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Clinical use of B-mode and color-Doppler ultrasonography to evaluate preovulatory follicle status in mares
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Abstract
This review will focus on the main findings of studies that used B-mode and color-Doppler ultrasonography during the preovulatory period to evaluate the morphological and blood flow/perfusion changes of the preovulatory follicle in mares. The topics to be addressed herein will be: B-mode ultrasonographic characteristics of the preovulatory follicle; blood flow and perfusion changes of the follicle wall; signs of impending ovulation; prediction of impending ovulation; types of preovulatory follicle outcomes such as ovulation, septated evacuation, hemorrhagic anovulatory follicle, atresia; vascularity of the preovulatory follicle versus fertility; and relationships between preovulatory follicle and corpus luteum blood flow in mares.

Keywords: Preovulatory follicle, blood flow, ultrasonography, ovulation, equine.

Introduction
Recently, transrectal Doppler ultrasonography has been utilized increasingly for research and clinical studies of ovarian and follicle hemodynamics in large farm animals. High-resolution ultrasonographic machines with B-mode (gray-scale) and color-, power-, and spectral-Doppler modes have brought a powerful dimension to the evaluation of the equine preovulatory follicle during recent years. These technologies have permitted the development of more in-depth scientific and clinical studies with regard to the characteristics of the preovulatory follicle and the ovulation process. Results of recent studies have demonstrated the potential of Doppler ultrasonography for providing clinical information on the status and future success of a follicle to ovulate and its oocyte to become fertilized and to generate an embryo/pregnancy. The equine model allows hypotheses testing using the three Doppler technologies for examining the ovaries and may provide additional information that can also be considered useful for other farm animal species and in human clinical medicine.

The use of color-Doppler technology to evaluate the vascularity of the follicle wall in mares started in 2004. Follicle blood-flow assessment by Doppler ultrasonography has been used in mares to study: (a) follicle selection, (b) anovulation during transitional seasons, (c) first versus later ovulations of the year, (d) follicle maturity and proximity to ovulation, (e) oocyte recovery rate, maturity, and quality, (f) normal versus abnormal ovulation (septated evacuation) or anovulation (hemorrhagic anovulatory follicles [HAFs] or luteinized unruptured follicles [LUFs]), (g) the relationship of systemic human chorionic gonadotropin (hCG) antibodies on follicle vascularity, maturity, and oocyte qualities, (h) age-related effects, (i) the relationship between preovulatory follicle and corpus luteum (CL) blood flow, and (j) potential for pregnancy establishment. Greater vascularity of the preovulatory follicle has been associated with greater follicle diameter (women, mares, heifers), retrieval rate of oocytes (women, mares, heifers), retrieval rate of mature oocytes (mares), in vitro fertilization rate (women, heifers), pregnancy rate (women, mares, heifers), and a lower incidence of triploidy (women). In addition, follicles with greater blood flow have resulted in better embryos and more pregnancies after embryo transfer in women.

This review article will summarize the main findings of experiments that used B-mode and color-Doppler ultrasonography during the preovulatory period to study the morphological and blood flow/perfusion changes of the preovulatory follicle in mares. Portions of this article have been adapted from Gastal 20111,2 and Gastal and Gastal 2011.3 This review is directed to equine theriogenologists and scientists who are involved in monitoring, managing, and manipulating ovarian function in mares.
**B-mode ultrasonographic characteristics of the preovulatory follicle**

**Follicle wall echotexture and thickness**

Ultrasonographically detectable changes in gray-scale echotexture and color-Doppler signals of blood flow can be noticed in the wall of the future dominant follicle as early as one or two days before the beginning of follicle diameter deviation or selection in mares.\(^4\)\(^5\) Thereafter, as the follicle matures and ovulation approaches, several ultrasonographic changes can be seen in the wall of the preovulatory follicle.

