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Follicle and gonadotropin relationships during the beginning of the anovulatory season in mares

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Abstract

The initial portion of the anovulatory season of mares consists of a follicle-receding phase followed by a mid-anovulatory phase (follicles ≤ 20 mm). Follicle dynamics were studied in a control group (n = 6) and a follicle-ablation group (n = 8), using the last ovulatory period of the season and three 20-day anovulatory periods (Days 11–30, 31–50, and 61–80; last ovulation of the season = Day 0). In the ablation group, all follicles ≥ 6 mm were ablated 10 days after the second last ovulation of the year and on Days 10, 30, and 60. The diversity in follicle dynamics within each group was considerable, including a follicle-receding phase that terminated in a mid-anovulatory phase (10 mares), a major anovulatory wave (dominant follicle ≥ 30 mm) during Days 11-30 (eight mares), multiple major anovulatory waves (two mares), and a short anovulatory season (< 80 days; two mares). Four additional mares were excluded from the study, owing to continuous ovulatory intervals throughout the 80 days. In each of 11 control and follicle-ablated mares that did not have a short anovulatory season or multiple major anovulatory waves, the diameter of the largest follicle was greater during Days 11–30 than during Days 31–50. Growth rate of the largest follicle for 2–10 days after ablation was greater ($P < 0.05$) during the last ovulatory period (2.3 ± 0.2 mm/day) or during Days 11–30 (1.9 ± 0.2 mm/day) than during Days 31–50 (0.6 ± 0.2 mm/day) or 61–80 (0.8 ± 0.2 mm/day). Concentrations of FSH and LH increased ($P <0.05$) following ablation before the ovulatory period and on Day 10. The LH concentrations further increased during the ovulatory period a few days before ovulation, but decreased during a corresponding time of the first anovulatory period (Days 11–30). Concentrations of gonadotropins did not increase following ablation on Days 30 or 60. The number of periods with a post-ablation statistically identified FSH surge was different ($P < 0.02$) among the ovulatory period (five of five), Days 11–30 (eight of eight), Days 31–50 (three of eight) and Days 61–80 (three of seven). Results indicated: 1) diversity in follicle dynamics among mares was considerable during the first 80 days of the anovulatory season in association with diversity in FSH and LH concentrations and 2) an increase in mean diameter of the largest follicle occurred following ablation on Days 30 and 60, despite the absence of a detected FSH increase. The hypothesis that concentrations of LH during the follicle-receding phase are greater in mares with major anovulatory waves than with minor waves was supported.

Keywords: Anovulatory season, follicles, gonadotropins, mares

Introduction

A seasonal reproductive pattern occurs in mares; the monthly incidence of ovulation is minimal or absent during winter, transitionally increasing during spring, maximal during summer, and transitionally decreasing during autumn (Ginther *et al*., 2004a). The low incidence of ovulation for the winter months reflects anovulatory seasons of different lengths among individual mares, combined with uninterrupted ovulations throughout the year by some mares. Although not critically studied, the monthly distribution of ovulations seems to apply for all latitudes, even for those of the Torrid Zone. In a slaughterhouse study done in southern Mexico (15ºN–22ºN), a definitive seasonal ovulatory pattern was found; about 10% of the 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). Although the spring transition has been studied extensively (Nagy *et al*., 2000; Ginther *et al*., 2004a), only a few critical studies have been done during the autumn transition. The first portion of the anovulatory season has been called the follicle-receding phase (Ginther *et al*., 2004a). The receding phase ends and a mid-anovulatory phase begins after the winter equinox. During the mid-anovulatory phase, follicles do not grow to more than 20 mm in diameter (mean, 16 mm; Donadeu and Ginther, 2002).

Follicle diameter deviation begins during an ovulatory follicular wave when the largest follicle reaches a mean of 22.5 mm (Gastal *et al*., 1997; 1999). Deviation is characterized by continued growth of the

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developing dominant follicle and diminished growth and regression of subordinate follicles (Ginther *et al*., 2004b). Dominant and subordinate follicles develop during both the ovulatory and anovulatory seasons (Watson *et al*., 2002**;** Donadeu and Ginther, 2003a). A major anovulatory follicular wave is defined by a largest follicle attaining the diameter of a dominant follicle (≥ 30 mm), and has been reported to occur during early diestrus before the ovulatory follicular wave in about 25% of mares (Ginther, 1993; Ginther *et al.,* 2004b). The remaining nonovulatory waves (no deviation and no dominant-sized largest follicle) are defined as minor waves.

