

Minireview

Follicle Selection in Monovular Species

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ABSTRACT

Follicle deviation is proposed to be the eminent event in follicle selection in monovular species. At deviation, the largest follicle establishes dominance apparently before the second-largest follicle can reach a similar diameter. In cattle, based on diameters of the two follicles at the beginning of deviation, the mechanism becomes established in <8 h. An FSH:follicle-coupling hypothesis has been supported as the essence of follicle selection. According to the hypothesis, the growing follicles cause the FSH decline from the peak of the wave-stimulating FSH surge until deviation, even though the follicles continue to require FSH (two-way functional coupling involving multiple follicles). During multiple-follicle coupling, inhibin is the primary FSH suppressant. Near the beginning of deviation, the largest follicle secretes increased estradiol, and apparently both estradiol and inhibin contribute to the continuing FSH decline; only the more-developed largest follicle is able to utilize the low FSH concentrations (single-follicle coupling). Deviation is encompassed by a transient elevation in LH in heifers and by a component, often distinct, of the long ovulatory LH surge in mares. In heifers, receptors for LH appear in the granulosa cells of the future dominant follicle about 8 h before the beginning of deviation. The LH stimulates the production of estradiol and insulin-like growth factor-1. These intrafollicular factors and perhaps others account for the responsiveness of the largest follicle to the low concentrations of FSH. The smaller follicles have not reached a similar developmental stage and because of their continued and close dependency on FSH become susceptible to the low concentrations. Thereby, follicle selection is established.

estradiol, follicle, follicle-stimulating hormone, growth factors, luteinizing hormone

INTRODUCTION

Follicle selection in monovular species (e.g., cattle, horses, humans) is the process wherein one follicle develops from a wave of growing follicles and becomes the only follicle with ovulatory capacity. Transrectal and transvaginal ultrasonography have accelerated research progress on the nature of follicle selection by providing a means for tracking individual follicles from examination to examination in control and treated animals and for ablating, sampling, and treating individual follicles at specific times [1].

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Although progress is being made, we are also learning that nature is tenaciously guarding the final secrets of follicle selection. We can now, however, better appreciate the awesome complexity underlying the phenomenon.

This minireview considers cattle and horses as comparative research models for humans. Cattle and horses have similar follicle-selection characteristics, despite an approximately 2.5-fold greater diameter of the follicles in horses throughout a follicular wave (e.g., preovulatory diameter, 40 versus 16 mm). Limited initial reports suggest the presence of similar selection characteristics during the ovulatory wave in humans [2–4], with follicles a few millimeters larger than in cattle (e.g., preovulatory diameter, 21 mm). This minireview integrates the research findings from cattle, horses, and humans. The report will be focused on the mechanism whereby one follicle establishes dominance over other follicles in a relatively short time (e.g., in an average of <8 h in cattle [5]). Earlier reviews can be consulted for broader information in farm species [5–10] and humans [11–13].

DEVIATION AND THE SIZE ADVANTAGE

Follicle emergence is defined by the smallest diameter used for generating growth profiles by sonography and often is represented by diameters of 6 mm in mares [5] and 4 mm in heifers [5] and women [3]. The future dominant follicle emerged in heifers a mean of 6 [14] or 7 h [15] earlier and in mares a mean of 1 day [16] earlier than the future largest subordinate follicle. After emergence, the follicles of a wave enter a common-growth phase involving reported means of 7–11 follicles in the three species [14, 16, 17]. Follicle selection occurs at the end of the common-growth phase; the dominant follicle grows at a continuing rate, and the remaining or subordinate follicles regress or temporarily grow at a reduced rate and then regress. This partitioning has been called deviation [14]. The deviation mechanism must prevent the continued growth of the future subordinate follicles because they, as well as the future dominant follicle, are capable of dominance as indicated by studies involving ablation of specific follicles in cattle and horses [5]. Furthermore, administration of FSH early in a wave in cattle and horses [5] or humans [11] or an inhibin antiserum in cattle and horses [5] delayed or prevented deviation as indicated by the development of more than one dominant follicle. These findings demonstrated that the predeviation capabilities of follicles necessitated the evolution of the deviation mechanism, if the species were to become monovular.

The beginning of observed deviation in the diameter profiles of individual follicles occurs at the ultrasound examination preceding the first examination with an apparent

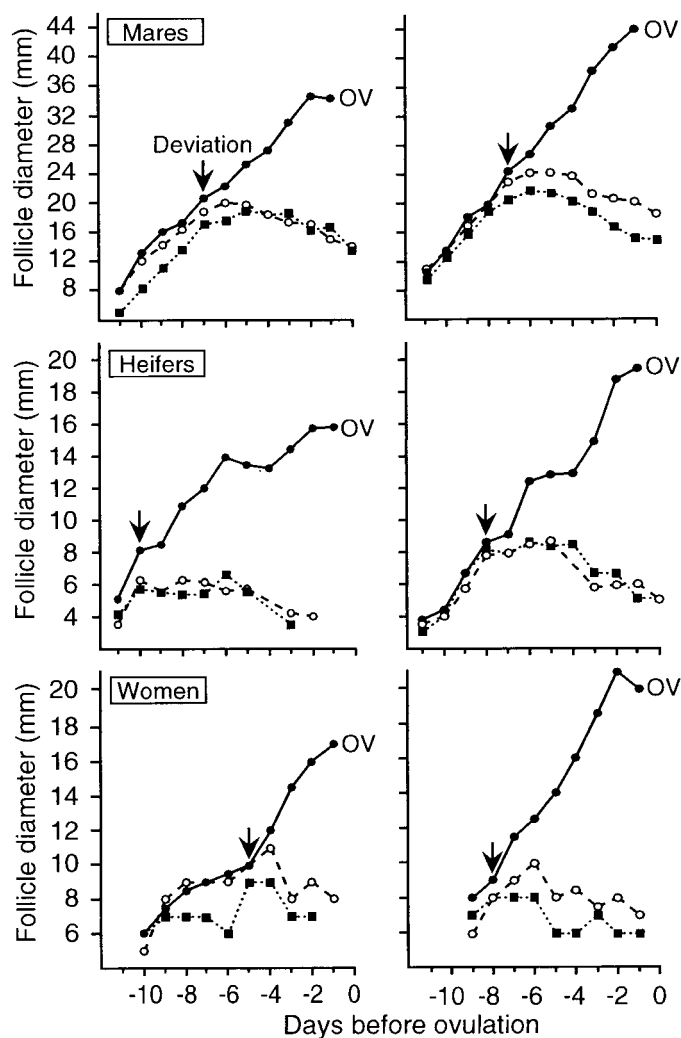


FIG. 1. Profiles of the three largest follicles of the ovulatory wave tracked from day-to-day by sonography in two individuals for each of the three species. The profiles for women are courtesy of A.R. Hess and R.A. Pierson, University of Saskatchewan, and are part of an ongoing study [3]. The indicated beginning of deviation (arrow) for each individual was judged by us and is based on the changes in the growth profiles of all three follicles. OV, Ovulation.

