



## Recent advances and new concepts on follicle and endocrine dynamics during the equine periovulatory period

E.L. Gastal<sup>1</sup>

Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison WI 53706, USA.

Eutheria Foundation, Cross Plains, WI 53528, USA.

### Abstract

During the recent years, mares have been shown as relevant follicle-related research comparative model for women because of similarities in the number and nature of ovarian follicular waves, a constant relative diameter of the largest follicle between the two species at definable events throughout the ovulatory wave, and similar ultrasonographic characteristics and changes of the preovulatory follicle. In the mare, as in other monovular species (cattle, women), usually only one dominant follicle develops as a result of the deviation mechanism. However, occasionally two or more dominant follicles may also occur. Few studies in mares have addressed the relationships between periovulatory circulating hormone concentrations and single versus multiple dominant/preovulatory follicles. Temporal and mechanistic studies performed recently (years 2005 to 2008) have contributed to elucidation of intriguing relationships among the dominant follicle and circulating hormones and will be the focus of this review. The main topics discussed herein are: (i) development of one versus two dominant follicles with a single ovulation; (ii) development of one versus multiple ovulatory follicles; (iii) conversion of two dominant follicles to double ovulations; (iv) role of hormones in development of double ovulations; (v) interrelationships of periovulatory reproductive hormones; (vi) repeatability of preovulatory follicle diameter and hormones; and (vii) factors that affect preovulatory follicle diameter and hormones, such as breeds and types of mares, season, body condition, and aging.

**Keywords:** follicles, gonadotropins, mares, ovarian steroids, ovulation.

### Introduction

Information on many aspects of equine folliculogenesis is needed for clinical and scientific purposes. Recently, the mare has been considered a relevant comparative research model for follicle studies because of striking similarities with women in regard to follicle dynamics and hormonal changes during the interovulatory interval (IOI; Ginther *et al.*, 2004a, c, 2005) and the ultrasonographic changes of the preovulatory follicle before ovulation (Martinuk *et al.*, 1992; Pierson and Chizen, 1994; Gastal *et al.*, 1998, 2006a, b). Therefore, this has provided encouragement for the use of the mare as a pertinent animal experimental model for the study of folliculogenesis and reproductive

aging in women (Carnevale, 2008; Ginther *et al.*, 2008d, f).

Changes in circulating concentrations of hormones during the estrous cycle began to be characterized in the early 1970s, however the classical descriptions are still being progressively refined. In mares, only few studies have considered the relationships between follicle diameter and hormonal changes during the periovulatory period. Attention to this aspect of reproductive physiology is needed due to the pivotal role of ovulation in cyclicity and in reproductive management. Temporal and mechanistic studies performed recently (years 2005 to 2008) in our laboratory have elucidated intriguing relationships among the preovulatory follicle and circulating hormones. The focus of this review will be on aspects related to the development of the ovulatory follicle and the associated systemic and intrafollicular hormones. B-mode ultrasonographic characteristics of the preovulatory follicle preceding normal (Gastal *et al.*, 2006a, b) and atypical ovulations (Ginther *et al.*, 2007a), and biochemical, molecular and cellular mechanisms involved in ovulation (Hunter, 2003) will not be addressed herein. This review emphasizes the contribution of recent findings and is directed to equine theriogenologists and scientists who are involved in monitoring, managing, and manipulating the mare reproductive system during the periovulatory period.

### Follicle development during the ovulatory wave

Waves of several follicles that emerge and initially grow in synchrony characterize ovarian follicle development in the mare. Various numbers and types of follicular waves develop during an equine IOI (Ginther, 1995). In a major wave, the largest follicle attains the diameter of a dominant follicle ( $\geq 28$ -30 mm), whereas in minor waves, the largest follicle does not become dominant (Ginther *et al.*, 2004a). The ovulatory wave emerges midway during an IOI of 21-24 days. After emergence at 6 mm (Gastal *et al.*, 1997), the follicles of a wave develop in a common-growth phase for several days (Gastal *et al.*, 2004a). At the end of the common-growth phase, a distinctive change in growth rates begins. This process is called deviation and in mares begins when the diameters of the two largest follicles on average are 22.5 mm and 19.0 mm (Gastal *et al.*, 1997, 1999; Ginther *et al.*, 2004a). The deviation in growth rates between the future dominant and subordinate

<sup>1</sup>Corresponding author: egastal@svm.vetmed.wisc.edu



follicles is a key event during the selection of the ovulatory follicle. After deviation, the developing dominant follicle maintains a constant growth rate until one or two days before ovulation (Gastal *et al.*, 2006a, c) and the remaining follicles (subordinate follicles) grow at a reduced rate and regress. Additional information about folliculogenesis in mares and the associated reproductive hormones may be found in recent reviews (Ginther *et al.*, 2004a, b; Beg and Ginther, 2006).

### Development of one versus two dominant follicles with a single ovulation

In the mare, as in other monovular species (cattle, women), usually only one dominant follicle develops as a result of the deviation mechanism. However, occasionally two dominant follicles also occur, based on two follicles reaching  $\geq 28$ -30 mm in mares (Ginther, 1993; Ginther *et al.*, 1996, 2004a; Gastal *et al.*, 1997). Single or double dominant follicles

can be associated to either one or two deviations (Fig. 1) in the same equine ovulatory wave. In 8/11 ovulatory waves with two dominant follicles, a second deviation occurred between the largest and second largest follicle on 2.5 days after the first deviation (Jacob *et al.*, 2008a). The incidence of two dominant follicles in an ovulatory wave has varied from 20% in Breton horses (Ginther *et al.*, 2004c) to about 30% in large ponies (Ginther *et al.*, 2008g; Jacob *et al.*, 2008a). This incidence seems to be lower in Miniature mares, since no double dominant follicles ( $\geq 28$  mm) were observed in 36 preovulatory periods (Gastal *et al.*, 2008b). Studies have not found a hormonal basis for the development of two dominant follicles in mares. Waves with one versus two dominant follicles (but no double ovulations) had similar plasma concentrations of LH, estradiol, and immunoreactive inhibin, however concentrations of FSH were lower in mares with two dominant follicles (Jacob *et al.*, 2008a; Fig. 2). Therefore, the factors that lead to the development of two dominant follicles or two deviations are still unknown in mares.

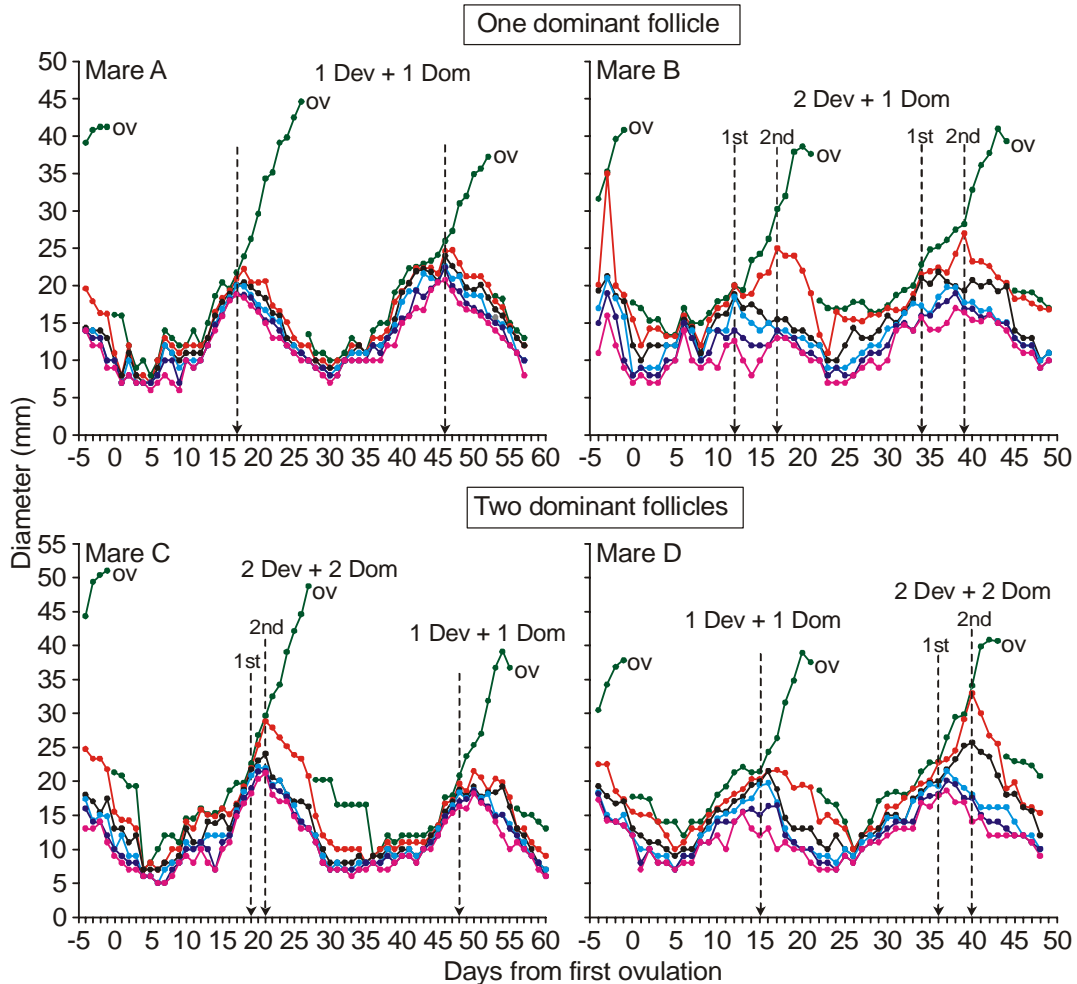


Figure 1. Profiles of the six largest follicles for individual mares with one (1 dom) or two (2 dom) dominant follicles ( $\geq 28$  mm) and one (1 dev) or two (2 dev) deviations in two sequential ovulatory waves. First (1st) and second (2nd) deviations are indicated in waves with two deviations. Dashed line with arrow indicates the day of follicle deviation in each wave.

