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# Uterine and Luteal hemodynamic evaluation of the non pregnant mare

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**ABSTRACT**

***Background*:** Studies with Doppler ultrassonography started at the end of the 90s for the determination of physiological and pathological alterations in the reproductive tract of the mare. Uterine alterations caused by inflammation, response from seminal plasma infusion, hormonal variations during estrous and diestrus, pregnancy and action of various vasoactive factors influence on the vascular perfusion detected by Doppler ultrasound. The development of efficient methods for uterine quality evaluation is of big importance for field equine reproduction veterinarians, once uterine environment is responsible for pregnancy maintenance.

***Review*:** Nowadays, the most used methods of uterine evaluation are the mode B ultrassonography, cytology, culture and biopsy. Hemodynamic evaluation of the uterus can be done by spectral data collected from large vessels, as A. uterine and its ramifications, or from subjective or objective evaluations from endometrium, miometrium and mesometrium attachment, which provide data referent to local and specific alterations of the evaluated area. Alterations in uterine vascular perfusion has been detected during estrous cycle, during pregnancy and in cases of infusion of inflammatory substances. These alterations happen because of vasoactive substances that act in the uterus during these events, however, most of these vasoactive substances are probably not even known. Also, important hemodynamic alterations in old mares, as an increase in vascular resistance, have been described. This increase might result from fibrosis of the uterus and in women it is considered to be a cause of infertility. In mares, periglandular fibrosis of the endometrium is considered to be the major diagnosable cause of embryonic and fetal loss in older mares. For the CL, ovarian artery of the mare supplies the ovary as well as the oviduct and therefore can be used for evaluation of these areas. The CL evaluation can also be done by the percentage of luteum area with colored signals as an indicator of the extent of blood flow. The percentage of the CL area with colored signals is determined subjectively by images observations in real time and/or by a freezing Power Doppler cross-section image with the maximum number of color pixels taped and the total number of color pixels is assessed by a computer analysis system. Therefore, a high correlation between plasmatic progesterone and CL vascularization also allows the CL evalution by this technique. In a first report, CL circulation reached its maximum on D5, the progesterone concentration in peripheral blood increased until D7 and in a posterior report, maximum perfusion was achieved two days after the maximum progesterone concentration (D8). Blood flow reduced between D10 - D14 some days before the plasma progesterone decrease and, during the luteolytic period (D15 - D17), the decline in CL blood-flow area was greater than blood flow decrease.

***Conclusion*:** Doppler ultrassonography add knowledge about uterine viability and CL functionality can be easily used by veterinarians in the field. It is a noninvasive method that provides real time results. However, because of the short time studies in this area have been done, many other answers still need to be found until normal and pathological patterns will be established.

**Keywords:** Doppler ultrassonography, vascular perfusion, uterine hemodynamic, CL hemodynamic, mare, reproduction.

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## I. INTRODUCTION

In the end of the 90s, studies utilizing Doppler ultrassonography started to be developed aiming to determine physiologic and pathologic alterations in the reproductive tract of the mare.

Uterine, follicle and corpus luteum vascular perfusion is the focus of the most studies. The objective of this review is to show the Doppler findings so far detected in the uterus and in the corpus luteum of non pregnant mares.

## II. LITERATURE REVIEW

### 2.1 Uterine evaluation

The development of efficient methods for uterine quality evaluation is of big importance for field equine reproduction veterinarians, once uterine environment is responsible for pregnancy maintenance. Nowadays, the most used methods of uterine evaluation are the mode B ultrassonography, cytology, culture and biopsy.

Doppler ultrassonography is a noninvasive method that brings information about vascular perfusion in real time. By this reason, it is noticed an increasing interest on the development of techniques applied to field for selection and detection of important uterine alterations about mare fertility.

Hemodynamic evaluation of the uterus can be done by spectral data collected from large vessels, as A. uterine and its ramifications [4], or from subjective or objective evaluations from endometrium, miometrium and mesometrium attachment, which provide data referent to local and specific alterations of the evaluated area [16].

In 1998 [4], a study aiming to develop a technique for measurement of the blood flow from spectral data (resistence index) from A. uterine in mares was done. The authors also evaluated changes in blood flow between mares, during the estrous cycle and between subsequent cycles.