change in diameter differences between the two largest follicles [14]. The beginning of expected deviation can be based on diameters obtained in previous studies of observed deviation and is used for experimental procedures that may interfere with or terminate deviation. Averaged over several reports [5], observed deviation for the first anovulatory wave in heifers began when the largest follicle was a mean of 8.5 mm and for the ovulatory wave in mares was a mean of 22.5 mm. In women, reports on the growth of follicles are consistent with a deviation phenomenon during the ovulatory wave [2–4]. However, deviation has not been described, specifically. In one study [2], the dominant or ovulatory follicle was first recognized by its larger diameter when the two largest follicles were means of 9.9 and 5.9 mm, respectively. The 4-mm difference suggests that presumed deviation would have begun at least 1 day earlier or between 8 and 9 mm. Two examples of ovulatory waves in women [3] and in each of the other two species and our judgment on the beginning of deviation are shown (Fig. 1). Mean follicle-diameter profiles normalized to de-

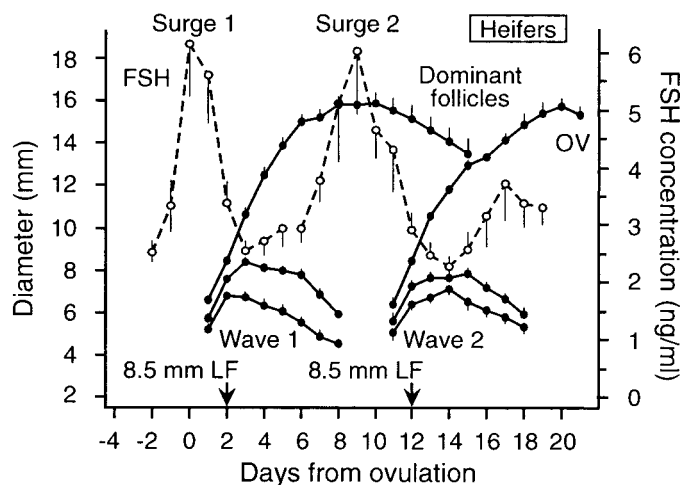


FIG. 2. Surges of FSH and development of the three largest follicles (mean \pm SEM) during 13 bovine estrous cycles with two follicular waves [44]. The data for each wave are normalized to the mean day of the interovulatory interval that the largest follicle (LF) reached 8.5 mm (expected beginning of deviation). OV, Ovulation.

viation (heifers) and emergence (mares) are depicted (Figs. 2 and 3).

Between emergence and deviation in heifers [14] and mares [16], the growth rates of the two largest follicles seem parallel in about 50% of individual waves; examples of the variation in growth profiles have been published [6, 14, 16]. The imprecision in measuring the sometimes irregularly shaped follicles [1] can be expected to contribute to fluctuations in data growth profiles. In addition, the time used in completing deviation varies, as suggested by variation in the interval between the beginning of deviation and terminated growth of the subordinate follicles. For these reasons, the beginning of deviation is indistinct in about 20% of waves (unpublished results). The follicle that first reached 8.0 mm in heifers (examinations every 8 h) and 20.0 mm in mares (examinations every day) became the dominant follicle in 90% of 51 waves and 93% of 29 waves, respectively. These diameters occurred on average at the examination before the beginning of deviation. The

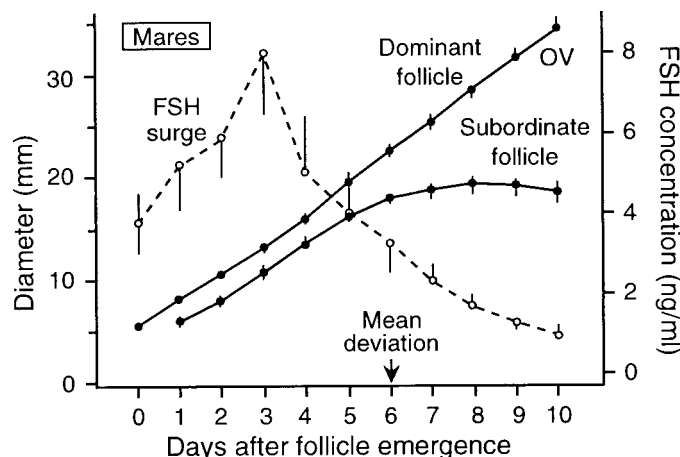


FIG. 3. Surge of FSH and development of the two largest follicles (mean \pm SEM) in 14 equine ovulatory waves normalized to the day of follicle emergence. The future dominant follicle emerged a mean of 2 days after ablation of all follicles at 10 days after ovulation [16]. Only two follicles were allowed to develop (two-follicle model). OV, Ovulation.