In the initial report on the follicle-receding phase in ponies (Snyder *et al*., 1979), follicle development for the two weeks following the last ovulation of the year was similar to that during the previous diestrus. Follicle development increased in most mares during a time equivalent to the follicular phase of an estrous cycle. However, follicle development throughout the remainder of the follicle-receding phase has not been characterized. In a recent study (Ginther *et al*., 2003), all follicles ≥ 6 mm were ablated at various times in the autumn during the first two months of the anovulatory season (same as the two months after the last ovulation of the ovulatory season); minor follicular waves developed following ablation, except that a major anovulatory wave developed in two of six pony mares near the expected time of an ovulation if the ovulatory season had continued.

Plasma concentrations of FSH and number of follicles were not significantly different between a 31-day period after the second-last ovulation of the year 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. Other studies have indicated that FSH concentrations apparently remain unchanged until well into the anovulatory season (Irvine *et al*., 2000; Nequin *et al*., 2000). In the autumn follicle-ablation study (Ginther *et al*., 2003), FSH surges following follicle ablation during the follicle-receding phase were similar regardless of whether the associated follicular waves were major or minor. In addition, similar FSH surges were statistically detected during the intervening periods between ablations, often in the absence of associated follicular waves. An inadequate or absent preovulatory surge of LH and final growth of the preovulatory follicle has been reported to account for the absence of ovulation at the onset of the anovulatory season (Snyder *et al*., 1979**;** Irvine *et al*., 2000; Nequin *et al*., 2000). In the autumn follicle-ablation study (Ginther *et al*., 2003), the preliminary observation was made that concentrations of LH were greater in association with major anovulatory waves than with minor waves. Apparently, a minor wave developed into a major wave when the largest follicle reached a diameter characteristic of the beginning of deviation in the presence of an adequate LH stimulus for continued growth of a dominant follicle. Concentrations of LH began to increase during the major waves several days before an increase in estradiol.

The present study investigated the changes in diameter of the largest follicle during successive waves of the follicle-receding phase and into the mid-anovulatory phase in follicle-intact mares and follicle-ablated mares. Post-ablation changes in circulating FSH and LH concentrations were studied in association with differences in follicle response. Based on a preliminary observation (Ginther *et al*., 2003), the hypothesis was tested that concentrations of LH during the follicle-receding phase are greater in association with major anovulatory waves than with minor waves.

Materials and Methods

Animals and groups

Non-lactating, small draft-type, crossbred Breton mares between 3–13 years of age and weighing 390–550 kg were used. Mares were kept under natural daylight in the Southern Hemisphere (latitude, 21ºS). Feed consisted of pasture and stall-fed napier grass, with free access to water and mineralized salt. The score for body condition for all mares was high throughout the experiment (score ≥ 5.5; Henneke *et al*., 1983).

Monitoring of the ovaries began in April toward the mean end of the ovulatory season (Ginther *et al*., 2004a); April is equivalent to October in the Northern Hemisphere (NH). Ovaries were scanned with an Aloka SSD-500V (Aloka, Wallingford, CT USA) equipped with a 5 MHz linear-array transrectal transducer. Mares were assigned randomly to a control group (n = 10) or an ablation group (n = 10); however, two mares with unsuitable temperaments were later removed from the control group. Four of 18 mares (22%) ovulated throughout the study and were not considered further, leaving six and eight mares in the control and ablation groups, respectively. Mares maintained their group assignment throughout the experiment. In the ablation group, all follicles ≥ 6 mm were ablated on designated days by transvaginal ultrasonography as described (Gastal *et al*., 1997). Follicles in the control group were not ablated.

Ultrasonography and end points

Ovaries were monitored daily by ultrasonography to determine, retrospectively, the day of the last ovulation of the season. Ten days after each ovulation daily follicle measurements and blood sampling began to assure that data were available from 10 days after the second-last ovulation to the last ovulation (last ovula-

tory period; Fig. 1). For follicle data, the diameters of the two largest follicles on each day were measured without regard to day-to-day identity. Mares that did not ovulate within 30 days after the previous ovulation were considered to have entered the anovulatory season (Gastal *et al*., 1999), thereby defining the last ovulation of the ovulatory season (Day 0; Fig. 1). Daily follicle data and blood samples were used for the retrospectively identified ovulatory period and for three 20-day anovulatory periods (Days 11–30, Days 31–50, Days 61–80). The same protocol was used for the ablation group, except that follicle ablation was done 10 days after each ovulation so that follicle data and blood samples were available for the last ovulatory period of the year. After the last ovulation (Day 0), ablations were done on Days 10, 30, and 60 (Fig. 1).

