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Seasonal influence on equine follicle dynamics

O. J. Ginther[1,3], E. L. Gastal[2], M. O. Gastal[2], M. A. Beg[1]

Eutheria Foundation, Cross Plains, Wisconsin 53528 USA
[1]Animal Health and Biomedical Sciences, 1656 Linden Drive, University of Wisconsin, Madison WI 53706.
[2]Departments of Veterinary and Animal Science, Federal University of Viçosa, Viçosa, Brazil.

Abstract

Recent innovations in ultrasonographic techniques have advanced our knowledge on the effects of seasonality on the changing populations of antral follicles in mares. The seasonal patterns in the monthly incidence of ovulations is at least partly a function of daylength, even at latitudes as low as 10º where the longest day may be less than 13 hours. Follicle dynamics involves a mid-anovulatory phase in winter when the follicles may not exceed 16 or 17 mm. A minor follicular wave (no dominant follicle) ends the mid-anovulatory phase and begins a resurging phase, raising the diameter of the largest follicle to at least 21 mm. The largest follicle of subsequent minor waves reaches a similar diameter forming an approximate plateau. However, in some mares, the later portion of the resurging phase or the entire phase may consist of a period of major anovulatory waves in which the largest follicle approaches or is similar to the diameter of a dominant ovulatory follicle. In one study, the length of the resurging phase was a mean of 52 days and consisted of minor waves, unless a period of major anovulatory waves occurred. Each minor or major wave is stimulated by an FSH surge, and the surges are similar regardless of the diameter reached by the largest follicle. Results of a Doppler study has led to the hypothesis that the future dominant-sized (≥ 30 mm) follicles of the period of major anovulatory waves develop during an LH deficient environment beginning when the follicles are as small as 15 mm. These future dominant-sized anovulatory follicles are already dysfunctional at about 20 mm as indicated by an increase in circulating FSH. Despite an aberrant vasculature and low concentrations of estradiol, free IGF-1, inhibin-A, and VEGF, the follicles continue to expand at a rate comparable to the growth of an ovulatory follicle. The effects of daylength on follicle dynamics continue through the ovulatory season. For example, follicular activity of the estrous cycle is greater during the first half of the season as indicated by more follicles ≥ 20 mm, greater diameter of largest follicle, and greater incidence of minor and major anovulatory waves. After the last ovulation of the year, most mares, during a receding phase, have an increase in follicle activity at the time ovulation would have occurred. A major anovulatory wave or a minor wave develops during the increased follicle activity. Concentrations of LH are greater in association with major waves than with minor waves, but the wave-stimulating FSH surges are similar. A persistent corpus luteum forms from the site of the last ovulation of the year in about 25% of mares. In these mares, a period of major anovulatory waves may ensue until the corpus luteum regresses.

Keywords: follicles, mares, seasonality

Introduction

The ovarian antral follicles of the nonpregnant mare herald the changing reproductive stages across the seasons. Studies involving the hypothalamus, pineal gland, and concentrations of circulating hormones have been given productive consideration for many years. However, the changing population of follicles were not considered or were only a superficial aspect of the research protocols. Ultrasonography for monitoring the follicles either was not available or the technique was not judiciously applied. Recently, several productive ultrasound adaptations have been made to better consider the follicles as targets or end points in seasonality studies. These innovations include: 1) transvaginal ultrasound-guided ablation of follicles appropriate to a given hypothesis, 2) transvaginal sampling and experimental treatment of targeted follicles, and 3) color Doppler ultrasound for assessing the changing vascularity of the follicle wall. These advances in technology have produced results that encourage a review of the

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[3] Corresponding author: ginther@svm.vetmed.wisc.edu, FAX: 608-262-7420

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current status of follicle dynamics and seasonality.

This review will consider seasonal effects on the dynamics and interrelationships among the changing population of follicles throughout the twelve months that encompass the anovulatory season, the transition from anovulatory to ovulatory seasons, the ovulatory season, and the transition from ovulatory to anovulatory seasons. This is not a historical or comprehensive review, but the cited reports will provide information on other studies. A comprehensive review on equine follicles (Ginther, 1992) and reviews on equine seasonality (Nagy *et al.*, 2000) and photostimulation (Guillaume *et al.*, 2000) can also be consulted.

A. Effects of Daylength and Other Factors on Follicle Dynamics

A1. Evolution of reproductive seasonality

The evolution of equine reproductive seasonality amalgamated both the 11-month gestation period and the need for parturition to occur when food supply and environmental conditions are optimal for survival and development of the young. Thus, conception in late spring (beginning of the ovulatory season) results in foaling during early spring beyond the climatic hazards of winter and at a time when potentially there will be many months of abundant feed ahead. This consideration implies that horses evolved in a temperate zone. Apparently, however, this striking compatibility with the environment is so well engrained that the ovulatory seasons of today's world-wide horse population seem approximately synchronized regardless of latitude, including those of the torrid zone. This concept has not been studied critically, but is suggested by seasonality reports from diverse latitudes.

A2. Seasonal ovulatory pattern

Examples of studies on the monthly changes in the annual distribution of ovulations are shown (Fig. 1). These studies were done in the northern or southern temperate zones in an approximate range of latitudes of 30º to 45º. The general seasonal pattern is that the incidence of ovulation is minimal or absent during the winter, transitionally increasing during the spring, maximal during the summer, and transitionally decreasing during the fall. The low incidence of ovulation for the winter months reflects anovulatory seasons of different lengths among individual mares combined with uninterrupted ovulation throughout the year by some mares. Except for a few studies in the past few years, data on the seasonal distribution of ovulations have been obtained from the northern and southern temperate zones, as in the examples. These zones begin at approximate latitudes of 23ºN and 23ºS, respectively, where the difference in length between the longest and shortest day is about two hours (Fig. 1). In a slaughterhouse study done in southern Mexico (15ºN–22ºN), a definitive seasonal ovulatory pattern was found; about 10% of the reproductive tracts had indications of ovulation in January and February and close to 100% in July and August (Saltiel *et al.*, 1982). The slight seasonal daylength variation at 10ºN in Venezuela (longest daylength, 12 h and 46 min) also was associated with a seasonal reproductive pattern (Quintero *et al.*, 2000).

A3. Role of daylength

The annual pattern of changes in monthly ovulation rate follows minimal, increasing, maximal, and decreasing daylengths (Fig. 1). Changing daylength is the primary controller of the follicle dynamics of seasonal reproductive rhythms (reviewed in Ginther, 1992). The effects of changes in daylength on reproductive seasonality involve a neural pathway from eyes to pineal gland, with involvement of the hypothalamus and pituitary. These mechanisms have been reviewed (Ginther, 1992; Nagy *et al.*, 2000; Daels *et al.*, 2000) and will not be considered herein, except to note the relationships between daylength and circulating concentrations of FSH and LH. The effects of changing daylength on circulating levels of FSH and LH have been demonstrated by removing ovarian influences by ovariectomy; the annual gonadotropin profiles (Freedman *et* al., 1979b) were similar to the profile of monthly percentages of ovulating mares. The high seasonally imposed LH levels during the ovulatory season are cyclically modified during an estrous cycle by depression during the progesterone phase and stimulation above the seasonal influence during the estrogen phase (Section C2; Freedman *et al.*, 1979b; Ginther, 1992). The monthly output of FSH is constant throughout the year in ovarian intact mares (Turner *et al.*, 1979); the high levels stimulated by daylength during the ovulatory season are modified by follicular factors (Section C2).