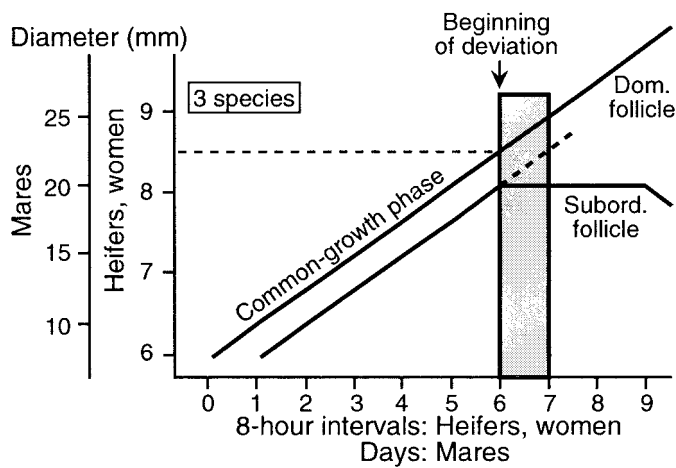


FIG. 4. Schematic two-follicle model illustrating the size advantage of the future dominant follicle. Based on limited information, the diameter scale for women is speculated to be similar to the scale for heifers. The extent and duration of parallelism between the two follicles during the common-growth phase varies considerably among individuals and are exaggerated in the illustration. On average, the common-growth phase ends and deviation begins when the largest follicle reaches the indicated diameters. Deviation is established before the next largest follicle can reach a similar diameter (represented by the width of the vertical bar).

corresponding data when the largest follicle reached 7.0 and 17.5 mm (approximately 1 day earlier) were 71% and 81%.

The difference in diameter between the two largest follicles at the end of the common-growth phase (beginning of deviation) is postulated [14] to allow the largest follicle to establish dominance before the next largest follicle can reach a similar diameter (Fig. 4). In this regard, the mean difference in diameter between the two follicles provides an apparent indication of the mean time required for the establishment of deviation (heifers, 0.5 mm, equivalent to 8 h of growth [15]; mares, 3 mm, equivalent to 1 day of growth [16]). We know of no other adequately supported interpretation, other than size or developmental advantage, that could account for the ability of one follicle to rapidly establish dominance. Despite this simple assumption, the mechanism for utilization of the size advantage in the establishment of deviation is complex.

FOLLICLE-STIMULATING HORMONE:FOLLICLE-COUPLING HYPOTHESIS

A close two-way functional coupling between FSH and the follicles has been proposed to be an integral component of the deviation mechanism (Fig. 5 [18]). The FSH surge that stimulates emergence of a wave begins to decline when the largest follicle is 4–5 mm in heifers and about 13 mm in mares (Fig. 3 [5]). The interval from the beginning of the FSH decline to the beginning of deviation is about 3 days in the two farm species. After the beginning of deviation, the concentrations of FSH continue to decline for 10–20 h in heifers and for several days in mares. During the interval from the beginning of the FSH decline until the beginning of deviation, multiple follicles contribute to the FSH decline in heifers [19] and mares (unpublished results). Paradoxically, the decline in FSH is imposed by the follicles, yet the follicles continue to require the FSH. The continued dependency of the follicles on FSH was indicated by a study [18] in cattle; experimental reduction of FSH below the concentrations at the middle of the FSH decline

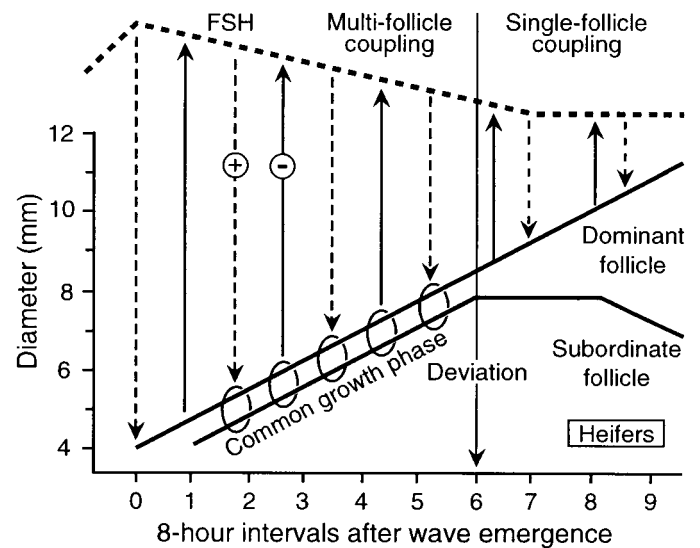


FIG. 5. Schematic illustration of two-way functional coupling between FSH and the follicles. Both follicles contribute to the decline in the wave-stimulating FSH surge, and the declining FSH is utilized by both follicles (two-way coupling). Deviation represents a change from multiple-follicle to single-follicle coupling. The more-developed larger follicle continues the FSH-follicle coupling by continuing to depress FSH while at the same time utilizing the reduced concentrations of FSH. The less-developed smaller follicles are unable to maintain follicle coupling because the declining concentrations of FSH have reached a level below their requirements.

was associated with a decrease in diameter of the three largest follicles.

The FSH:follicle relationship changes from multiple- to single-follicle coupling at the beginning of deviation (Fig. 5). The coupling from the single follicle to FSH has been demonstrated by ablating the largest follicle at the expected beginning of deviation; FSH concentrations increased soon after ablation in heifers (Fig. 6) and mares [20]. An FSH increase also occurs in women after ablation of the dominant follicle [21]. The coupling in the opposite direction (from FSH to the single follicle) has been demonstrated in heifers. Experimentally depressing the FSH concentrations below the prevailing concentrations at the expected beginning of deviation interfered with growth of the largest follicle [18, 22]. It was concluded that the more-developed largest follicle not only withstood but also required the low FSH concentrations associated with deviation. Experimental prevention of the postdeviation FSH decline suggested that the FSH concentrations at the beginning of deviation were already below the requirements of the smaller follicles and the continued FSH decline usually was not needed for the continuation of deviation (unpublished results). Consistent with this result, the smaller follicles were not further inhibited when FSH was experimentally reduced below the concentrations in controls at the expected beginning of deviation [18]. These findings indicated that a developmental change occurs in the largest follicle by the beginning of deviation so that it is responsive to a concentration of FSH that is inadequate for the smaller follicles, thereby heralding the beginning of deviation. In this regard, cultured granulosa cells from women are more responsive to low doses of FSH when obtained from larger follicles (>8 mm [12]). In monkeys, mature follicles thrive in the presence of FSH concentrations that are too low to support less-mature follicles [23].