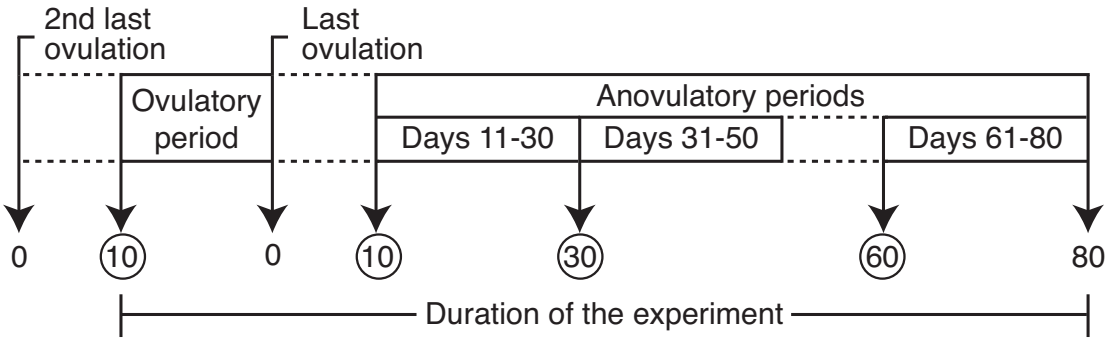

Figure 1. Schematic presentation of the experimental design. The design was similar for control and ablation groups, except for ablation of all follicles ≥ 6 mm on the encircled days in the ablation group.

The control group was used to determine whether follicle ablation affected the incidence of ovulation throughout the study (anovulatory season not detected) and the incidence of major anovulatory waves for each anovulatory period. The control group was not used for direct study of the interrelationships of follicles and hormones because of the inability to identify individual follicles from intermingling of growing and regressing follicles. The number of statistically identified FSH surges was compared among the four periods in the ablation group; one mare was excluded for Days 61–80 period because of an early beginning of a new ovulatory season. The growth rate of the largest follicle for 2–10 days after ablation was compared among periods. The post-ablation diameters of the largest follicle and concentrations of FSH, LH, and estradiol were analyzed among periods, with number of days truncated to the day the first mare ovulated during the ovulatory period. Plasma progesterone was assayed 10 and 20 days after both the second-last ovulation and last ovulation of the season in both groups. This was done to consider whether maintenance of the corpus luteum may have occurred in some individuals. Mares in the ablation group for Days 11–30 were subgrouped into those with a post-ablation ovulatory wave, a major anovulatory wave, and a minor wave. For these subgroups, diameter of the largest follicle and concentrations of FSH and LH were centered on the day the largest follicle was nearest to 22.5 mm (expected beginning of deviation; Gastal *et al*., 1999).

Blood samples and hormone assays

Jugular blood samples were collected into heparinized tubes and centrifuged (500 x g for 10 min), decanted, and stored (-20ºC) until assay. Daily plasma samples were assayed for FSH (Freedman *et al*., 1979**)** and LH (Whitmore *et al*., 1973) by radioimmunoassay as validated and modified (Donadeu and Ginther, 2002) in our laboratory. The intra- and interassay coefficients of variation (CV) and mean sensitivity, respectively, were 5.5%, 5.5%, and 0.8 ng/ml for FSH and 6.6%, 7.9%, and 0.2 ng/ml for LH. Progesterone concentrations in plasma samples were determined using a competitive ELISA that has been described previously (Rasmussen et al., 1996). The intra-assay and inter-assay CVs were 3.8% and 2.0%, respectively, and the sensitivity was 0.04 ng/ml. Plasma concentrations of estradiol were measured every 3 days by a double-antibody radioimmunoassay kit (Double Antibody Estradiol, Diagnostic Products Corporation, Los Angeles) after extracting the samples with ether, as described for mare samples (Gastal *et al*., 1999). Intra-assay CV was 19.5%, and the sensitivity was 0.08 pg/ml.

Statistical analyses

Sequential diameters of largest follicle and plasma concentrations of gonadotropins and estradiol were analyzed to determine effects of period or subgroup, day, and the interaction, using the SAS MIXED procedure with a repeated statement to account for the autocorrelation between sequential measurements (8.2 Version; SAS, Institute Inc., Cary, NC). When the interaction was significant, the main effects were not considered. If a significant effect of period or period-by-day interaction was detected, unpaired *t*-tests were used to locate differences in means between periods or subgroups. Single point data were analyzed by one-way or factorial ANOVA. Surges in FSH within a mare were identified by the coefficient of variation for the values composing the ascending and descending portions of the suspected surge; an accepted surge required a coefficient that was at least four times higher than the intra-assay coefficient (Donadeu and Ginther, 2002). Frequency of outcomes were analyzed by chi-square. A probability of $P \leq 0.05$ indicated that a difference was significant, and probabilities between $P > 0.05$ and $P = 0.1$ indicated that a difference approached significance.