The observation that left-right differences in RI were not related to the side of ovulation [4]. Later studies confirmed these findings, there is no difference of perfusion between large uterine arteries [3] neither between vessels of the mesometrium attachment [16] of the horns ipsi and contralateral to ovulation (Figure 1). However, differences in uterine blood flow are seen during the estrous cycle [3,4]. Not inseminated mares showed increased vascular perfusion [reduction in the resistence index (RI)] at D5 (D0 = ovulation) that was related to the importance of a high blood supply at the time of entry of embryo into the uterus. Around two or three days before ovulation and at D0, an increase in perfusion (redu-

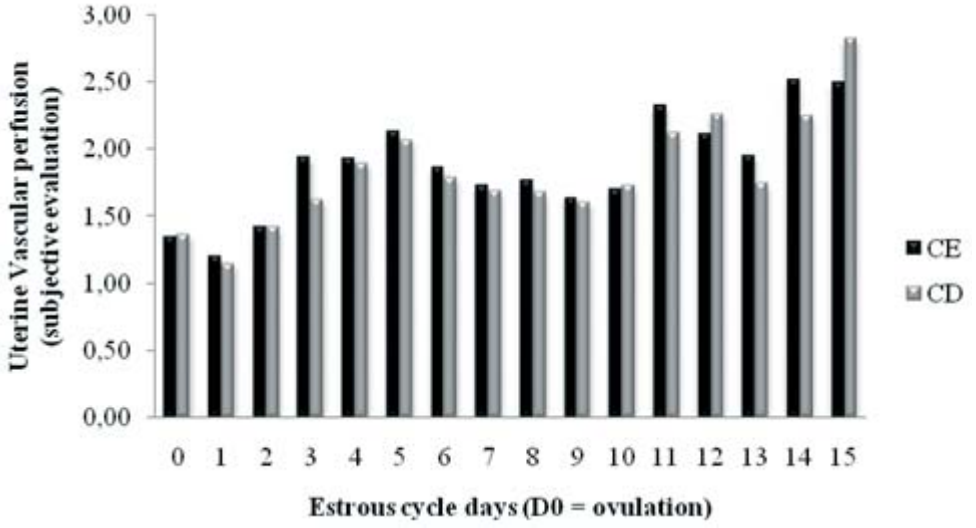


**Figure 1.** Values of uterine vascular perfusion from real time subjective evaluation graduated from 1 to 4 by Power Doppler mode of the left (CE) and right (CD) horns during estrous cycle days in mares used as embryo recipients.

ction on RI) followed by reduction (increase on RI) until ovulation were detected, probably because of the increase and decrease in estrogen (vasodilatory effect on uterine vessels) during these days. A perfusion reduction were also detected at D15 (end of diestrus) not related to estrogen levels but by other vasoative compounds that might be acting [4]. Therefore, alterations on the uterine perfusion are correlated to plasmatic concentrations of estrogen during estrous but not during diestrus [3].

In 2005, Silva *et al.* [16] described a subjective evaluation of the endometrium and myometrium and a objective evaluation from spectral data collected from vessels of the mesometrium attachment to evaluate uterine vascular perfusion alterations [16], different of the until now used [2,4]. In this experiment all mares were inseminated and the uterine perfusion of pregnant and non pregnant was compared. There was no detected difference in vascular perfusion between pregnant and non pregnant mares until D11, but from this day pregnant mares showed a gradual increase in vascular perfusion.

A maximum value of mares with inadequate Doppler signals occurred on Days 4-6, which was attributed to a low RI. However, the presence or absence of an embryo on Days 1-8 cannot be considered, because embryonic loss could have occurred in the group with no embryo detected by D12 [16].

It is important to point that hemodynamic alterations that indicate an increase on vascular perfusion during the beginning of diestrus (D4 – D6) have been described [3,9,14]. Non pregnant and no inseminated mares have shown a reduced RI from the spectral evaluation of A. uterine [3]. Using the same technique as Silva *et al*. [16], recent studies showed a gradual increase on RI and PI from D3 in pregnant mares [9] and reduction on PI at D5 (Figure 2) in mares used as embryo recipients [14]. In this later report, mares receiving embryos at D4 - D6 demonstrated uterine blood flow increase on both

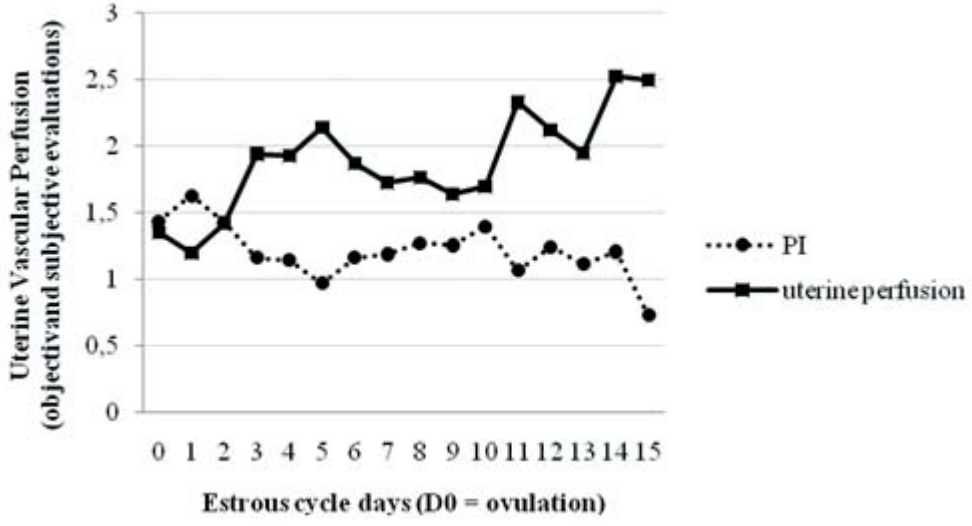


**Figure 2.** Values of uterine vascular perfusion from real time subjective analysis graduated from 1 to 4 by Power Doppler (uterine perfusion, means of both horns) and objective analysis by pulsatility index values (PI) from spectral mode of mesometrial attachment vessels during the estrous cycle days in mares used as embryo recipients.