A4. Modifiers of the effect of daylength

Food supply and body condition have a well documented modifying role in the follicle dynamics of equine reproductive seasonality (Fig. 1; reviewed in Ginther, 1992). For example, the interval from the winter solstice to the first ovulation was about one month longer for mares that lost weight than for mares that gained weight (Ginther, 1974). The greater the loss in weight, the longer the interval to the beginning of the ovulatory season. High body weight and percentage body fat (Fitzgerald and McManus, 2000) or high body-condition scores (Gentry *et al.*, 2002) were associated with greater reproductive activity during the winter. Green pasture hastened the onset of the ovulatory season in mares placed on pasture when a 30-mm follicle developed (Carnevale *et al.*, 1997). The pro-

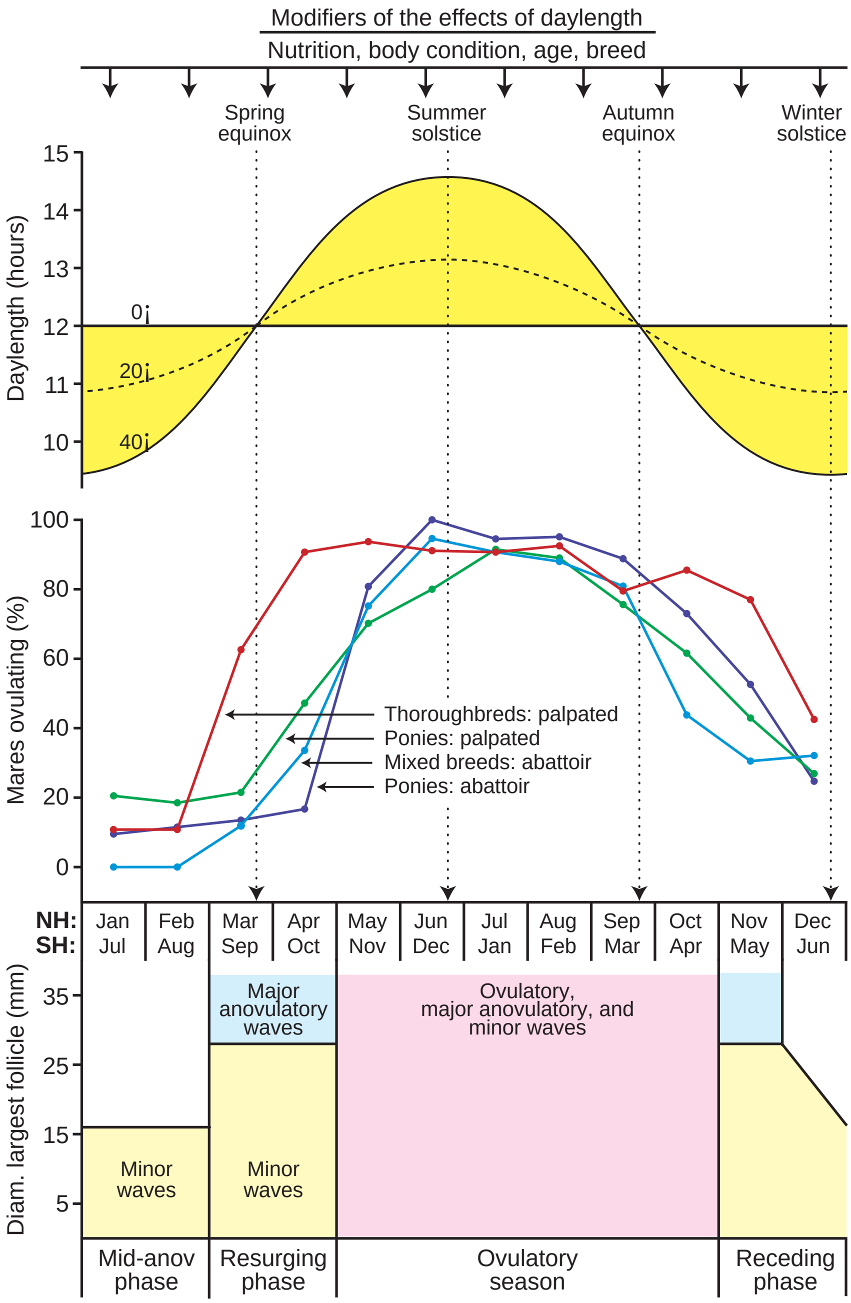

Figure 1. Percentage of mares ovulating per month based on examination of thousands of reproductive tracts at an abattoir for mixed breeds (Osborne, 1966) and ponies (Wesson and Ginther, 1981a) and on transrectal palpation in Thoroughbreds and ponies (data courtesy of D. C. Sharp, University of Florida). The corresponding changes in daylength are shown for 0º, 20º, and 40º latitudes. The approximate maximum diameters of the largest follicles are shown for the phases of the anovulatory season (lower panel). Minor waves develop during the mid-anovulatory (Mid-anov) and resurging phases. The largest follicles of the resurging phase are larger than during the mid-anovulatory phase, and the transition between these two phases is abrupt. In many mares, the minor waves of the resurging phase develop into major anovulatory waves as the ovulatory season approaches. The initial portion of the receding phase may also consist of a period of major anovulatory waves.

found modifying effect of nutrition and body condition likely has confounded reported results on the effects of photoperiod on reproductive seasonality and may account for some of the differences among reports.

Age also alters reproductive seasonality. In fillies, puberty (first ovulation of life) occurred at the same time as the first ovulation of the year in mature mares, but the ovulatory season was shorter (Wesson and Ginther, 1981b). In a slaughterhouse survey (Wesson and Ginther, 1981a), younger mares (2 to 5 years) had about a 2-month earlier cessation of the ovulatory season, whereas older mares (> 15 years) had lower total yearly ovulatory productivity, resulting from an approximately 2-month later start of the ovulatory season. These age effects involved the average number of follicles < 20 mm and not diameter of the largest follicle. Number of follicles gradually increased between puberty and 5 years of age, plateaued over 6 to 15 years, and declined after 15 years. Apparently, therefore, the negative effects of old age involve reduced numbers of available follicles, rather than the response of the largest follicle to hormone stimulation.

There are differences among breeds and types in the length of the ovulatory season. In this regard, the illustrated (Fig. 1) and other reviewed studies (Ginther, 1992) indicate that the mean ovulatory season begins earlier and ends later in horses than in ponies. There are indications that there is an intrinsic annual rhythm of reproductive function as well as the seasonally related extrinsic rhythms. That is, some individuals have broken away and the species is beginning to break away from the dominance of changing daylength. For example, 50% of mares ovulated in the summer despite a fixed 9-hour experimental photoperiod, beginning in late autumn of the previous year (reviewed in Ginther, 1992).