Results

The months required for all mares to complete the experiment (10 days after second last ovulation of the year to 80 days after the last ovulation; Fig. 1) extended from April–June (October–December, NH) to July–September (January–March, NH). There was no difference in plasma progesterone concentrations between the control and ablation groups on either 10 days or 20 days after either the second-last or last ovulations (data not shown). Progesterone concentrations (combined for the two groups) were greater ($P < 0.001$) at 10 days (2.2 ± 0.1 ng/ml) than at 20 days (0.06 ± 0.01 ng/ml) after the second-last ovulation and were greater ($P < 0.0001$) at 10 days (2.0 ± 0.2 ng/ml) than at 20 days (0.10 ± 0.04 ng/ml) after the last ovulation.

Data profiles for follicle diameters for each mare are shown for the control group (Fig. 2) and the ablation group (Fig. 3). Complete data for the ovulatory period were missed for three mares in the ablation group (Mares H, I, M). Follicle data for anovulatory Days 60–80 were not available for two control mares, owing to ovulation (Mare E) and the masking effect of consecutive major anovulatory waves (Mare A). The control mare E had a short anovulatory season, with a major anovulatory wave during Days 31–50, followed by the first ovulation of the subsequent ovulatory season. Also, one mare in the ablation group (Mare G) had a short anovulatory season, involving sequential major anovulatory waves after ablation on both Days 10 and 30 and an ovulatory wave after ablation on Day 60. No other mares ended the anovulatory season during the experiment. The frequency of mares with a major anovulatory wave during Days 11–30 was not different between the control group (three of six mares) and the ablation group (five of eight). Only one mare in each group had multiple major anovulatory waves (Mares A, G). All or part of a mid-anovulatory phase (largest follicle ≤ 20 mm) was apparent for 10 mares (C, D, E, F, H, I, J, L, M, N). The end of the mid-anovulatory phase or the beginning of the resurging phase was apparent in one mare (Mare L). In each of 11 control and ablated mares, the diameter of the largest follicle was greater during Days 11–30 than during Days 31–50; three mares were not considered because of a short anovulatory season or multiple major anovulatory waves.

Post-ablation diameters of the largest follicle and concentrations of FSH, LH, and estradiol for the four periods are shown (Fig. 4). The interaction was significant for the largest follicle, and the first day that the diameter for one period exceeded ($P < 0.05$) the diameter for another period is indicated. The growth rate of the largest follicle from 2 to 10 days after ablation was greater ($P < 0.005$) for the ovulatory period (2.3 ± 0.2 mm/day) and for ablation at Day 10 (1.9 ± 0.2 mm/day) than for ablation at Day 30 (0.6 ± 0.2 mm/day) or Day 60 (0.8 ± 0.2 mm/day). The period-by-day interaction was significant for FSH, LH, and estradiol and the first day of a difference between two periods is shown. When postablation data for each of the four periods were analyzed separately, there were significant increases for FSH, LH, and estradiol for the ovulatory period and Days 11–30 but not for Days 31–50 and Days 61–80. The frequency of periods with postablation identified surges of FSH was different ($P < 0.02$) among the ovulatory period (five of five), Days 11–30 (eight of eight), Days 31–50 (three of eight), and Days 61–80 (three of seven).

When data of the ablation group were centralized to the day the largest follicle was nearest to 22.5 mm and subgrouped into the ovulatory period, Days 11–30 with a major wave, and Days 11–30 with a minor wave, there was a significant subgroup-by-day interaction for the largest follicle and for LH (Fig. 5). The days of significant differences among subgroups are shown. Analyses of the concentrations of FSH indicated only a main effect of day.