In regard to the follicular wall, the granulosa layer is identifiable as an echoic band enclosing the antrum.\(^6\) The two layers of theca can be assumed on the basis of position, but the boundary between the theca externa and interna is indistinct. In the initial equine B-mode ultrasonographic study,\(^7\) granulosa thickness increased as the interval to ovulation decreased. In another study,\(^8\) mean scores of echogenicity and thickness of the granulosa increased during the 24 hours before ovulation, and changes occurred in 60% and 70% of individuals, respectively. In addition to an increase in echogenicity of the granulosa, prominence of an anechoic band in the expected area of the theca layers increased daily and progressively over three days before ovulation.\(^6\)\(^9\) Follicle diameter and the two echotexture characteristics were more prominent earlier than later in the season.\(^6\) Early in the season, both characteristics were at the maximal score in 70% (33/47) of follicles on Day -1 (ovulation = Day 0). The anechoic band is located in the area of the thecal layers and its characteristics have been described.\(^4\)\(^6\) A similar anechoic band has been described in women.\(^10\)\(^11\) Color-Doppler signals of blood flow are dispersed within the anechoic band, indicating that the band includes the fluid of blood vessels, presumably venules as well as arterioles. In another study,\(^12\) several mean pixel values increased in an approximately linear fashion during the 14 hours before ovulation in hCG-treated mares. In a more recent study,\(^13\) mares with and without hCG treatment have been compared. An increase in granulosa thickness and echogenicity, percentage of follicle wall with color-flow signals and prominence of the signals, and a decrease in circulating estradiol during the 36 hours post-treatment were greater in the hCG group than in the controls. During 36 to 12 hours before ovulation, the granulosa thickness and echogenicity, and percentage of follicle wall with color-flow signals and prominence of the signals increased in both groups. However, four hours before ovulation, the two groups showed similar decreases in prominence and percentage of wall with an anechoic band and prominence and percentage of wall with color-flow signals. This study indicated that the ultrasonographic changes of the wall of the preovulatory follicle were not associated temporally with changes in estradiol concentrations and prominence of an anechoic band, and color-Doppler signals decreased during the last few hours before ovulation. In regard to age-related effects on preovulatory follicle echotexture and vascularity, a recent study has not found differences among young, intermediate, and old mares for granulosa thickness and echogenicity, anechoic band prominence, and blood flow during 4 days before ovulation.\(^14\)

**Follicle shape and turgidity**

Changes in follicle shape from spherical to nonspherical can be noticed from three days before ovulation, with the highest frequency occurring within 24 to 12 hours before ovulation (reviewed in Gastal et al. 1998\(^6\)\(^). In a recent study, loss of spherical shape was associated to decreased follicle turgidity, indicated during transducer pressure, and occurred mostly between the last 24 to 12 hours before ovulation.\(^15\) The subjectivity aspect for evaluation of the previous characteristics can produce different results between operators that can be at least partly attributable to the criteria used to differentiate between spherical and nonspherical follicles and variations with transducer pressure.

**Indicators associated with impending ovulation**

The above studies indicate that quantitative ultrasonic characteristics of the granulosa and anechoic band are useful for sequential assessment of the developmental progress of the preovulatory follicle. However, assessing progress requires judgment on relative changes either subjectively or by computerized pixel analyses. Discrete (nonquantitative) B-mode characteristics have been described for the preovulatory follicle and may be useful for predicting time of ovulation without the necessity for
judging quantitative progress. These characteristics include the following: 1) decreased turgidity of the follicle under transducer pressure in mares and women, 2) loss of spherical shape in mares and women, 3) irregular inner surface of the granulosa termed crenation in women and serration in mares (see below for details), 4) stigma, thin apical cone-shaped, or nipple-like protrusion of the follicle (future ovulation site) in mares and women, 5) apparent detached segments of granulosa or rent in the wall in mares and women, and 6) echoic spots floating in the antrum in mares and women.