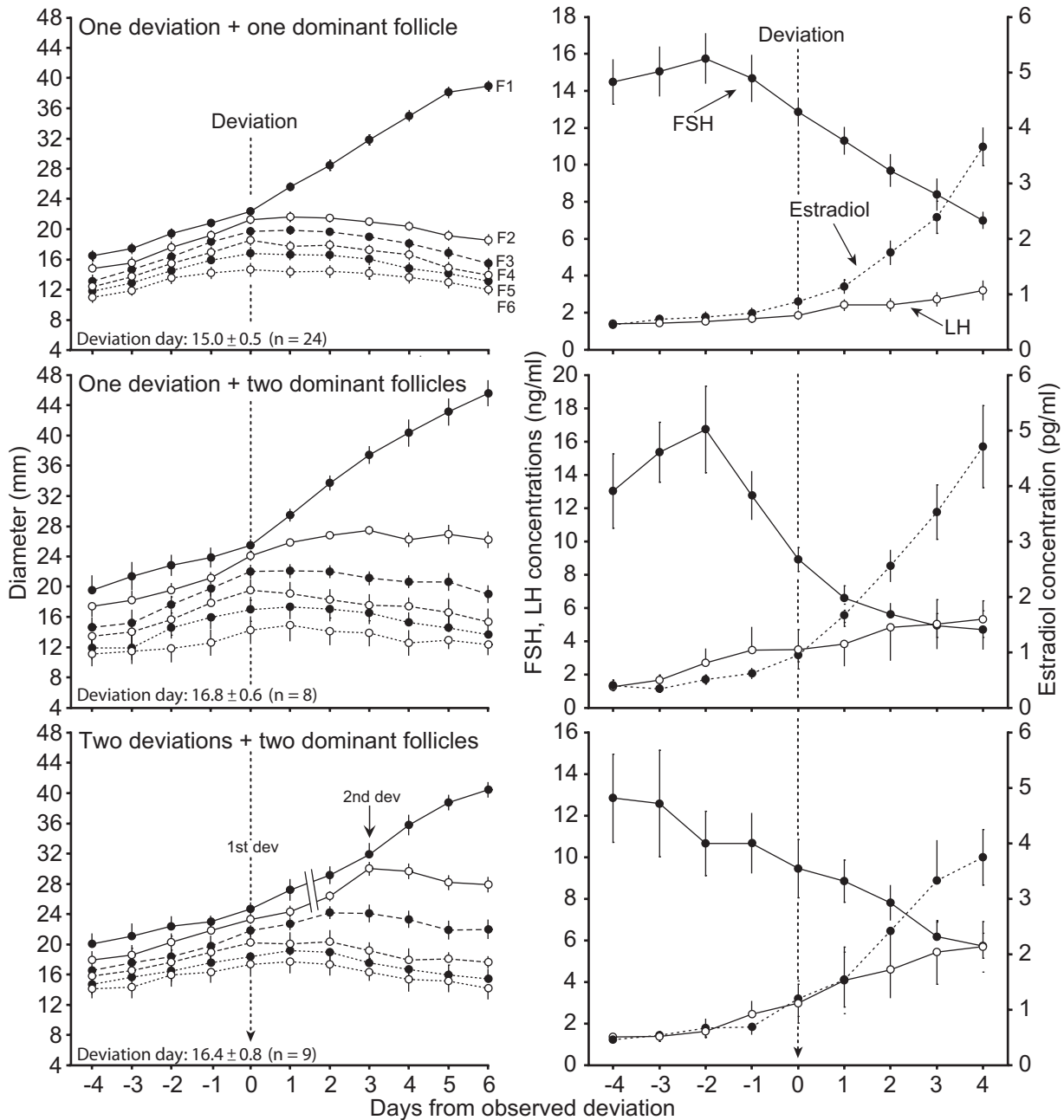


Figure 2. Means ( $\pm$  S.E.M.) for diameters of the six largest follicles and FSH, LH, and estradiol concentrations for estrous cycles of mares with one or two deviations associated to the presence of one or two dominant follicles. Data were normalized to the beginning of deviation and to the first deviation (1st dev) in cycles with two deviations. In the group with two deviations, the second deviation (2nd dev) was normalized to the mean day on which it occurred relative to the first deviation. The beginning of deviation was designated Day 0 and preceded the first increase in the differences in diameter between the largest and second largest follicles in the one dominant follicle group and between a combination of the two largest follicles versus the third largest follicle in the two dominant follicle group. A second deviation was examined between the two largest follicles. Figure produced from original data of Jacob *et al.* (2008a).

#### Development of one versus multiple ovulatory follicles

Follicle ablation-induced waves with one (33 waves, 72%) and multiple (13 waves, 28%) ovulatory follicles have been recently compared in large pony

mares (Ginther *et al.*, 2008g). Multiple ovulatory follicles had later emergence and were preceded by more follicles  $\geq 20$  mm at the beginning of deviation, higher LH preceding deviation, lower concentrations of FSH on the day of deviation and thereafter, and higher estradiol by 2 days after deviation (Fig. 3). During the



periovulatory period, systemic hormone concentrations for waves with multiple ovulations involved higher estradiol before ovulation, lower FSH before and after ovulation, and both higher progesterone and lower LH beginning on the day after ovulation (Fig. 3). These

findings are compatible with the concept that higher LH concentration in certain mares preceding deviation favors the development of multiple estrogen-competent follicles  $\geq 20$  mm at deviation, leading to the development of multiple ovulations.

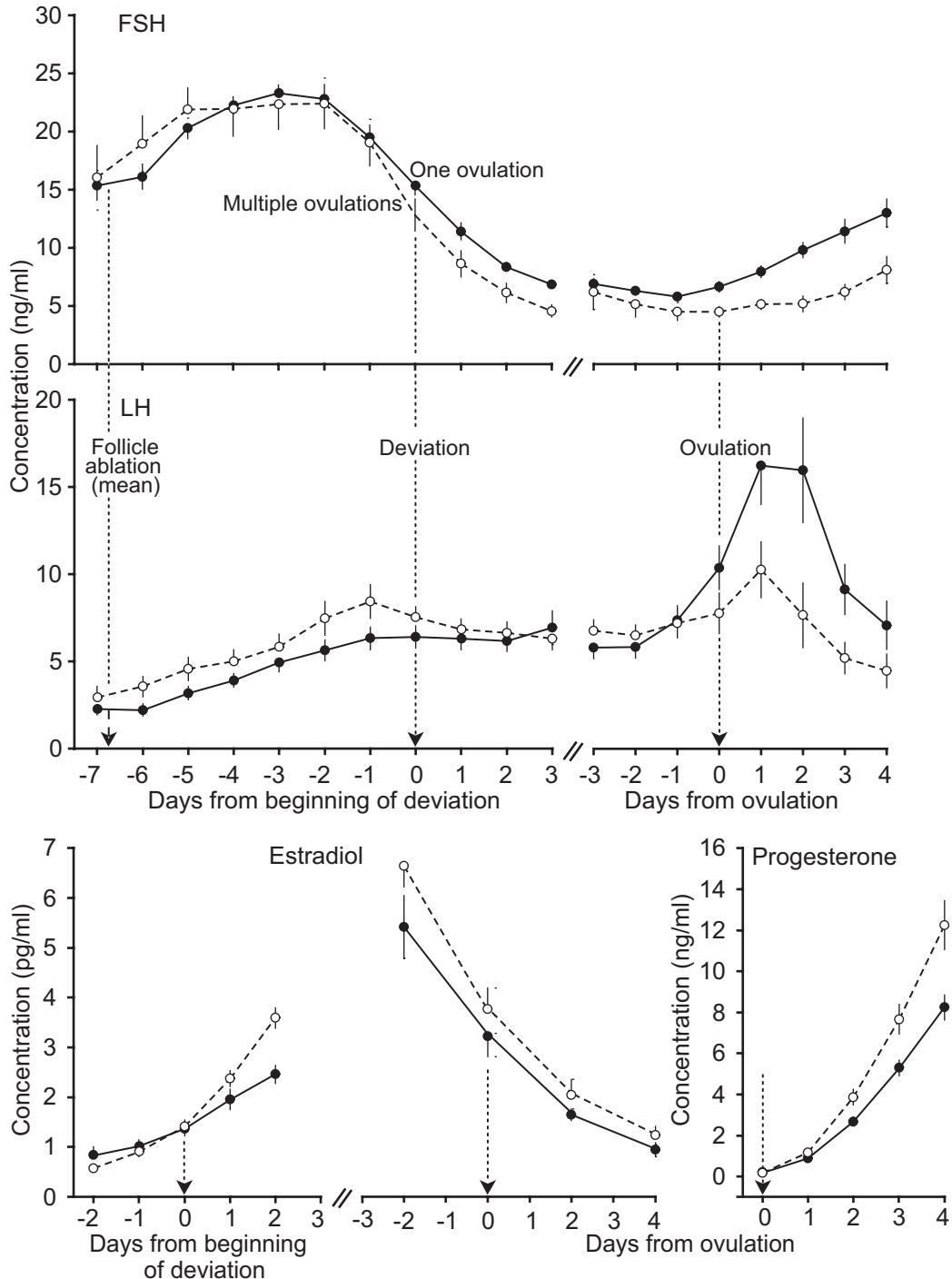


Figure 3. Means ( $\pm$  S.E.M.) for systemic concentrations of FSH, LH, estradiol, and progesterone in induced ovulatory waves with one versus multiple ovulations. Data were normalized to the beginning of deviation and to ovulation. Number of waves for the one and multiple ovulations are 33 and 13 for FSH and LH, 12 and 9 for estradiol, and 20 and 12 for progesterone, respectively. Data were adapted from Ginther *et al.* (2008g).