uterine horns during these days [14]. All the results show a vascular perfusion increase during the beginning of diestrus, however, results are still note conclusive if the increase is a result of entry of the embryo into the uterus or if it is part of a uterine preparation for a possible entry of the embryo.

Important hemodynamic alterations in old mares, as an increase in vascular resistance [4,9], have been described. This increase might result from fibrosis of the uterus [4] and in women it is considered to be a cause of infertility [5]. In mares, periglandular fibrosis of the endometrium is considered to be the major diagnosable cause of embryonic and fetal loss in older mares [15]. Recently, a comparison between mares with and without uterine cysts (a common ultrasound finding in mares with degenerative chronic

endometriosis) found a reduced uterine vascular perfusion in mares with uterine cysts and a positive association between size of the cystic are and disturbed uterine hemodynamics [8].

So far, only one report has evaluated the uterine hemodynamic in recipient mares and because partial results were published it is not yet concluded which parameters should be followed to the selection of mares [14]. The results from the evaluation of the beginning of pregnancy are in accordance to the ones reported before for inseminated and pregnant mares [9,14].

Few are known about the hemodynamic uterine response to the infusion of inflammatory substances, such as semen. To evaluate the uterine inflammatory response and hemodynamic, Bollwein et al. (2003) [2] injected in one group of mare raw semen and in the other seminal plasma. Cytological evaluation showed that, compared to raw semen, intrauterine infusion of seminal plasma caused only mild endometritis, but both led to a remarkable increase in uterine blood flow. Nitric oxide (NO) is a potent vasodilator released during endometrial inflammation [17]. However, the result suggests that not only uterine inflammation, but also other factors are responsible for increased uterine blood flow, such as prostaglandins and estrogens present in seminal plasma [7]. Further investigations are necessary to determine whether changes in uterine and ovarian perfusion after insemination are related to fertility in mares.

#### 2.2 CORPUS LUTEUM (CL) EVALUATION

Ovarian artery of the mare supplies the ovary as well as the oviduct and therefore can be used for evaluation of these areas. The CL evaluation can also be done by the percentage of luteum area with colored signals as an indicator of the extent of blood flow. The percentage of the CL area with colored signals is determined subjectively by images observations in real time [12] and/or by a freezing Power Doppler cross-section image with the maximum number of color pixels taped and the total number of color pixels is assessed by a computer analysis system [1].

The first evaluation of the ovarian hemodynamic was reported in 2002 from data collected of the right and left ovarian arteries [3]. In contrast to the uterine arteries, ovarian arteries revealed significant differences between sides in blood supply. PI values were sifnificantly lower in the ovarian artery ipsilateral to the corpus luteum. In this side, the resistance to blood flow declined after a short rise until D6 and increased afterwards again until the end of diestrus [3].

During the estrous cycle an extensive angiogenesis takes place in the CL [13] which is essential for the substrate supply needed for the biosynthesis and secretion of progesterone [6]. Vascular perfusion and progesterone concentrations showed high correlation, both parameters rose noticeably in the first days after ovulation [1,10]. In a first report, the CL circulation reached its maximum on D5, the progesterone concentration in peripheral blood increased until D7 [1] and in a posterior report, maximum perfusion was achieved two days after the maximum progesterone concentration (D8) [10]. Blood flow reduced between D10 - D14 some days before the plasma progesterone decrease [1, 10] and, during the luteolytic period (D15 - D17), the decline in CL blood-flow area was greater than blood flow decrease [10].

During the luteolytic period, blood flow was constant during the ascending portion and decreased during the descending portion of the PGFM pulses [11].

## III. FINAL CONSIDERATIONS

Uterine alterations caused by inflammation, response from seminal plasma infusion, hormonal variations during estrous and diestrus, pregnancy and action of various vasoactive factors influence on the vascular perfusion detected by Doppler ultrasound. As well as a high correlation between plasma progesterone concentration and CL blood flow allows the evaluation of the CL in this technique.

Doppler ultrassonography add knowledge to uterine viability and CL functionality and it can be easily used by field veterinarians. It is a non invasive technique and which supplies a real time result. However, because of the short time, further investigations are still necessary to the establishment of normal and pathological patterns.

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