Recently, we observed that both surfaces of the granulosa (interfaces with the antrum and with the theca interna) became irregular or with a notched appearance as ovulation approached and termed the phenomenon “serration of granulosa”. In this study, the following discrete end points were recorded as present or absent: 1) serration of granulosa, 2) decreased turgidity, 3) loss of spherical shape, 4) apical area, and 5) echoic spots floating in the antrum. When records were examined for 24 and 12 hour intervals, serration was detected at the last examination before ovulation in 37% and 59%, respectively. Decreased turgidity at the last 12-hour examination was detected concomitantly with serration, but was detected alone in 9 to 12% of previous examinations. Serration and decreased turgidity were present at each examination until ovulation five hours later. Loss of spherical shape initially occurred less frequently than decreased turgidity, but the incidence increased from 50 to 100% during six to one hours before ovulation. The incidence of an apical area reached 100% and echoic spots increased to 50% during one hour before ovulation. The results indicated that serration of granulosa and the other discrete characteristics were useful for predicting the time of ovulation within hours, but optimal efficiency would require examinations every few hours. In this regard, owing to the appearance of serration within the last 12 hours before ovulation and a decrease in follicle blood flow during the last hours before ovulation (e.g., six to four hours) we have proposed a model for follicle maturity and impending ovulation in the mare. The working hypothesis was that during a few hours before follicle evacuation, the blood flow becomes concentrated to the base of the follicle and vessels protrude throughout the wall, causing the appearance of serration in the follicular wall opposite to the future site of follicle rupture. Although the presence or absence of an indicator of impending ovulation required judgment, the presence of discrete indicators would likely be more readily evaluated than the progression in thickness and echogenicity of the granulosa and prominence of an anechoic band described in other studies. Serration of granulosa was the most useful indicator of impending ovulation, considering its distinctive appearance and consistent development only during the late preovulatory period. Future work is needed to develop a system that utilizes serration for predicting the interval to ovulation in mares.

**Follicle diameter**

**Repeatability of preovulatory follicle diameter.** Repeatability of follicle diameters has been recently studied in mares. Significant correlations were found for the diameter of the preovulatory follicle during the three days before ovulation in spontaneous waves and for the maximal diameter of the preovulatory follicle in induced waves. When induced waves with only one ovulation were considered, there were stronger and significant correlations for diameter of the preovulatory follicle at maximum (r = + 0.70) and on the day before ovulation (r = + 0.66). In another study, most mares (85%) ovulated from follicles that were within 3 mm of the diameter during the previous estrous cycle. The finding that the preovulatory follicle tends to reach a diameter that is characteristic of the mare may be a useful knowledge in equine breeding programs. The significant positive correlations for diameter of the preovulatory follicle recently found occurred during the three days before ovulation. These days encompass the day that the follicle first reaches ≥35 mm in approximately 70% of the interovulatory intervals (IOIs), and ≥35 mm is a common diameter for administration of an ovulation-inducing drug. These observations suggest that knowledge of the mare’s history on the diameter preceding ovulation may be useful for estimating the optimal follicle diameter for a given mare for ovulation induction, as well as for the optimal time for breeding before spontaneous ovulation.

**Factors that affect preovulatory follicle diameter.** Preovulatory follicles in horses and large ponies generally reach 40-45 mm the day before ovulation. The preovulatory follicle was larger in
Quarter Horses (43 mm) than in Arabians (40 mm),\textsuperscript{29} larger in one type of Thoroughbred than in another (44 vs. 41 mm),\textsuperscript{30} and 3.1 mm larger for Thoroughbreds in Australia than in England.\textsuperscript{31} The diameter or growth rate of the ovulatory follicle before ovulation was similar between ponies and Quarter horses.\textsuperscript{32} However, the maximum diameter of the preovulatory follicle was approximately 3 mm larger in French saddle horses than in Welsh ponies.\textsuperscript{33} Limited data suggested that preovulatory follicles were about 5 mm smaller in miniature mares and 10 mm larger in Clydesdales than in Quarter horses and large ponies.\textsuperscript{34}

In a recent study, follicular dynamics was considered in miniature mares.\textsuperscript{35} The mean diameter of the ovulatory follicle at maximum (37.3 ± 0.5 mm) was greater than on Day -1 (35.9 ± 0.6 mm). A reduction in growth rate of the ovulatory follicle between maximum diameter (1 or 2 days before ovulation) and ovulation was seen in the miniature ponies and is consistent with that reported for large ponies\textsuperscript{13} and horses.\textsuperscript{36-38} Knowledge on the diameter of the preovulatory follicle in miniature mares is useful in comparison of breeds and types, considering the extremely small body size.