B. Follicle Dynamics during the Transition from Anovulatory to Ovulatory Seasons

B1. Mid-anovulatory phase

The term spring transitional period is used for follicle events leading to the onset of the ovulatory season. This term is meaningful for general use but more specific terms will be used herein to highlight and focus on various events and phases. Follicle activity is minimal during approximately the two winter months of January and February (Northern Hemisphere; Fig. 1) and has been called the inactive phase of the anovulatory season (Ginther, 1992). Follicles are active throughout

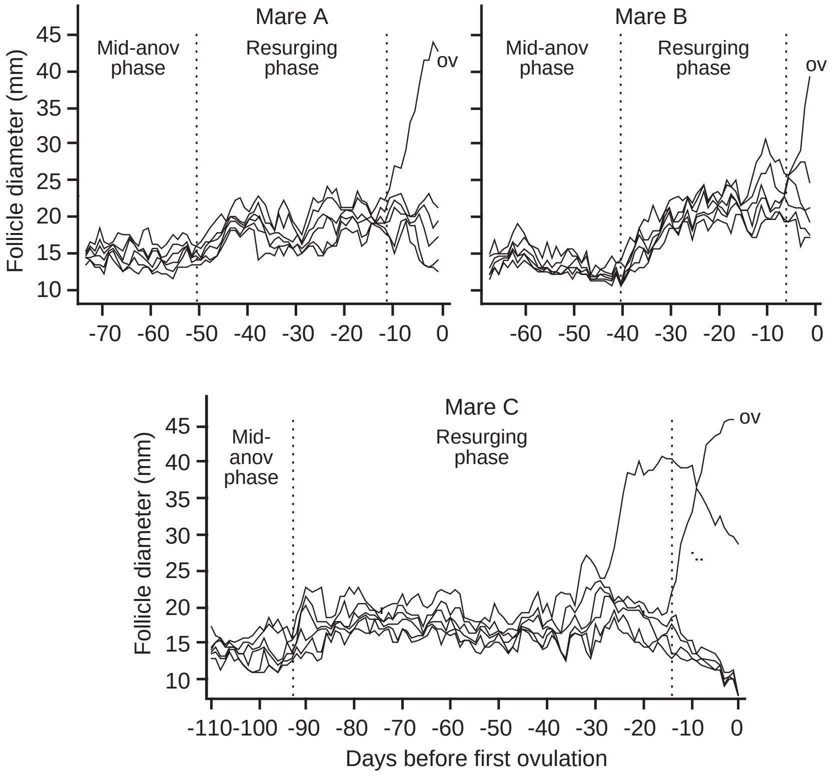

Figure 2. Diameters of the six largest follicles in three ponies. Diameters are for each day without regard to day-to-day identity. Note the apparent wave-like patterns of minor waves, including a consistent prominent minor wave at the onset of the resurging phase. The resurging phase includes a major anovulatory wave preceding the ovulatory wave in Mares B and C. The dominant anovulatory and ovulatory follicles originate from a wave of follicles. ov = ovulation. Adapted from Donadeu and Ginther, 2002.

Figure 2. OJ Ginther-1

However, this is not an appropriate term because the follicles are active throughout the year, although at widely different maximum diameters. In this regard, histologic study (Driancourt *et al*., However, this is not an appropriate term because the the year, although at widely different maximum diameters. In this regard, histologic study (Driancourt *et al*., 1983) measured the extent of division of the granulosa cells (mitotic index) in January. All aspects of folliculogenesis were present except for the development of follicles > 15 mm. A recommended term is mid-anovulatory phase (Donadeu and Ginther, 2002). Recent study has statistically identified both the emergence of minor follicular waves (mean diameter of largest follicle, 16 mm) and FSH surges (mean number, 0.8/10 days) during the mid-anovulatory phase (Fig. 2; Donadeu and Ginther, 2002). Ablation of all follicles > 5 mm during the mid-anovulatory period resulted in an immediate surge in FSH and growth of a minor follicular wave; the largest follicle reached a mean of 17.0 mm (Donadeu and Ginther, 2003a). In this regard, parturition during the last portion of the anovulatory season (January–March) stimulated an FSH surge and transient follicle growth, but did not stimulate early onset of the ovulatory season (Ginther *et al*., 1994). The decline in FSH concentrations after the peak of the post-ablation surge was associated with an increase in inhibin.

B2. Resurging phase

Diameter of the largest follicle, gradually increases as daylength increases (Ginther, 1979; Carnevale *et al*., 1997) and is a convenient indicator of the reproductive status of the mare during the last half of the anovulatory season. The gradual increase begins at the end of the mid-anovulatory phase and results from averaging diameters for a group of mares on the basis of calendar days. However, in individuals, a distinct increase in follicle activity or a follicular wave terminates the mid-anovulatory phase and is followed by a resurging phase (Donadeu and Ginther, 2002). This delineating wave occurs on various calendar days among individuals and therefore is obscured in the means for days. The diameter of the largest follicles increases from a mean of about 16 mm to a mean of at least 21 mm for the delineating wave and subsequent waves, resulting in an approximate plateau in maximum diameters. In some mares, the later portion of the resurging phase or the entire phase consists of a period in which the largest follicle of each sequential wave approaches or is similar to the diameter of a dominant follicle but does not ovulate (Section B3).

The resurging phase, including its relationship to the mid-anovulatory phase has been studied in follicle-intact ponies (Donadeu and Ginther, 2002) and in ponies with follicles ablated to initiate a new wave (Donadeu and Ginther, 2003a). The resurging phase began on a mean of 52 days before ovulation, ranging from 31–93 days. The six largest follicles for each day are shown for three mares (Fig. 2), and daily means for follicle end points for 34 days before and after the beginning of the resurging phase also are shown (Fig. 3). Two or three minor follicular waves per mare were detected statistically in all mares during the resurging phase, including the wave that initiated the phase. Inspection of profiles of the six largest follicles suggested that several additional minor waves were present in each mare but were not detectable statistically because of the intermingling of regressing and growing follicles (Fig. 2). The largest follicle of the minor waves during the resurging phase usually was ≥ 21 mm.

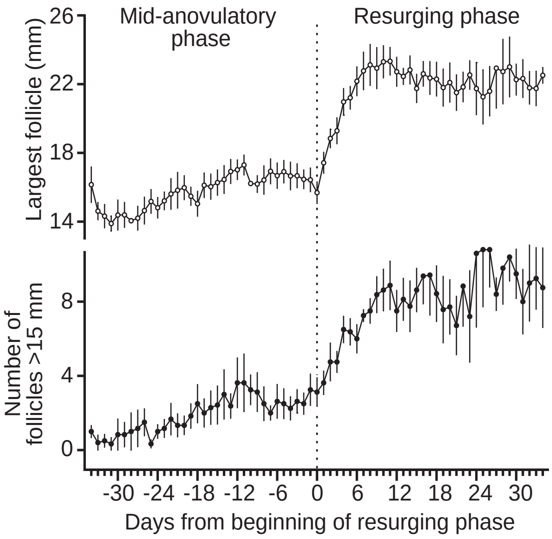

Figure 3. Mean ± SEM for diameters of the largest follicle and number of follicles for 34 days before and after the beginning of the follicular wave that demarks the end of the mid-anovulatory phase and beginning of the resurging phase. Adapted from Donadeu and Ginther, 2002.

