." Reproduction in Domestic Animals = Zuchthygiene 49 (6). https://doi.org/10.1111/rda.12434.
**Gracia-Calvo, L.A., C. Ortega Ferrusola, and J. Ezquerra.** 2014. "Laparoscopic Closure of the Inguinal Rings in Horses: Literature Review." Journal of Equine Veterinary Science 34 (10). https://doi.org/10.1016/j.jevs.2014.07.003.
**Kelley, Dale, Michelle M. LeBlanc, Lori K. Warren, and Christopher J. Mortensen.** 2014. "Influence of L-Arginine Supplementation on Reproductive Blood Flow and Embryo Recovery Rates in Mares." Theriogenology 81 (5). https://doi.org/10.1016/j.theriogenology.2013.12.012.
**Love CC, Garcia MC, Riera F, and Kenney RM.** 1991. "Evaluation of Measures Taken by Ultrasonography and Caliper to Estimate Testicular Volume and Predict Daily Sperm Output in the Stallion ." Journal of Reproduction and Fertility. Supplement 44: 99–105.
**Marrama, P., G.F. Baraghini, C. Carani, M.F. Celani, P. Giovenco, F. Grandi, and V. Montanini.** 2009. "Further Studies on the Effects of Pentoxifylline on Sperm Count and Sperm Motility in Patients with Idiopathic Oligo-Asthenozoospermia." Andrologia 17 (6). https://doi.org/10.1111/j.1439-0272.1985.tb01728.x.
**Mebarki, M., R. Kaidi, A. Azizi, and M. Basbaci.** 2019. "Comparative Efficacy of Two-Dimensional Mode and Color Doppler Sonography in Predicting Gender of the Equine Fetus." Veterinary World 12 (2). https://doi.org/10.14202/vetworld.2019.325-330.
**Mesa, A.M., L.K. Warren, J.M. Sheehan, D.E. Kelley, and C.J. Mortensen.** 2015. "L-Arginine Supplementation 0.5% of Diet during the Last 90 Days of Gestation and 14 Days Postpartum Reduced Uterine Fluid Accumulation in the Broodmare." Animal Reproduction Science 159 (August). https://doi.org/10.1016/j.anireprosci.2015.05.011.
**Nieto-Olmedo, P., F.E. Martín-Cano, G. Gaitskell-Phillips, J.M. Ortiz-Rodríguez, F.J. Peña, and C. Ortega-Ferrusola**. 2020. "Power Doppler Can Detect the Presence of 7–8 Day Conceptuses Prior to Flushing in an Equine Embryo Transfer Program." Theriogenology 145. https://doi.org/10.1016/j.theriogenology.2020.01.015.
**Oliva, Alejandro, Alejandro Dotta, and Luc Multigner**. 2009. "Pentoxifylline and Antioxidants Improve Sperm Quality in Male Patients with Varicocele." Fertility and Sterility 91 (4). https://doi.org/10.1016/j.fertnstert.2008.09.024.
**Oristaglio Turner, Regina M**. 2007. "Pathogenesis, Diagnosis, and Management of Testicular Degeneration in Stallions." Clinical Techniques in Equine Practice 6 (4). https://doi.org/10.1053/j.ctep.2007.09.006.
**Ortega-Ferrusola, C, LA Gracia-Calvo, J Ezquerra, and FJ Pena.** 2014b. "Use of Colour and Spectral Doppler Ultrasonography in Stallion Andrology." Reproduction in Domestic Animals 49 (October). https://doi.org/10.1111/rda.12363.
**Ortiz-Rodriguez, J.M., L. Anel-Lopez, P. Martin-Munõz, M. Lvarez, G. Gaitskell-Phillips, L. Anel, P. Rodriguez-Medina, F.J. Penã, and C. Ortega-Ferrusola.** 2017. "Pulse Doppler Ultrasound as a Tool for the Diagnosis of Chronic Testicular Dysfunction in Stallions." PLoS ONE 12 (5). https://doi.org/10.1371/journal.pone.0175878.
**Pinggera, Germar-Michael, Michael Mitterberger, Georg Bartsch, Hannes Strasser, Johannes Gradl, Friedrich Aigner, Leo Pallwein, and Ferdinand Frauscher.** 2008. "Assessment of the Intratesticular Resistive Index by Colour Doppler Ultrasonography Measurements as a Predictor of Spermatogenesis." BJU International 101 (6). https://doi.org/10.1111/j.1464-410X.2007.07343.x.
**Pozor, M.A., M. Nolin, J. Roser, S. Runyon, M.L. Macpherson, and A. Kelleman.** 2014. "Doppler Indices of Vascular Impedance as Indicators of Testicular Dysfunction in Stallions." Journal of Equine Veterinary Science 34 (1). https://doi.org/10.1016/j.jevs.2013.10.021.
**Pozor, Malgorzata**. 2005. "Diagnostic Applications of Ultrasonography to Stallion's Reproductive Tract." Theriogenology 64 (3). https://doi.org/10.1016/j.theriogenology.2005.05.007.
**Pozor, Malgorzata A., Jennifer Muehlhaus, Alana King, Margo L. Macpherson, Mats H. Troedsson, and Christopher S. Bailey**. 2011. "Effect of Pentoxifylline Treatment on Testicular Perfusion and Semen Quality in Miniature Horse Stallions." Theriogenology 76 (6). https://doi.org/10.1016/j.theriogenology.2011.05.005.
**Pugliesi, Guilherme, Gabriela Dalmaso de Melo, Gilmar Arantes Ataíde, Carlos Augusto Gontijo Pellegrino, Júlio Barboza Silva, Cecília Constantino Rocha, Igor Garcia Motta, José Luiz Moraes Vasconcelos, and Mario Binelli**. 2018. "Use of Doppler Ultrasonography in Embryo Transfer Programs: Feasibility and Field Results." Animal Reproduction 15 (3). https://doi.org/10.21451/1984-3143-AR2018-0059.