In the same study\textsuperscript{35,39} described above, a preliminary comparison was made among miniature ponies, large ponies, and Breton horses. The miniature ponies and the large ponies had a longer IOI than the Breton horses, agreeing with previous comparisons between large ponies and horses (reviewed in Ginther 1992\textsuperscript{40}). The miniature ponies had fewer growing follicles ≥10 mm per ovulatory wave and more ovulatory waves with only one growing follicle ≥10 mm than for large ponies and horses. The diameter of the ovulatory follicle was smaller at maximum and on Day -1 in the Miniature ponies than in the horses, but was not different from the diameter in large ponies. The growth rate (approximately 3 mm/day) of the preovulatory follicle, prior to the cessation or reduction in growth, was similar among the three types of mares and agrees with reported studies in ponies and horses (reviewed in Ginther 1995\textsuperscript{34}). Therefore, differences in diameter of the preovulatory follicle among breeds and types of mares have to be considered and incorporated in the reproductive management. These findings demonstrate that when body size difference is large (e.g., Miniature and draft horses), follicle size difference might be more pronounced among breeds and types of mares. However, these differences are small when contrasted to the great difference in body size.

Mares are seasonally ovulatory with the transition between the anovulatory and ovulatory seasons occurring in the spring. The diameter of the preovulatory follicle on the day before ovulation was greater before the first than before the second ovulation of the year.\textsuperscript{41-43}

The effects of inadequate nutrition or poor body condition on follicle dynamics and reproductive hormones during the equine ovulatory season are not fully understood. Although, there is evidence that inadequate nutrition or body condition has been associated with delayed onset of the breeding season, decreased pregnancy rate, increased embryo loss, and increased gestation length in mares.\textsuperscript{44-46} During the winter, mares with low body condition had fewer medium (11 to 19 mm) and large (≥20 mm) follicles than mares with high body condition.\textsuperscript{47} The mechanisms by which feed restriction and low body condition modify the reproductive axis are also not well known. Apparently, glucose, insulin, leptin, growth hormone, and fatty acids seem to be involved, at some level, in the regulation of the reproductive axis.

A study in our laboratory compared follicle activity between mares of similar age with low and high body conditions.\textsuperscript{48} Examinations began during the anovulatory season (August 14, Southern Hemisphere) and continued until the second ovulation of the year. Low body condition, compared to high body condition, was associated significantly with the following: longer interval to first ovulation; smaller diameter of the preovulatory follicle at maximum (45 vs. 51 mm, combined for both ovulations) and at Day -1 (45 vs. 50 mm); small diameter of the four largest follicles; fewer medium (11-19 mm) and large (≥20 mm) follicles; and fewer total number of follicles ≥5 mm. The body condition score was positively correlated with the preovulatory follicle at maximum diameter and at Day -1.

A recent study of spontaneous ovulatory waves in mares compared the effects of age (5 to 6 yr, young; 10 to 14 yr, intermediate; ≥18 yr, old) on follicle dynamics during an IOI (n = 46).\textsuperscript{49} The old mares were not approaching senescence, as indicated by regular lengths of IOIs. The length of the IOI was approximately one day longer in the old group than in the younger groups and was attributable to slower growth rate of the ovulatory follicle and lower luteinizing hormone (LH) concentrations. The old
group had diminished follicle activity, as indicated by significantly smaller and fewer follicles. The diameters of the preovulatory follicle on Day -1 and at maximum were smallest in the old group. In an early study of age in mares, the preovulatory follicle grew more rapidly in young mares (5 to 7 yr) than in mares ≥15 yr. The results of the recent study are also consistent with the finding of fewer follicles ≤20 mm in older ponies, although some of the mares in the early reports were approaching senescence. These studies indicate the importance of age as a potential confounding factor for development of theriogenology programs (e.g., optimal time to breed and superovulation regimes) and in equine research protocols.