One to five FSH surges were identified during the resurging phase. Following ablation of all follicles ≥ 5 mm, the postablation FSH surge and associated LH concentrations were not altered between phases, but the number of 13-mm follicles and mean diameter of the largest follicle were greater when ablation was done during the resurging phase (11.5 follicles and 21.3 mm) than when done during the mid-anovulatory phase (3.2 follicles and 17.0 mm). A difference in the profiles of FSH surges therefore did not account for the greater follicle response at the beginning of the resurging phase. The declining portion of FSH surges during the resurging phase as well as during the mid-anovulatory phase is attributable to the production of inhibin (probably inhibin-A; Watson *et al*., 2002a) by the growing follicles, rather than estradiol as determined from the studies with follicles intact and follicles ablated. In the latter portion of the resurging phase, the increase in circulating inhibin from the growth of follicles to > 21 mm counteracts the stimulating effects mediated by season, noted above, and therefore the FSH surges are unaltered. There was a slight

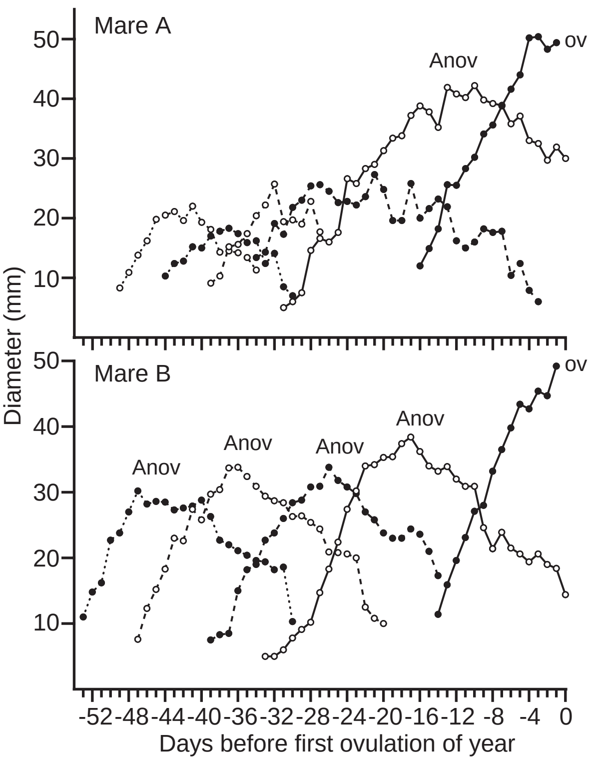

Figure 4. Largest follicle of waves during the resurging phase after omitting other follicles. Follicle data for earlier in the anovulatory season were not available. In Mare A, the known resurging phase consisted of four minor waves and one major anovulatory wave (Anov). In Mare B, the known resurging phase consisted of a period of four major anovulatory waves, with a largest follicle of ≥ 30 mm. ov = ovulation.

but significant mean increase in LH over the mid-anovulatory and resurging phases. It is not known if this LH increase accounted for the difference in follicle response; there was no abrupt change in LH between the two phases. Furthermore, LH concentrations associated with wave emergence were not different between the mid-anovulatory phase and beginning of the resurging phase in follicle-intact and follicle-ablated mares. An increase in LH pulse frequency occurs during the last 2 months of the anovulatory season (Fitzgerald *et al.*, 1987), but it is not known if frequency changes would have overlapped the onset of the resurging phase. Elucidation of the reason for the abrupt beginning of the resurging phase in all mares but at widely varying times among mares will require focused study.

B3. Spring period of major anovulatory waves

The later portion or all of the resurging phase may consist of a period of waves in which the largest follicle reaches a diameter much larger than 21 mm. Most striking and familiar to breeding farm veterinarians is the development of major anovulatory waves during the resurging phase in which the largest follicle attains the diameter of a dominant follicle (≥ 28 mm; Figs. 1, 2, 4). Consideration of the ovulatory potential of 35-mm follicles during the period of major anovulatory waves is important on breeding farms because at that time a breeding decision may be needed. In some mares, a period of major anovulatory waves does not occur and in others the period is of various lengths depending on the number of successive anovulatory waves (Fig. 4). To further illustrate the extremes in individual variation, a period of major anovulatory waves may encompass the entire anovulatory period or may be absent. In this regard, ovulation throughout the year has been stated to involve 20–25% of mares (Hughes *et al.*, 1980); other studies indicate an incidence of 2 of 16 (11%) light horses (King et al., 1993) and 5 of 19 (26%) Brazilian Bretons (unpublished). Good body condition and high percentage of body fat favor ovulation throughout the year (Section A4). The incidence of major anovulatory waves is greatly influenced by breed and type. For example, published reports on the equivalent of periods of major anovulatory waves cite incidences as high as 15 of 15 horses (Ginther, 1990) and as few as 3 of 8 ponies (Donadeu and Ginther, 2002). In a study (unpublished) of 16 small (390 to 550 kg), draft-type, crossbred Breton mares in Brazil (latitude, 21º S), 10 of 16 mares had a period of major anovulatory waves. The number of waves per mare was one (five mares), two or three (two mares), and four or five (three mares).

Owing primarily to the practical importance of the period of major anovulatory waves, many studies of the underlying mechanisms have been done. The wave-stimulating FSH surge is similar for minor waves, major anovulatory waves, and ovulatory waves (Donadeu and Ginther, 2002; 2003a; 2003b). Intrafollicular production of estradiol by dominant-sized follicles, as indicated by tissue culture and follicular-fluid assays, is minimal during the equivalent of early resurgence and increases during late resurgence (Watson *et al.*, 2002b; Davis and Sharp, 1991). However, circulating estradiol concentrations are very low during the anovulatory season (Donadeu and Ginther, 2002) and during the last anovulatory wave (Donadeu and Ginther, 2003b); a detectable increase does not occur until the end of the anovulatory season during the development of the first ovulatory wave (Donadeu and Ginther, 2003b; Watson *et al.*, 2002b; Peltier *et al.*, 1996; Seamans and Sharp, 1982). Circulating LH concentrations are low during the anovulatory season and increase before the first ovulation (Donadeu andGinther, 2002; Donadeu and Ginther, 2003b; Freedman *et al.*, 1979b; Fitzgerald *et al.*, 1987). Administration of estradiol during transition increases the production of LH (Sharp *et al.*, 1991; 2001). However, an increase in LH at the end of the anovulatory season occurs even in the absence of the ovaries (Freedman *et al.*, 1979b; Peltier *et al.*, 1996; Fitzgerald *et al.*, l987; Affleck *et al.*, 1991), and the initial portion of the LH surge before the first ovulation is attributable to seasonal effects rather than to estradiol (Donadeu and Ginther, 2003b). These results indicate that the LH surge associated with the first ovulation of the year, as well as with later ovulations (Section C2), is initially a function of season followed by a positive effect of estradiol (Ginther, 1992).