### **Conversion of two dominant follicles to double ovulations**

Although the importance of knowing the mechanisms involved in production of double or multiple ovulatory follicles in farm animals and women, the percentage of two dominant follicles that spontaneously become double ovulations apparently has not been reported previously for any monovular species, except for recent reports in mares. In a review (Ginther, 1992) of many reports, it was concluded that the incidence of double ovulations varied extensively but was lower in ponies (2%) than in horses (7 to 25%). This incidence seems to be very low in Miniature mares, since no double ovulations have been reported (Gastal *et al.*, 2008b). In addition to equine breed or type, the variability in double-ovulation rate reflects the effects of reproductive status, age, and repeatability in individuals. In six cases of double dominant follicles in Breton horses, ovulation occurred from either one follicle (five mares) or both follicles (one mare; Ginther *et al.*, 2004c). The incidence of double dominant follicles becoming double ovulations has varied from 9 to 43% in large pony mares (Ginther *et al.*, 2008c, h; Jacob *et al.*, 2008a). A combination of an ovulation and a hemorrhagic anovulatory follicle (HAF) from the largest remaining follicle can also occur in some mares with double dominant follicles (Ginther *et al.*, 2008e).

The incidence of two or multiple dominant follicles in our recent studies was similar between ablation-induced and spontaneous ovulatory waves; however double or multiple dominant follicles ovulated more frequently in induced waves (Ginther *et al.*, 2008g, h; Jacob *et al.*, 2008a). In the induced waves, both the day of the FSH surge and day of deviation were more synchronized, FSH and LH concentrations were greater before and after deviation, and estradiol concentrations were greater after deviation (Ginther *et al.*, 2008h). FSH, LH, and estradiol concentrations did not differ between induced and spontaneous waves near ovulation. It can be speculated that the uniform prominent wave-stimulating FSH surge or the post-ablation LH increase during the common-growth phase in induced waves enhanced the ovulatory capabilities of the future dominant follicles. Although further studies are indicated, this concept is compatible with the greater estradiol production of the double follicles before dominance ( $\geq 28$  mm) was established, leading to increased ovulatory capacity of both dominant follicles.

### **Role of hormones in development of double ovulations**

Single ovulators and double ovulators have similar length for the IOI (Urwin and Allen, 1983; Ginther *et al.*, 2008c). Diameter of the preovulatory follicle is larger for single than double ovulations

(Ginther, 1995) and the growth rate of the preovulatory follicle preceding a single or double ovulation diminishes on the day before ovulation in mares (Gastal *et al.*, 2006a, b; Ginther *et al.*, 2008c). The mechanism involved in the reduced growth of the follicle before ovulation apparently begins when the ovulatory LH surge reaches a critical level; this has been demonstrated by an immediate reduction in follicle growth and estradiol production when an ovulatory dose of hCG was given (Gastal *et al.*, 2006a, c). Although the small diameter of double preovulatory follicles in one ovary can be attributed, speculatively, to a crowding effect, a reduction in diameter also occurs with bilateral ovulations, indicating the involvement of other factors.

Few studies in mares have tried to address the relationship between periovulatory circulating hormone concentrations and double preovulatory follicles leading to double ovulations. Furthermore, it is not known in any of the monovular species whether such a relationship would reflect a role for the hormones in the development of double ovulatory follicles or an effect of the follicles on the hormones. Previous studies (Urwin and Allen, 1983; Squires *et al.*, 1987) in mares did not report significant differences in gonadotropin concentrations between single and double ovulators; however, due to either the experimental design utilized or the data presented, the results seemed to be inconclusive. The relationships between follicle diameters and hormone concentrations have been recently compared between single ovulator and double ovulator mares (Ginther *et al.*, 2008c; Fig. 4). In double ovulators, compared to single ovulators, the largest follicle was smaller, FSH was lower, and estradiol was higher on most days, but LH and ir-inhibin were not different. A relatively greater depression of FSH after double ovulations than after one ovulation was seen and may have been caused by inhibin from multiple follicles. After double ovulations, lower concentrations of LH were detected and likely reflected, at least in part, the greater concentrations of progesterone on the corresponding days. Results from this study indicated that smaller preovulatory follicles in double ovulators are a consequence of lower FSH, owing to higher estradiol from two preovulatory follicles and that the preovulatory differences in hormone concentrations between single and double ovulators are an effect rather than a cause of the double ovulations.

### **Interrelationships of periovulatory reproductive hormones**

The understanding of the hormonal changes during the periovulatory period in mares has been considerably advanced with the contribution of recent studies (Ginther *et al.*, 2008a, b, c, g, h; Jacob *et al.*,



2008b). Briefly, as depicted in Fig. 5, the concentrations of LH during the ovulatory LH surge initially increase slowly, followed by a more rapid increase beginning on Day -2 (ovulation = Day 0). The surge reaches a peak on Day 1. Concentrations of FSH begin increasing on Day -2, forming an early diestrus surge that precedes a surge that stimulates emergence of the next ovulatory follicular wave. The preovulatory estradiol surge reaches a peak on Day -2. Progesterone increases slightly but significantly by Day 0. The interrelationships among the changing hormone concentrations during the periovulatory period have been considered in two studies discussed ahead.

When hormone concentrations from a large number of periovulatory periods ( $n = 66$ ) were evaluated every 24 hour, mean concentrations of FSH began to increase and estradiol began to decrease in synchrony on Day -2 (Fig. 5; Jacob *et al.*, 2008b). These two changes are likely related, given that exogenous estradiol has a negative effect on FSH concentration in mares (Donadeu and Ginther, 2003). The FSH increase between Days -2 and -1 was interrupted by a transient plateau in FSH between Days -1 and 0, consistent with a discharge of hormone-laden follicular fluid into the peritoneal cavity during ovulation that occurred sometime between Day -1 and Day 0. In this regard, an increase in inhibin concentrations on Day 0 (Bergfelt *et al.*, 1991; Roser *et al.*, 1994; Nambo *et al.*, 2002) has been related to absorption of inhibins into the circulation after the discharge of follicular fluid into the abdomen (Nambo *et al.*, 2002). A similar indication that estradiol of the discharged follicular fluid alters the profile of plasma estradiol has not been apparent. Thus, the absorption of inhibin from the follicular fluid in the peritoneal cavity accounts at least partially for the transient suspension in the FSH increase between Days -1 and 0. An increase, apparently a rebound, in FSH occurred between Days 0 and 1. This FSH response likely reflects the release of FSH from the suppressing effects of inhibin during the absorption of inhibin from the peritoneal cavity. In this regard, a rebound in FSH began 24 h after administration of a proteinaceous fraction of follicular fluid in ovariectomized mares (Miller *et al.*, 1979) and ovarian-intact mares (Bergfelt and Ginther, 1985). The rapid decrease in estradiol concentrations on Days -2 to 1 and a slower decrease on Days 1 to 4 resulted in an abrupt change in the rate of decrease on Day 1 in synchrony with the mean day of the LH peak. The rapid decrease in estradiol is attributable to a negative effect of the rapidly increasing LH on estradiol (Gastal *et al.*, 2006a; Ginther *et al.*, 2008c), and the slower decrease is attributable to a diminishing negative effect of the decreasing LH. The LH decrease after the peak of the surge on Day 1 is related to a negative effect of the postovulatory increase in progesterone.

