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Mare's Folliculogenesis: Assessment of ovarian and perifollicular vascular perfusion by Doppler ultrasound

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ABSTRACT

***Background*:** Ultrasound Doppler is a new technology that has recently been used in large animal reproduction. As the conventional ultrasound (B-mode) it is a noninvasive technique, but with the advantage of allows the assessment of the hemodynamic of reproductive tract in real time. The observation of important features of the vascularization and changes that occur during physiological processes that were not previously seen on B-mode encourage a reassessment of the concepts already established on the events of the reproductive physiology of animals and their applications.

***Review*:** In attempt to re-understand the equine reproductive physiology and finding practical uses to this new technique, authors showed that, during the follicular deviation, features are observed by Doppler before being observed under B-mode ultrasound like changes in the speed of the blood flow two days before deviation of the follicle size and one day before the increase in blood flow area of the follicular wall. According to another study ovulation is characterized by a decreased blood flow of the follicular wall in the last four hours preceding it, as well as the serration of the granulosa layer and formation of a non vascularized apex, but in our ongoing study, the ability to decrease the vascularity was not found. Very vascularized follicles are associated with higher rates of oocyte maturation and pregnant that does less vascularized follicles in the preovulatory phase. Those follicles that have septated evacuation (or prolonged) showed more vascularization and serration of the granulosa one hour before ovulation than follicles that ovulate normally, and this vascularization includes the apex of the follicle, the follicular wall portion that is not vascularized in normal ovulation. Another study reported that hemorrhagic follicles have better vascularization of the follicular wall on the days preceding ovulation than follicles destined to ovulate. Some authors also showed that anovulatory follicles grow in size at the same rate as ovulatory follicles, but the percentage of vascularization of its wall is much smaller at 35 mm. Another study characterized that the vascular wall of the follicle that results in the first ovulation of the year is much smaller on the day before ovulation than the number of vessels present in a follicle that will ovulate in the middle of the breeding season. In these cases, the use of Doppler ultrasound can help to prevent economic losses as insemination of mares in cycles that are not able of resulting in pregnancy. This review aims to gather the information found in the literature about the characteristics of follicular hemodynamic of mares taking into account moments of deviation in follicle size, ovulation, ovulation failure and follicular viability.

***Conclusion*:** The Doppler technology has the potential to provide important information about the follicular environment and thus be used in practice in search of the perfect equine reproductive management, achieving better utilization of genetic material and increasing the financial return. The use of this new tool opens a large area for several interesting studies that will contribute to the knowledge of the physiological events of the mare for that this technique can soon be effectively applied.

***Keywords*:** mare, follicle, doppler, blood flow, follicular wall, ovulation.

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I. INTRODUCTION

Few, however exciting, are studies in the literature with use of Doppler in equine reproduction, this has been used for studying vascularization of the preovulatory follicle [5,13], predict oocyte maturation [13], follicular and oocyte quality check [14] and establishment of a pregnancy [15] and more.

The characteristics of the ovarian antral follicles of non pregnant mares have relation with changing in their reproductive stages. Studies involving the hypothalamus, pineal gland, and concentrations of circulating hormones have been given productive consideration for many years. Recently, several productive ultrasound adaptations have been made to better consider the follicles as targets or end points in seasonality studies. These innovations include: 1) transvaginal ultrasound-guided ablation of follicles appropriate to a given hypothesis, 2) transvaginal sampling and experimental treatment of targeted follicles, and 3) color Doppler ultrasound for assessing the changing vascularity of the follicle wall. These advances in technology have produced results that encourage a review of the current status of follicle dynamics and seasonality [9].

II. DOPPLER U.S. TO STUDY DEVIATION AND FOLLICULAR DOMINANCE

Deviation is preceded by a common growth phase of several days. During this phase, the follicles grow at an approximately similar rate and each follicle has the capacity for future dominance [4]. During follicle growth, an extensive vascular plexus develops in the thecal layer surrounding the avascular basement membrane and granulosa layer. It has been suggested that the preferential delivery of gonadotropins and nutrients via a more highly developed vascular system in individual follicles plays a role in the selection and growth of the dominant follicle and that insufficient vascular support contributes to follicle atresia [16]. In this regard deviation in mares is indicated morphologically not only by differential growth rate between the developing dominant and subordinate follicles, but also by an apparent expansion of the anechoic ultrasonic layer, as expressed subjectively, surrounding the granulosa of the dominant follicle [4]. This echotexture change distinguished the future dominant follicle from the future largest subordinate follicle about one day earlier than the beginning of diameter deviation and was attributed to increased vascularization. In another study, Acosta et al. [2] observed that even before the change in blood flow area can be viewed in color mode, the spectral analysis showed that the peak systolic velocity and time-average maximum velocity of blood flow begins to be higher in the dominant follicle in 6mm average (equivalent to 2 days) before deviation in follicle diameter. Although the concentrations of vascular endothelial growth factor in follicular fluid were higher in the largest follicle one day after the expected beginning of deviation [10], the original ratio of VEGF with the deviation is not known.