**Color-Doppler ultrasonographic characteristics of the preovulatory follicle**

Follicle wall blood flow and perfusion changes

Recently, transrectal Doppler ultrasonography has been utilized increasingly for research and clinical studies of ovarian and follicle hemodynamics in large farm animals. Follicle blood-flow assessment by Doppler ultrasonography has been used in mares to study follicle selection, anovulation during transitional seasons, first versus later ovulations of the year, follicle maturity and proximity to ovulation, oocyte recovery rate, maturity, and quality, effects of circulatory hCG antibodies on follicle and oocyte, age-related effects, and pregnancy establishment. The use of color-Doppler technology to evaluate the vascularity of the follicle wall in mares started in 2004. In a study of the vascular changes associated with the beginning of follicle deviation, the future dominant follicle was evaluated by transrectal color-Doppler ultrasonography until the follicle was about 30 mm, four days before ovulation. The results demonstrated that deviation in the blood flow between the two largest follicles occurred one to two days before diameter deviation during follicle selection in mares. This conclusion is compatible with an earlier demonstration that an anechoic band surrounding the granulosa of the dominant follicle begins to expand differentially between the two largest follicles one day before the beginning of diameter deviation. These results provided the first evidence in any species that differential blood-flow changes between future dominant and subordinate follicles begin early in the ovulatory wave and precede diameter deviation during follicle selection.

Color-Doppler ultrasonography also has the potential for judging the status (future ovulatory or anovulatory) of dominant follicles during the transitional period. During the anovulatory transitional season, vascular changes in the follicle walls of both a future dominant anovulatory follicle and a future ovulatory follicle were studied from 25 mm until seven days after the follicle was 30 mm. Blood-flow area was already lower for dominant-sized anovulatory follicles than for ovulatory follicles at the time blood-flow determinations began at 25 mm. A hypothesis for anovulation that involves hormones and follicle angiogenesis during the transitional period has been discussed elsewhere. In this regard, preovulatory vascular changes have been compared between the first and later ovulations of the year in 40 pony mares for six days preceding ovulation. Although follicle blood-flow area increased towards the ovulation day in both groups, results demonstrated that follicle vascularization and the LH surge were attenuated preceding the first ovulation of the year, with no indication that estradiol was involved in the differences between the first and later ovulations.

In regard to preovulatory follicle blood flow, recent studies have shown a daily increase in vascularity of the wall of the dominant follicle as it matures and approaches the day of ovulation. However, on the day of ovulation, a few hours before evacuation an abrupt decrease in blood perfusion in the wall of the preovulatory follicle has been detected. Prediction of ovulation: preliminary results

The ability to control or predict ovulation time has practical implications for the equine industry and for research purposes and is a desirable goal. Currently, there are hormonal agents such as hCG, gonadotropin-releasing hormone (GnRH), and recombinant equine LH (reLH) that can induce ovulation in most (60 to 90%) mares within a predictable time after treatment (e.g., 24 to 48 hours); however, there is a proportion (10 to 40%) of mares that do not respond in a timely fashion to the administration of these hormones, and presumably ovulate on their own. Therefore, regardless of the use or disuse of hormonal
treatment, there are several practical situations that need the prediction of impending ovulation within 24 hours or even within a few hours. These scenarios can be exemplified by cases of horse breeder associations that allow only natural mating, clinics and farms that use cooled or frozen semen, limited semen quality or quantity as a result of stallion age or morbidity, assisted reproductive techniques, and research protocols. Therefore, the use of the main ultrasonographic signs of impending evacuation of the preovulatory follicle (see previous section) can be helpful to predict the time of ovulation in mares.

Few ultrasonographic studies of the preovulatory follicle have been done with a specific design to validate a methodology to predict the time of ovulation in mares. In a detailed study, echogenicity of the granulosa layer and prominence of an anechoic band beneath the granulosa reached a maximum score in 70% of mares on the day before ovulation. The efficiency of these two echotexture characteristics was compared with follicle diameter as criteria for initiating breeding early in the ovulatory season. Results indicated that the ultrasonographic echotextural characteristics were superior to diameter in identifying the optimal breeding day in mares. Another study has concluded that the slopes of regression lines for the same previous characteristics were useful in predicting impending ovulation within 24 to 48 hours.

Recently, two studies were conducted to allow prediction of ovulation within different hours in hCG and non-hCG treated mares. Prediction of ovulation in each experiment was carried out by two different operators without the knowledge of either the previous B-mode ultrasound scan record or day of the estrous cycle of each animal. In the first experiment, ultrasonographic scanning was done every six hours after a \( \geq 35 \) mm follicle in four groups of mares. In the second experiment, the frequency of scanning was every 1, 12, or 24 hours after a \( \geq 32 \) mm follicle. Attempted predictions based on each scan within each group for both studies were classified as correct or incorrect. The mean percentage of correct predictions for both experiments was 92%. However, in about 36% of the mares prediction could not be attempted due to insufficient follicular wall characteristics. These results, although limited by the number of animals, demonstrate that the degree of certainty for correct diagnosis can be high for independent operators; however, there are several mares that do not show the combination of adequate ultrasonographic follicular wall signs to be judged as impending ovulation. Therefore, studies to predict impending ovulation with B-mode and color-Doppler ultrasonography using different combinations of follicular wall characteristics (e.g., serration of granulosa and decrease in blood flow) are warranted.