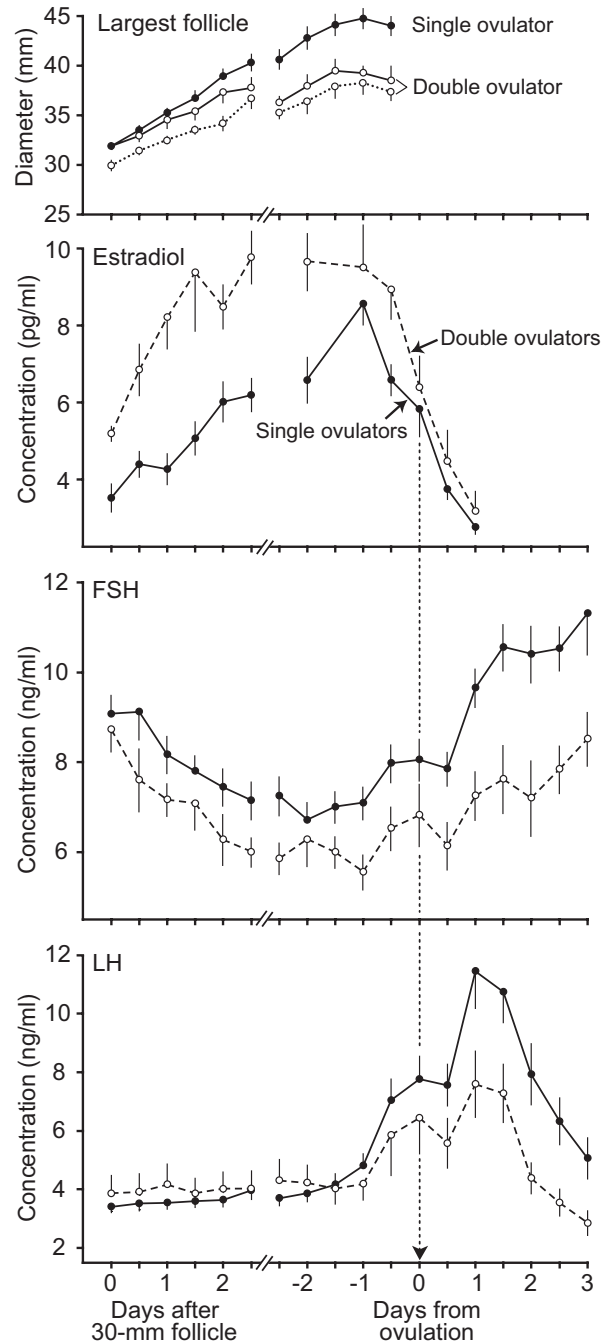


Figure 4. Means ( $\pm$  S.E.M.) for diameters of ovulatory follicles and concentrations of estradiol, FSH, and LH in single ( $n = 18$ ) and double ( $n = 6$ ) ovulating mares. Data were analyzed for 2.5 days after the first follicle reached  $\geq 30$  mm and separately for the 2.5 days before ovulation (follicles and hormones) and 3 days after ovulation (hormones) to correspond to a mean 5-day interval between a  $\geq 30$ -mm follicle and ovulation. The ovulating follicle for single ovulators first became significantly larger than the largest follicle in double ovulators on Day -2.5. Owing to a negative effect of estradiol, FSH was lower throughout the preovulatory period, resulting in smaller follicles in the double ovulators. Adapted from Ginther *et al.* (2008c).

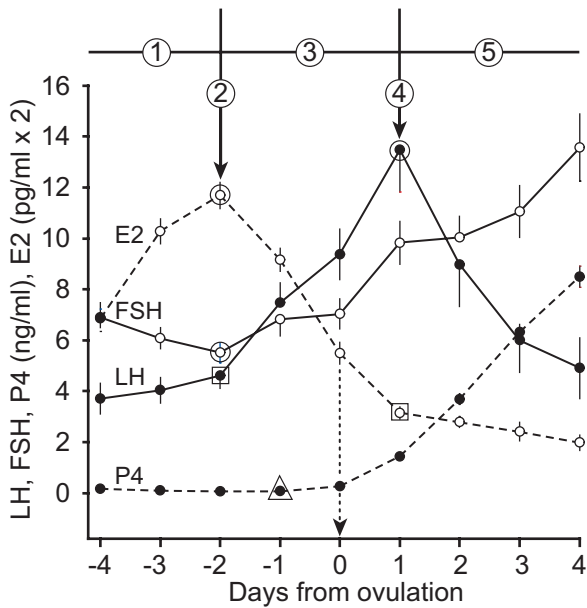


Figure 5. Means ( $\pm$  S.E.M.) for periovulatory systemic concentrations of estradiol (E2), LH, FSH, and progesterone (P4). Estradiol is shown at twice the actual concentrations. A circle around a mean indicates the day of a transition from a significant increase to a significant decrease (LH, E2) or from a decrease to an increase (FSH). A square indicates the day of change in the rate of a significant increase (LH) or rate of decrease (E2). A triangle indicates the beginning of a significant increase (P4). The circled numbers refer to the following: (1) days of continuous growth of the preovulatory follicle, decreasing FSH concentrations, and reduced rate of increase in LH, owing to a negative effect of the increasing E2; (2) day of acquisition of a negative effect of LH on E2 and apparently on cessation of follicle growth at the peak of E2; (3) days of rapidly decreasing E2 from the increasing negative effect of the rapidly increasing LH, resulting in turn in a lessening of the negative effect of E2 on LH and FSH and therefore a greater rate of production of LH and an increase in FSH; (4) day of transition between increasing and decreasing LH when P4 reaches a critical concentration; and (5) days of reduction in LH from increasing P4, with a lessening of the negative effect of LH on E2 and therefore a slower decrease in E2. Data from Jacob *et al.* (2008b) and figure adapted from Ginther *et al.* (2008a).

Using collection of blood samples and ultrasound scanning every 12 hours, the periovulatory hormonal interrelationships were considered in 18 single-ovulating mares in another recent study (Ginther *et al.*, 2008b). Results for Days -1 to 2 are relevant to the previous report (Jacob *et al.*, 2008b) and are shown (Fig. 6). The gradual preovulatory mean increase in each gonadotropin was temporarily disrupted at ovulation. Concentration of LH and FSH increased significantly between Days -1 to -0.5 and Days 0.5 to 1

but not between Days -0.5 to 0.5. Concentration of estradiol decreased significantly between Days -1 and -0.5 and between Days 0 and 0.5, but the decrease between Days -0.5 and 0 was not significant. In this regard, estradiol and inhibin have a synergistic effect on suppression of FSH (Miller *et al.*, 1981; Donadeu and Ginther, 2003), and estradiol has a negative effect on LH (Miller *et al.*, 1981; Ginther *et al.*, 2007b). Therefore, the estradiol content of the discharged follicular fluid seems to contribute to the disruption in the LH increase at the time of ovulation, as well as to the FSH disruption. The estradiol content of a 44-mm follicle (80  $\mu$ g; unpublished) is adequate for disruption of the LH surge, based on a titration study (Ginther *et al.*, 2007b), and contributes to disruption of the FSH increase.

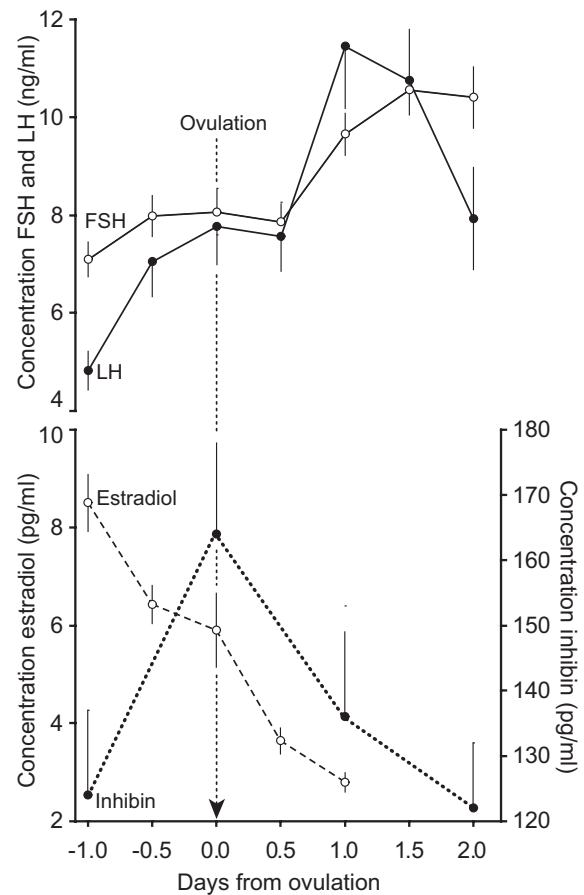


Figure 6. Means ( $\pm$  S.E.M.) for periovulatory concentrations of FSH, LH, estradiol, and immunoreactive inhibin. The increases in concentrations of FSH and LH are disrupted in temporal association with ovulation. Significant increases in FSH and LH occurred between Days -1 and -0.5 and between Days 0.5 and 1, but not between -0.5 and 0.5. A significant decrease in estradiol occurred between adjacent half days, except between Days -0.5 and 0. Inhibin reached a peak on Day 0. These data are consistent with the discharge of follicular fluid with its estradiol and inhibin content into the peritoneal cavity, resulting in a disruption in the gonadotropin surges. Adapted from Ginther *et al.* (2008b).



### Repeatability of preovulatory follicle diameter and hormones

Although the occurrence of double ovulations within individual mares has been known for many years (Ginther, 1992), only recently the repeatability of follicle diameters and hormone concentrations has been studied in mares (Ginther *et al.*, 2008g; Jacob *et al.*, 2008b). Several end points were significantly correlated between consecutive ovulatory waves within mares, indicating animal repeatability. Significant correlations were found for the diameter of the preovulatory follicle during the three days before ovulation in spontaneous waves (Jacob *et al.*, 2008b) and for the maximal diameter of the preovulatory follicle in consecutive induced waves (Ginther *et al.*, 2008g). When consecutive 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 (Cuervo-Arango and Newcombe, 2008), most mares (85%) ovulated from follicles that were within 3 mm of the diameter during the previous estrous cycle. Significant correlations as indicators of repeatability in concentrations of FSH and LH were found at specific events throughout the consecutive induced ovulatory waves (Ginther *et al.*, 2008g) and are consistent with the previously reported repeatability on many days during the estrous cycle (Jacob *et al.*, 2008b). Repeatability within mares in both LH concentrations and double ovulations is compatible with the concept that mares with relatively high concentrations of LH are more likely to develop double ovulations. Clinically, predicting the time of occurrence of an event (e.g., ovulation) on the basis of another (e.g., attainment of a given LH concentration) is not likely to be successful without knowledge of the history for each individual.