The tightness of the FSH/follicle coupling has been dem-

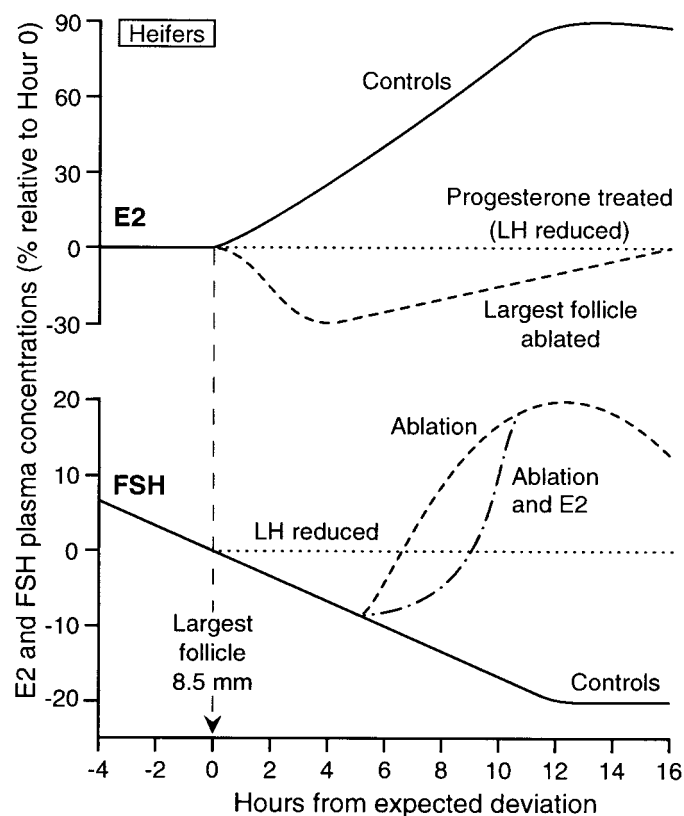


FIG. 6. Composite schematic of the results of several experiments on the role of circulating estradiol (E2) from the largest follicle and circulating LH on the continued decline in FSH after the expected beginning of deviation (largest follicle, 8.5 mm). In the controls, circulating E2 began to increase at the expected beginning of deviation [30]. However, E2 decreased when the largest follicle was ablated at 8.5 mm [30] and remained at basal levels when LH was reduced by progesterone treatment beginning before expected deviation (unpublished results). In the controls, FSH concentrations continued to decrease as the estradiol increased after the beginning of deviation. In the treated groups, FSH increased when the largest follicle was ablated at 8.5 mm (loss of E2 source [24, 30]). The postablation FSH increase was delayed when E2 was given [30]. The FSH did not decline after deviation when E2 was maintained at basal levels by experimental reduction of LH (unpublished results).

onstrated in heifers by the following results: 1) an FSH suppressant given near the middle of the FSH decline reduced both the FSH concentration and diameter of the three largest follicles by 8 h after treatment [18], 2) an FSH suppressant given at the expected beginning of deviation reduced the diameter of the largest follicle by 6 h after the experimental reduction in FSH concentrations [18, 22, 24], and 3) ablation of the largest follicle at expected deviation was followed by an increase in FSH concentrations beginning 4 or 5 h after ablation [18, 24]. These results satisfied the postulated minimal interval of <8 h in heifers from the beginning of deviation to the establishment of deviation (Fig. 4) and represent the basis of the FSH/follicle coupling hypothesis.

The coupling hypothesis is consistent with an earlier interpretation that the decline in the wave-stimulating FSH surge plays a role in follicle selection in cattle [25]; exogenous FSH interfered with selection. The hypothesis is compatible with the threshold/window terminology developed for humans [12]. That is, the duration (window) that FSH concentrations of the wave-stimulating surge are above a threshold level is short for development of a single large follicle and prolonged for development of multiple

large follicles. Exogenous FSH widens the window and thereby induces the growth of multiple dominant follicles (10 mm or larger [17]). The coupling hypothesis differs from a proposal [26] that selection occurs in humans when the follicles are 2–4 mm. These diameters are equivalent to the time of an FSH increase in women [2, 12, 27], presumably at the stimulation of a wave. The period of the FSH increase was likened to a gate through which only the selected follicle can pass because it alone is at a favorable developmental stage [26]. In contrast, according to the FSH:follicle-coupling hypothesis, selection occurs days later from the continued close dependency of the follicles on FSH, resulting in rapid deprivation of the less-developed smaller follicles when the FSH concentrations have decreased to a critical level at the beginning of deviation.

FOLLICULAR FSH SUPPRESSANTS

Inhibin

Certain forms of inhibin are involved systemically in deviation through a suppressant action on circulating FSH concentrations. In this report, the term inhibin will be used when the specific form is not known. An FSH-suppressing activity of bovine and equine follicular fluid has been demonstrated by an increase in FSH concentrations after ablation of follicles [5] and a decrease in FSH after treatment with a proteinaceous fraction of follicular fluid [22]. In addition, administration of an inhibin antiserum increased plasma FSH concentrations and increased the number of large follicles and ovulations in both species [5]. In cattle, circulating concentrations of total inhibin were reported to increase concurrently with the emergence of waves [28] and with an increase in estradiol [29]. The time of an estradiol increase is equivalent to the beginning of deviation [30]. Treatment with an anti-inhibin 5 days after estrus (apparently after the beginning of deviation) resulted in an increase in FSH [29], suggesting that inhibin plays a role in the continued postdeviation FSH decrease in cattle.

In mares, total inhibin concentrations during the estrous cycle increase in the circulation at the time FSH concentrations are declining [31–33]. In a recent study (unpublished results), it was concluded that the first 2 days of the FSH decrease was caused by inhibin, based on the positive relationship between the number of experimentally retained follicles and the extent of the increase in circulating total inhibin concentrations and the corresponding decrease in FSH (Fig. 7). Near the expected day of deviation, total inhibin remained at elevated concentrations and likely contributed to the continuing FSH decline. In this regard, treatment with serum containing inhibin antibodies on the day the largest follicle was 20 mm and on the next day resulted in an increase in the number of 30-mm follicles [34]. A 20-mm largest follicle is close to a mean of 22.5 mm at the beginning of deviation [16]. The results therefore suggested that inhibin continues to be a postdeviation FSH suppressant in mares.