**Resende, H. L., M. T. Carmo, C. Ramires Neto, and M. A. Alvarenga**. 2014. "Determination of Equine Fetal Sex by <scp>D</Scp> Oppler Ultrasonography of the Gonads." Equine Veterinary Journal 46 (6). https://doi.org/10.1111/evj.12213.

**Savaş, Çağrı, Hüseyin Dindar, Tülin Aras, and Selçuk Yücesan**. 2002. "Pentoxifylline Improves Blood Flow to Both Testes in Testicular Torsion." International Urology and Nephrology 33 (1). https://doi.org/10.1023/A:1014469323448.

**Schill, W B.** 1982. "[Therapy of Idiopathic Astheno- and Oligozoospermia with Pentoxifylline]." Fortschritte Der Medizin 100 (15).

**Takhtfooladi, Hamed Ashrafzadeh, Saeed Hesaraki, Foad Razmara, Mohammad Ashrafzadeh Takhtfooladi, and Hadi Hajizadeh.** 2016. "Effects of N-Acetylcysteine and Pentoxifylline on Remote Lung Injury in a Rat Model of Hind-Limb Ischemia/Reperfusion Injury." Jornal Brasileiro de Pneumologia 42 (1). https://doi.org/10.1590/S1806-37562016000000183.

**Takhtfooladi, Mohammad Ashrafzadeh, Fariborz Moayer, and Hamed Ashrafzadeh Takhtfooladi.** 2015. "Beneficial Effect of Pentoxifylline into the Testis of Rats in an Experimental Model of Unilateral Hindlimb Ischemia/Reperfusion Injury." International Braz j Urol 41 (3). https://doi.org/10.1590/S1677-5538.IBJU.2014.0263.

**Ward, Alan, and Stephen P. Clissold.** 1987. "Pentoxifylline." Drugs 34 (1). https://doi.org/10.2165/00003495-198734010-00003.

**Zelli, R., A. Troisi, A. Elad Ngonput, L. Cardinali, and A. Polisca**. 2013. "Evaluation of Testicular Artery Blood Flow by Doppler Ultrasonography as a Predictor of Spermatogenesis in the Dog." Research in Veterinary Science 95 (2). https://doi.org/10.1016/j.rvsc.2013.04.023.