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 on the three days before ovulation. These days encompass the day that the follicle first reaches  $\geq 35$  mm in approximately 70% of the IOIs and  $\geq 35$  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. The reliability and practicality for the use of these findings would require a specific study. The present results in mares may lead to investigations in women.

### Effect of breeds and types of mares on preovulatory follicle diameter and hormones

Differences in diameter of the preovulatory follicle among breeds and types of mares have to be considered and incorporated in the reproductive management and in research experimental designs. In horses and large ponies the preovulatory follicle generally reaches 40-45 mm at the day before ovulation. The diameter or growth rate of the ovulatory follicle before ovulation is similar between ponies and Quarter horses (Bergfelt and Ginther, 1996). However, the maximum diameter of the preovulatory follicle is approximately 3 mm larger in French saddle horses than in Welsh ponies (Palmer, 1987). Limited data suggest that preovulatory follicles are about 5 mm smaller in Miniature mares and 10 mm larger in Clydesdales than in Quarter horses and large ponies (Ginther, 1995). The preovulatory follicle is larger in Quarter Horses (43 mm) than in Arabians (40 mm; Dimmick *et al.*, 1993), larger in one type of Thoroughbred than in another type (44 vs 41 mm; Vivo and Vinuesa, 1993), and 3.1 mm larger for Thoroughbreds in Australia than in England (Newcombe, 1994).

Follicular dynamics in Miniature ponies has been recently characterized (Gastal *et al.*, 2008b). The mean diameter of the ovulatory follicle at maximum ( $37.3 \pm 0.5$  mm) was greater than on Day -1 ( $35.9 \pm 0.6$  mm); data combined from two experiments ( $n = 34-36$  ovulatory waves). 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 (Gastal *et al.*, 2006a) and horses (Palmer and Driancourt, 1980; Koskinen *et al.*, 1989; Gastal *et al.*, 2006c). A hormonal mechanism possibly involved in the reduction in growth rate of the preovulatory follicle before ovulation has been discussed earlier. The growth profile of the ovulatory follicle from Days -6 to -1 did not differ between left and right ovaries. However, the frequency of an ovulatory follicle and future CL in the right ovary (61%) tended to be greater than in the left ovary (39%). This latter result in the Miniature mares seems to conflict with the findings for larger breeds where ovulation was more frequent from the left ovary, especially in maiden mares (Ginther, 1992). These apparently contrasting results indicate that Miniature ponies are a potential comparative model for studying the factors that favor ovulation from the ovary on a given side of the body. 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.





A preliminary comparison among Miniature ponies, large ponies, and Breton horses was made in the same study (Gastal *et al.*, 2008a, b) described above. 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). The Miniature ponies had fewer growing follicles  $\geq 10$  mm per ovulatory wave and more ovulatory waves with only one growing follicle  $\geq 10$  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 (Fig. 7). 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 (Fig. 7) and agrees with reported studies in ponies and horses (reviewed in Ginther, 1995). 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 compared to the great difference in body size.

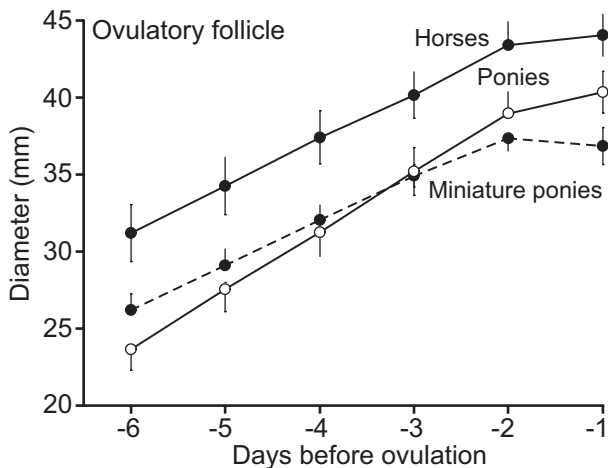


Figure 7. Means ( $\pm$  S.E.M.) for diameter of the ovulatory follicle from Days -6 to -1 in Miniature ponies, large ponies, and horses ( $n = 12$  mares per type). Figure adapted from Gastal *et al.* (2008b).

#### Effect of season on preovulatory follicle diameter, blood flow, and hormones

Recent innovations in ultrasonographic techniques have advanced our knowledge on the effects of seasonality on the changing populations and characteristics of antral follicles in mares. Studies involving the hypothalamus, pineal gland, and concentrations of circulating and intrafollicular hormones have contributed for the understanding of the effect of season on equine ovarian activity. Mares are seasonally ovulatory with the transition between the

anovulatory and ovulatory seasons occurring in the spring. The spring transition has been studied extensively because of its practical importance and because comparisons between the events preceding the first ovulation and a later ovulation of the year provide insights into preovulatory endocrinology (reviewed in Ginther, 1992; Ginther *et al.*, 2004b). The diameter of the preovulatory follicle on the day before ovulation is about 5 mm greater before the first than before the second ovulation of the year (Pierson and Ginther, 1987; Ginther, 1990). Mean and peak plasma estradiol concentrations are similar between the first and second preovulatory periods of the year from the day the preovulatory follicle reaches 25 mm and the day of ovulation (Watson *et al.*, 2003). The LH surge associated with the first ovulation of the year is of lower magnitude than the surge associated with subsequent ovulations (Oxender *et al.*, 1977; Freedman *et al.*, 1979; Fitzgerald *et al.*, 1987; Sharp *et al.*, 1991). It has been concluded that the attenuated LH surge before the first ovulation is a consequence of incomplete recrudescence of the hypothalamic-pituitary axis (Silvia *et al.*, 1986; Silvia and Fitzgerald, 1991). Considering the profound differences in magnitude of the LH surge associated with the first versus later ovulations of the year, more studies are needed to determine if differences in LH concentrations between the two ovulations are associated with differences in estradiol concentrations. Such information may be related to the effects of estradiol on LH or the effects of LH on estradiol (Gastal *et al.*, 2006a; Ginther *et al.*, 2007b), as discussed earlier.

The temporal relationships among LH, estradiol, and follicle vascularization preceding the first versus a later ovulation of the year have been studied recently in 40 pony mares for 6 days preceding ovulation (Gastal *et al.*, 2007). Diameter of the preovulatory follicle was greater for the first ovulation of the year than for the second ovulation and for a later ovulation (Fig. 8). Concentrations of LH were greater during the second preovulatory period than during the first preovulatory period, whereas concentrations of estradiol were not different between periods. The blood-flow area, based on color-Doppler signals (Fig. 9), in the wall of the preovulatory follicle increased at a reduced rate during the first preovulatory period. The vascularized area was similar between the preovulatory periods on Day -6, but the reduced rate of increase before the first ovulation resulted in a lower area on Day -1 than before a later ovulation. In conclusion, the first preovulatory period of the year has a lower magnitude of LH surge, similar estradiol concentrations, and lower rate of vascularization of the follicle wall than a later preovulatory period. These findings demonstrate that factors other than estradiol are involved in the differences between first and later ovulations of the year in the magnitude of the LH surge and the extent of follicle vascularization.

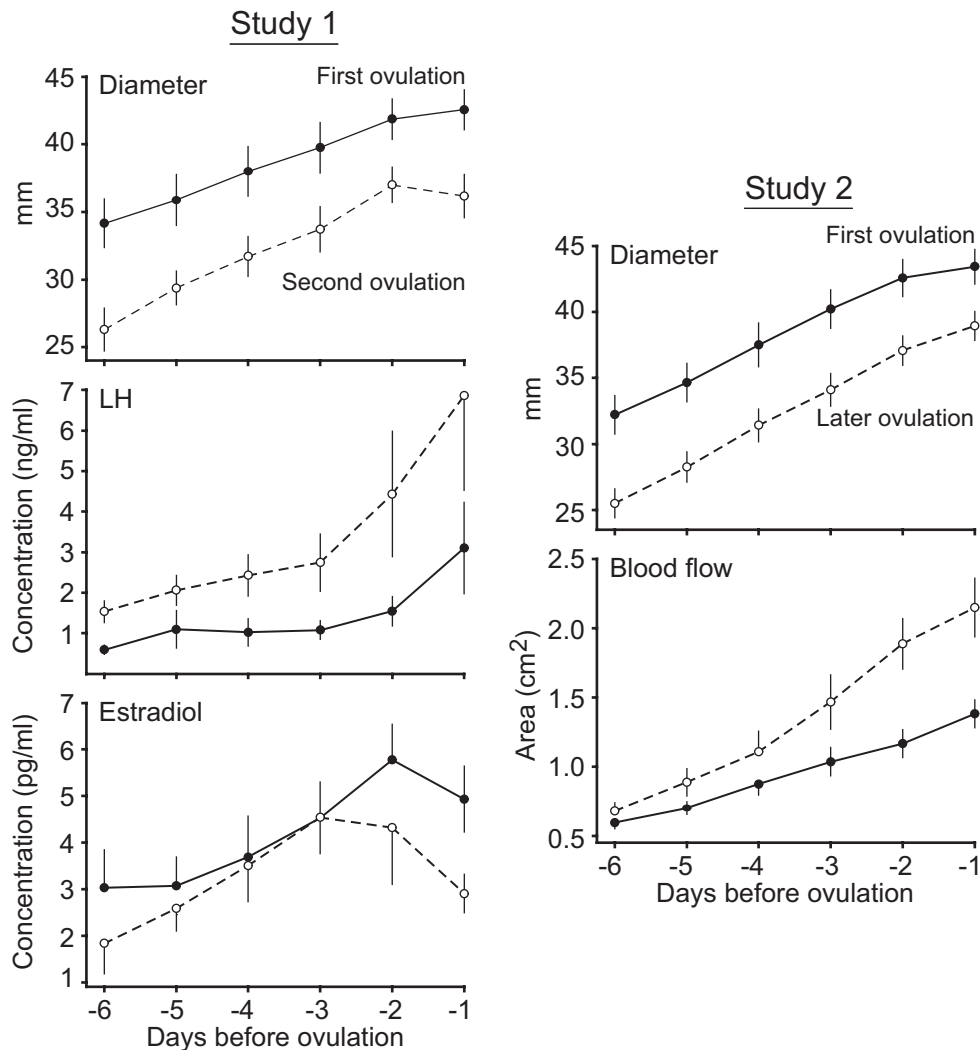


Figure 8. Means ( $\pm$  S.E.M.) for diameter of the ovulatory follicle and plasma concentrations of LH and estradiol for the 6 days preceding the first ( $n = 8$ ) and second ( $n = 6$ ) ovulation of the year (left panel; Study 1) and for diameter of the ovulatory follicle and area of the follicle wall with color-Doppler signals for blood flow for the 6 days preceding the first ovulation of the year (spring;  $n = 14$ ) and a later (summer;  $n = 12$ ) ovulation (right panel; Study 2). Figures adapted from Gastal *et al.* (2007).