**Types of preovulatory follicle outcomes**

**Ovulation**

*Normal evacuation.* As ovulation approaches, a bulge at the apex of the follicle can be detected at the ovulation fossa by laparoscopy or by ultrasonographic imaging. The apex is a thin-walled and relatively avascular portion of the preovulatory follicle that separates an infundibular fluid pocket from the follicular antrum. During follicle evacuation at ovulation the follicular fluid enters the infundibular fluid accumulation and the majority of the discharged follicular fluid is drained into the abdomen.

The first chance observations of continuous follicle evacuation by transrectal ultrasound were made in mares. In subsequent planned studies, two distinctive evacuation patterns (abrupt and gradual) were observed during continuous monitoring with B-mode ultrasound. In about 50% of ovulations, evacuation of follicular fluid from the preovulatory follicle was an abrupt process ranging from five to 90 seconds, with approximately 15% of the initial fluid remaining in the antrum. In the other 50%, release of follicular fluid was a slow and gradual process taking six to seven minutes to evacuate about 90% of the initial volume. Complete loss of detectable follicular fluid from the antrum and the extravarian space usually takes minutes or hours, but may last as long as two days. In some occasions, residual antral fluid may not be lost before blood or transudate begins to collect within the antral cavity. Evacuation can be suspected to be under way when the follicle is reduced in size and irregular in shape. Examination a few minutes later and sometimes a few seconds later, if early in the process, may indicate further evacuation and confirm that ovulation is under way or has occurred. Other reports described evacuations that were considered atypical (see septated evacuation).
Septated evacuation. Follicle evacuations with atypical sites on the day of ovulation (estimated decrease in antrum) were compartmentalized with irregular echoic septa and contained apparent follicular fluid. Whether complete evacuation occurred was not determined, owing to a daily interval between examinations. Atypical sites occurred in approximately 7% of hCG-treated and nontreated mares and were less common in young (3 to 7 years; 2%, 1/64) than in intermediate (15 to 19 years; 8%, 4/48), and old mares (≥20 years; 16%, 6/38). Preliminary observations in our previous research projects, based on hourly ultrasonographic examinations before ovulation, suggested that septated evacuations were prolonged and may be related to the location of blood vessels at the periphery of the follicle.

A recent study was performed using color-Doppler and B-mode film clips taken of the preovulatory follicle one hour or every hour during 12 hours preceding the beginning of evacuation in normal and septated evacuators. Locations of serrated granulosa and color-flow signals were determined by clock-face positions with the apex of the follicle (future ovulation site) at 12 o’clock. Mares were divided into a group with normal follicle evacuation (completion within 1 hour; n = 21 mares) and a group with septated evacuations (completion of evacuation in ≥3 hours and formation of echoic trabeculae in the antrum during evacuation; n = 5 mares). The percentage of follicle circumference with color-flow signals was greater one hour before the beginning of evacuation in the septated group (76%) than in the normal group (37%). For mares with hourly data available before evacuation (n = 8), there was a greater decrease in percentage of follicle circumference with color-flow signals beginning six hours before ovulation in the normal group than in the septated group. In the normal-evacuation group, serration and blood-flow signals were located at the basal hemisphere of the follicle directly opposite to the apex. The apical area was devoid of both serration and color-flow signals. In the septated evacuation group, color-flow signals and serration were detected at every clock-face position in each mare at the hour before ovulation. Results supported the hypothesis that prolonged septated follicle evacuation is associated with vascularization and serration of a greater circumference of the follicle than for normal evacuation, and vascularization includes the apical area. The results also indicated that detectable blood-flow signals were lost from the apical pole over the few hours before ovulation in the normal evacuations but not in the septated evacuations. The mechanism involved in the loss of blood-flow signals from the apical area in the normal evacuations is unknown, but may have been associated with the approach of a narrower apex toward the ovulation fossa. These findings suggest that when a reduction in vascularization at a broad apex does not occur and ovulation occurs in the presence of apical vessels, the evacuation is prolonged and trabeculae form in the antrum during evacuation. Studies are necessary to evaluate if the oocyte is released from the follicle during prolonged septated evacuation and determine the fertility of this type of ovulation.

