Effect of number and diameter of follicles on plasma concentrations of inhibin and FSH in mares

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The role of the number of follicles and circulating immunoreactive inhibin in the decrease in plasma FSH concentrations that occurs during development of a follicular wave was studied in mares. All follicles ≥ 6 mm in diameter were ablated by ultrasound-guided transvaginal aspiration of follicular fluid on day 10 after ovulation. During the subsequent wave, all follicles, the three largest follicles (three follicle group), the largest follicle (single follicle group) or no follicles were retained and the remaining follicles were ablated before they reached > 10 mm in diameter ($n = 10$ – 11 mares per group). Ablation of new follicles was continued until the day on which the largest follicle of the new wave reached 25 mm in diameter (day 18 after ovulation in the 'no follicle' group). Diameters of retained follicles were measured once a day by transrectal ultrasonography. Plasma samples were taken once a day and analysed by radioimmunoassay for concentrations of FSH and immunoreactive inhibin (includes dimeric inhibin as well as free α -subunit forms). Data were normalized to the day of the expected start of the decrease in plasma FSH concentrations (day 0: largest follicle 13 mm in diameter in the follicle-retained groups).

A simultaneous increase in circulating concentrations of FSH ($P < 0.05$) and immunoreactive inhibin ($P < 0.05$) occurred before the largest follicle reached 13 mm in diameter, which indicates that immunoreactive inhibin produced by follicles < 13 mm in diameter did not suppress FSH. Plasma concentrations of FSH decreased ($P < 0.05$) and immunoreactive inhibin concentrations increased ($P < 0.05$) after day 0 in the follicle-retained groups. A slower decrease in FSH concentrations was associated temporally with a delay in the increase in immunoreactive inhibin concentrations in the 'single follicle' group relative to the 'three follicle' and 'all follicle' groups. All follicle-retained groups had similar plasma concentrations of FSH and immunoreactive inhibin after the expected beginning of deviation in growth rates between the two largest follicles (largest follicle 22–23 mm in diameter). These results indicated that the decrease in plasma FSH concentrations from the start of the decrease until the expected day of deviation was a function of multiple follicles of a wave and was attributable to the secretion of inhibin. Thereafter, the largest follicle alone accounted for the continued FSH suppression.

Introduction

Follicular growth occurs in waves in mares, as in cattle (for review, see Ginther (2000)). The ovulatory wave begins (as determined by ultrasonography) at the middle of the interovulatory interval. Initially, the follicles of a wave grow as a cohort. Thereafter, the largest follicle continues to grow (dominant or ovulatory follicle) while smaller follicles regress (subordinate follicles). The dissociation into a dominant follicle and subordinate follicles is termed follicle deviation and occurs when the largest follicle reaches 22–23 mm in diameter (Gastal *et al.*, 1997, 1999a). The initiation of a follicular wave is associated temporally with a surge in plasma FSH concentrations (Bergfelt and Ginther, 1993). A decrease in the FSH surge begins when the largest follicle of the resulting wave is a mean 13 mm in diameter (Gastal *et al.*, 1997). The decrease in plasma FSH concen-

trations coincides initially with the growth of all follicles of a wave and continues during growth of the dominant follicle after deviation (Bergfelt and Ginther, 1993). The FSH decrease is necessary for the occurrence of deviation and regression of the subordinate follicles of a wave (Ginther *et al.*, 2000). In cattle, it has been concluded that the growing follicles cause the decrease in circulating FSH concentrations and that multiple follicles of a wave ≥ 5 mm in diameter contribute to the decrease (Gibbons *et al.*, 1997). The relationship between the number of follicles in a wave and the decrease in plasma FSH concentrations has not been studied in mares.

A negative feedback effect of follicles on FSH secretion in mares has been demonstrated by ovariectomy (Freedman *et al.*, 1979a; Driancourt and Palmer, 1984), administration of follicular fluid (Miller *et al.*, 1979, 1981; Bergfelt and Ginther, 1985, 1986), and follicular ablation (Hinrichs *et al.*, 1991; Gastal *et al.*, 1999b). The suppressive effects of follicles on FSH secretion have been attributed to a non-steroidal, proteinaceous fraction of follicular fluid (Miller *et al.*, 1979) and to oestradiol (Burns and Douglas, 1981;