#### Effect of body condition on preovulatory follicle diameter and hormones

There are evidences that inadequate nutrition or body condition is associated with delayed onset of the breeding season, decreased pregnancy rate, increased embryo loss, and increased gestation length in mares (Henneke *et al.*, 1983, 1984; Hines *et al.*, 1987). However, the effects of inadequate nutrition or poor body condition on follicle dynamics and reproductive hormones during the equine ovulatory season are not fully understood. During the winter, mares with low body condition had fewer medium (11 to 19 mm) and large ( $\geq 20$  mm) follicles than mares with high body condition (Gentry *et al.*, 2002). 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.

Follicle activity and gonadotropin concentrations, between mares of similar age with low and high body conditions, have been compared in a study in our laboratory (Gastal *et al.*, 2004b). 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 significantly associated to: 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); smaller diameter of the four largest follicles; fewer medium (11-19 mm) and large ( $\geq 20$  mm) follicles; and smaller total number of

follicles  $\geq 5$  mm. The score for body condition was positively correlated with the preovulatory follicle at maximum diameter and at Day -1. There were no differences between groups in growth rate of the ovulatory follicle or in concentrations of FSH and LH preceding both ovulations. The finding for the gonadotropins agrees with previous studies in mares subjected to feed-restriction or with low body condition. Acute (McManus and Fitzgerald, 2000) or chronic (Gentry *et al.*, 2002) feed restriction did not alter the plasma concentrations of FSH and LH. However, low plasma concentrations of leptin, IGF1 (insulin-like growth factor 1), and prolactin were observed in mares with low score for body condition during the middle of the anovulatory season (Gentry *et al.*, 2002). Results

from the previous study (Gastal *et al.*, 2004b) indicated for the first time in mares that low-body condition was associated with reduced follicle development, including diameter of the ovulatory follicle, during the transition between the anovulatory and ovulatory seasons and during the first interovulatory interval of the ovulatory season. These results were not attributable to altered circulating concentrations of FSH and LH. More studies are needed to evaluate the interrelationships between systemic and intrafollicular hormones and body condition in mares. These studies should be specifically designed and take into account also the age of the mares as a factor, since recent studies (Ginther *et al.*, 2008d, f) have shown the influence of aging in follicle dynamics and reproductive hormones (see discussion ahead).

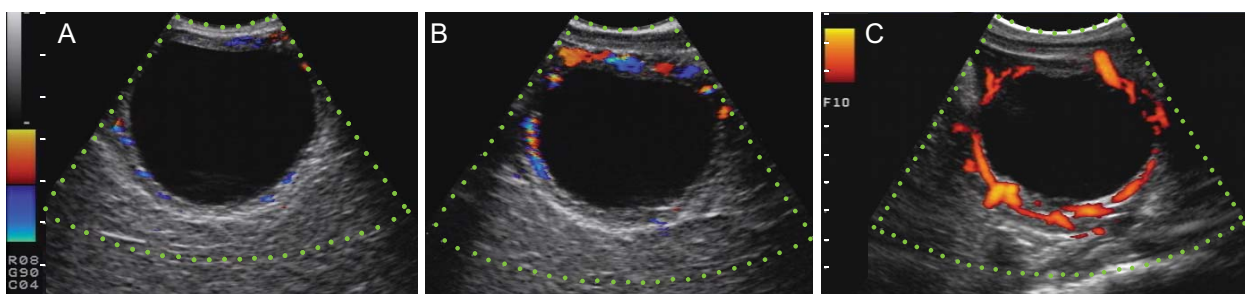


Figure 9. Ultrasonograms from different mares illustrating various degrees of blood-flow signals in the wall of preovulatory follicles. Images were captured in color- (A and B) and power-Doppler (C) modes 24 hours before the first (A; low blood flow) or later (B and C; high blood flow) ovulations of the season.

#### Effect of aging on preovulatory follicle diameter, blood flow, and hormones

Recently, the mare (Carnevale, 2008; Ginther *et al.*, 2008d, f) and the cow (Malhi *et al.*, 2005) have been advocated as relevant comparative research models for the study of reproductive aging in women. A recent study of spontaneous ovulatory waves in mares compared the effects of age (5 to 6 yr, young; 10 to 14 yr, intermediate;  $\geq 18$  yr, old) on follicle and hormone dynamics during an interovulatory interval (IOI;  $n = 46$ ) and on concentrations of follicular-fluid factors (Ginther *et al.*, 2008f). The old mares were not approaching senescence, as indicated by regular lengths of IOIs. The length of the IOI was about 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 LH concentrations (Fig. 10). The old mare 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 (Carnevale *et al.*, 1993), the preovulatory follicle grew more rapidly in young mares (5 to 7 yr) than in mares  $\geq 15$  yr. The results of the recent study are also consistent with the finding of fewer follicles  $\leq 20$  mm in older ponies (Wesson and Ginther, 1981; Carnevale *et*

*al.*, 1993), although some of the mares in the early reports were approaching senescence. Concentrations of FSH did not differ among age groups (Fig. 10), except that the maximum concentration was greater in the old group. A striking finding was a greater concentration of LH in the young group than in the other two groups throughout the ovulatory LH surge (Fig. 10) and this may have played a role in a shorter interval from maximum diameter of the preovulatory follicle to ovulation. In a previous study, LH was lower only on the day of the peak of the ovulatory surge in mares approaching senescence ( $\geq 20$  yr) than in younger mares ( $\leq 19$  yr) during one ovulatory period but not during the previous period (Carnevale *et al.*, 1993). In the recent study, maximum circulating concentration of estradiol during the preovulatory surge was greatest in the young group (Fig. 10). Concentrations of ovarian steroids in the preovulatory follicular fluid were not affected by mare age, but the concentrations of free IGF1 were greater in the old group. In another study, we have used the induction of an ovulatory wave with prostaglandin F<sub>2</sub> $\alpha$  treatment and ablation of follicles on Day 10 to evaluate the effects of age on the induced wave using the three previous groups of mares (Ginther *et al.*, 2008d). During the common-growth phase (Days 12 to 17) of induced waves, the diameter of the future ovulatory follicle was not different among ages, but the



young group had more follicles that reached  $\geq 10$  mm. Concentrations of LH increased in all age groups during Days 12 to 17, but were greatest in the young group and continued to be greater throughout the ovulatory LH surge. During several days before Day -1, there were no age-related effects on systemic estradiol concentrations, diameter of the preovulatory follicle, and B-mode echotexture and color-Doppler signals of blood flow in the follicle wall. Results suggested that greater number of follicles in the young group reflected a greater follicle reserve and that greater LH concentrations

throughout the ovulatory surge in the young group reflected a more positive response to an extraovarian/environmental influence after removal of the negative effect of progesterone. These studies indicate the importance for consideration of age in development of theriogenology programs (e.g., optimal time to breed and superovulation regimes), for consideration of age as a potential confounding factor in equine research protocols, and to investigate the role of the mare as a comparative research model for age effects.