Surgically collected dominant-sized follicles, presumed to be seasonally anovulatory, had lower follicular-fluid concentrations of estradiol, progesterone, and inhibin (Watson et al., 2002b); lower expression of mRNAs for steroidogenic enzymes (Watson et al., 2004); less vascularization (Watson and Al-zi'abi, 2002); and lower vascular endothelial growth factor (VEGF) protein expression and poor development of the theca and granulosa layers (Watson and Al-zi'abi, 2002).

In a recent study in ponies (Acosta *et al.*, 2004), dominant-sized anovulatory follicles were distinguished from dominant ovulatory follicles by daily color Doppler determinations of blood flow area, and comparisons were made between mares with future dominant-sized (≥ 30 mm) anovulatory versus ovulatory follicles. In the group, with a future dominant-sized anovulatory follicle, LH concentrations were reduced, beginning when the follicle was about 15 mm or about an average of 6 days before the follicle was 30 mm (Fig. 5). Concentrations of FSH and number of FSH surges were greater in the anovulatory group, beginning when the follicle was about 20 mm and encompassing a 30-mm follicle. Blood flow area was less for dominant-sized anovulatory follicles than for ovulatory follicles by the time blood flow determinations began at 25 mm (Fig. 5). In a separate group of sampled mares, follicular-fluid concentrations in 30-mm anovulatory follicles were lower than in 30-mm ovulatory follicles for estradiol, free IGF-1, inhibin-A, and VEGF. Concentrations of free insulin-like growth factor-1 (IGF-1) were only about 35% of the concentrations in ovulatory follicles of similar size. The early LH deficiency accounted for the reduced follicular-fluid concentrations of estradiol, inhibin-A, free IGF-1, and VEGF (reviewed in Ginther *et al.*, 2003a). Estradiol and inhibin-A are FSH suppressors. Their limited production accounted for the increased plasma FSH, beginning at 20 mm or when the wave-stimulating FSH surge would otherwise have continued to decline. The low concentrations of free IGF-1 and estradiol can be expected to have resulted in retarded follicle development and the low concentrations of VEGF to have resulted in retarded vascular development. A working hypothesis is that future dominant-sized anovulatory follicles develop during an LH-deficient environment, resulting in generalized dysfunction of the follicle during its expansion to a future dominant-sized anovulatory follicle (Fig. 6).

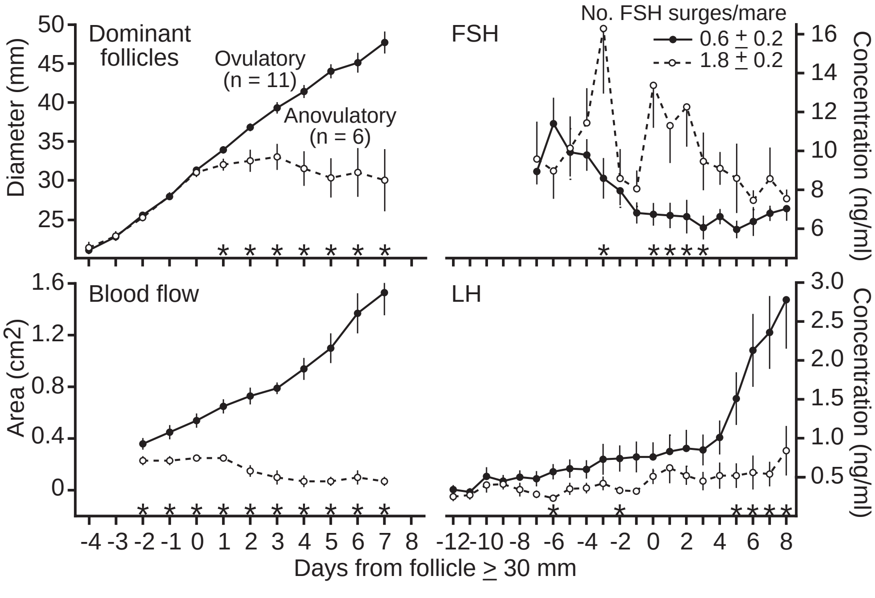

Figure 5. Mean ± SEM follicle diameters, blood flow areas of the follicle wall, and plasma gonadotropin concentrations for mares with dominant (30-mm) anovulatory and ovulatory follicles. The asterisks indicate days when the difference between follicle groups was significant. The indicated number of FSH surges/mare was greater for the anovulatory group than for the ovulatory group. From Acosta *et al.*, 2004.

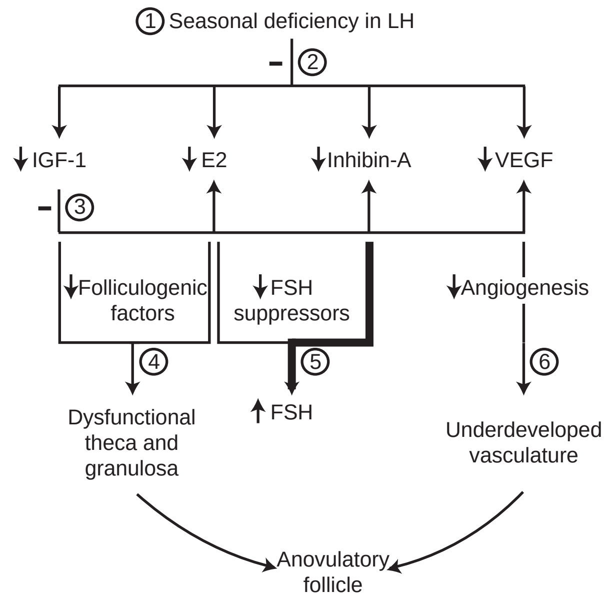

Figure 6. Schematic hypothesized mechanism underlying the development of dominant-sized (≥ 30 mm) anovulatory follicles. 1) A deficiency of circulating LH occur when the future dominant-sized anovulatory follicle is only about 15 mm. 2) The LH deficiency results in low concentrations of follicular-fluid factors in the 30-mm follicle. 3) The low concentrations of free IGF-1 contributes to the low concentrations of other factors. 4–6) The low concentrations of estradiol and inhibin account for greater circulatory FSH concentrations. Low intrafollicular concentrations of growth factors and estradiol leads to underdeveloped theca and granulosa layers, and the shortage of VEGF lead to an underdeveloped vasculature.

The growth of a dominant ovulatory follicle is associated with a follicular wave and a deviation process, wherein the developing dominant follicle continues to grow and the subordinate follicles begin to regress. In a study in ponies (Donadeu and Ginther, 2003b), it was concluded that follicles of many major anovulatory waves also display an apparent deviation process. The diameter of the two largest follicles at the beginning of apparent deviation was similar among the last major anovulatory wave and the first two ovulatory waves. Two days later, the diameter of the largest follicle was smaller in the anovulatory waves. Circulating estradiol and LH did not increase during the anovulatory wave. These results are, in retrospect, compatible with the results of the color Doppler study, discussed above. During the growth of follicles from 20 to 30 mm in the color Doppler study, the dominant-sized anovulatory follicles expanded at the same rate as ovulatory follicles. Even after 30 mm was reached, the anovulatory follicles that continued to expand did so at a rate similar to the diameter increase during growth of the ovulatory follicles (Fig. 7). Apparently, expansion occurred at a given rate without regard to the extent of the diminished plasma LH, aberrant vascularity, and diminished concentrations of the follicular-fluid factors. These observations indicate that the growth or expansion rate of a future dominant-sized anovulatory follicle is not, in itself, an indicator of the status or health of the follicle. For this reason, deviation observed by ultrasonography during major anovulatory waves may not represent true deviation.

