Rev. Bras. Reprod. Anim., Belo Horizonte, v.35, n.2, p.239-252, abr./jun. 2011. Disponível em www.cbra.org.br

Equine preovulatory follicle: blood flow changes, prediction of ovulation and fertility*

Folículo pré-ovulatório equino: mudanças circulatórias, predição de ovulação e fertilidade

E.L. Gastal[1], M.O. Gastal

Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale, Carbondale, IL 62901, USA.
[1]Corresponding author: egastal@siu.edu

Abstract

This review will focus on the main findings of our 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. The topics to be addressed herein will be: ultrasonographic characteristics of the preovulatory follicle; B-mode echotextural changes of the follicle wall; 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, and atresia; follicle blood flow during evacuation; early corpus luteum blood flow; and vascularity of the preovulatory follicle versus fertility.

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

Resumo

Esta revisão tem como foco os principais achados de nossos experimentos que utilizaram ultra-sonografia em modo B e Doppler colorido durante o período pré-ovulatório para estudar mudanças morfológicas e de fluxo e perfusão sanguíneos do folículo pré-ovulatório em éguas. Os tópicos a serem aqui abordados serão: características ultra-sonográficas do folículo pré-ovulatório; alterações ultra-sonográficas na ecotextura da parede folicular; mudanças sanguíneas de fluxo e perfusão na parede do folículo; sinais de ovulação iminente; predição de ovulação; possíveis destinos do folículo pré-ovulatório, tais como ovulação, evacuação septada, folículo hemorrágico anovulatório e atresia; fluxo sanguíneo durante a evacuação folicular; fluxo sanguíneo no corpo lúteo jovem; e vascularização do folículo pré-ovulatório versus fertilidade.

Palavras-chave: *equino, fluxo sanguíneo, folículo pré-ovulatório, ovulação, ultra-sonografia.*

Introduction

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 for providing clinical information on the status and future success of a follicle to ovulate and its oocyte to become fertilized and generate an embryo/pregnancy.

Ovulation in the mare seems to be a culmination of a complex series of events under elevating LH concentrations that lead to rupture of a preovulatory follicle (e.g., >30 mm) at the ovulation fossa and the extrusion of follicular fluid, granulosa cells, and the cumulus-oocyte complex. This process usually occurs 1 or 2 days before the end of estrus. Controlling or predicting the time of ovulation within hours is important, and has practical implications for the equine industry and for research protocols that require a close interval between breeding and ovulation. Insemination close to the time of ovulation (e.g., within 6 to 12 hours) is important to prevent rebreeding mares and to maximize conception rates when using frozen semen or perhaps semen from stallions with limited sperm longevity. In addition, prediction of the time of ovulation is desired for equine oocyte retrieval programs that use assisted reproductive technologies.

During recent years the mare has become an increasingly productive research model in the area of folliculogenesis (reviewed in Gastal, 2009, 2011a, b). The striking similarities between mares and women in follicle dynamics and hormonal changes during the interovulatory interval and the ovulatory follicular wave (Ginther et al., 2004a, c, 2005; Mihm and Evans, 2008; Baerwald, 2009), in ultrasonographic changes of the preovulatory follicle before ovulation (Martinuk et al., 1992; Pierson and Chizen, 1994; Gastal et al., 1998, 2006a, b), and in reproductive aging processes (Carnevale, 2008; Ginther et al., 2008b, 2009) provide rationale for the use of and highlight the importance of the mare as an experimental model for the study of folliculogenesis in women. The equine model allows hypothesis testing using invasive technologies and may provide additional information that can also be considered useful for other farm animal species and in human clinical medicine.

*Portions of this manuscript have been adapted from Gastal EL. Ovulation: Part 2. Ultrasonographic morphology of the preovulatory follicle. In: McKinnon AO, Squires EL, Vaala WE, Varner DV (Eds.). Equine Reproduction. 2nd ed. Ames, IA: Wiley-Blackwell, 2011. p.2032-2054.

Palestra apresentada no XIX Congresso Brasileiro de Reprodução Animal, Recife, PE, Brasil, 25 a 27 de maio de 2011.

This review is directed to those who are interested in monitoring, managing, and manipulating the mare reproductive system during the periovulatory period. Details about other aspects of ovulation in mares not presented herein can be found in previous reviews (Ginther, 1992, 1995, 2007; Pierson, 1993; Carnevale, 1998; Bergfelt and Adams, 2007).

Ultrasonographic characteristics of the preovulatory follicle

In mammals, a preovulatory follicle consists of a fluid-filled antrum encapsulated progressively (from inside to outside) by granulosa, theca interna, and theca externa layers (Hunter, 2003). The avascular stratum granulosum is separated from the vascular thecal layers by a basement membrane. The theca interna is a layer of glandular cells mingled with a rich capillary plexus. The theca externa is more fibrous but contains a prominent network of arterioles and venules. As the follicle matures, expansion of the theca vessels and capillaries involves formation of new vessels as well as dilation of existing vessels (Familiari et al., 1991).

Ultrasonography has brought a powerful dimension to the evaluation of the equine preovulatory follicle. High-resolution ultrasonographic machines with B-mode (gray-scale) and color- and power-Doppler modes have been used recently in studies of preovulatory follicle characteristics with different goals (Gastal et al., 2006a, b, 2007a, b; Silva et al., 2006; Ginther et al., 2007a, b; Siddiqui et al., 2009b). These technologies have the potential for providing clinical information on the status and future success of a follicle and its oocyte. The main ultrasonographic characteristics of the equine preovulatory follicle with clinical and basic importance will be addressed in the next sections.

B-mode echotextural changes of the follicle wall

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 1 or 2 days before the beginning of follicle diameter deviation or selection in mares (Gastal et al., 1999; Acosta et al., 2004b). Thereafter, as the follicle matures and ovulation approaches, several ultrasonographic changes can be seen in the wall of the preovulatory follicle (Figs. 1-4). Changes in follicle shape from spherical to nonspherical can be noticed from 3 days before ovulation, with the highest frequency occurring within 24 to 12 hours before ovulation (reviewed in Gastal et al., 1998). In a recent study, loss of spherical shape was associated to decreased follicle turgidity, indicated by transducer pressure, and occurred mostly between the last 24 to 12 hours before ovulation (Gastal et al., 2006b). 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 in transducer pressure. In regard to the follicular wall, the granulosa layer is identifiable as an echoic band enclosing the antrum (Gastal et al., 1998; Fig. 1). 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 (Pierson and Ginther, 1985), granulosa thickness increased as the interval to ovulation decreased. In another study (Carnevale, 1998), 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 (Fig. 1) in the expected area of the theca layers increased daily and progressively over 3 days before ovulation (Gastal et al., 1998; Chan et al., 2003). Follicle diameter and the two echotexture characteristics were more prominent early compared to late in the season (Gastal et al., 1998). 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 (Gastal et al., 1998, 1999). A similar anechoic band has been described in women (Picker et al., 1983; Jaffe and Ben-Aderet, 1984). 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 (Fig. 1). In another study (Carnevale et al., 2002), several mean pixel values increased in an approximately linear fashion during the 14 hours before ovulation in human chorionic gonadotropin (hCG)-treated mares. In a more recent study, mares with and without hCG treatment have been compared (Gastal et al., 2006a). 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, 4 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 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 (Ginther et al., 2009).