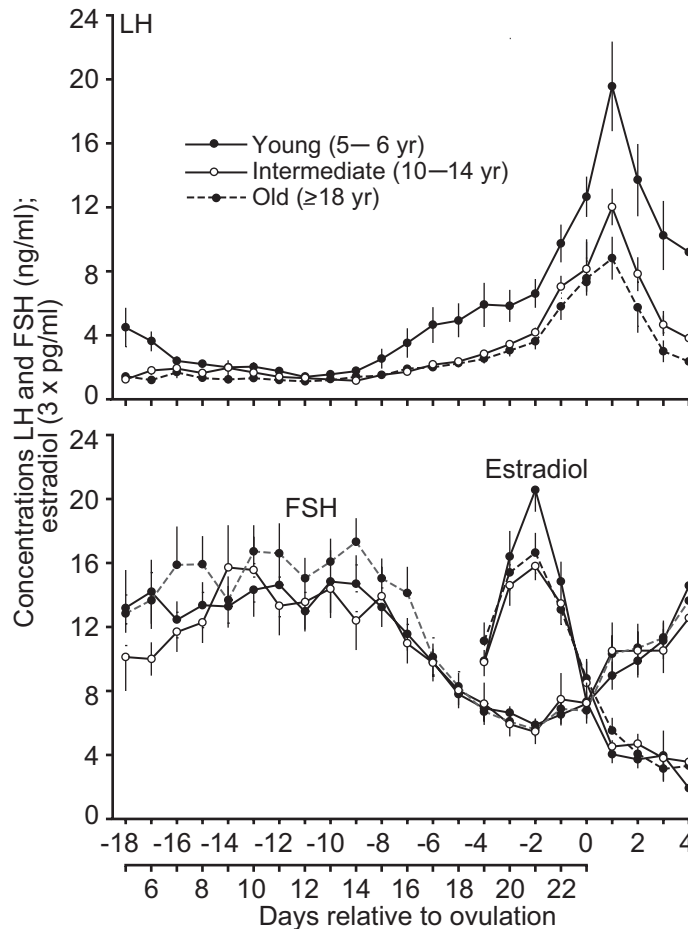


Figure 10. Means ( $\pm$  S.E.M.) for LH, FSH, and estradiol concentrations for young ( $n = 14$ ), intermediate ( $n = 16$ ), and old ( $n = 16$ ) groups of mares. Greater LH concentration in the young group was seen throughout the LH surge. Concentrations of estradiol are shown at three times the actual means to improve the presentation balance between estradiol and FSH. Concentrations of FSH for all groups were lower on the day of estradiol peak. Estradiol concentration at the peak was greatest in the young group. Adapted from Ginther *et al.* (2008d).

### Concluding remarks

In summary, during the recent years the mare has become an increasingly productive research model in the area of folliculogenesis. The striking similarities between mares and women in follicle dynamics and hormonal changes during the interovulatory interval and the ovulatory follicular wave, preovulatory follicle characteristics, and during the aging process provide

encouragement for the use and importance of the mare as a relevant experimental model for study of folliculogenesis in women. The equine model allows hypothesis testing using invasive technologies and may provide additional information that can be also considered for other farm animal species and in human clinical medicine.

During the periovulatory period estradiol and inhibin have a synergistic effect of suppression of FSH,



and estradiol has a negative effect on LH. Studies have not found a hormonal basis for the development of two dominant follicles in mares. Systemic hormone concentrations for waves with multiple ovulations involved higher estradiol before ovulation, lower FSH before and after ovulation, and both higher progesterone and lower LH beginning on the day after ovulation. Higher LH concentration in certain mares preceding deviation seems to favor the development of multiple estrogen-competent ovulatory follicles. The variability in double-ovulation rate reflects the effects of breed or type, reproductive status, age, and repeatability in individuals. Double or multiple dominant follicles ovulate more frequently in follicle ablation-induced waves. The uniform prominent wave-stimulating FSH surge or the post-ablation LH increase during the common-growth phase in induced waves seems to enhance the ovulatory capabilities of the future dominant follicles. Smaller preovulatory follicles in double ovulators are a consequence of lower FSH, owing to higher estradiol from two preovulatory follicles. The preovulatory differences in hormone concentrations between single and double ovulators are an effect rather than a cause of the double ovulations. Repeatability of follicle diameters and hormone concentrations has been demonstrated for several end points between consecutive ovulatory waves within mares. The repeatability within mares in both LH concentrations and double ovulations is compatible with the concept that mares with relatively high concentrations of LH are more likely to develop double ovulations. The first preovulatory period of the year has a lower magnitude of LH surge, similar estradiol concentrations, and lower rate of vascularization of the follicle wall than a later preovulatory period. Factors other than estradiol seem to be involved in the differences between first and later ovulations of the year in the magnitude of the LH surge and the extent of follicle vascularization. Low-body condition is associated with reduced follicle development, including diameter of the ovulatory follicle, but these results do not seem attributable to altered circulating concentrations of FSH and LH. Aging has a pronounced negative effect on follicle development and reproductive hormones during the periovulatory period in mares.

#### Acknowledgments

I would like to thank Dr. O.J. Ginther for the opportunity to work in his laboratory for many years and for funding the majority of the work discussed herein. I am also sincerely grateful to the following colleagues who have participated during the collection of data in many of the experiments: TJ Acosta, MA Beg, D Cooper, FX Donadeu, MO Gastal, JC Jacob, AP Neves, BL Rodrigues, MA Siddiqui, LA Silva, and MD Utt.

#### References

- Beg MA, Ginther OJ.** 2006. Follicle selection in cattle and horses: role of intrafollicular factors. *Reproduction*, 132:365-377.
- Bergfelt DR, Ginther OJ.** 1985. Delayed follicular development and ovulation following inhibition of FSH with equine follicular fluid in the mare. *Theriogenology*, 24:99-108.
- Bergfelt DR, Ginther OJ.** 1996. Ovarian, uterine and embryo dynamics in horses versus ponies. *J Equine Vet Sci*, 16:66-72.
- Bergfelt DR, Mann BG, Schwartz NB, Ginther OJ.** 1991. Circulating concentrations of immunoreactive inhibin and FSH during the estrous cycle of mares. *J Equine Vet Sci*, 11:319-322.
- Carnevale EM, Bergfelt DR, Ginther OJ.** 1993. Aging effects on follicular activity and concentrations of FSH, LH, and progesterone in mares. *Anim Reprod*, 31:287-299.
- Carnevale EM.** 2008. The mare model for follicular maturation and reproductive aging in the woman. *Theriogenology*, 69:23-30.
- Cuervo-Arango J, Newcombe JR.** 2008. Repeatability of preovulatory follicular diameter and uterine edema pattern in two consecutive cycles in the mare and how they are influenced by ovulation inductors. *Theriogenology*, 69:681-687.
- Dimmick MA, Gimenez T, Schalager RL.** 1993. Ovarian follicular dynamics and duration of estrus and diestrus in Arabian vs Quarter Horse mares. *Anim Reprod*, 31:123-129.
- Donadeu FX, Ginther OJ.** 2003. Suppression of circulating concentrations of FSH and LH by inhibin and estradiol during the initiation of follicle deviation in mares. *Theriogenology*, 60:1423-1434.
- Fitzgerald BP, Affleck KJ, Barrows SP, Murdoch WL, Barker KB, Loy RG.** 1987. Changes in LH pulse frequency and amplitude in intact mares during the transition into the breeding season. *J Reprod Fertil*, 79:485-493.
- Freedman LJ, Garcia MC, Ginther OJ.** 1979. Influence of photoperiod and ovaries on seasonal reproductive activity in mares. *Biol Reprod*, 20:567-574.
- Gastal EL, Gastal MO, Bergfelt DR, Ginther OJ.** 1997. Role of diameter differences among follicles in selection of a future dominant follicle in mares. *Biol Reprod*, 57:1320-1327.
- Gastal EL, Gastal MO, Ginther OJ.** 1998. The suitability of echotexture characteristics of the follicular wall for identifying the optimal breeding day in mares. *Theriogenology*, 50:1025-1038.
- Gastal EL, Bergfelt DR, Nogueira GP, Gastal MO, Ginther OJ.** 1999. Role of luteinizing hormone in follicle deviation based on manipulating progesterone concentrations in mares. *Biol Reprod*, 61:1492-1498.
- Gastal EL, Gastal MO, Beg MA, Ginther OJ.** 2004a.