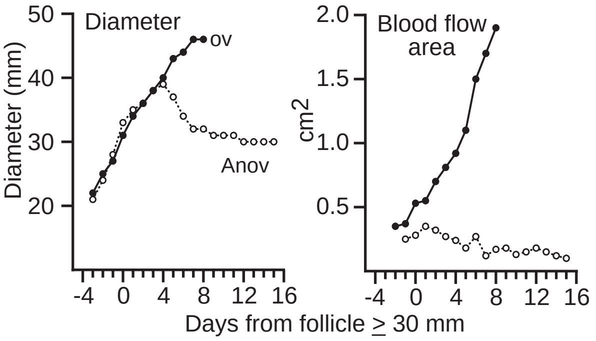

Figure 7. Example of expansion of an anovulatory follicle to 38 mm, despite the greatly reduced blood-flow area of the follicle wall and the associated early deficiencies depicted in Figure 6.

Administration of GnRH (Johnson, 1987; Ginther and Bergfelt, 1990) to horse mares during the anovulatory season stimulated growth and ovulation of follicles. The treatments not only stimulated growth of small follicles but also stimulated seasonal anovulatory follicles that were ≥ 25 mm. Based on the results of the color Doppler study, the stimulation of the larger follicles may have represented rescue of follicles that were already impaired, but this assumption requires specific study. The studies on the use of GnRH preparations for stimulation of growth of anovulatory follicles were done in horses, whereas the studies on massive dysfunction of dominant-sized anovulatory follicles were done in ponies. There is a need to resolve whether dysfunction of dominant-sized anovulatory follicles is similar between ponies and horses and whether dysfunctional follicles can be rescued.

C. Seasonal Effects on Follicle Dynamics during the Interovulatory Interval

C1. Follicle responses

The effects of daylength on equine follicle dynamics and the estrous cycle do not end and begin at the first and last ovulation of the year but rather continue throughout the year (reviewed in Ginther, 1992). Estrus associated with the first ovulation can be quitelong in early spring (e.g., 25 days), decrease progressively by late spring (e.g., 7 days), become shorter and stabilized during the summer, and increase progressively by the end of autumn (e.g., 9 days). Prolonged first estrus of the ovulatory season is more closely related to influences associated with month (daylength) than to its position as first estrus. These behavioral data seem consistent with a report that length of the follicular phase (progesterone < 1 ng/ml) was shorter in the summer (7.8 days) than in the autumn (9.8 days; Weedman et al., 1993). Lengthened follicular phase and larger follicle size in the autumn were associated with lower circulating estradiol. Follicular activity during an estrous cycle is greater during the first half of the ovulatory season than during the second half, as indicated by greater diameter of the preovulatory follicle (Ginther and Pierson, 1989), more follicles > 20 mm, greater diameter of the largest follicle (Fig. 8; Pierson and Ginther, 1987), and greater incidence of minor and major anovulatory follicular waves (Ginther, 1993).

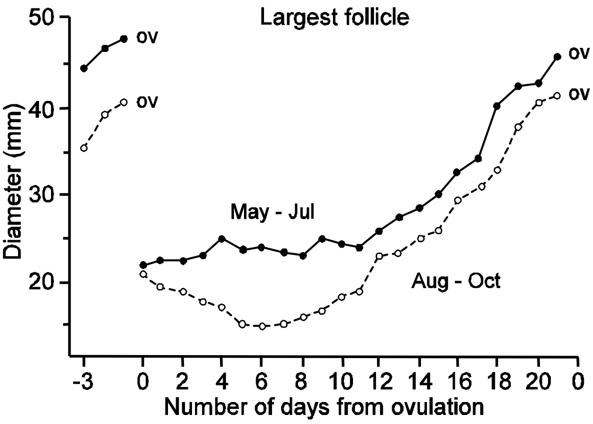

Figure 8. Mean diameter of the largest follicle on each day of an interovulatory interval during the first months (May-Jul) and last months (Aug-Oct) of the ovulatory season. The follicle was significantly larger during the first 3 months. Adapted from Pierson and Ginther, 1987.

Breed has surprisingly little effect on follicle diameters, despite the great differences in body size, extending from pony to draft mares. In a comparison of ponies and Quarter horses by a single operator, no differences were found in the diameter or growth rate of the tracked ovulatory follicle, extending from the expected beginning of deviation to the day before ovulation (Bergfelt and Ginther, 1996). Limited data suggest that the preovulatory follicle is about 5 mm smaller in Miniatures and 10 mm larger in Clydesdales iameter of the follicle on the day before ovulation were significantly greater in mares with a high score than in Quarter Horses (Ginther, 1995). The of factors other than daylength and breed on follicle diameters during the ovulatory season has received limited attention. However, in a recent study in our laboratories (unpublished), the number of follicles > 10 mm andfor body condition (10.0 ± 0.4 and 50.2 ± 0.9 mm) than in mares with a low score (7.4 ±0.3 and 44.6 ± 1.0 mm).

C2. Endocrinology of seasonal effects

The effect of seasonal influences on the ovaries in regulation of equine LH and FSH concentrations during the ovulatory season is an exquisite example of the interplay of environmental and internal controls. Only brief consideration can be given to the subject in this review. The following comments are based on a comprehensive review (Ginther, 1992). During the first months of the ovulatory season, LH concentrations increase progressively in ovariectomized mares, and the maximum LH concentrations in successive ovulatory surges in intact mares also increase progressively. During the summer months, the LH concentrations are continually high in ovariectomized mares but intermediate between the concentrations of diestrus and estrus in ovarian-intact mares (Fig. 9). That is, the influence of the ovaries on seasonally controlled LH concentrations is negative during diestrus and positive during estrus, reflecting changes in the progesterone:estrogen ratio. Concentrations of FSH are also high during the ovulatory season in ovariectomized mares. In ovarian-intact mares, ovarian factors continually suppress the positive seasonal influence as shown (Fig. 10); however, the negative impact of ovarian products (estrogen and inhibin) is greater during estrus than during diestrus. In this regard, the concentrations in ovariectomized mares have not been compared to the concentration at the peak of an FSH surge. Circulating concentrations of progesterone and estradiol decreased during the last few estrous cycles of the ovulatory season, and LH concentrations decreased about 50% during the last ovulatory surge in 6 of 8 mares (Nequin et al., 2000b).