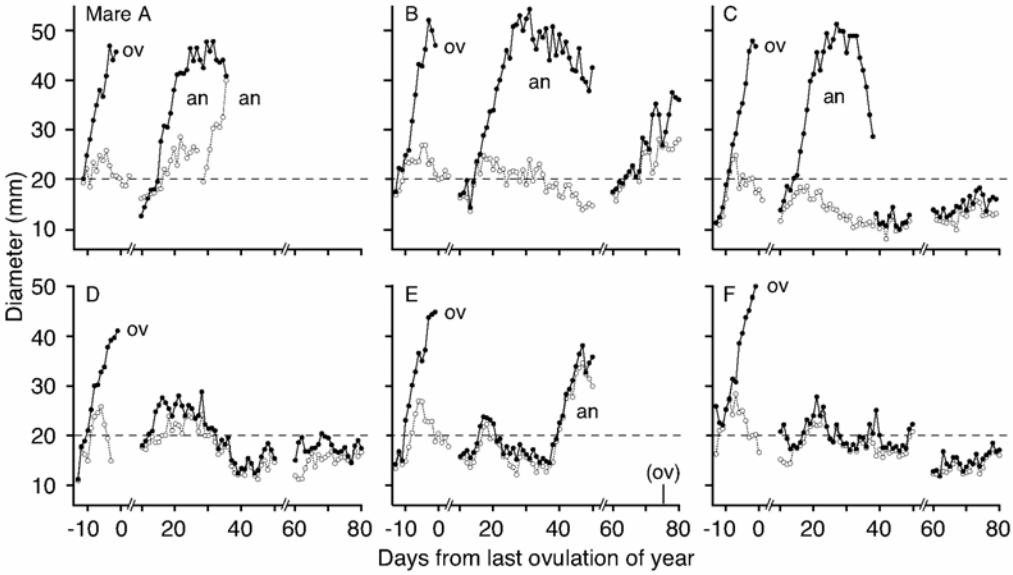

Figure 2. Daily diameters of the two largest follicles in the six control mares (Mares A–F) for the last ovulatory period and the subsequent receding and anovulatory phases. Follicle data are missing after Day 40 in Mare A because of the masking effect of consecutive major anovulatory waves. When the largest follicle of a wave did not exceed 20 mm (broken line), the mare was defined as being in the mid-anovulatory phase. An = major anovulatory follicle. Ov = ovulation.

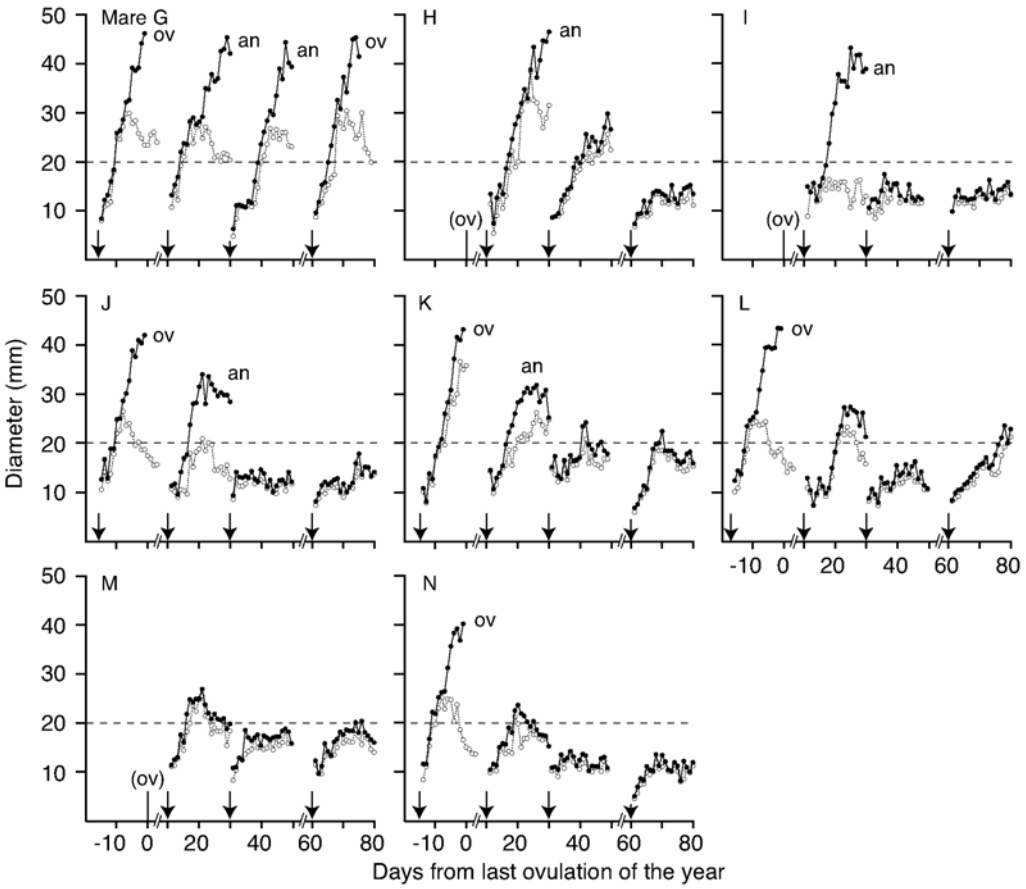

Figure 3. Daily diameters of the two largest follicles in the eight follicle-ablation mares (Mares G–N). The arrows indicate days when all follicles ≥ 6 mm were ablated. When the largest follicle of a post-ablation period did not exceed 20 mm (broken line), the mare was defined as being in the mid-anovulatory phase. An = major anovulatory follicle. Ov = ovulation.