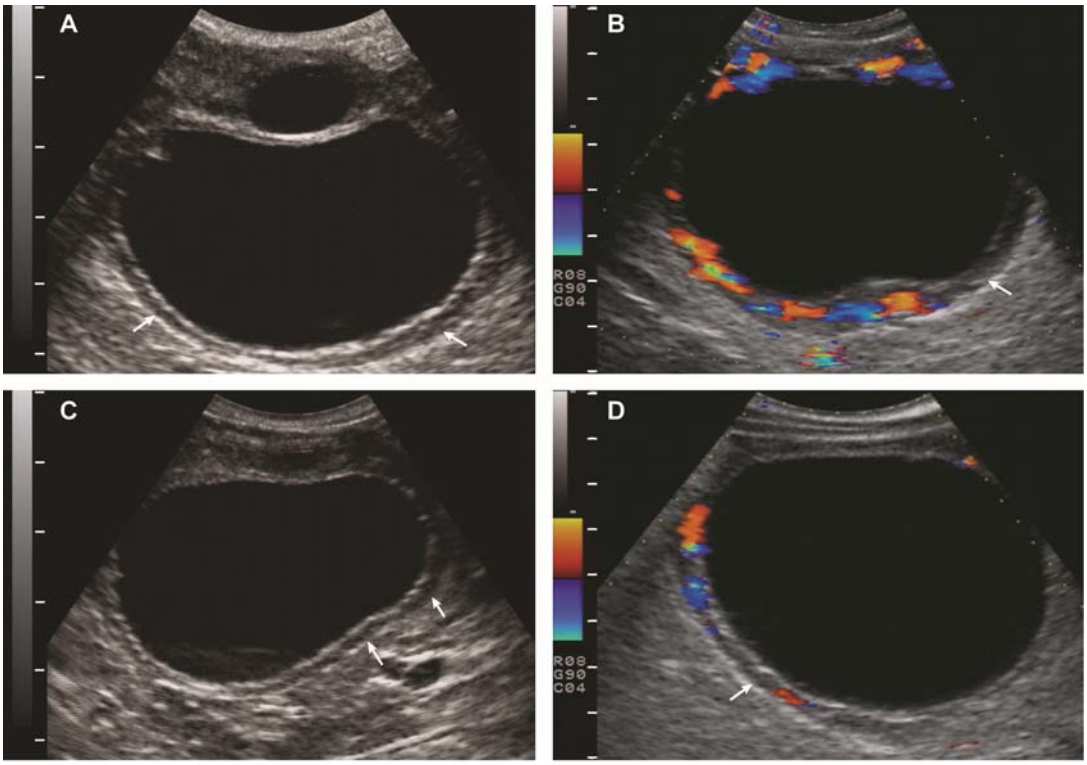

Figure 1. B-mode ultrasonograms (A, C) of preovulatory follicles of two mares at 2 days after the largest follicle reached ≥35 mm and 1 day before ovulation. Note the presence of the anechoic band (arrows). Color-Doppler ultrasonograms (B, D) of preovulatory follicles of two mares at 4 days after the largest follicle reached ≥35 mm. Images were taken at 2 days (B) and 3 hours (D) before ovulation. Note the anechoic band (arrows) and color-Doppler signals located within or extending beyond the width of the anechoic band. All images have been magnified to improve visibility of the anechoic band. Adapted from Gastal et al. (2006a).

Blood flow and perfusion changes of the follicle wall

Recently, transrectal Doppler ultrasonography has been utilized increasingly for research and clinical studies of ovarian and follicle hemodynamics in large farm animals (Ginther and Utt, 2004; Miyamoto et al., 2006; Ginther, 2007; Herzog and Bollwein, 2007). Follicle blood-flow assessment by Doppler ultrasonography has been used in mares to study follicle selection (Acosta et al., 2004b), anovulation during transitional seasons (Acosta et al., 2004a), first versus later ovulations of the year (Gastal et al., 2007a), follicle maturity and proximity to ovulation (Gastal et al., 2006a, b; Palmer et al., 2006; Ginther et al., 2007a), oocyte recovery rate, maturity, and quality (Ginther et al., 2007b), effects of circulatory hCG antibodies on follicles and oocytes (Siddiqui et al., 2009b), age-related effects (Ginther et al., 2009), and pregnancy establishment (Silva et al., 2006; Siddiqui et al., 2009a).

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 (4 days before ovulation; Acosta et al., 2004b). The results demonstrated that deviation in the blood flow between the two largest follicles occurred 1 or 2 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 1 day before the beginning of diameter deviation (Gastal et al., 1999). 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 (Acosta et al., 2004a; Palmer et al., 2006). 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 7 days after the follicle was 30 mm (Acosta et al., 2004a). Blood-flow area was already less for dominant-sized anovulatory follicles than for ovulatory follicles by 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

(Ginther et al., 2004b). In this regard, preovulatory vascular changes have been compared between the first and later ovulations of the year in 40 pony mares for 6 days preceding ovulation (Gastal et al., 2007a; Fig. 2). 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.

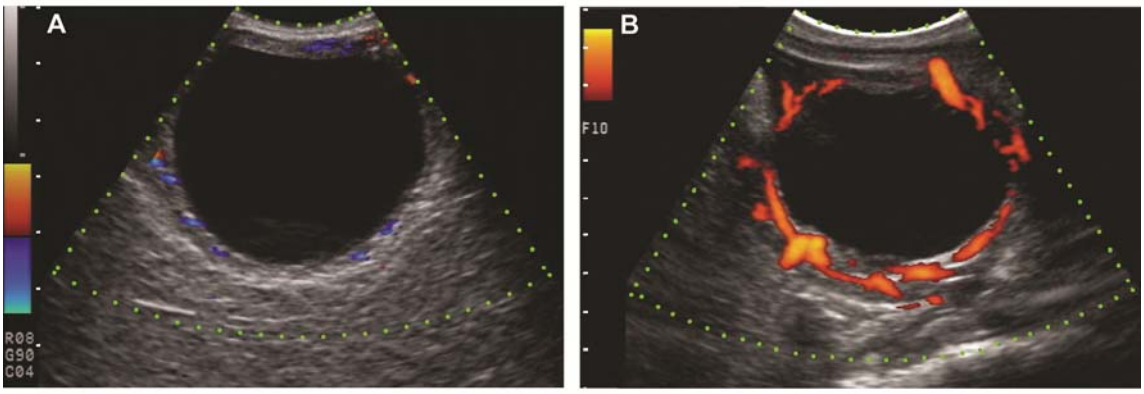

Figure 2. Color- (A) and power-Doppler (B) ultrasonograms from two mares illustrating different degrees of blood-flow signals in the wall of preovulatory follicles. Images were captured 24 hours before the first (A; low blood flow) or later (B; high blood flow) ovulations of the season. Adapted from Gastal (2009).

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 (Gastal et al., 2006a, 2007a; Palmer et al., 2006; Ginther et al., 2009; Fig. 3). 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 (Gastal et al., 2006a; Ginther et al., 2007a), as described in detail in this review (see sections B-mode echotextural changes of the follicle wall, Signs of impending ovulation, and Septated evacuation).