Hemorrhagic anovulatory follicle

Extravasation of blood into follicles, ovulation sites, and corpora lutea is common in mares. A hematoma that forms in the antrum instead of ovulation has been termed a hemorrhagic anovulatory follicle (HAF). Similar structures also have been termed hemorrhagic follicles, hemorrhagic follicles, persistent anovulatory follicles, and autumn follicles. The economic importance of HAFs as a breeding-management problem in mares has been noted and reflects anovulation of a follicle after the mare has been bred. The extent of the problem is indicated by a 5% and 20% incidence during the early and late ovulatory season, respectively (reviewed in Ginther et al. 2007). Moreover, the syndrome seems to be more common in old mares and may occur repeatedly in an individual, sometimes encompassing much or all of the breeding season.

The morphology and vascularity of HAFs in mares and the endocrinology immediately preceding HAF formation have been studied in control and HAF groups. The day of ovulation and the first day of HAF formation, as indicated by cloudiness of follicular fluid, have been defined as Day 0. The frequency of discrete gray-scale ultrasonic indicators of impending ovulation and follicle diameter on Day -1 did not differ between future ovulating and HAF groups. On Day -1, the circumference of the follicle wall of future HAFs had more color-Doppler signals of blood flow than in the control mares. In regard to hormones, higher estradiol concentrations occurred a few days before HAF formation, but systemic LH,
follicle stimulating hormone (FSH), and progesterone were not altered during conversion of a preovulatory follicle into an HAF. In a recent comparative study of induced waves using prostaglandin F2α (PGF) treatment on Day 10 and ablation of follicles ≥6 mm and spontaneous ovulatory waves, an unexpected high incidence of HAFs (24%; n = 21) occurred in the induced waves and none in the spontaneous waves. The induced ovulatory waves differed considerably from spontaneous waves, including greater LH concentrations during much of the induced wave and greater growth rate of a smaller ovulatory follicle. In this regard, results of another recent study indicated that the high incidence of HAFs after PGF/ablation was associated with later follicle emergence and immediate and continuing greater LH concentration after PGF treatment, apparently augmented by an inherently high pretreatment LH concentration. A retrospective study found a significant association between the use of prostaglandin analogues and HAF formation over a 20-yr period. Pharmacological induction of HAFs and LUFs in mares has been recently obtained by systemic treatment with flunixin-meglumine, a cyclooxygenase-2 (COX-2) inhibitor, given during the preovulatory period. Furthermore, a recent histological and immunohistochemical study was unable to determine conclusively the participation of several angiogenic factors or the LH receptor in HAF formation. Studies of HAF formation and the underlying mechanisms in mares may be of comparative importance for other animal species and women that have similar ultrasonographic structures.

Atresia

The process of atresia of a preovulatory follicle during an expected major ovulatory wave is not a common finding during the natural equine breeding season, but it occurs quite often during follicular waves in the spring and fall transitional seasons. A postulated hormonal mechanism for anovulation of a preovulatory follicle in mares has been proposed and its effects may possibly lead to changes in the follicular wall that could be appreciated through ultrasonography during a transitional season.