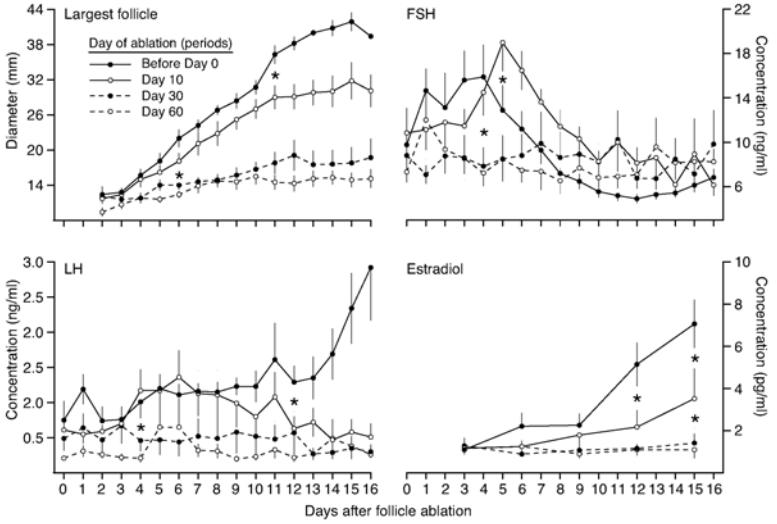

Figure 4. Mean ± SEM for diameter of largest follicle and concentrations of FSH, and LH, and estradiol following ablation of all follicles ≥ 6 mm relative to the last ovulation of the year (Day 0). Follicle ablations before Day 0 (ovulatory period) were done 10 days after the second-last ovulation. Post-ablation data are for four periods beginning with the day of ablation. The period-by-day interaction was significant ($P < 0.0005$) for all end points. An asterisk between two periods indicates the first day the two periods were different ($P < 0.05$).

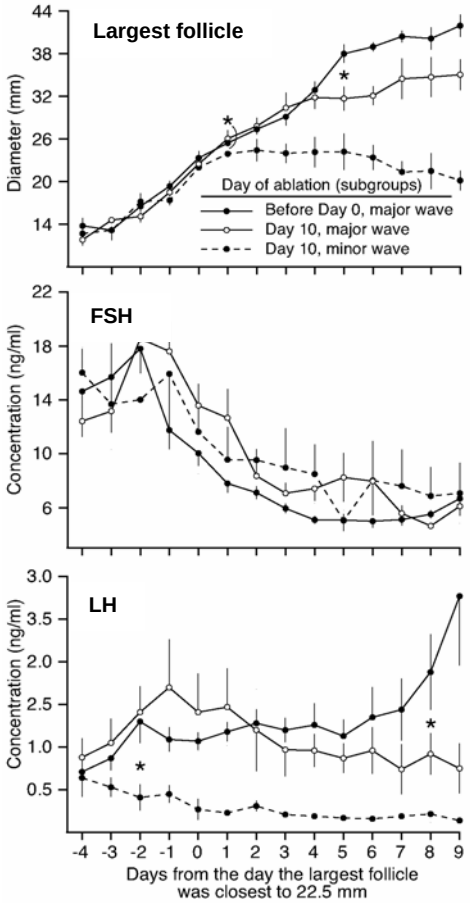

Figure 5. Mean ± SEM for diameter of largest follicle and concentrations of FSH and LH following ablation of all follicles ≥ 6 mm relative to the last ovulation of the year (Day 0) and the formation of major and minor follicular waves. Follicle ablations before Day 0 were done 10 days after the second-last ovulation. The ablation group was divided into subgroups as shown. The major waves following ablation before Day 0 were ovulatory, and the major waves following ablation on Day 10 were anovulatory. The subgroup-by-day interaction was significant for follicle diameter ($P < 0.0001$) and LH concentration ($P < 0.004$). Only the day effect was significant ($P < 0.0001$) for FSH concentration. An asterisk between two subgroups indicates the first day the two subgroups were different ($P < 0.05$).