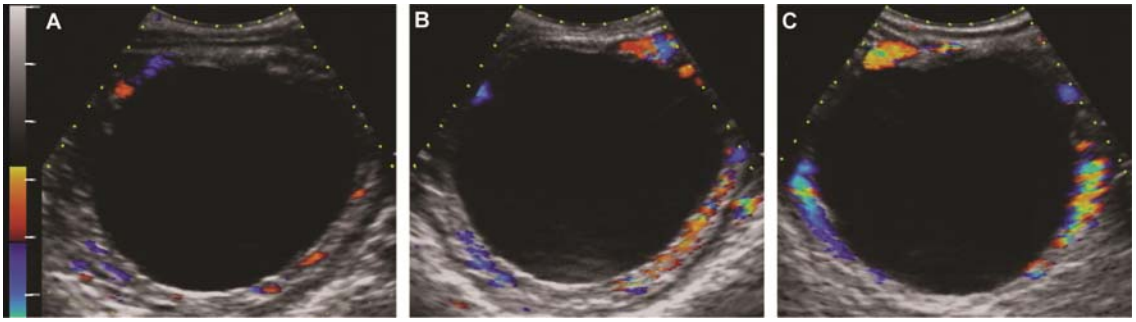

Figure 3. Sequential color-Doppler ultrasonograms of preovulatory follicles of one mare at 48 (A), 40 (B), and 24 (C) hours before ovulation. Note the increase in color-Doppler signals (amount of color dots and intensity) as the follicle matured (left to right). Most of the color-Doppler signals are located within or extend beyond the width of the anechoic band of the follicle wall. Adapted from Gastal et al. (2006a).

Signs of 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 the time of ovulation without the necessity of judging quantitative progress. These characteristics include the following: 1) decreased turgidity of the follicle under transducer pressure in mares (Carnevale et al., 1988) and women (Hanna et al., 1994), 2) loss of spherical shape in mares (Pierson and Ginther, 1985; Carnevale et al., 1988; Townson and Ginther, 1989a) and women (Lenz, 1985; Martinuk et al., 1992; Hanna et al., 1994), 3) irregular inner surface of the granulosa termed crenation in women (Picker et al., 1983; Martinuk et al., 1992) and serration in mares (Gastal et al., 2006b; Ginther et al., 2007a; see below for details), 4) stigma or thin apical cone-shaped or nipple-like protrusion of the follicle (future ovulation site) in mares (Pierson and Ginther, 1985; Carnevale et al., 1988) and women (Hanna et al., 1994), 5) apparent detached segments of granulosa or rent in the wall in mares (Carnevale et al., 1988; Townson and Ginther, 1989b) and

women (Bourne et al., 1991), and 6) echoic spots floating in the antrum in mares (Carnevale et al., 1988) and women (Mendelson et al., 1985; Fleischer and Kepple, 1995).

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" (Gastal et al., 2006b; Fig. 4). 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 5 hours later. Loss of spherical shape initially occurred less frequently than decreased turgidity, but the incidence increased from 50 to 100% during 6 to 1 hours before ovulation. The incidence of an apical area reached 100% and echoic spots increased to 50% during 1 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., 6 to 4 hours; Gastal et al., 2006a, 2007a), we have proposed a model (Fig. 5) for follicle maturity and impending ovulation in the mare. The working hypothesis was that during the few hours before follicle evacuation, the blood flow becomes concentrated to the base of the follicle (Figs. 6B, C) 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.

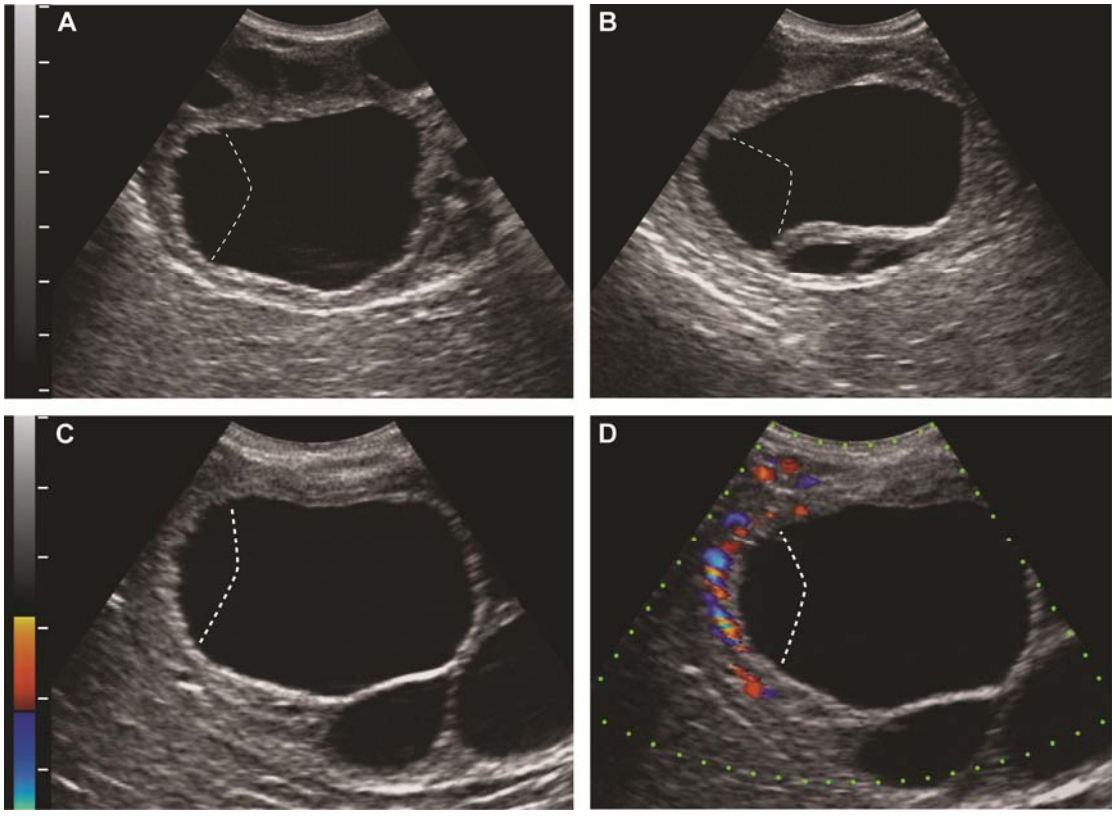

Figure 4. B-mode (A, B, C) and color-Doppler (D) ultrasonograms from three different mares illustrating the presence of serration of granulosa. The images were taken at 4 (A) and 1 (B, C, D) hours before ovulation. As ovulation approaches, both surfaces of the granulosa (interfaces with the antrum and with the theca interna) become irregular. Note the follicles with a distinct serrated granulosa (dashed line). A color-Doppler image (D) was taken from the same follicle (C) at approximately the same plane. The apex is to the right but is not apparent in this plane. Adapted from Gastal et al. (2006a) and Ginther et al. (2007a).

