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The Effect of the Manipulation of Follicle-Stimulating Hormone (FSH)-Peak Characteristics on Follicular Wave Dynamics in Sheep: Does an Ovarian-Independent Endogenous Rhythm in FSH Secretion Exist?[1]

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ABSTRACT

We designed three experiments to investigate the relationship between FSH peaks and ovarian follicular waves and to examine whether an endogenous rhythm of FSH peaks exists in sheep. In experiment 1, anestrous ewes were treated with ovine FSH (oFSH) or vehicle (6 ewes per group) at the expected time of an endogenous FSH peak, to double the FSH-peak amplitude in treated ewes. In experiment 2, anestrous ewes were treated with either oFSH or vehicle (6 ewes per group) at the expected time of two consecutive interpeak nadirs, such that the treated ewes had 5 FSH peaks in the time frame of 3 FSH peaks in control ewes. In experiment 3, to measure FSH concentrations, daily blood samples were collected from 5 cyclic ewes for a control period during the estrous cycle and then for three 17-day periods after ovariectomy. Daily blood samples were collected from another group of 8 ovariectomized ewes that were treated with estradiol-releasing implants and intravaginal progestogen sponges. Doubling the FSH-peak amplitude did not alter the characteristics of the following follicular wave. Increasing the frequency of FSH peaks stimulated the emergence of additional follicular waves, but did not alter the rhythmic occurrence of FSH peaks and follicular wave emergence. Endogenous follicular waves in oFSH-treated ewes emerged and grew in the presence of the growing largest follicle of the induced follicular waves. Finally, based on the observation of serum FSH concentrations in ovariectomized ewes, it appears that there exists an endogenous rhythm for peaks in daily serum FSH concentrations, which is, at least in part, independent of regulation by ovarian follicular growth patterns.

endogenous rhythm, estradiol, follicle, follicle-stimulating hormone, FSH peaks, ovarian follicle, ovary, pituitary, sheep

INTRODUCTION

Ovarian antral follicular growth occurs in a wave-like pattern in sheep, both during the breeding season [1–8] and seasonal anestrus [9, 10]. The emergence of follicular waves is preceded by a transient peak in circulating FSH concentrations [2, 3, 6, 8–14]. However, the relationship between the characteristics of FSH peaks and the emergence of follicular waves in ewes has not been studied [15]. Treatment of ewes with a superovulatory dose of FSH results in increased numbers of follicles growing to an ovulatory diameter [16–19]; however, the effects of changes in the amplitude of FSH peaks within a physiological range have not been tested. In a recent study from our laboratory [12], we demonstrated that the presence of the largest follicle of a wave induced by a treatment with ovine FSH (oFSH) did not postpone the occurrence of the next endogenous FSH peak. However, it is not known whether an increase in the frequency of FSH peaks, using repeated injections of FSH, would disrupt the rhythmic occurrence of endogenous FSH peaks and the regular emergence of follicular waves. As injection of oFSH induced a follicular wave that did not disrupt the normal rhythm of FSH peaks and follicular waves [12], a question arose as to the regulation of the peaks in FSH secretion that precede follicular waves. Could there be an ovarian-independent endogenous rhythm of FSH secretion (i.e., periodic peaks in serum FSH concentrations) in the ewe?

We designed a series of three experiments to examine the relationship between FSH peaks and follicular wave dynamics in nonprolific Western White Face ewes. Experiment 1 was intended to monitor the effect of a doubling of the FSH peak amplitude on the growth of antral follicles in the following follicular wave. Experiment 2 was designed to monitor the effect of increasing the frequency of peaks in FSH concentrations (peaks at an interval of 2 to 2.5 days compared with the normal interval of 4 to 5 days) on the rhythmic occurrence of endogenous FSH peaks and the emergence of follicular waves. The aim of experiment 3 was to determine whether periodic peaks in serum FSH concentrations were present in ovariectomized ewes, similar to those in ovary-intact ewes.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Hormone Preparation

One milligram of the oFSH preparation (NIDDK-oFSH-18) used in the present study has a biological potency of 65.6× NIH-oFSH-S1, or 1640 IU; and 0.1× NIH-oLH-S1, or 106 IU. The oFSH for injection was prepared in saline with 0.05% BSA (w/v; Sigma Chemical Co., St. Louis, MO) and 50% polyvinylpyrrolidone (w/v; Sigma) [12].