In women, circulating inhibin-A and inhibin-B have different concentration profiles during the menstrual cycle [27]. A rise in inhibin-B began near the peak of the FSH surge [27] and was high during the early follicular growth phase [4, 27]. In contrast, inhibin-A was low during the early growth phase [27] and began to increase approximately as estradiol increased or the largest follicle was first recognized by its largest diameter [4, 17]. Using the terminology of this minireview, inhibin-B apparently in-

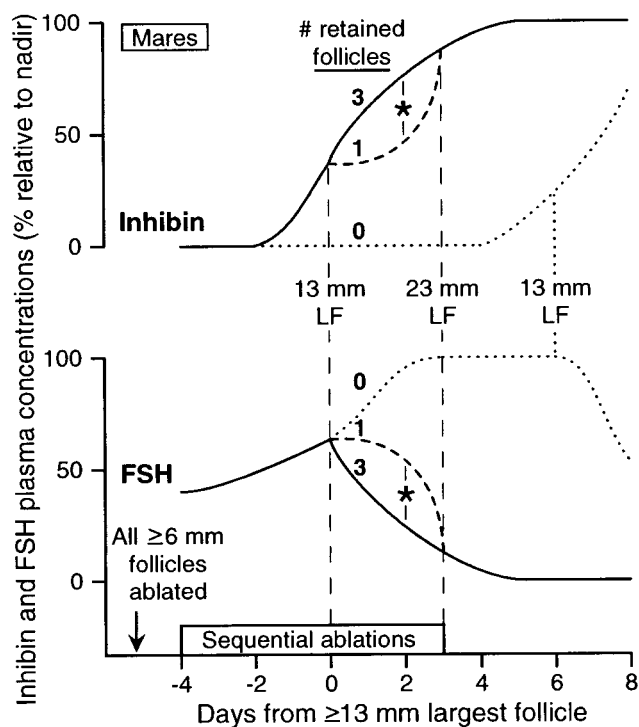


FIG. 7. Schematic presentation of the results of an experiment (unpublished) on the relationships among number of follicles and concentrations of inhibin and FSH during the decline in the FSH surge that stimulated emergence of a follicular wave. All follicles were ablated 10 days after ovulation. Various numbers of follicles ≥ 10 mm in the new wave were established by sequential ablations as needed. Three groups with zero, one, or three retained follicles are shown for each hormone. Data were normalized to the day the largest follicle (LF) reached 13 mm. Three retained follicles were more effective than one retained follicle in increasing the inhibin concentrations and decreasing the FSH concentrations. This differential effect ceased at the expected beginning of diameter deviation or when the largest follicle was 23 mm. A star indicates a difference ($P < 0.05$) in hormone concentrations between the groups with one and three retained follicles. The significant difference occurred on the same day for the two hormones.

creased during the common-growth phase and inhibin-A near or after the beginning of presumptive deviation.

Estradiol

In cattle, estradiol begins to increase in the circulation at the beginning of deviation [15, 30]. Experimentally increasing or decreasing the circulating estradiol concentrations at the expected beginning of deviation resulted in a decrease or increase, respectively, in FSH concentrations (Fig. 6), indicating a functional relationship between the two hormones. The largest follicle at the expected beginning of deviation (8.5 mm) had greater FSH-suppressing power than the largest follicle at 7.5 mm [18]. The added capacity of an 8.5-mm follicle for suppressing systemic FSH may reflect the combined action [35, 36] of an inhibin and estradiol.

In mares, estradiol begins to increase in the circulation the day before the beginning of deviation [37]. Thus, on a temporal basis, increased estradiol apparently does not contribute to the FSH decline until the day before deviation. Ablation of the largest follicle at the expected beginning of deviation prevented the continuing estradiol increase and resulted in an associated FSH increase [20]; an FSH increase did not occur when the second-largest follicle was ablated. Both estradiol and inhibins would be lost upon

ablation of the largest follicle and a decrease in either or both could account for the resulting increase in FSH.

In women, an increase in circulating estradiol occurs at about the time the dominant follicle is first identifiable by its larger diameter (>9 mm [4, 12, 38]). In monkeys, exogenous estradiol reduces the concentrations of FSH [39], and an antiestrogen increases FSH concentrations and causes maturation of multiple follicles [40, 41].

Summary

Inhibin from the wave of growing follicles apparently is the principal, if not the only, systemic FSH suppressant during most of the common-growth phase in the three species. Circulating estradiol concentrations increase near the beginning of deviation, and it appears that both inhibin and estradiol contribute to the subsequent continuation of the FSH decline. The estradiol:deviation relationships in cattle and apparently in women are similar to those in mares, except for a longer interval in mares between increased estradiol secretion into the circulation and the beginning of deviation. A schematic diagram of the systemic relationships among FSH, total inhibin, estradiol, and follicle development is shown for mares (Fig. 8).

ROLE OF LH

In heifers, LH concentrations increased between 32 and 24 h before deviation [15, 42], remained elevated for about 48 h, and then decreased [22, 43]. A small, but significant, transient elevation in LH was characterized for both the first and second follicular waves of the estrous cycle, with data normalized to the expected beginning of deviation [44]. The profile for elevated LH concentrations was similar for the two waves, but the concentrations were about twofold greater for wave 1 than for wave 2. In mares, elevated LH concentrations were temporally associated with deviation and often (58% of waves) were represented by a distinct part of the prolonged ovulatory LH surge in this species [16]. In women, an LH elevation in association with presumptive deviation has not been reported, and its detection, if present, may require normalizing the LH concentrations to the beginning of deviation.

In cattle, the granulosa cells had greater LH receptor (LHr) activity on the day after expected deviation than on the day before deviation [45, 46]. In a recent study [47], an increase in the differences between the two largest follicles in granulosa LHr mRNA expression occurred 8 h before an increase in the differences between the two follicles in diameter and in follicular-fluid estradiol concentration. The content of LHr protein in equine granulosa cells was greater when the follicles were 15–19 mm than 10–14 mm, and aromatase content was greater when the follicles were 20–24 mm than 15–19 mm [48]. In conclusion, the granulosa cells of the future dominant follicle acquire LHrs in heifers and apparently in mares just before the beginning of deviation, providing a pathway for a functional LH effect of the early portion of the transient LH elevation. Thus, the early portion of the LH elevation followed by the expression of LHr in the granulosa cells are the first known events that can be assigned to the cascade of events leading to deviation during the continuing FSH decline.