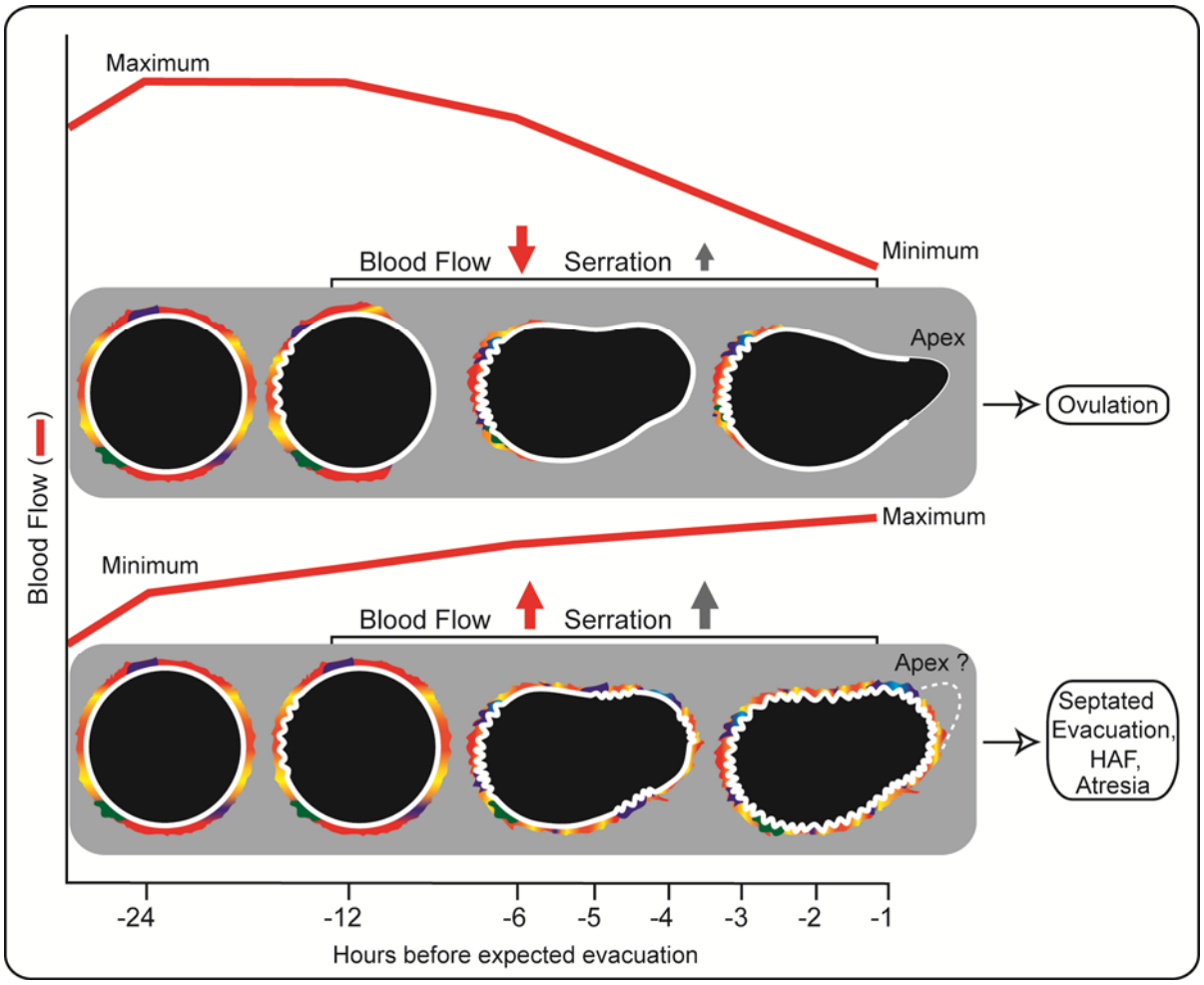

Figure 5. Diagrammatic representation of a proposed working model to illustrate the relationship between preovulatory follicle blood flow and serration of granulosa for mares with a normal evacuation versus a prolonged septated evacuation. In normal evacuators, follicle blood flow reaches its maximum 24 hours before ovulation and starts to decrease significantly over the 6 hours before evacuation. During the hours before evacuation, serration of granulosa appears in the wall and is associated with blood flow signals at the base of the follicle and opposite to a thin and avascular apical area. In septated evacuators, follicle blood flow continues to increase between 24 and 1 hours before the beginning of evacuation. Serration and color-flow signals are dispersed throughout the periphery of the follicle, including a potential apical area.

Prediction of ovulation: preliminary results

Prediction of ovulation in mares is a desirable goal for the equine industry and for research purposes. 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 not) 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 (Gastal et al., 1998). 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 (Chan et al., 2003). Recently, we designed two studies to allow prediction of ovulation within different hours in hCG and non-hCG treated mares (Gastal et al., 2010). 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 6 hours after a ≥35 mm follicle was present in four groups of mares. In the second experiment, the frequency of scanning was every 24, 12, or 1 hours after a ≥32 mm follicle was present. 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 and infundibular fluid

As ovulation approaches, a bulge at the apex of the follicle (Figs. 6B, C, F) can be detected at the ovulation fossa by laparoscopy (Witherspoon and Talbot, 1970) or by ultrasonographic imaging (Carnevale et al., 1988; Gastal et al., 2006a, b). This thin-walled and relatively avascular portion of the preovulatory follicle (Ginther et al., 2007a) separates an infundibular fluid pocket and the follicular antrum. A preovulatory collection of fluid external to the ovary in the infundibular area has been detected by transrectal ultrasonography (Townson and Ginther, 1989b). In a recent detailed study, an accumulation of fluid in the infundibular area was discovered by transrectal ultrasonographic imaging and was studied daily in both oviducts of 12 mares from Day -10 to Day 10 (Day 0 = ovulation), and from Day -6 to Day 6 during 35 estrous cycles of young, intermediate, and old mares. The infundibulum was identified by processes (fimbriae) and folds in the pocket of fluid (5 to 20 mm in diameter). Frequency of detection of fluid in the infundibular area increased between Day -10 (46% of oviducts) and Day -3 (88%), and decreased between Day -3 and Day 7 (8%; Gastal et al., 2007b). The source of the fluid has not been determined but presumably originates from the oviduct, peritoneum, or both. The main findings of this study were that a substantial amount of fluid accumulated in the infundibular area in mares and that the amount of accumulation depended on the stage of the estrous cycle and the side of the preovulatory follicle or ovulation, but was independent of age. The changes in the amount of fluid accumulation in the infundibular area and endometrial edema were similar and related to previously reported changes in systemic concentrations of estradiol. 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 (Ginther and Pierson, 1984). In subsequent planned studies, two distinctive evacuation patterns (abrupt and gradual) were observed during continuous monitoring with B-mode ultrasound (Townson and Ginther, 1987, 1989b). In about 50% of ovulations, evacuation of follicular fluid from the preovulatory follicle is an abrupt process ranging from 5 to 90 seconds, with approximately 15% of the initial fluid remaining in the antrum. In the other 50%, release of follicular fluid is a slow and gradual process taking 6 to 7 minutes to evacuate about 90% of the initial volume. Complete loss of detectable follicular fluid from the antrum and the extraovarian space usually takes minutes or hours, but may last as long as 2 days (Townson and Ginther, 1989b; Ginther et al., 2007a). 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 (Fig. 7). Other reports (Carnevale et al., 1994; Ginther et al., 2007a) described evacuations that were considered atypical (see septated evacuation ahead).

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 (Carnevale et al., 1994). 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 (Figs. 6E-H) were prolonged and may be related to the location of blood vessels at the periphery of the follicle.