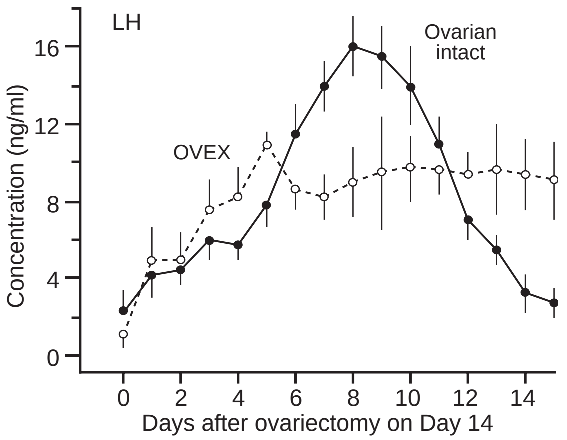

Figure 9. Mean ± SEM concentrations of LH following ovariectomy (OVEX) 14 days after ovulation. Concentrations were greater during the peak of the periovulatory LH surge in the ovarian-intact group than on the corresponding days in the ovariectomized group. Data courtesy of J. E. Fay and R. H. Douglas, University of Kentucky.

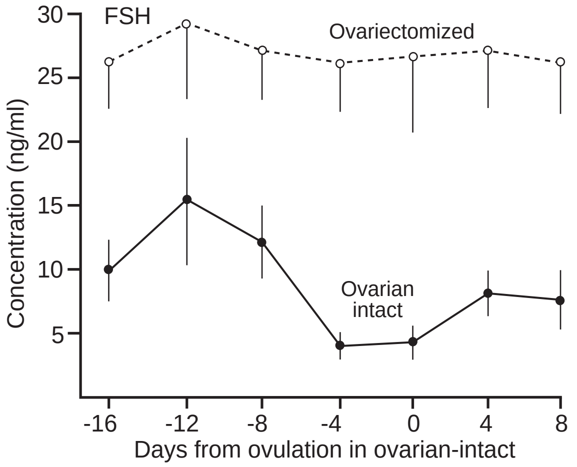

Figure 10. Mean ± SEM concentrations of FSH in ovariectomized and ovarian-intact mares during the middle of ovulatory season. Data for intact mares were normalized according to day of ovulation, and data for the ovariectomized mares were for the corresponding calendar days. Concentrations of FSH were significantly greater for each day in the ovariectomized group than in the intact group. Adapted from Freedman *et al.*, 1979a.

D. Follicle Dynamics during the Transition from Ovulatory to Anovulatory Seasons

The end of the ovulatory season may, by definition, be considered an abrupt event marked by the last ovulation of the year. However, most mares make an initial but futile attempt to maintain cyclic follicle dynamics with the associated behavioral and hormonal changes. The transition from the last ovulation of the ovulatory season to the beginning of the mid-anovulatory phase of the anovulatory season (Section B1) may be called the receding phase (Ginther, 1992). The initial portion of the receding phase may contain a transient period of major anovulatory waves similar to the concluding period that often occurs at the end of the resurging phase.

D1. Autumn period of major anovulatory waves

A period of major anovulatory waves occurred after the last ovulation of the year in two of six ponies (Ginther *et al.*, 2003b) and in 9 of 14 Bretons (small draft horses; unpublished). The incidence in Bretons was similar to the number of mares with a period of major anovulatory waves during the following spring (10/16; Section B3). However, in mares that had major anovulatory waves, the number per mare was less ($P < 0.04$) in the autumn (1.2 ± 0.1) than in the spring (2.3 ± 0.4). This is apparently the only available direct comparison between the receding and resurging phases on the characteristics of the period of major anovulatory waves. In a study in light horses, a period of major anovulatory waves occurred in 6 of 8 mares (Fig. 11; personal communication, E.M. Carnevale).

D2. Persistent corpus luteum

Persistent corpus luteum after the last ovulation of the year has been reported (Hughes *et al.*, 1972) and occurred in about 25% of light mares on the basis of ultrasonography and circulating progesterone concentrations of > 1 ng/ml for ≥ 30 days (Weedman *et al.*, 1993; King *et al.*, 1993; Nequin *et al.*, 2000b). This topic is germane to this review because the persistent corpora lutea were associated with altered follicle dynamics. The corpus luteum regressed at the expected time in the follicle profiles for the eight depicted mares (Fig. 11). However, four additional mares had luteal persistence (Nequin *et al.*, 2000b). Two of the four mares did not show the increased follicular activity at approximately 22 days after ovulation (personal communication, E. M. Carnevale). The other two mares had a prolonged period of six or greater than seven major anovulatory waves. The corpus luteum regressed during the last major anovulatory wave. The ovulation that led to luteal persistence in the two mares with the periods of major anovulatory waves occurred earlier in the year than for the last ovulation in the other 10 mares.

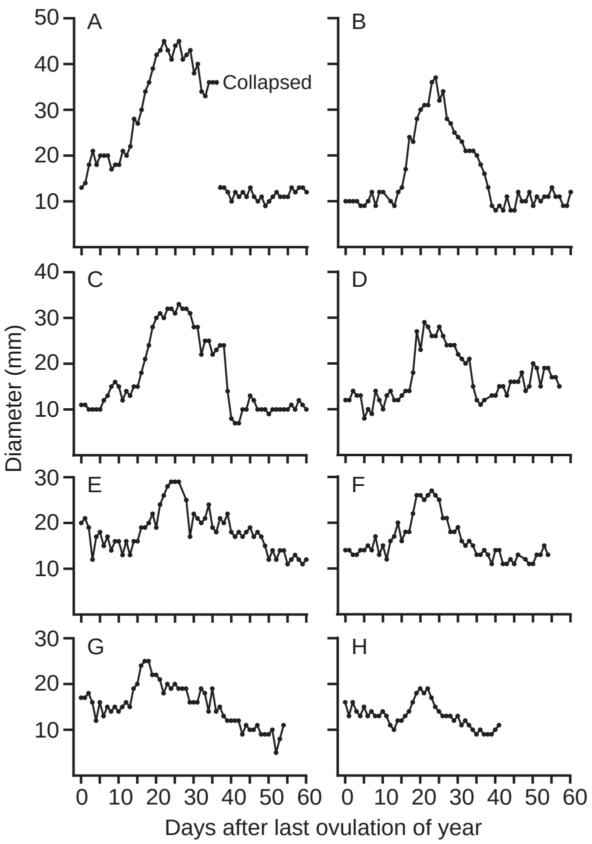

Fig. 11. Changing diameters of the largest follicle during the 2 months after the last ovulation of the year in eight mares. The largest follicle was measured each day without regard to day-to-day identity. Other follicles of the waves represented by the largest follicle have been removed. All eight mares had increased follicle activity on the approximate days that ovulation would have occurred during the ovulatory season. The increased diameter of the largest follicle is characteristic of a major anovulatory wave in mares A–E and a minor wave in mares F–H. Four additional mares in this set (not shown) had a persistent corpus luteum with altered follicle activity (see text). Data courtesy of E. M. Carnevale, Colorado State University.