In cattle, an experimental reduction of LH by progesterone treatment at the expected beginning of deviation was associated with a reduction in the follicular-fluid concentrations of estradiol, androstenedione, and insulin-like growth factor (IGF)-1 in the two largest follicles (unpub-

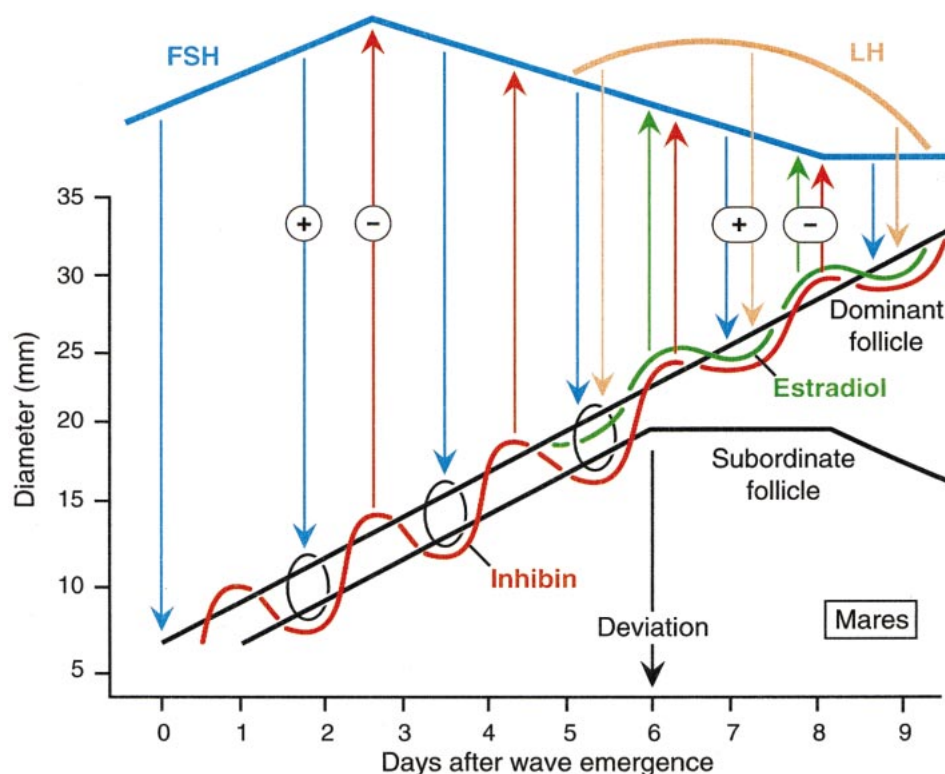


FIG. 8. Illustration of the circulating hormonal aspects of deviation using a two-follicle model. When the follicles reach about 13 mm, they both secrete increasing concentrations of an inhibin during the common-growth phase (before deviation). About a day before deviation, increased estradiol is secreted by the largest follicle under the influence of increased concentrations of LH. Apparently, the increasing estradiol acts in conjunction with inhibin to continue the reduction in FSH concentrations after deviation. The elevated LH continues to stimulate the production of estradiol by the developing dominant follicle and has a positive diameter effect on the dominant follicle within 2 days after the beginning of deviation.

lished results). This result indicated that these factors are stimulated by the early portion of the LH elevation associated with deviation. Experimental reduction of LH concentrations decreased the diameter of the dominant follicle beginning 1 or 2 days after the beginning of deviation in cattle [43] and mares [49, 50]. The smaller diameter of the dominant follicle in association with experimental LH reduction also occurs in cattle in association with natural LH reduction. That is, lower concentrations of the LH elevation for wave 2 compared to wave 1 (discussed above) were associated with smaller diameter of the dominant follicle beginning 32 h after the beginning of deviation [43]. Reduction of LH by progesterone treatment beginning when the largest follicle was 9.0 mm (about 8 h after the beginning of deviation) resulted in a reduced diameter and concentrations of estradiol and IGF-1 in the follicular fluid of the dominant follicle 24 h later [43]. These results indicated that an LH requirement for normal postdeviation growth and function of the dominant follicle represents an influence of the postdeviation portion of the LH elevation. The role of the transient LH elevation is included in the schematic models (Figs. 8 and 9).

INTRAFOLLICULAR AND CELLULAR ASPECTS

This section should be considered with reference to the accompanying schematic model (Fig. 9). The two-cell:two-gonadotropin model of follicular estrogen synthesis is well accepted for many species [51]. The enzyme cascade responsible for androgen biosynthesis in the theca cells is stimulated by LH, beginning in the early stages of follicle growth [52]. The androgens in turn are aromatized into estrogens in the granulosa cells by the cytochrome P450 aromatase (P450_{arom}) enzyme coupled to the FSH-cAMP signaling pathway [51]. In cattle, pieces of theca and attached granulosa from walls of the largest and second-largest follicles on Days 2 or 3 of a wave (approximately equivalent

to the beginning of deviation) secreted similar amounts of androstenedione in culture [53]. Furthermore, the concentrations of androstenedione were similar in follicular fluid of the largest and second-largest follicles. The stimulatory effect of LH was equivalent for both follicles at the beginning of deviation, based on a similar reduction in follicular-fluid androstenedione following experimental reduction in circulating LH (unpublished results). There was no difference in LHR mRNA expression in the theca cells of the future dominant follicle and subordinate follicles [54]. It is concluded that LH binds to the LHR of the theca cells and stimulates the production of androgens to an equivalent extent in both the future dominant and the future subordinate follicles. Therefore, it is not likely that differential androgen concentrations between the two largest follicles are involved in initiating deviation.