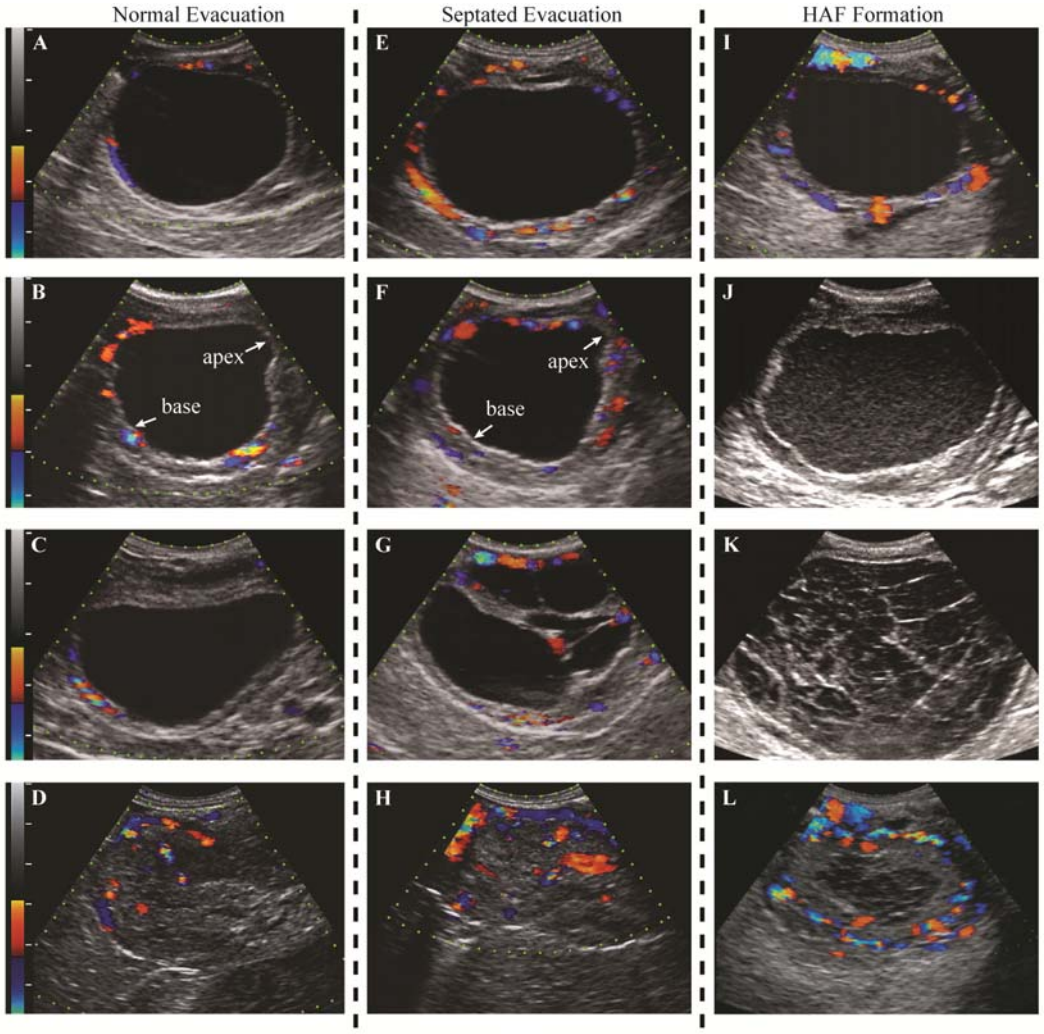

Figure 6. ***Normal Evacuation (A, B, C, D)***. Color-Doppler ultrasonograms showing preovulatory follicles 1 hour before a normal evacuation (A, B, C) with color signals concentrated only at the base. The apex is to the right but is not apparent in the image (A). Color signals are limited to the base of the corpus luteum 1 hour after completion of a normal evacuation (D). ***Septated Evacuation (E, F, G, H)***. Prolonged septated evacuation 1 hour before (E, F) and 1 hour after (G) the beginning of an evacuation. The apex is to the right but is not apparent in this plane (E). Color signals are dispersed throughout the periphery (E, F) and in the septum (G). The septum is an apparent crease in the evacuating follicle. The apex and the septa within the antrum contain color-flow signals. Color signals are dispersed throughout the corpus luteum (H) 1 hour after completion of a prolonged septated evacuation (required 9 hours). ***HAF Formation (I, J, K, L)***. Color-Doppler ultrasonogram (I) showing blood-flow signals in the wall of the preovulatory follicle on the day before anovulation or beginning of the formation of an HAF (Day -1). Gray-scale (J, K) and color-Doppler (L) ultrasonograms of the same mare illustrate the most common sequential events in formation of an HAF. Day 0 (equivalent to the day of ovulation): excessive floating specks in antrum (J). Day 2: network of strands that quivered upon ballottement (K). Color signals are dispersed throughout the periphery of the follicle (I; Day -1) and luteinized structure (L; Day 6).

A recent study was performed using color-Doppler and B-mode film clips taken of the preovulatory follicle 1 hour or every hour during 12 hours preceding the beginning of evacuation in normal and septated evacuators (Ginther et al., 2007a). 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 (Figs. 6B, F). 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; Figs. 6E-H). The percentage of follicle circumference with color-flow signals was greater 1 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 6 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 (Figs. 4, 6B, C). 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 within 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 (Figs. 5, 6F, G). 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.

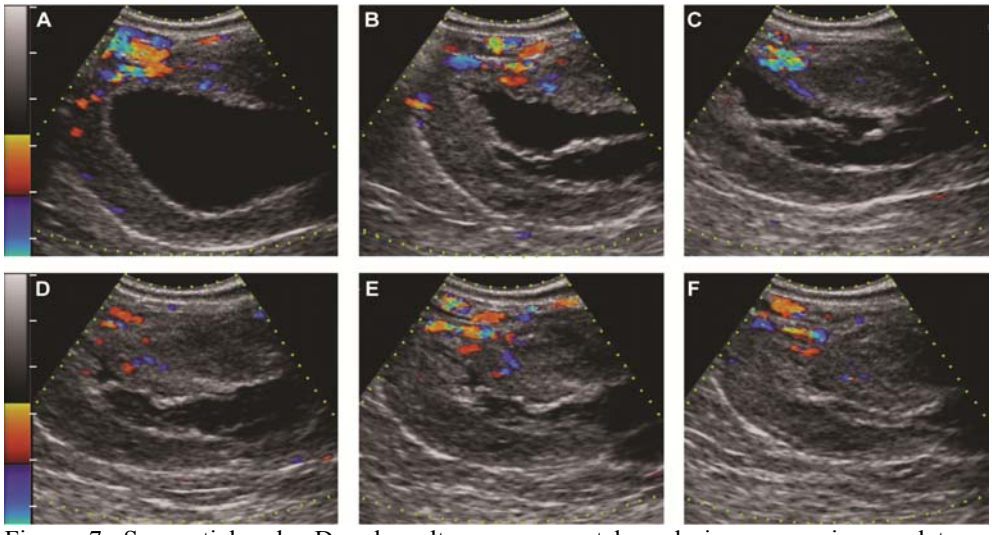

Figure 7. Sequential color-Doppler ultrasonograms taken during an equine ovulatory process. (A) Preovulatory follicle approximately 60 minutes before the onset of evacuation; (B) Evacuating follicle shortly after the onset of rupture of the follicle wall; and (C–F) Evacuating follicle taken 2.1, 2.2, 2.4, and 3.2 minutes after image B. The sequence of images illustrates the blood flow concentrated at the basal hemisphere of the follicle directly opposite to the apex or rupture point during the evacuation process.

Follicle blood flow during evacuation

As mentioned above, preliminary results from these studies also indicate that close to the beginning of follicle wall rupture and fluid evacuation, the blood flow is concentrated at the base of the structure and associated with serration of the granulosa layer (Figs. 5, 6B, C, 7A). During the evacuation process in normal ovulators, the middle and apical parts of the ovulation site usually do not have noticeable blood flow (Fig. 7). However, during slow-septated evacuations, the blood flow and serration are spread around the follicle and future ovulation site (see above; Figs. 5, 6E, F).