Discussion

None of 26 monitored interovulatory intervals in the control mares and in mares that did not have an anovulatory season in the present study and in 10 mares in a previous study (Gastal *et al*., 2000) were ≥ 30 days in length, indicating that a 30-day lapse in ovulations realistically defined the beginning of the anovulatory season. Maintenance of the corpus luteum sometimes occurs in association with the onset of the anovulatory season and can affect follicle dynamics (Ginther *et al*., 2004a). There was no indication of luteal maintenance in any mare in the present study, as indicated by the low progesterone concentrations on Day 20 after the last ovulation of the year; the highest concentration in an individual at Day 20 (0.17 pg/ml) was well below the Day 10 concentration.

Incorporating control and follicle-ablated mares into the research protocol has been done during the resurging phase (last portion of anovulatory season; Donadeu and Ginther, 2003b). However, the present study is the first to utilize both controls and follicle-ablated mares to study the follicle-receding phase of the anovulatory season. The ablation approach was valuable because of the complexity inherent in the intermingling of growing and regressing follicles in control mares. For this reason, the interrelationships of follicles and hormones was based on the follicle-ablation group and not on the controls. In this regard, the recording of diameters of the two follicles began at 6–10 mm in the ablation group (Fig. 3), but not until about 20 mm in some of the controls (Fig. 2). The diversity in follicle dynamics among mares during the receding phase was similar between control and follicle-ablated mares, including the frequency of mares with major anovulatory waves during Days 11–30. In addition, the number of mares without a follicle-receding phase (periodic ovulation throughout the year) was similar between the two groups. The decrease in diameter of the largest follicle between Days 11–30 and Days 31–50 occurred in both groups. These results indicated that the repeated ablation procedures did not alter follicle dynamics during the receding phase.

In individual mares, the experiment began after the next to the last ovulation of the year and ended 80 days after the last ovulation. Thus, the experiment was expected to encompass the follicle-receding phase (Ginther *et al*., 2004a) and to end during the mid-anovulatory phase (Donadeu and Ginther, 2002). The experiment ended during July–September (January–March, NH). In a study beginning on January 29 (NH), the mid-anovulatory phase was underway in all mares and extended on average until March 8; during this phase, the largest follicle per day did not grow above 20 mm (mean, 16 mm; Donadeu and Ginther, 2002). In an earlier study, diameter of the largest follicle on average did not reach > 20 mm until mid-March (NH; Turner *et al*., 1979). In the present experiment, the 20-mm criterion was used to define the mid-anovulatory phase. The experiment encompassed or ended during an apparent mid-anovulatory phase in 10 of 14 mares. In the remaining four mares, a defined mid-anovulatory phase did not occur during the 80 days after the last ovulation of the year. The apparent end of the mid-anovulatory phase or the beginning of the follicle-resurging phase, as shown for Mare L, has been reported to be a distinctive event in most individual mares (Donadeu and Ginther, 2002); the event was characterized by a follicular wave in which the largest follicle exceeded 20 mm and was followed by a plateau of similar waves, until the development of major anovulatory waves or the ovulatory wave.

Wide diversity in follicle dynamics occurred among individuals, as illustrated by the follicle profiles and as described in the Results. The diversity during the first portion of the expected anovulatory season was further illustrated by the four additional mares that were removed from the analyses because they ovulated at regular intervals throughout the length of the study. These mares likely represented mares that did not have an anovulatory season, consistent with other reports (reviewed in Ginther *et al*., 2004a). The examinations were not continued beyond September (March, NH) in two mares. The other two mares were used in a subsequent experiment and continued to ovulate.

Whether the diversity in follicle dynamics represents repeatability in individuals or is a response to unknown external factors is not known. In this regard, mares in good body condition in January (NH) had more follicles ≥20 mm than mares in poor body condition (Gentry *et al.*, 2002). In the present study, all mares maintained good body condition throughout the study, precluding study of the effect of body condition on follicle diameters. Other studies on follicle development in follicle-intact mares during the initial portion of the anovulatory season focused on the time that ovulation was expected to occur or did not assess the diversity in follicle dynamics during the follicle-receding phase (Snyder *et al*., 1979; King *et al*., 1993; Nequin *et al*., 2000; Ginther *et al*., 2003). The present study of the receding phase together with the previous study of the mid-anovulatory and resurging phases (Donadeu and Ginther, 2002) have given initial consideration to follicle dynamics for the entire anovulatory season. Nevertheless, there remains a need for a complete uninterrupted study of follicle dynamics during the anovulatory season, considering more than the largest follicles, and including successive anovulatory seasons in individual mares. Successive seasons will be needed to investigate the repeatability of follicle patterns in individual mares.