- Interrelationships among follicles during the common-growth phase of a follicular wave and capacity of individual follicles for dominance in mares. *Reproduction*, 128:417-422.
- Gastal MO, Gastal EL, Spinelli V, Ginther OJ.** 2004b. Relationships between body condition and follicle development in mares. *Anim Reprod*, 1:115-121.
- Gastal EL, Gastal MO, Ginther OJ.** 2006a. Relationships of changes in B-mode echotexture and colour-Doppler signals in the wall of the preovulatory follicle to changes in systemic oestradiol concentrations and the effects of human chorionic gonadotrophin in mares. *Reproduction*, 131:699-709.
- Gastal EL, Gastal MO, Ginther OJ.** 2006b. Serrated granulosa and other discrete ultrasound indicators of impending ovulation in mares. *J Equine Vet Sci*, 26:67-72.
- Gastal EL, Silva LA, Gastal MO, Evans MJ.** 2006c. Effect of different doses of hCG on diameter of the preovulatory follicle and interval to ovulation in mares. *Anim Reprod*, 94:186-190.
- Gastal EL, Gastal MO, Donadeu FX, Acosta TJ, Beg MA, Ginther OJ.** 2007. Temporal relationships among LH, estradiol, and follicle vascularization preceding the first compared with later ovulations during the year in mares. *Anim Reprod*, 102:314-321.
- Gastal EL, Gastal MO, Beg MA, Neves AP, Petrucci BPL, Mattos RC, Ginther OJ.** 2008a. Miniature ponies: similarities and differences from larger breeds in follicles and hormones during the estrous cycle. *J Equine Vet Sci* 28:508-517.
- Gastal EL, Neves AP, Mattos RC, Petrucci BPL, Gastal MO, Ginther OJ.** 2008b. Miniature ponies: 1. Follicular, luteal, and endometrial dynamics during the oestrous cycle. *Reprod Fertil Dev*, 20:376-385.
- Gentry LR, Thompson Jr. DL, Gentry Jr. GT, Davis KA, Godke RA, Cartmill JA.** 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J Anim Sci*, 80:2695-2703.
- Ginther OJ.** 1990. Folliculogenesis during the transitional period and early ovulatory season in mares. *J Reprod Fertil*, 90:311-320.
- Ginther OJ.** 1992. *Reproductive Biology of the Mare, Basic and Applied Aspects*. 2<sup>nd</sup> ed. Cross Plains, WI: Equiservices Publishing.
- Ginther OJ.** 1993. Major and minor follicular waves during the equine estrous cycle. *J Equine Vet Sci*, 13:18-25.
- Ginther OJ.** 1995. *Ultrasonic Imaging and Animal Reproduction: Horses, Book 2*. Cross Plains, WI, USA: Equiservices Publishing.
- Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K.** 1996. Selection of the dominant follicle in cattle. *Biol Reprod*, 55:1187-1194.
- Ginther OJ, Beg MA, Gastal MO, Gastal EL.** 2004a. Follicle dynamics and selection in mares. *Anim Reprod*, 1:45-63.
- Ginther OJ, Gastal EL, Gastal MO, Beg MA.** 2004b. Seasonal influence on equine follicle dynamics. *Anim Reprod*, 1:31-44.
- Ginther OJ, Gastal EL, Gastal MO, Bergfelt DR, Baerwald AR, Pierson RA.** 2004c. Comparative study of the dynamics of follicular waves in mares and women. *Biol Reprod*, 71:1195-1201.
- Ginther OJ, Beg MA, Gastal EL, Gastal MO, Baerwald AR, Pierson RA.** 2005. Systemic concentrations of hormones during development of follicular waves in mares and women: a comparative study. *Reproduction*, 130:379-388.
- Ginther OJ, Gastal EL, Gastal MO.** 2007a. Spatial relationships between serrated granulosa and vascularity of the preovulatory follicle and developing corpus luteum. *J Equine Vet Sci*, 27:20-27.
- Ginther OJ, Utt MD, Beg MA, Gastal EL, Gastal MO.** 2007b. Negative effect of estradiol on LH throughout the ovulatory LH surge in mares. *Biol Reprod*, 77:543-550.
- Ginther OJ, Gastal EL, Gastal MO, Beg MA.** 2008a. Dynamics of the equine preovulatory follicle and periovulatory hormones: What's new? *J Equine Vet Sci*, 28:454-460.
- Ginther OJ, Gastal EL, Gastal MO, Beg MA.** 2008b. Passage of postovulatory follicular fluid into the peritoneal cavity and the effect on concentrations of circulating hormones in mares. *Anim Reprod*, 107:1-8.
- Ginther OJ, Gastal EL, Rodrigues BL, Gastal MO, Beg MA.** 2008c. Follicle diameters and hormone concentrations in the development of single versus double ovulations in mares. *Theriogenology*, 69:583-590.
- Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA.** 2008d. Age-related dynamics of follicles and hormones during an induced ovulatory follicular wave in mares. *Theriogenology*. (in press).
- Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Beg MA.** 2008e. Induction of haemorrhagic anovulatory follicles in mares. *Reprod Fertil Dev*, 20:947-954.
- Ginther OJ, Gastal MO, Gastal EL, Jacob JC, Siddiqui MAR, Beg MA.** 2008f. Effects of age on follicle and hormone dynamics during the oestrous cycle in mares. *Reprod Fertil Dev*, 20:955-963.
- Ginther OJ, Jacob JC, Gastal MO, Gastal EL, Beg MA.** 2008g. Development of one versus multiple ovulatory follicles and associated systemic hormone concentrations in mares. *Reprod Dom Anim*, doi: 10.1111/j.1439-0531.2008.01109.x
- Ginther OJ, Jacob JC, Gastal MO, Gastal EL, Beg MA.** 2008h. Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. *Anim Reprod*, 106:181-187.
- Henneke DR, Potter GD, Kreider JL, Yeats BF.** 1983. Relationship between body condition score, physical measurements and body fat percentage in mares. *Equine Vet J*, 15:371-372.
- Henneke DR, Potter GD, Kreider JL.** 1984. Body condition during pregnancy and lactation and



- reproductive efficiency of mares. *Theriogenology*, 21:897-909.
- Hines KK, Hodge SL, Kreider JL, Potter GD, Harms PG.** 1987. Relationship between body condition and levels of serum luteinizing hormone in postpartum mares. *Theriogenology*, 28:815-825.
- Hunter RHF.** 2003. *Physiology of the Graafian Follicle and Ovulation*. Cambridge, UK: Cambridge University Press.
- Jacob JC, Gastal EL, Gastal MO, Beg MA, Ginther OJ.** 2008a. Follicle deviation in ovulatory follicular waves with one or two dominant follicles in mares. *Reprod Domest Anim*, doi: 10.1111/j.1439-0531.2007.01048.x
- Jacob JC, Gastal EL, Gastal MO, Carvalho GR, Beg MA, Ginther OJ.** 2008b. Temporal relationships and repeatability of follicle diameters and hormone concentrations within individuals in mares. *Reprod Domest Anim*, doi: 10.1111/j.1439-0531.2007.01003.x
- Koskinen E, Kuntsi H, Lindeberg H, Katila T.** 1989. Predicting ovulation in the mare on the basis of follicular growth and serum oestrone sulphate and progesterone levels. *J Vet Med*, 36:299-304.
- Malhi PS, Adams GP, Singh J.** 2005. Bovine model for the study of reproductive aging in women: follicular, luteal, and endocrine characteristics. *Biol Reprod*, 73:45-53.
- Martinuk SD, Chizen DR, Pierson RA.** 1992. Ultrasonographic morphology of the human preovulatory follicle wall prior to ovulation. *Clin Anat*, 5:339-352.
- McManus CJ, Fitzgerald BP.** 2000. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. *Domest Anim Endocrinol*, 19:1-13.
- Miller KF, Wesson JA, Ginther OJ.** 1979. Changes in concentrations or circulating gonadotropins following administration of equine follicular fluid to ovariectomized mares. *Biol Reprod*, 21:867-872.
- Miller KF, Wesson JA, Ginther OJ.** 1981. Interaction of estradiol and a nonsteroidal follicular fluid substance in the regulation of gonadotropin secretion in the mare. *Biol Reprod*, 24:354-358.
- Nambo Y, Nagaoka K, Tanaka Y, Nagamine N, Shinbo H, Nagata S, Yoshihara T, Watanabe G, Groome N, Taya K.** 2002. Mechanisms responsible for increase in circulating inhibin levels at the time of ovulation in mares. *Theriogenology*, 57:1707-1717.
- Newcombe JR.** 1994. A comparison of ovarian follicular diameter in Thoroughbred mares between Australia and the UK. *J Equine Vet Sci*, 14:653-654.
- Oxender WD, Noden PA, Hafs HD.** 1977. Estrus, ovulation, and serum progesterone, estradiol, and LH concentrations in mares after an increased photoperiod during winter. *Am J Vet Res*, 38:203-207.
- Palmer E, Driancourt MA.** 1980. Use of ultrasonic echography in equine gynecology. *Theriogenology*, 13:203-216.
- Palmer E.** 1987. New results on follicular growth and ovulation in the mare. In: Roche JF, O'Callaghan D (Eds.). *Follicular Growth and Ovulation Rate in Farm Animals*. Dublin: Martinus Nijhoff Publishers. pp. 237-255.
- Pierson RA, Ginther OJ.** 1987. Follicular population dynamics during the estrous cycle of the mare. *Anim Reprod*, 14:219-231.
- Pierson RA, Chizen DR.** 1994. Transvaginal ultrasonographic assessment of normal and aberrant ovulation. In: Jaffe R, Pierson RA, Abramowicz JS (Eds.). *Imaging in Infertility and Reproductive Endocrinology*. Philadelphia: J.B. Lippincott Company. pp. 129-142.
- Roser JF, McCue PM, Hoye E.** 1994. Inhibin activity in the mare and stallion. *Domest Anim Endocrinol*, 11:87-100.
- Sharp DC, Grubaugh WR, Weithenauer J, Davis SD, Wilcox CJ.** 1991. Effects of steroid administration on pituitary luteinizing hormone and follicle-stimulating hormone in ovariectomized pony mares in the early spring: pituitary responsiveness to gonadotropin-releasing hormone and pituitary gonadotropin content. *Biol Reprod*, 44:983-990.
- Silvia PJ, Squires EL, Nett TM.** 1986. Changes in the hypothalamic-hypophyseal axis of mares associated with seasonal reproductive recrudescence. *Biol Reprod*, 35:897-905.
- Silvia PJ, Fitzgerald BP.** 1991. Determinants of attenuated LH-release associated with the first ovulation of the equine breeding season. *Domest Anim Endocrinol*, 8:255-264.
- Squires EL, McKinnon AO, Carnevale EM, Morris R, Nett TM.** 1987. Reproductive characteristics of spontaneous single and double ovulating mares and superovulated mares. *J Reprod Fertil Suppl*. 35:399-403.
- Urwin VE, Allen WR.** 1983. Follicle stimulating hormone, luteinising hormone and progesterone concentrations in the blood of Thoroughbred mares exhibiting single and twin ovulations. *Equine Vet J*, 15:325-329.
- Vivo RR, Vinuesa SM.** 1993. Diameter of the preovulatory follicle and early embryonic vesicle in Arab and Spanish Thoroughbreds. *Arch Zootec*, 42:263-267.
- Watson ED, Thomassen R, Nikolakopoulos E.** 2003. Association of uterine edema with follicle waves around the onset of the breeding season in pony mares. *Theriogenology*, 59:1181-1187.
- Wesson JA, Ginther OJ.** 1981. Influence of season and age on reproductive activity in pony mares on the basis of a slaughterhouse survey. *J Anim Sci*, 52:119-129.