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Thompson *et al.*, 1983; Wiest *et al.*, 1987). The involvement of oestradiol in the suppression of circulating FSH concentrations in mares has been demonstrated in association with follicle deviation (Gastal *et al.*, 1999a,b). Inhibin is a follicular protein found in different molecular mass forms that include dimeric inhibin (bound α - and β -subunits) and free α -subunit variants (Moore *et al.*, 1994). Studies in cows (Robertson *et al.*, 1986, 1989; Knight *et al.*, 1989) have shown that dimeric inhibin forms, but not free α -subunit, suppress FSH secretion. The involvement of inhibin in suppression of FSH in mares is indicated by the observations that: (i) circulating concentrations of FSH and immunoreactive inhibin are related reciprocally during the oestrous cycle (Bergfelt *et al.*, 1991; Roser *et al.*, 1994; Nagamine *et al.*, 1998; Nagaoka *et al.*, 1999); and (ii) passive immunization against inhibin results in increased plasma FSH concentrations (Nambo *et al.*, 1998; Briant *et al.*, 2000). These results are similar to those obtained in other species (Mann *et al.*, 1989, 1990; Glencross *et al.*, 1994; Kaneko *et al.*, 1995, 1997; Arai *et al.*, 1996).

Studies have shown that immunization against inhibin increases the number of preovulatory follicles and ovulations in mares (McCue *et al.*, 1992; McKinnon *et al.*, 1992; Nambo *et al.*, 1998). However, the temporal relationships among changes in circulating concentrations of inhibin, concentrations of FSH and growth of the follicles of a wave have not been elucidated in mares.

The aims of the present study in mares were: (i) to characterize the involvement of different numbers of growing follicles > 10 mm in diameter in the decrease in plasma FSH concentrations associated with a follicular wave; and (ii) to evaluate the role of follicular inhibin in the decrease in FSH concentrations. All follicles ≥ 6 mm in diameter were ablated at mid-cycle and specific numbers of follicles (none, one, three or all) of the following wave were retained. The temporal relationships between follicular growth and circulating concentrations of FSH and immunoreactive inhibin were then examined. The following hypotheses were tested: (i) that the decrease in plasma FSH concentrations would be a function of the number of follicles retained in a wave; and (ii) that within each follicle group, changes in circulating concentrations of FSH would have a temporal, inverse relationship to changes in immunoreactive inhibin concentrations.

Materials and Methods

Experimental animals

Non-lactating Pony mares ($n = 42$) of mixed breeding, aged 3–15 years, body weight 230–460 kg, were used from March to August in the northern hemisphere. The mares were kept outdoors and had been exposed to artificial lights (15 h per day) during the previous winter. The mares were fed alfalfa and grass hay and had free access to water and mineralized salt. Before the experiment, ovulations were

synchronized by administration of 10 mg dinoprost i.m. (Lutalyse; Pharmacia and Upjohn Co, Kalamazoo, MI; Ginther, 1992) to all mares. Follicles > 30 mm in diameter were monitored once a day by ultrasonography to establish the day of ovulation. Mares double-ovulating in the previous cycle or with apparent uterine pathology (fluid collections; Ginther, 1995) before the start of the experiment were not used.

Follicle ablation

Ablation of specified follicles was performed by ultrasound-guided transvaginal aspiration of follicular contents as described by Gastal *et al.* (1997). Follicles that refilled with fluid and grew to 10 mm in diameter were re-ablated. All follicles ≥ 6 mm in diameter were ablated on day 10 after ovulation in all mares to eliminate follicles from previous waves and to facilitate tracking of individual follicles of the subsequent or new wave. The mares were then assigned randomly to four groups. When a follicle of the new wave first reached 10 mm in diameter, various numbers of follicles 6–10 mm in diameter were ablated so that no follicles ('no follicle' group, $n = 11$), the largest follicle ('single follicle' group, $n = 10$), the three largest follicles ('three follicle' group, $n = 10$) or all follicles ('all follicle' group, $n = 10$) were retained. Follicles of the same diameter were chosen randomly for retention. When a new follicle 10 mm in diameter was detected during subsequent daily examinations, all follicles ≥ 6 mm were ablated, except for the designated retained follicles. Periodic ablations were continued until the largest follicle reached 25 mm in diameter in the follicle-retained groups or on day 18 after ovulation in the no follicle group (expected day the largest follicle would have reached 25 mm in diameter; Gastal *et al.*, 1997). Animals with fewer than three follicles ≥ 6 mm in diameter when the first 10 mm follicle of the new wave was detected were not used, so that all mares would have an equal chance to be included in any of the four groups.