Estradiol

In heifers, follicular-fluid estradiol concentrations began to increase in the largest follicle at the beginning of deviation [47, 55]. In mares, an increase in estradiol occurred in the follicular fluid [37], and echogenic changes characteristic of estrogen stimulation occurred in the wall [56] of the future dominant follicle the day before the beginning of deviation. In women, an increase in follicular-fluid estradiol occurred about the time the dominant follicle was first identifiable by its larger diameter [4, 12, 57]. Thus, for all three species, the beginning of increased local availability of estradiol approximately coincides with the beginning of deviation. Reported autocrine and paracrine activities of estradiol in the granulosa cells include enhancing aromatase enzyme activity, promoting expression of LHR, and enhancing the sensitivity to FSH and LH [58]; all of these functions seem relevant to the deviation mechanism. However, most of the studies on the role of estrogens in folliculogenesis were done in rodents. Specific study is

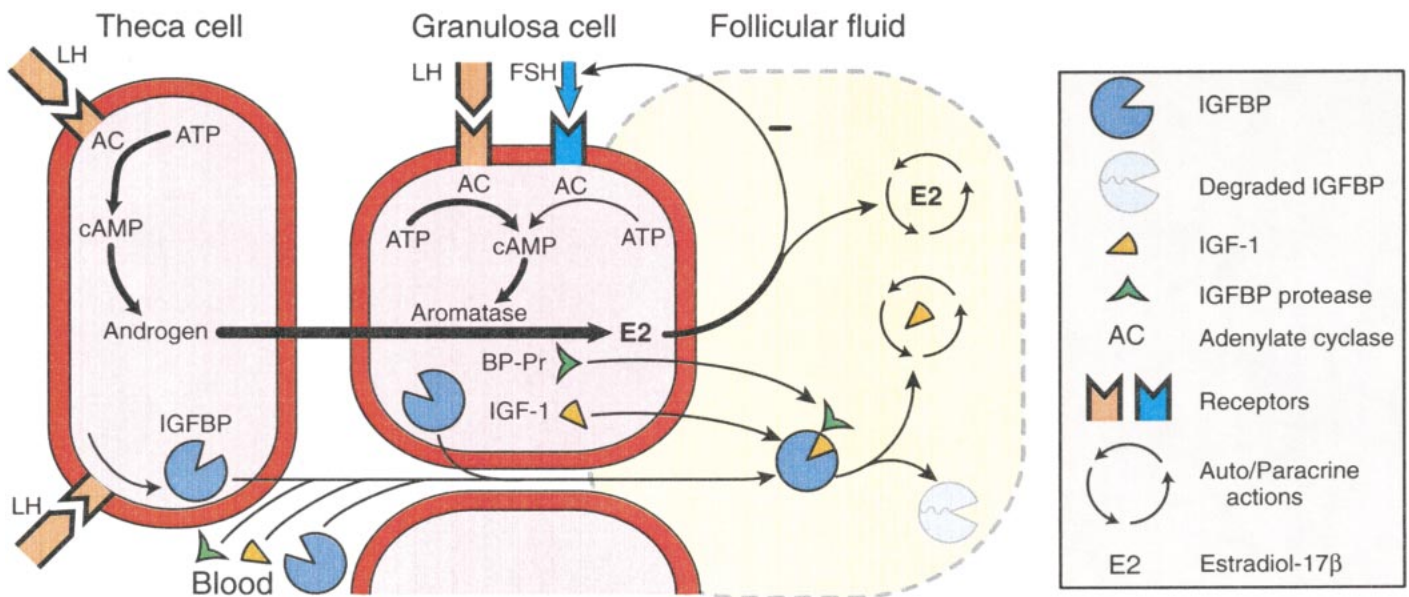


FIG. 9. Schematic model of the proposed interactions of the theca and granulosa cells of the future dominant follicle at the beginning of diameter deviation. The depicted activities allow the more-developed largest follicle to thrive despite the low levels of FSH. The second-largest follicle (not shown) has not reached this developmental level of activity and therefore is inhibited by the low levels of FSH, preventing the follicle from attaining similar status. It is intended that reference will be made to this model during reading of the text on cellular aspects of deviation.

needed in the monovular species because the local activities of estradiol may be crucial to the deviation mechanism. Increased responsiveness of the future or established dominant follicle to FSH could also occur through an increase in the number of FSH receptors in the granulosa cells; however, reported results in cattle on the differential acquisition of FSH receptors by the largest follicle versus the second-largest follicle seem inconclusive or inconsistent [46, 53, 54, 59].

The increased estradiol produced by the developing dominant follicle near the beginning of deviation may be attributed to the transient elevation in LH concentrations and the increased expression of LHR by the granulosa cells. The cellular model (Fig. 9) assumes that the increased LHR expression in the granulosa cells provides for direct LH-regulated activity of the cAMP system within the granulosa. In this regard, an LH-induced increase in cAMP and aromatase activity and estradiol synthesis occurred in cultured granulosa cells in humans [60]. In cattle, P450_{arom} enzyme expression was higher in the granulosa of cells of follicles averaging 10.8 mm than in follicles averaging 7.8 mm [61]. However, direct LH-induced secretion of estradiol within the granulosa apparently has not been demonstrated in either cattle [51] or horses [62]. Further study is needed, using cells obtained near the time of deviation.

Insulin-Like Growth Factor System

The IGF system consists of IGF-1 and IGF-2, IGF receptors, and a family of binding proteins (IGFBPs) and IGFBP proteases [7]. The paracrine-autocrine activities of IGF-1 and -2 include increasing cell growth, increasing estradiol production, and enhancing the sensitivity of the granulosa cells to FSH in cattle [63, 64] and humans [13]. Theca cells possess IGF-1 receptors [65] and LH-stimulated synthesis of androgen by cultured theca cells is synergistically enhanced by the presence of IGF-1 [66]. Apparently, IGF-1 has the potential to exert positive paracrine action on the growth and steroidogenic function in both theca and granulosa cells [63, 67]. The role of the IGF system in

deviation has been studied only in cattle. The levels of free IGF-1 did not change in the largest follicle while growing from 7.5 to 11.2 mm [47]. However, the concentrations of the free IGF-1 in the second-largest follicle decreased progressively during the same time span and was associated with continuously lower concentrations than in the largest follicle. These results suggested that the IGF system may be important to the deviation phenomenon. However, concentrations of free IGF-1 in the follicular fluid were not different between the two largest follicles when the largest follicle was a mean of 8.7 mm (near the beginning of expected deviation) [43]. In another study (unpublished results), the concentrations of free IGF-1 were not different between the two largest follicles at the expected time of deviation in 11 cattle. In the same study, estradiol concentrations were higher in the largest follicle, suggesting that changes in estradiol preceded changes in IGF-1. More study will be needed to clarify whether IGF-1 concentrations differ between the two largest follicles relative to the beginning of deviation and the increased production of estradiol.