III. DOPPLER US TO DETECT THE TIME OF OVULATION

Currently, the imminence of ovulation in mares is estimated on B-mode ultrasound by the combination of thickness and echogenicity of the granulosa cell layer, decreased turgidity, loss of spherical shape of the follicle, granulosa detaching and echogenic spots in antrum, associated with decreased uterine edema, and can predict ovulation 24 h before [3]. Likewise, an anechoic band seen on B-mode gradually increases in the theca layer during the days preceding ovulation representing the blood flow of the follicle [5].

In a recent study, Gastal *et al*. [5] observed in ultrasound Doppler that in the last 4 h before ovulation occurs a decrease in the percentage of follicle circumference with color display and a decrease in the intensity of colors. The two surfaces of the granulosa (facing the antrum and the theca interna) become irregular during the last few hours prior to ovulation [6]. This phenomenon has been termed serration of the granulosa. Serration seemed most prominent at the base of the follicle opposite to the apex (future ovulation site). In this study, the authors noted also a formation of a not vascularized apical

area recognized by a alteration on the formerly spherical follicle a few hours before ovulation, indicating the future rupture point. However, in ongoing study in our laboratory (Uliani *et al*, unpublished data) there was no decrease in the amount of vascularization at the time immediately preceding ovulation.

In mares, increased blood flow and increase in diameter of the preovulatory follicle between the time of ovulation induction and 30 h after HCG injection was associated with higher rates of pregnancy [15]. Moreover, signs of more intense intraovarians signals of color indicated a decrease in rates of resistance (increased vascular perfusion) and increase in blood velocity in mares that became pregnant [15]. However the author showed the necessity to use more number of animals to confirm these findings. Ginther *et al*. [13] conducted a study in which follicles were aspirated 30 h after treatment with hCG and oocyte maturation rate was assessed. They observed that blood flow was better for the group of oocytes not recovered than for recovered oocytes and better for the group of mature oocyte than for immature oocyte. Spectral analysis revealed no differences between groups. These results are inconsistent with previous studies and therefore were considered inconclusive. In another study, Siddiqui *et al*. [14] evaluated blood flow in the follicular wall after treatment with hCG of mares whom had antibodies against hCG and mares that did not have antibodies. Follicles were aspirated 30 h after and were not differences between groups regarding the recovery rate, but showed significantly less blood flow in the follicular wall of mares antibody-negative than antibody-positive mares. Spectral analysis showed no differences between groups.

Disturbances in ovulation may be submitted in the form of septed evacuations with prolonged discharge and no ovulation with the subsequent formation of a hemorrhagic anovulatory follicle (HAF). Septated evacuations are associated with better serration and vascularization of the follicular wall one hour prior to ovulation when compared to normal evacuation, also including the apical area [12]. Serration and vascularization in mares with normal ovulation were not found in the apical pole. If the oocyte was included in the extended discharge is unknown [12]. Studying the HAFs, Ginter *et al*. [11] noted that follicular diameter was not different on the day before ovulation between the follicles that ovulated and those that formed one HAF. The results also indicated that high concentrations of systemic estradiol few days before the expected ovulation and better vascularization of the follicle on the day before ovulation are involved in the conversion of a viable preovulatory follicle into a HAF, but results are inconsistent because assessments were conducted with long intervals of 24 hours.

IV. CHARACTERISTICS OF FOLLICULAR DYNAMICS OBSERVED BY DOPPLER U.S. IN THE SPRING TRANSITION

The transition between the anovulatory and ovulatory seasons occurs in the spring and often is characterized by the formation of one or several anovulatory dominant follicles until a dominant ovulatory follicle terminates the anovulatory season [1]. The characteristics of the deviation are similar between both the phases. During the growth of follicles from 20 to 30 mm, the anovulatory follicles expanded at the same rate as the ovulatory follicles, being size of the dominant follicles unable to identify their health. The color Doppler ultrasound technology distinguished between future anovulatory and ovulatory dominant follicles with reasonable accuracy. On the day that the follicle reached 35mm, vascularized area was 0.48 to 0.81 cm^2 in the ovulatory group and from 0.12 to 0.28 cm^2 in anovulatory group [1].

The diameter of the preovulatory follicle on the day before ovulation is about 5 mm greater before the first than before the second ovulation of the year [8]. However, in one study, Gastal *et al*. [7] observed a much reduced rate of increase in vascularity preceding the first ovulation (day 0) of the year, The vascularized area (cm^2) of the follicle wall was similar between the preovulatory groups on Day -6, but the area was much smaller on Day -1 preceding the first ovulation, this characteristic may be used to identify the health of the follicle and thus prevent economic losses.

V. CONCLUSION

Doppler technology is a new tool for assessment of follicular characteristics and better understand the physiological changes of the estrous

cycle. However a wider range of studies is needed looking for the real applications of this technique aiming its use by veterinarians to increase fertility and improve the efficiency of biotechnologies such as artificial insemination.

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