Data collection

Ovaries were monitored once a day with an ultrasound scanner equipped with a 5 MHz linear-array transducer (Aloka SSD-500V; Aloka, Wallingford, CT), starting on day 8 after ovulation and extending until the next ovulation. At each scanning session, the diameter of follicles > 5 mm in diameter was estimated by comparison with the graduation marks on the scanner screen. After the follicles reached 10 mm in diameter, the four largest follicles or the designated retained follicles were tracked from examination to examination and measured with electronic callipers (Ginther, 1995). Follicles were measured by taking the mean width and length from a frozen image. After the follicles reached 15 mm in diameter, two frozen images were used.

Blood samples were taken every day from day 8 after ovulation until the next ovulation. Samples were taken

via jugular venepuncture into heparinized tubes and immediately centrifuged at 1500 *g* for 6 min. The plasma fraction was separated and stored at -20°C until hormone assays were performed.

Hormone assays

Plasma FSH concentrations were measured by a double antibody radioimmunoassay validated previously for mares by Freedman *et al.* (1979b). Intra- and interassay coefficients of variation, determined from a pool of plasma containing 1.7–3.4 ng FSH ml^{-1} , and sensitivity ($n = 8$ assays) were 14.05%, 7.13% and 0.44 ng FSH ml^{-1} , respectively. For FSH, as well as for immunoreactive inhibin, assay sensitivity was calculated by subtracting two SD from the mean maximum percentage binding and the value obtained was averaged over all assays.

Concentrations of immunoreactive inhibin in plasma were measured by a radioimmunoassay kit (Institute of Reproduction and Development, Monash Medical Center, Clayton, Victoria). The kit included inhibin as a 32 kDa fraction of bovine follicular fluid for iodination and anti-inhibin (Pool B, 1989) generated against a 31 kDa fraction of bovine follicular fluid. The crossreactivity of this assay in mares has been described by Roser *et al.* (1994); the antibody recognizes all dimeric inhibin forms, as well as full-length α -subunit and pro- αC forms. Assay procedures were similar to those described by Roser *et al.* (1994), except that iodination was done using Iodogen (Matteri *et al.*, 1987) and a different reference standard was used (recombinant 32 kDa bovine inhibin; IP-1095; Peninsula Laboratories Europe Ltd, St Helens). Assay of serial dilutions of the standard (5–250 ng ml^{-1}) and two different pools of plasma from oestrous and dioestrous mares (5–100 μl each) in total volumes of 100 μl resulted in displacement curves that were similar. Intra- and interassay coefficients of variation, calculated from a pool of plasma containing 27.1–37.5 ng ml^{-1} , were 10.5 and 2.9%, respectively, and the sensitivity was 7 ng ml^{-1} ($n = 6$ assays).

Statistical analyses

Previous data from our laboratory (Gastal *et al.*, 1997) indicated that the plasma FSH decrease associated with the development of a follicular wave began when the largest follicle reached a mean of 13 mm in diameter. Therefore, follicular and hormonal data were normalized to the day on which the largest follicle of the new wave reached 13 mm in diameter (day 0). Data from the 'no follicle' group were normalized to the mean day on which the largest follicle reached 13 mm in diameter in the other three groups (mean, day 15 after ovulation). Follicular and hormonal data were also normalized to the day of ablation of all follicles ≥ 6 mm in diameter (day 10 after ovulation). In each instance, the longest interval of time for which data from all mares were available was used in the statistical analyses; data were truncated to the day on which the first mare ovulated.

In contrast to circulating immunoreactive inhibin, FSH data did not follow a normal distribution, as assessed by a Kolmogorov–Smirnov test (level of significance, $P < 0.05$); therefore, FSH data were log-transformed. Thereafter, for each hormone, extreme values were tested using the extreme standardized deviate (Pearson and Hartley, 1976). Under these criteria, two distinctly high values for immunoreactive inhibin from different mares were excluded from the statistical analyses. Hormonal and follicular data were then analysed by the SAS MIXED procedure taking the animal (group) as the random effect and using a first order autoregressive structure to account for the autocorrelation among samples taken over time. Main effects of group and day and the interaction of group by day were determined. When the group effect or the interaction were significant, Duncan's multiple range test was used to detect differences among individual means. Single-point measurements for follicular end points were analysed by one-way ANOVA. The level of significance was $P < 0.05$.