Early corpus luteum blood flow

After ovulation, the basement membrane between the granulosa and thecal layers breaks down in association with the invasion of blood vessels from theca interna into the developing corpus luteum. The spatial relationship between the vascularity of the preovulatory follicle and developing corpus luteum has been recently studied in mares (Ginther et al., 2007a) and for the first time in any species. Color-Doppler and B-mode film clips from 26 mares were taken of the preovulatory follicle 1 hour before the beginning of ovulation. The pattern

of the progressive development of blood-flow signals in various portions (basal third, middle third, and apical third) of the developing corpus luteum was evaluated every 12 hours until Day 6. Color-flow signals were determined by clock-face positions with the apex of the follicle (future ovulation site) or corpus luteum at 12 o'clock (Figs. 6D, H). Mares with normal follicle evacuation and prolonged septated evacuation were considered. Results indicated that vascularization of the ovulation site in the normal-evacuating mares and subsequent vascularization of the corpus luteum began at the base of the structure (Fig. 6D). This was shown by a greater percentage of blood-flow signals in the basal third than middle and apical thirds of the newly forming corpus luteum. This process involved the incorporation of at least some of the vessels from the follicle base into the collapsed basal ovulation site. There was a progressive increase in blood-flow signals from base to apex of the corpus luteum over the 6 days after ovulation. This was shown by the beginning of a successive increase in blood-flow signals over the basal, middle, and apical areas. This finding is compatible with previous studies (Bollwein et al., 2002; Ginther et al., 2006b) that showed a progressive increase in plasma progesterone concentrations and percentage of corpus luteum with blood-flow signals near maximal levels during the first week after ovulation in normal evacuators. In the slow-septated evacuation group, peripheral vessels seemed to contribute to the vessels of the newly forming corpus luteum uniformly in all parts (basal, middle, apical) of the structure (Fig. 6H).

Hemorrhagic anovulatory follicle

Extravasation of blood into follicles, ovulation sites, and corpora lutea is common in mares (Ginther, 1992, 1995). A hematoma that forms in the antrum instead of ovulation has been termed a hemorrhagic anovulatory follicle (HAF; Ginther and Pierson, 1984, 1989; Figs. 6J, K). Similar structures also have been termed hemorrhagic follicles (Ginther, 1995), anovulatory hemorrhagic follicles (Carnevale et al., 1989; Lefranc and Allen, 2003), persistent anovulatory follicles (McCue and Squires, 2002), and autumn follicles (Knudsen and Weiert, 1961). The economic importance of HAFs as a breeding-management problem in mares has been noted (McKinnon, 1998; Pycock, 2000; McCue and Squires, 2002) 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., 2007b). 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 (Ginther et al., 2006a). The day of ovulation and the first day of HAF formation, as indicated by cloudiness of follicular fluid (Fig. 6J), have been defined as Day 0. The frequency of discrete gray-scale ultrasonic indicators of impending ovulation (Gastal et al., 2006b) 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 (Figs. 6A, I). In regard to hormones, higher estradiol concentrations occurred a few days before HAF formation, but systemic LH, 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 $\geq$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 (Ginther et al., 2008c). 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 (Ginther et al., 2008a) 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. 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 (Ellenberger et al., 2009). 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 a 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 (Ginther et al., 2004b) and its effects may possibly lead to changes in the follicular wall that could be appreciated through ultrasonography during the 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: Bhal et al., 1999; mares: Silva et al., 2006; heifers: Siddiqui et al., 2009a), retrieval rate of oocytes (women: Bhal et al., 1999; mares: Ginther et al., 2007b; heifers: Siddiqui et al., 2009c), retrieval rate of mature oocytes (mares: Ginther et al., 2007b; Siddiqui et al., 2009b, d), in vitro fertilization rate (women: Bhal et al., 1999; heifers: Siddiqui et al., 2009d), pregnancy rate (women: Bhal et al., 1999, Coulam et al., 1999; Huey et al., 1999; Bhal et al., 2001; Du et al., 2006; Shrestha et al., 2006; mares: Silva et al., 2006; heifers: Siddiqui et al., 2009a), and lower incidence of triploidy (women: Bhal et al., 2001). In addition, follicles with greater blood flow resulted in better embryos and more pregnancies after embryo transfer in women (Chui et al., 1997; Bhal et al., 1999, 2001; Coulam et al., 1999; Du et al., 2006).

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 (Ginther et al., 2007b). The vascularity of the preovulatory follicle tended to be greater for follicles that contained a mature oocyte. Follicles with a recovered oocyte had a significantly higher frequency of serration of granulosa and a higher 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 in our former laboratory 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 follicle blood flow was greater in mares (Silva et al., 2006) and heifers (Siddiqui et al., 2009a) that became pregnant. 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 (Silva et al., 2006), 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 mares (Siddiqui et al., 2009b) 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 (Siddiqui et al., 2009a), demonstrating that highly vascularized preovulatory follicles are more likely to be associated with higher pregnancy rates, as previously seen for mares and women.

Acknowledgments

We would like to thank the Eutheria Foundation, Cross Plains, WI, University of Wisconsin, Madison, WI, and Dr. OJ Ginther. Appreciation is also expressed to the following colleagues who have participated during the collection of data in some of the experiments: TJ Acosta, M Almamun, MA Beg, D Cooper, FX Donadeu, MO Gastal, JC Jacob, BL Rodrigues, MA Siddiqui, LA Silva, and MD Utt.

References

Acosta TJ, Beg MA, Ginther OJ. Aberrant blood flow area and plasma gonadotropin concentrations during the development of dominant-sized transitional anovulatory follicles in mares. *Biol Reprod*, v.71, p.637-642, 2004a.

Acosta TJ, Gastal EL, Gastal MO, Beg MA, Ginther OJ. Differential blood flow changes between the future dominant and subordinate follicles precede diameter changes during follicle selection in mares. *Biol Reprod*. v.71, p.502-507, 2004b.

Baerwald AR. Human antral folliculogenesis: what we have learned from bovine and equine models. *Anim Reprod*, v.6, p.20-29, 2009.

Bergfelt DR, Adams GP. Ovulation and corpus luteum development. In: Samper JC, Pycock JF, McKinnon AO (Ed.). *Current therapy in equine reproduction*. St. Louis, MO, USA: Saunders Elsevier, 2007. p.1-13.

Bhal PS, Pugh N, Chui D, Gregory L, Walker SM, Shaw RW. The use of transvaginal power Doppler ultrasonography to evaluate the relationship between perifollicular vascularity and outcome in in-vitro fertilization treatment cycles. *Hum Reprod*, v.14, p.939-945, 1999.

Bhal PS, Pugh ND, Gregory L, O'Brien S, Shaw RW. Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: a study using transvaginal power Doppler. *Hum Reprod*, v.16, p.1682-1689, 2001.

Bollwein H, Mayer R, Weber F, Stolla R. Luteal blood flow during the estrous cycle in mares. *Theriogenology*, v.57, p.2043-2051, 2002.

Bourne TH, Jurkovic D, Waterstone J, Campbell S, Collins WP. Intrafollicular blood flow during human ovulation. *Ultrasound Obstetr Gynecol*, v.1, p.53-59, 1991.