Although there currently is ambiguity about the temporal relationship between IGF-1 concentrations and the beginning of deviation in cattle, results have consistently shown high concentrations of free IGF-1 in the postdeviation developing dominant follicle [43, 47]. Concentrations of free IGF-1 were higher in the dominant follicle when it was a mean of 10.8 mm than in the second largest follicle [43]. In this regard, granulosa cells of 9-mm follicles expressed mRNA for IGF-1 [68]. The postdeviation increase in IGF-1 apparently is important for optimal growth and function of the developing dominant follicle.

Experimental reduction of circulating LH concentrations was associated with lower levels of free IGF-1 in the follicular fluid of the two largest follicles near the expected beginning of deviation (largest follicle, 8.7 mm) and after deviation in cattle (largest follicle, 10.8 mm) (unpublished results), indicating another role for the transient elevation in LH. An LH-stimulated increase in estradiol production by the granulosa (discussed above) also could be secondary

to the stimulation of the IGF system as suggested in cattle by the following: 1) the follicular-fluid concentrations of both IGF-1 and estradiol decreased when the circulating concentrations of LH were reduced near the time of deviation (unpublished results) and 2) the addition of IGF-1 to a granulosa culture system resulted in increased production of estradiol [69].

Insulin-like growth-factor binding proteins may play a role in deviation by decreasing the bioavailability of IGF-1 and thereby interfering with the growth of the subordinate follicles. The granulosa cells expressed mRNA for IGFBP-2, but thecal cells did not [70]. Concentrations of IGFBP-2 were similar between the largest and second-largest follicles at the expected beginning of deviation [47]. The differential changes in the concentrations of IGFBP-2 did not occur until the largest follicle had reached 9.5–10.5 mm; the levels were higher in the second-largest follicle. Experimental reduction of LH concentrations resulted in higher levels of IGFBP-2 in the two largest follicles, indicating an additional function of the transient LH elevation (unpublished results). In addition to synthesis in the follicles, the changes in IGFBPs and IGF-1 content of follicular fluid reflect an uptake from the blood.

Expression of IGFBP-4 mRNA by theca cells in cattle was similar for small (1–4-mm), medium (4–8-mm), and large (>8-mm) follicles; granulosa cells did not express IGFBP-4 mRNA [70]. In addition, LH stimulated the expression of IGFBP-4 by bovine theca cells *in vitro*. Nevertheless, the concentrations of IGFBP-4 were higher in the follicular fluid of the second- and third-largest follicles than in the largest follicle when it reached a mean of 7.6 mm [71]. Changes in IGFBP-4 levels in the follicular fluid have been attributed to IGFBP-4 specific protease in cattle [70] and humans [72]. The protease frees IGF-1 from the IGFBP-4:IGF-1 complex by degrading IGFBP-4. The protease was found in higher concentration in the largest follicle than in the second-largest follicle in heifers [73, 74]; however, the specimens were obtained after deviation, as indicated by mean estradiol concentrations in the largest follicle and second-largest follicle of 865 versus 5 ng/ml. An increase in IGFBP-4 protease and a decrease in IGFBP-4 reflect the availability of more IGFs to support the continued growth of the dominant follicle. In this regard, IGFBP-4 protease is regulated by IGF-1 in bovine and equine preovulatory follicles [75], and production of IGFBP-4 protease is stimulated by FSH in human granulosa cells [76]. At an unknown point relative to deviation, the largest follicle may have higher IGFBP-4 protease activity because of greater responsiveness of the largest follicle than the smaller follicles to low levels of FSH. The lower responsiveness of the smaller follicles to the declining levels of FSH would result in less IGFBP-4 proteolytic activity, increased IGFBP-4, and decreased IGF-1 in the follicular fluid.

Inhibin

An intrafollicular role for inhibin and other proteinaceous factors in deviation has not been demonstrated and is not included in the schematic model (Fig. 9). However, forms of activin and inhibin from the granulosa cells are involved in androgen production from the theca [77]. Activin stimulates cAMP, aromatase activity, estradiol production, and an increase in gonadotropin receptors. The production of inhibins (α and β_A -subunits) is stimulated by LH *in vitro*, apparently through the cAMP pathway [78];

however, LH stimulation of inhibin apparently has not been studied *in vivo*. In cattle, follicular-fluid concentrations of follistatin, inhibin-A, and various molecular-weight forms of inhibin were similar among the three largest follicles when the largest follicle was a mean of 7.6 mm [71]. In a study [47] using expected deviation as a reference, no differential change in total inhibin or inhibin-A was found between the largest follicle and the second-largest follicle before or at the beginning of deviation. In mares, inhibin α , β_A , and β_B subunits were detected in granulosa cells of follicles >30 mm [33], but studies have not focused on deviation that is expected to occur at 22.5 mm.

In women, inhibin concentrations in the ovarian venous blood did not differ between the ovary with the dominant follicle and the opposite ovary [12]. However, during follicle growth, the follicular-fluid environment changes from higher levels of activin to higher levels of inhibin-A:follistatin [4]. Follicular-fluid inhibin-A, but not inhibin-B, concentrations increased in the two largest follicles as both diameter and maturity increased [4]. These workers concluded that a transition from an activin follicular environment to an inhibin-A:follistatin environment is critical for dominant follicle development; however, it is not known whether the change to an inhibin-A:follistatin environment is involved in the deviation mechanism or only in the maturation of the dominant follicle after deviation has been established.

Summary

The transient elevation in LH at deviation stimulates the IGF system, as well as the steroid system. At the beginning of deviation, the largest follicle is able to utilize the low concentrations of FSH, presumably enhanced by local effects of estradiol and IGF-1. The second-largest follicle has not developed to a similar extent and therefore cannot withstand the low FSH concentrations. Thereby, deviation is established before the second-largest follicle can develop to a stage that would allow it to assume the biochemical characteristics of a future dominant follicle.

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