Results

Twelve mares were removed from the experiment and replaced for the following reasons: follicles were ablated mistakenly or left unablated in eight mares; two mares developed an apparent minor wave (Ginther and Bergfelt, 1992); follicles of one mare did not grow to 10 mm in diameter by week 2 of the experiment; and one mare had a dominant follicle that grew at a very low rate (1.25 mm day^{-1}). The interovulatory interval of the latter mare was exceptionally long (34 days) which, among the follicle-retained groups, was found to be an outlier by the method of the extreme standardized deviate (Pearson and Hartley, 1976). Data from two mares that had double ovulations were included in the statistical analyses.

There were no differences among groups before the establishment of the experimental groups (largest follicle of the new wave reaches 10 mm in diameter) in the following follicular characteristics: (i) number of follicles ≥ 6 mm in diameter on day 10 after ovulation (combined mean: 11.1 ± 0.9 follicles); (ii) diameter of the largest follicle on day 10 after ovulation (16.1 ± 0.7 mm); (iii) day of detection of the first 10 mm follicle of the new wave (3.4 ± 0.2 days); and (iv) number of follicles ≥ 6 mm in diameter when the largest follicle of the new wave reached 10 mm in diameter (4.9 ± 0.3 follicles). A mean 5.4 ± 0.6 follicles grew to > 10 mm in diameter in the 'all follicle' group from the time of ablation of all follicles on day 10 after ovulation until the largest follicle of the new wave reached 25 mm in diameter. There were no differences in maximum diameter (38.5 ± 0.9 mm) or growth rate (3.0 ± 0.1 mm day^{-1}) of the dominant follicle among the follicle-retained groups. All mares ovulated from the dominant follicle of the post-ablation wave, and the interovulatory interval was longer ($P < 0.01$) in the 'no follicle' group (29.7 ± 1.3 days) than in the groups with follicles retained (24.2 ± 0.3 days).

Averaged over all groups, plasma concentrations of FSH increased after the ablation of all follicles ≥ 6 mm in diameter on day 10 after ovulation (Fig. 1). Plasma immunoreactive inhibin concentrations decreased initially after ablation, followed by an increase after day 2 after ablation (day 12 after ovulation).

Diameter of the largest follicle and concentrations of plasma FSH and immunoreactive inhibin, normalized to the day on which the largest follicle reached 13 mm in diameter (day 0), are shown (Fig. 2). The diameter of the largest follicle was similar among the groups with follicles retained. Exceptionally high circulating FSH concentrations in a single mare on day -1 accounted for the high mean concentrations in the 'all follicle' group on that day. Plasma FSH concentrations were lower continuously in each of the follicle-retained groups than in the 'no follicle' group beginning on day 1. Plasma FSH concentrations were higher in the 'single follicle' group than in the 'three follicle' or 'all follicle' groups on day 2. Thereafter, no significant differences in FSH concentrations were detected among the follicle-retained groups. Compared with the 'no follicle' group, plasma immunoreactive inhibin concentrations were higher by day 1 in the 'three follicle' and 'all follicle' groups, and by day 3 in the 'single follicle' group. Immunoreactive inhibin concentrations in the 'single follicle' group were lower on day 2 than in the 'three follicle' and 'all follicle' groups. There were no significant differences in plasma immunoreactive inhibin concentrations among the follicle-retained groups starting on day 3.

Fig. 1. Concentrations of FSH (●) and immunoreactive inhibin (○) in plasma, and diameter of the largest follicle (■) after ablation of all follicles ≥ 6 mm in diameter on day 10 after ovulation. Values were averaged over all groups ($n = 41$ mares) and are presented as mean $\pm$ SEM. Day effect was significant for FSH ($P < 0.0005$), inhibin ($P < 0.001$) and diameter of the largest follicle ($P < 0.001$). ^{ab}For FSH and immunoreactive inhibin, means with different letters are significantly different ($P < 0.05$).