Vascularity of the preovulatory follicle versus fertility

The degree of vascular perfusion of the ovary and follicles, assessed by color- or power-Doppler ultrasonography, has been used as a potential new technology for research and clinical studies of ovarian and follicle hemodynamics and to predict fertility in horses, cattle, and humans. Increased follicle blood flow, along with a rapid increase in LH at the terminal stage of follicle maturation has been associated with meiosis resumption and completion of oocyte maturation. Greater vascularity of the preovulatory follicle has been associated with greater follicle diameter (women, mares, heifers), retrieval rate of oocytes (women, mares, heifers), retrieval rate of mature oocytes (mares, heifers), in vitro fertilization rate (women, heifers), pregnancy rate (women, mares, heifers) and lower incidence of triploidy (women). In addition, follicles with greater blood flow resulted in better embryos and more pregnancies after embryo transfer in women. The relationships of oocyte maturity, 30 hours after hCG treatment, to blood flow (color-Doppler mode) and ultrasonographic characteristics of impending ovulation (B-mode) of the preovulatory follicle have been recently studied in mares. The vascularity of the preovulatory follicle tended to be greater for follicles that contained a mature oocyte. Follicles with an oocyte recovered had a significantly greater frequency of serration of granulosa and greater frequency of an apical area (indicators of impending ovulation). The frequency of serration of granulosa and decreased turgidity was greater in follicles that contained a mature oocyte. Results indicated that recovery of an oocyte and maturity of the recovered oocytes are both dependent upon the rate of follicle maturity in response to the hCG treatment.

Recent studies have tested the hypothesis that a higher pregnancy rate is associated with greater blood flow to the preovulatory follicle before breeding. The studies used color- and power-Doppler ultrasonography and indicated that pregnancy rates were greater in mares and heifers that displayed increased follicle blood flow. In both of these studies, mares and heifers were previously treated with hCG and GnRH, respectively, to induce ovulation. Follicle blood flow was evaluated at the time of treatment and natural breeding or artificial insemination. Although the results of both studies are preliminary, since they involved a limited number of animals, important statistical differences were found.
between animals that became pregnant versus nonpregnant in each study. In the mare study,⁶⁰ B-mode echogenicity and thickness of the granulosa layer and prominence of the anechoic band beneath the granulosa increased similarly in both pregnant and nonpregnant groups. An increase in follicle diameter and percentage of follicle circumference with color-Doppler signals was greater between the time of hCG treatment (hour 0) and artificial insemination (hour 30) in the pregnant group than in the nonpregnant group. Spectral-Doppler measurements were made at the most prominent intraovarian color signal. Decreases in resistance and pulsatility indices were greater between hours 0 and 30 in the pregnant group than in the nonpregnant group, indicating increased vascular perfusion downstream from the spectral measurement in the pregnant group. Relative peak systolic velocity and time-averaged maximum velocity of blood flow at the point of spectral assessment was greater in the pregnant group.

Aiming to evaluate fertility with a different perspective, a recent study in mares⁵⁹ investigated the effect of an ovulation-inducing dose of hCG in the presence versus absence of hCG antibodies on blood flow of the preovulatory follicle and maturity and quality of recovered oocytes at 30 hours post-treatment. The percentage of the follicle wall with blood-flow signals was less in the antibody positive group than in the negative group. The oocyte recovery rate (62%, 37/60) between hCG antibody-positive (44%) and negative mares (68%) tended to be different. The antibody-positive group had fewer mature (MII) and more atypical oocytes than the antibody-negative group.

Although preliminary, our recent studies in mares supported the hypothesis that greater blood flow to the preovulatory follicle is associated with higher follicle and oocyte maturation rates, oocyte recovery and quality rates, and pregnancy rates. Similar results were found in the heifer study,⁶¹ demonstrating that highly vascularized preovulatory follicles are more likely to be associated with higher pregnancy rates, as previously seen for mares and women.

**Relationships between preovulatory follicle and corpus luteum blood flow in mares**

In a recent study,⁶⁴ the diameter and blood flow of the preovulatory follicle were greater (P<0.05) than for those follicles that underwent atresia in single and double ovulator mares. The preovulatory follicle diameter and the blood flow were positively correlated in ovulatory (r=0.51; P<0.0001) and in atretic (r=0.32; P<0.02) follicles. In double ovulator mares, preovulatory follicle diameter and blood flow increased (P<0.0006) during five days before ovulation with no difference between the two follicles in the same cycle for each parameter. The preovulatory follicle blood flow was positively correlated (r=0.32; P<0.0009) with CL vascularity during the first periovulatory period (Days -7 to +6) of the season. Furthermore, a positive correlation (r=0.58; P<0.01) was obtained between the maximum vascularity of the preovulatory follicle and its subsequent CL. The results of this study demonstrated that: 1) potential atretic preovulatory follicles had low blood flow; 2) double preovulatory follicles had similar vascularity; and 3) greater preovulatory follicle blood flow was associated with higher CL vascularity.

**References**


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