D3. Receding phase

In the initial study in ponies (Snyder *et al.*, 1979), follicular activity for the two weeks following the last ovulation of the year was similar to that during the previous diestrus. At a time equivalent to a follicular phase of the estrous cycle, activity nevertheless increased. Eleven of 14 mares manifested increased follicle activity. It was concluded that most of the mares initially attempted to develop an ovulatory follicle but gradually receded into the anovulatory season. Similarly, all of the eight mares shown in Fig. 11 had inreased follicle activity at the expected time that ovulation would have occurred.

D4. Endocrinology of the receding phase

Plasma concentrations of FSH and number of follicles were not significantly different between a 31-day period after the second-last ovulation and an equivalent period after the last ovulation (Snyder *et al.*, 1979). It was concluded that follicle numbers and FSH concentrations likely were not involved in the termination of the ovulatory season. In more recent studies, FSH concentrations apparently remained substantially unchanged until well into the anovulatory season (Nequin *et al.*, 2000a; Irvine *et al.*, 2000). In contrast, LH concentrations decrease during the receding phase. An inadequate or absent preovulatory surge of LH and final growth of the preovulatory follicle accounted for the absence of ovulation at the onset of the anovulatory season (Snyder *et al.*, 1979; Irvine *et al.*, 2000; Nequin *et al.*, 2000a).

In a recent study in ponies (Ginther *et al.*, 2003b), follicle growth and circulating hormone concentrations were compared between an interovulatory interval and the first 60 days of the anovulatory season, using ablation of follicles ≥ 6 mm 10 days after an ovulation that initiated an interovulatory interval, ablation 10 days after the last ovulation of the year, and ablation 60 days after the last ovulation of the year. The growth of the largest follicle was similar for the groups with ablation during the interovulatory interval and 10 days after the last ovulation until the follicle was about 20 mm; the means then decreased in the latter group (Fig. 12). The percentage of mares with major postablation waves for the three groups was 100%, 33%, and 0%, respectively, and the percentage with minor waves was 0%, 67%, and 100%. Minor waves also were detected in 83% of anovulatory mares between days 20 and 60 after the last ovulation. Results confirmed that a period of major anovulatory waves developed in some mares early in the anovulatory season but also showed that minor waves developed throughout the first two months. It can be concluded that minor waves develop throughout the anovulatory season (Fig. 1). Growth of the largest follicle after ablation 10 days after the last ovulation of the year was similar for major waves and minor waves but only until the beginning of deviation in the major waves. Post-ablation FSH surges were similar for all groups (Fig. 12) and for surges statistically detected during days 20–60. It was concluded that FSH surges with similar patterns occur during early diestrus and throughout the anovulatory season.

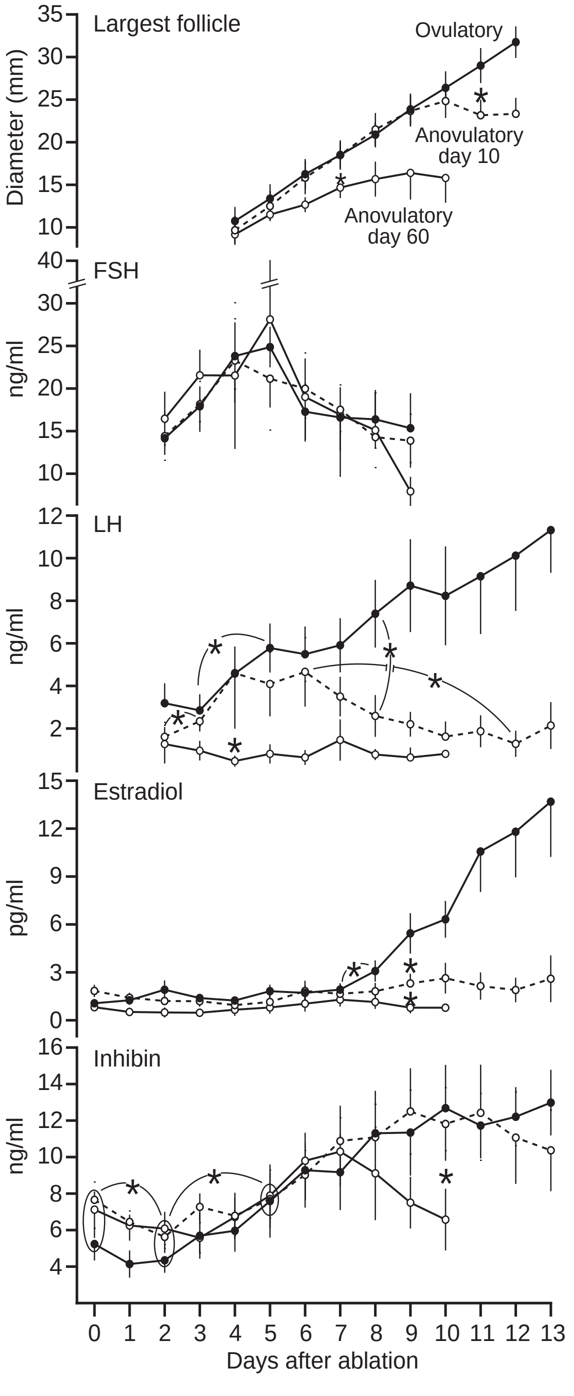

Figure 12. Mean ± SEM diameters of largest follicle and concentrations of circulating hormones in mares in which follicles of ≥ 6 mm in diameter were ablated at day 10 after ovulation in the ovulatory and anovulatory-10 groups and at day 60 after ovulation in the anovulatory-60 group. Asterisks indicate a significant difference between the indicated groups within a day or between days within a group.

Detectable follicular waves frequently do not occur in association with these surges, and waves that do occur may differ profoundly in the extent of follicle development. During both ovulatory and anovulatory seasons, approximately 0.5 FSH pulses occurred per day, as determined by sampling every 4 or 6 hours (Irvine *et al.*, 1998; 2000). These pulses presumably are superimposed on the FSH surges that were based on samples obtained daily (Bergfelt and Ginther, 1993**;** Donadeu and Ginther, 2002**;** 2003a). The irregularities in the surges can be attributed, at least in part, to daily sampling at various times during a pulse.

Concentrations of LH were greater in association with major waves than with minor waves. A minor wave developed into a major wave when the largest follicle reached a diameter characteristic of the beginning of deviation (about 22.5 mm) in the presence of an adequate LH stimulus for continued growth of a dominant follicle. These finding are consistent with the concept that adequate circulating LH is needed for growth of the dominant follicle after the beginning of deviation during an ovulatory wave in mares (Ginther *et al.*, 2003a). Concentrations of LH began to increase during the major waves, whether ovulatory or anovulatory, several days before a detected increase in estradiol. This initial LH increase may have been a response to a decrease in progesterone (Gastal *et al.*, 1997). On a temporal basis, the subsequent mean plateau of LH may have reflected a negative effect of estradiol, based on the report that LH increases when the largest follicle is ablated at the expected beginning of deviation (Gastal *et al.*, 1999). The increase in LH after the plateau during the ovulatory major waves but not during anovulatory major waves occurred as the estradiol concentrations continued to increase. This portion of the LH/estradiol relationship is consistent with a positive feedback effect of estradiol. The intricacies of the apparent changes in the reciprocal relationships between estradiol and LH will require further study.

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