Results of this experiment and the previous experiment (Ginther *et al*., 2003) agree on the development of an FSH surge after follicle ablation during the last interovulatory interval and 10 days after the last ovulation. The higher concentrations of FSH beginning 5 days after ablation in the ablated group for the Days

11–30 period than for the ovulatory period reflected a one-day delay before the beginning of the FSH decrease. This FSH result may be related to the apparent one-day delay for the largest follicle to reach a mean of 16 mm for the Days 11–30 period, compared to the ovulatory period. The considerable reduction in growth rate after ablation on Days 30 and 60 was associated with nondetection of an FSH increase. The other indication of reduced FSH activity during the later portion of the receding phase and often including the mid-anovulatory phase was the approximately 60 % reduction in number of periods with statistically detected FSH surges during Days 31–50 and 61–80. The increase in follicle diameter after ablation on Days 30 and 60 may reflect growth independent of FSH or a minimal response to basal FSH concentrations. In a previous study, ablation 60 days after the last ovulation of the year was followed by an FSH surge (Ginther *et al*., 2003). This reported finding was not detected in the present study.

The positive LH response to follicle ablation agrees with previous results during the early anovulatory season (Ginther *et al*., 2003) and ovulatory season (Gastal *et al*., 2000; Bergfelt *et al*., 2001). Concentrations of LH initially increased similarly following ablation before the last ovulatory period and on Day 10 after the last ovulation; the concentrations later diverged between the two periods as indicated by an earlier apparently constant level during the anovulatory period. Concentration of LH did not change after ablation on Days 30 and 60, agreeing with the previous study for ablation on Day 60. The increase in estradiol several days after the LH increase following follicle ablation 10 days after the second-last and last ovulations confirms the results of the previous study (Ginther *et al*., 2003).

The purpose of subgrouping the ablation group into the ovulatory period, Days 11–30 with a major wave, and Days 11–30 with a minor wave was to examine the gonadotropin aspects of follicle deviation. For this reason, the data were centralized nearest to the day the largest follicle was 22.5 mm (expected beginning of deviation; Gastal *et al*., 1999). This represented a follow-up to a previously reported preliminary result of a similar ablation study (Ginther *et al*., 2003). Only two mares with a major anovulatory wave were available for the previous study. In the present study, the post-ablation FSH surge was similar among the subgroups. The diameter of the largest follicle increased similarly among subgroups until the diameter was close to 22.5 mm, consistent with the similarity in the FSH surge. Thereafter, however, diameter in the minor waves began to decrease relative to the major waves. Four days later, diameter for the major anovulatory waves began to plateau relative to the ovulatory wave. These diameter differences were temporally related to differences in LH concentrations. Results supported the hypothesis on the follicle:LH relationships that encompass follicle deviation. That is, similar FSH surges would account for the similar growth of the largest follicle among subgroups until the expected beginning of deviation. Thereafter, the LH increase in the two subgroups with major waves and the absence of a post-ablation LH response in association with minor waves likely accounted for the continued growth of the largest follicle in the major waves. The subsequent LH increase in association with the ovulatory major follicular waves but not for the anovulatory major waves likely accounted for the continued growth of the ovulatory follicle during the few days before ovulation. These findings are consistent with the report that an elevation in LH concentrations encompassed deviation in mares during the ovulatory season and that an additional increase occurred as ovulation approached (Gastal *et al*., 1997).

In conclusion, there was considerable diversity among mares in follicle dynamics during the first portion of the anovulatory season or follicle-receding phase. The diversity included a major anovulatory wave during Days 11–30 (last ovulation of season = Day 0) in eight of the 14 control and follicle-ablated mares, multiple major anovulatory waves (two mares), short anovulatory season with the new ovulatory season occurring before Day 80 (two mares), and a follicle-receding phase that terminated in a mid-anovulatory phase in 10 of 14 mares. In addition, four mares were removed from the analyses, owing to repeated ovulations throughout a comparable 80-day period. Growth of the largest follicle occurred during the 2–10 days following ablation on Days 30 and 60, but the rate was reduced compared to the growth rate after ablation on Day 10. Mean FSH concentrations did not increase after ablation on Days 30 and 60, but a distinct surge occurred after ablation on Day 10. Thus, the follicle growth after ablation on Days 30 and 60 occurred in the absence of a detected increase in FSH. Concentrations of LH increased similarly following ablation before the ovulatory period and on Day 10, but did not increase following ablation on Days 30 and 60. On a temporal basis, differences in LH concentrations during these two periods accounted for differences in follicle development at the time of expected deviation, despite the similarities in the FSH surge. Concentrations of LH during the ovulatory period further increased a few days before ovulation, whereas during a corresponding time of the first anovulatory period (Days 11–30) concentrations decreased.

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