Fig. 2. Mean (a) diameter of the largest follicle and plasma concentrations of (b) FSH and (c) immunoreactive inhibin normalized to the day on which the largest follicle of the new wave reached 13 mm in diameter (day 0) in mares with no follicles (○), one follicle (□), three follicles (■) or all follicles (●) of the wave retained ($n = 10$ – 11 mares per group). Data in the 'no follicle' group were normalized to the mean day on which the largest follicle reached 13 mm in diameter in the other three groups (day 15 after ovulation). Overall SEMs for each end point were as follows: largest follicle: 0.9; FSH: 0.9; and immunoreactive inhibin: 3.5. There was an effect of day ($P < 0.0001$) for diameter of the largest follicle. There were effects of day ($P < 0.0001$), group ($P < 0.0005$) and group $\times$ day ($P < 0.001$) for FSH and immunoreactive inhibin. ^{abc}For each end point, group means within a day with different letters are significantly different ($P < 0.05$).

Discussion

Follicular aspiration of the dominant follicle (Dippert *et al.*, 1995), the preovulatory follicle (Hinrichs *et al.*, 1991) and all follicles > 5 mm (Gastal *et al.*, 1997, 1999a,b) or > 8 mm (Duchamp *et al.*, 1995) in diameter by a transvaginal ultrasound-guided technique has been used previously for research purposes in mares. In the present study, maximal diameter and growth rate of the dominant follicle, and duration of the interovulatory interval in the follicle-retained groups were similar to those reported previously for intact Ponies and after periodic follicle ablations to retain only the two largest follicles of a wave (Gastal *et al.*, 1997, 1999a). The proportion of double ovulations (two of 41) was similar to results reported in Ponies by Ginther (1992). The consistent hormonal responses detected after follicular ablation among groups in the present study and in other studies (Hinrichs *et al.*, 1991; Gastal *et al.*, 1999b) indicate that this technique is effective in functionally ablating follicles. Clinical side effects after the repeated use of the procedure were not observed in this or in previous studies (Bracher *et al.*, 1993; Gastal *et al.*, 1997). Averaged over the follicle-retained groups, the plasma FSH decrease started when the largest follicle of the new wave was a mean 13.7 ± 0.6 mm in diameter, in agreement with the results of Gastal *et al.* (1997). Thus, normalization of data in this experiment to the day on which the largest follicle reached 13 mm in diameter was justified for comparing the effects of number of follicles on the decrease in FSH concentrations and to study the involvement of circulating inhibin in the decrease.

Examination of the follicular and hormonal changes before the largest follicle reached 13 mm in diameter indicated that ablation of all follicles ≥ 6 mm in diameter on day 10 after ovulation caused an immediate increase in plasma FSH concentrations and a concomitant but transient (during days 1 and 2 after ablation) decrease in immunoreactive inhibin concentrations. The 2 day transient decrease in plasma immunoreactive inhibin concentrations may have reflected the ablation of inhibin-producing follicles, but this could not be assessed critically. An increase in circulating immunoreactive inhibin occurred during days 2–4 after ablation concomitant with a continued increase in FSH concentrations. This result indicates that the increasing plasma inhibin concentrations did not have an FSH-suppressing effect while the follicles of the new wave were growing from means of 7.7 mm to 11.6 mm in diameter. Free inhibin α -subunit forms, which have no FSH-suppressing activity (Knight *et al.*, 1989; Robertson *et al.*, 1989), as well as dimeric inhibins, are measured by the radioimmunoassay that was used in the present study. The previous finding agrees with two to five times higher circulating FSH concentrations (days 2–6) in the 'no follicle' group, in which follicles were ablated before they exceeded 10 mm in diameter, than in the follicle-retained groups; this difference in plasma FSH concentrations is similar to that reported during the breeding season between ovariectomized and

intact mares (Freedman *et al.*, 1979a). The conclusion that follicles < 13 mm in diameter secrete inhibin forms that do not suppress plasma FSH concentrations is consistent with reports that granulosa cells of follicles < 10 mm in diameter contain inhibin α -subunits (Nagamine *et al.*, 1998; Goudet *et al.*, 1999), but not β -subunits (Nagamine *et al.*, 1998). Two free inhibin α -subunit variants have been isolated from equine follicular fluid (Moore *et al.*, 1994). Circulating immunoreactive inhibin with no FSH-suppressing activity has also been detected in mares during the second half of pregnancy (Nambo *et al.*, 1997).