Carnevale EM. Folliculogenesis and ovulation. In: Rantanen NW, McKinnon AO (Ed.). *Equine diagnostic ultrasound*. Baltimore, MD, USA: Williams & Wilkins, 1998. p.201-211.

Carnevale EM. The mare model for follicular maturation and reproductive aging in the woman. *Theriogenology*, v.69, p.23-30, 2008.

Carnevale EM, Bergfelt DR, Ginther OJ. Follicular activity and concentrations of FSH and LH associated with senescence in mares. *Anim Reprod Sci*, v.35, p.231-246, 1994.

Carnevale EM, Checura CM, Coutinho da Silva MA, Adams GP, Pierson RA. Use of computer-assisted image analysis to determine the interval before and after ovulation. *Proc Am Assoc Equine Pract*, v.48, p.48-50, 2002.

Carnevale EM, McKinnon AO, Squires EL, Voss JL. Ultrasonographic characteristics of the preovulatory follicle preceding and during ovulation in mares. *J Equine Vet Sci*, v.8, p.428-431, 1988.

Carnevale EM, Squires EL, McKinnon AO, Harrison LA. Effect of human chorionic gonadotropin on time to ovulation and luteal function in transitional mares. *J Equine Vet Sci*, v.9, p.27-29, 1989.

Chan JPW, Tsung-Hsiang H, Chuang ST, Cheng FP, Chen CL, Mao CL. Quantitative echotexture analysis for prediction of ovulation in mares. *J Equine Vet Sci*, v.23, p.397-402, 2003.

Chui DKC, Pugh ND, Walker SM, Gregory L, Shaw RW. Follicular vascularity - the predictive value of transvaginal power Doppler ultrasonography in an in-vitro fertilization programme: a preliminary study. *Hum Reprod*, v.12, p.196-196, 1997.

Coulam CB, Goodman C, Rinehart JS. Colour Doppler indices of follicular blood flow as predictors of pregnancy after in-vitro fertilization and embryo transfer. *Hum Reprod*, v.14, p.1979-1982, 1999.

Du B, Takahashi K, Ishida GM, Nakahara K, Saito H, Kurachi H. Usefulness of intraovarian artery pulsatility and resistance indices measurement on the day of follicle aspiration for the assessment of oocyte quality. *Fertil Steril*, v.85, p.366-370, 2006.

Ellenberger C, Muller K, Schoon HA, Wilsher S, Allen WR. Histological and immunohistochemical characterization of equine anovulatory haemorrhagic follicles (AHFs). *Reprod Domest Anim*, v.44, p.395-405, 2009.

Familiari G, Vizza E, Miani A, Motta PM. Ultrastructural and functional development of the theca interna. In: Familiari G, Makabe S, Motta PM (Ed.). *Ultrastructure of the ovary*. Boston, MA: Kluwer Academic, 1991. p.115-128.

Fleischer A, Kepple DM. *Transvaginal sonography: a clinical atlas*. 2.ed. Philadelphia, PA: J.B. Lippincott, 1995. p.186-189.

Gastal EL. Ovulation: Part 1. Follicle development and endocrinology during the periovulatory period. McKinnon AO, Squires EL, Vaala WE, Dickson DV (Eds.). *Equine reproduction*. 2.ed. Ames, IA: Wiley-Blackwell, 2011a. p.2020-2031.

Gastal EL. Ovulation: Part 2. Ultrasonographic morphology of the preovulatory follicle. In: McKinnon AO, Squires EL, Vaala WE, Varner DV (Eds.). *Equine reproduction*. 2.ed. Ames, IA: Wiley-Blackwell, 2011b. p.2032-2054.

Gastal EL. Recent advances and new concepts on follicle and endocrine dynamics during the equine periovulatory period. *Anim Reprod*, v.6, p.144-158, 2009.

Gastal EL, Donadeu FX, Gastal MO, Ginther OJ. Echotextural changes in the follicular wall during follicle deviation in mares. *Theriogenology*, v.52, p.803-814, 1999.

Gastal EL, Gastal MO, Donadeu FX, Acosta TJ, Beg MA, Ginther OJ. Temporal relationships among LH, estradiol, and follicle vascularization preceding the first compared with later ovulations during the year in mares. *Anim Reprod*, v.102, p.314-321, 2007a.

Gastal EL, Gastal MO, Ginther OJ. 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*, v.131, p.699-709, 2006a.

Gastal EL, Gastal MO, Ginther OJ. Serrated granulosa and other discrete ultrasound indicators of impending ovulation in mares. *J Equine Vet Sci*, v.26, p.67-73, 2006b.

Gastal EL, Gastal MO, Ginther OJ. The suitability of echotexture characteristics of the follicular wall for identifying the optimal breeding day in mares. *Theriogenology*, v.50, p.1025-1038, 1998.

Gastal EL, Jacob JCF, Gastal MO, Ginther OJ. Accumulation of fluid in the infundibulum during the estrous cycle in mares. *J Equine Vet Sci*, v.27, p.251-259, 2007b.

Gastal EL, Silva LA, Rocha Filho AN, Chiquetto D, Reis CP, Gastal MO. Predicting impending ovulation in mares: preliminary results from a field study using B-mode ultrasonography. *Anim Reprod Sci*, v.121S, p.S76-S78, 2010.

Ginther OJ. *Reproductive biology of the mare, basic and applied aspects*. 2.ed. Cross Plains, WI: Equiservices, 1992.

Ginther OJ. *Ultrasonic imaging and animal reproduction: book 2, horses*. Cross Plains, WI: Equiservices, 1995.

Ginther OJ. *Ultrasonic imaging and animal reproduction: book 4, color-doppler ultrasonography*. Cross Plains, WI: Equiservices, 2007.

Ginther OJ, Beg MA, Gastal MO, Gastal EL. Follicle dynamics and selection in mares. *Anim Reprod*, v.1, p.45-63, 2004a.

Ginther OJ, Beg MA, Gastal EL, Gastal MO, Baerwald AR, Pierson RA. Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. *Reproduction*, v.130, p.379-388, 2005.

Ginther OJ, Gastal EL, Gastal MO. Spatial relationships between serrated granulosa and vascularity of the preovulatory follicle and developing corpus luteum. *J Equine Vet Sci*, v.27, p.20-27, 2007a.

Ginther OJ, Gastal EL, Gastal MO, Beg MA. Conversion of a viable preovulatory follicle into a hemorrhagic anovulatory follicle in mares. *Anim Reprod*, v.3, p.29-40, 2006a.

Ginther OJ, Gastal EL, Gastal MO, Beg MA. Seasonal influence on equine follicle dynamics. *Anim Reprod*. 1, p.31-44, 2004b.

Ginther OJ, Gastal EL, Gastal MO, Bergfelt DR, Baerwald AR, Pierson RA. Comparative study of the dynamics of follicular waves in mares and women. *Biol Reprod*, v.71, p.1195-1201, 2004c.

Ginther OJ, Gastal EL, Gastal MO, Siddiqui MAR, Beg MA. Relationships of follicle versus oocyte maturity to ultrasound morphology, blood flow, and hormone concentrations of the preovulatory follicle in mares. *Biol Reprod*, v.77, p.202-208, 2007b.

Ginther OJ, Gastal EL, Gastal MO, Utt MD, Beg MA. Luteal blood flow and progesterone production in mares. *Anim Reprod*, v.99, p.213-220, 2006b.

Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA. Age-related dynamics of follicles and hormones during an induced ovulatory follicular wave in mares. *Theriogenology*, v.71, p.780-788, 2009.

Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA. Induction of haemorrhagic anovulatory follicles in mares. *Reprod Fertil Dev*, v.20, p.947-954, 2008a.

Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Siddiqui MA, Beg MA. Effects of age on follicle and hormone dynamics during the oestrous cycle in mares. *Reprod Fertil Dev*, v.20, p.955-963, 2008b.

Ginther OJ, Jacob JC, Gastal MO, Gastal EL, Beg MA. Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. *Anim Reprod Sci*, v.106, p.181-187, 2008c.

Ginther OJ, Pierson RA. Regular and irregular characteristics of ovulation and the interovulatory interval in mares. *J Equine Vet Sci*, v.9, p.4-12, 1989.

Ginther OJ, Pierson RA. Ultrasonic anatomy of equine ovaries. *Theriogenology*, v.21, p.471-483, 1984.

Ginther OJ, Utt M. Doppler ultrasound in equine reproduction: principles, techniques and potential. *J Equine Vet Sci*, v.24, p.516-526, 2004.

Hanna MD, Chizen DR, Pierson RA. Characteristics of follicular evacuation during human ovulation. *Ultrasound Obstet Gynecol*, v.4, p.488-493, 1994.

Herzog K, Bollwein H. Application of Doppler ultrasonography in cattle reproduction. *Reprod Domest Anim*, v.42, p.51-58, 2007.

Huey S, Abuhamad A, Barroso G, Hsu MI, Kolm P, Mayer J, Oehninger S. Perifollicular blood flow Doppler indices, but not follicular pO2, pCO2, or pH, predict oocyte developmental competence in in vitro fertilization. *Fertil Steril*, v.72, p.707-712, 1999.

Hunter RHF. *Physiology of the Graafian follicle and ovulation*. Cambridge, UK: Cambridge University Press. 2003.

Jaffe R, Ben-Aderet N. Ultrasonic screening in predicting the time of ovulation. *Gynecol Obstet Invest*, v.18, p.303-305, 1984.
Knudsen O, Weiert V. Ovarian oestrogen levels in the non-pregnant mare: relationship to histological appearance of the uterus and to clinical status. *J Reprod Fertil*, v.2, p.130-137, 1961.
Lefranc A, Allen WR. Incidence and morphology of anovulatory haemorrhagic follicles in the mare. *Pferdeheilkunde*, v.19, p.611-612, 2003.
Lenz S. Ultrasonic study of follicular maturation, ovulation and development of corpus luteum during normal menstrual cycles. *Acta Obstet Gynecol Scand*, v.64, p.15-19, 1985.
Martinuk SD, Chizen DR, Pierson RA. Ultrasonographic morphology of the human preovulatory follicle wall prior to ovulation. *Clin Anat*, v.5, p.339-352, 1992.
McCue PM, Squires EL. Persistent anovulatory follicles in the mare. *Theriogenology*, v.58, p.541-543, 2002.
McKinnon AO. Ovarian abnormalities. In: Rantanen NW, McKinnon AO (Eds.). *Equine diagnostic ultrasonography*. Baltimore, MD: Williams & Wilkins, 1998. p.233-251.
Mendelson EB, Friedman H, Neiman HL, Calenoff L, Vogelzang RL, Cohen MR. The role of imaging in infertility management. *Am J Roentgenol*, v.144, p.415-420, 1985.
Mihm M, Evans ACO. Mechanisms for dominant follicle selection in monovulatory species: comparison of morphological, endocrine and intraovarian events in cows, mares and women. *Reprod Domest Anim*, v.43, suppl.2, p.48-56, 2008.
Miyamoto A, Shirasuna K, Hayashi KG, Kamada D, Awashima C, Kaneko E, Acosta TJ, Matsui M. A potential use of color ultrasound as a tool for reproductive management: new observations using color ultrasound scanning that were not possible with imaging only in black and white. *J Reprod Dev,* v.52, p.153-160, 2006.
Palmer E, Chavatte-Palmer P, Verdonck E. Field trial of Doppler ultrasonography of the preovulatory follicle in the mare. *Anim Reprod Sci*, v.94, p.182-183, 2006. Abstract.
Picker RH, Smith DH, Tucker MH, Saunders DM. Ultrasonic signs of imminent ovulation. *J Clin Ultrasound*, v.11, p.1-2, 1983.
Pierson RA. Folliculogenesis and ovulation. In: McKinnon AO, Voss JL (Eds.). *Equine reproduction*. Philadelphia, PA: Lea & Febiger, 1993. p.161-171.
Pierson RA, Chizen DR. Transvaginal ultrasonographic assessment of normal and aberrant ovulation. In: Jaffe R, Pierson RA, Abramowicz JS (Eds.). *Imaging in infertility and reproductive endocrinology*. Philadelphia, PA: JB Lippincott, 1994. p.129-142.
Pierson RA, Ginther OJ. Ultrasonic evaluation of the preovulatory follicle in the mare. *Theriogenology*, v.24, p.359-368, 1985.
Pycock JF. Breeding management of the problem mare. In: Samper JC (Ed.) *Equine breeding management and artificial insemination*. Philadelphia, PA: WB Saunders, 2000. p.195-228.
Shrestha SM, Costello MF, Sjoblom P, McNally G, Bennett M, Steigrad SJ, Hughes GJ. Power Doppler ultrasound assessment of follicular vascularity in the early follicular phase and its relationship with outcome of in vitro fertilization. *J Assist Reprod Genet,* v.23, p.161-169, 2006.
Siddiqui MA, Almamun M, Ginther OJ. Blood flow in the wall of the preovulatory follicle and its relationship to pregnancy establishment in heifers. *Anim Reprod Sci*, v.113, p.287-292, 2009a.
Siddiqui MAR, Gastal EL, Gastal MO, Beg MA, Ginther OJ. Effect of hCG in the presence of hCG antibodies on the follicle, hormone concentrations, and oocyte in mares. *Reprod Domest Anim*, v.44, p.474-479, 2009b.
Siddiqui MAR, Gastal EL, Gastal MO, Beg MA, Ginther OJ. Relationship of vascular perfusion of the wall of the preovulatory follicle to in vitro fertilisation and embryo development in heifers. *Reproduction*, v.137, p.689-697, 2009c.
Siddiqui MAR, Gastal EL, Ju JC, Gastal MO, Beg MA, Ginther OJ. Nuclear configuration, spindle morphology and cytoskeletal organization of in vivo maturing horse oocytes. *Reprod Domest Anim*, v.44, p.435-440, 2009d.
Silva LA, Gastal EL, Gastal MO, Beg MA, Ginther OJ. Relationship between vascularity of the preovulatory follicle and establishment of pregnancy in mares. *Anim Reprod*, v.3, p.339-346, 2006.
Townson DH, Ginther OJ. Duration and pattern of follicular evacuation during ovulation in the mare. *Anim Reprod Sci*, v.15, p.131-138, 1987.
Townson DH, Ginther OJ. Size and shape changes in the preovulatory follicle in mares based on digital analysis of ultrasonic images. *Anim Reprod Sci*, v.21, p.63-71, 1989a.
Townson DH, Ginther OJ. Ultrasonic characterization of follicular evacuation during ovulation and the fate of the discharged follicular fluid in mares. *Anim Reprod Sci*, v.20, p.131-141, 1989b.
Witherspoon DM, Talbot RB. Ovulation site in the mare. *J Am Vet Med Assoc*, v.157 p.1452-1459, 1970.