The capacity for FSH suppression by growing follicles began when the follicles reached approximately 13 mm in diameter (day 0), as indicated by the onset of the decrease in plasma FSH concentrations. The results supported the two hypotheses on the relationships among number of follicles, a decrease in circulating FSH concentrations and an increase in inhibin concentrations. The plasma immunoreactive inhibin concentrations increased and the FSH concentrations decreased more rapidly in the 'three follicle' and 'all follicle' groups than in the 'one follicle' group, as indicated by significant differences on day 2. The changes in concentrations of the two hormones were similar between the 'three follicle' and 'all follicle' groups. This result indicated that three follicles were adequate for inhibin production and suppression of plasma FSH. It was not determined whether two follicles would be similarly effective. In cattle, more than two follicles of a wave were involved in the initial decrease in plasma FSH concentrations (Gibbons *et al.*, 1997).

During the initial FSH decrease (days 1 and 2), inhibin alone may have accounted for the FSH suppression. An increase from baseline circulating concentrations of oestradiol, the other known FSH-suppressant in mares, was not detected at this time in the same group of Pony mares (Gastal *et al.*, 1999a,b), or at the equivalent time in cattle (Gibbons *et al.*, 1999). Therefore, plasma oestradiol concentrations probably did not play a role in the initial FSH decrease. The role of follicular inhibin in the initial decrease in plasma FSH concentrations is consistent with the reported suppression of circulating FSH concentrations by a proteinaceous fraction of follicular fluid in ovariectomized mares (Miller *et al.*, 1979) and the increase of circulating FSH concentrations after treatment with inhibin antiserum in mares during dioestrus (Nambo *et al.*, 1998).

The mean diameter of the largest follicle on day 3 (23.9 mm) in the follicle-retained groups was close to the diameter reported by Gastal *et al.* (1997, 1999a) at the start of follicle deviation (22–23 mm). That is, a change from suppression of plasma FSH by multiple follicles to suppression by a single follicle occurred at the approximate expected start of deviation on day 3. Earlier reports in mares (Gastal *et al.*, 1999b) and cattle (Ginther *et al.*, 2000) indicated that the largest follicle alone is responsible for the continued decrease in circulating FSH concentrations after the start of deviation. This conclusion is supported in the present study by the suppression of FSH concentrations to a

similar extent in all of the follicle-retained groups, starting on day 3. Plasma immunoreactive inhibin concentrations remained increased in the 'three follicle' and 'all follicle' groups starting on day 2 and in the 'single follicle' group starting on day 3 and extending until the end of the experiment (day 6). However, the relative role of inhibin in the FSH decrease after deviation was not studied in this or previous experiments. Briant *et al.* (2000) indicated that an antiserum against inhibin after the largest follicle reached 20 mm in diameter increased plasma FSH concentrations. Although not focused on deviation, this reported finding is consistent with a continuing suppressing effect of inhibin on FSH after the expected start of deviation. Although the temporal relationship between an increase in circulating oestradiol and the beginning of deviation is well established (Gastal *et al.*, 1999a,b), the relative role of plasma oestradiol in the FSH decrease after deviation has not been determined. Miller *et al.* (1979) investigated a proteinaceous fraction of follicular fluid in ovariectomized mares and reported that circulating inhibin and oestradiol may have a synergistic effect on FSH suppression. A combined action of circulating inhibin and oestradiol in suppressing FSH has been demonstrated in other species, such as cattle (Kaneko *et al.*, 1995), sheep (Mann *et al.*, 1992) and rats (Arai *et al.*, 1996).

This is thought to be the first report in which the changes in follicle development and circulating concentrations of both immunoreactive inhibin and FSH in relation to developing follicular waves in mares have been characterized. On the basis of the temporal relationships among circulating concentrations of FSH, circulating concentrations of immunoreactive inhibin and number of retained follicles, it is concluded that the growing follicles of a wave acquire the ability to secrete inhibin with FSH-suppressing activity when they reach approximately 13 mm in diameter. The secretion of FSH-suppressing inhibin by multiple follicles of a wave induces the decrease in plasma FSH concentrations from its beginning (when the largest follicle is 13 mm in diameter) until the expected time of deviation; inhibin secretion by the three largest follicles is sufficient to induce the FSH decrease. After the expected time of deviation, the largest follicle appears to be solely responsible for continued suppression of plasma FSH concentrations. The relative roles of inhibin and other follicular factors, such as oestradiol, in the suppression of circulating FSH concentrations during and after deviation are not known.

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