Transrectal Ovarian Ultrasonography and Blood Sampling

In both experiments 1 and 2, the anestrous ewes underwent transrectal ovarian ultrasonography twice daily (0800 and 2000 h) until the largest follicle of the third follicular wave attained its maximum diameter (see below for details), using a real-time B-mode echo camera (Aloka SSD-900; Aloka Co., Ltd., Tokyo, Japan) connected to a 7.5-MHz transducer.

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During each examination, the relative position and diameter of all follicles ≥1 mm in diameter and corpora lutea were sketched onto ovarian charts. In addition, all ovarian images were recorded on high-grade video tapes (Fuji S-VHS, ST-120 N; Fujifilm, Tokyo, Japan) for retrospective analysis of ovarian data. Blood samples (10 ml) were collected from all ewes by jugular venipuncture into vacutainers (Becton Dickinson, Rutherford, NJ) just before each ultrasonographic examination.

Hormone Analyses

Blood samples were allowed to clot for about 24 h at room temperature and the resultant harvested serum was stored at −20°C until assayed. Serum concentrations of FSH [20] and estradiol [21] were determined by established radioimmunoassays. Serum concentrations of FSH are expressed in terms of oFSH-SIAFP-RP-2. The ranges of the standards for FSH and estradiol assays were 0.12 to 16.0 ng/ml and 1 to 100 pg/ml, respectively. The sensitivities of the assay (defined as the lowest concentration of hormone capable of significantly displacing labeled hormone from the antibody) were 0.1 ng/ml for FSH assays and 1 pg/ml for estradiol assays. The intraassay and interassay coefficients of variation (CVs) for FSH assays for sera with a concentration of 0.46 ng/ml or 1.37 ng/ml, were 6.5% and 9.1%, or 6.2% and 8.8%, respectively; and for estradiol assays, for sera with a concentration of 3.5 pg/ml or 12.0 pg/ml, the CVs were 16.5% and 7.1% or 14.2% and 8.9%, respectively.

Experiment 1

Twelve adult, anestrous (June–July) Western White-Face ewes (age, 2–3 yr; mean body weight, 90.0 ± 5.6 kg) were used in this experiment. The ewes were kept outdoors in sheltered pens and were fed daily maintenance rations of alfalfa hay, with water available ad libitum.

Treatment involved two s.c. injections of either oFSH (0.5 μg/kg) or vehicle given 8 h apart. This treatment regimen was based on preliminary trials and was shown to result in an FSH peak of physiological amplitude [12]. Six ewes were injected with oFSH (oFSH-treated group) and six control ewes were injected with vehicle only.

A 4-mm follicle that grew from the pool of 2- to 3-mm follicles was detected with ultrasonography, and it was designated as the wave 1 follicle. Such a follicle had to have emerged (and grown beyond the 2- to 3-mm stage) 3–5 days after the emergence of the previous follicular wave (i.e., at the normal time interval for follicular waves in ewes; [9]). The first injection of oFSH or vehicle was given 60 h after the detection of the 4-mm follicle, but only if the follicle had grown to ≥5 mm to establish a follicular wave; the second injection was given 8 h later. The timing of the treatment was designed to give oFSH or vehicle injections at the expected time of the peak in endogenous FSH concentration associated with the emergence of a follicle wave (designated as wave 2).

The mean serum FSH concentrations in FSH-treated and control ewes from 6 days before to 3 days after the injection of oFSH or vehicle (Day 0) are shown in Figure 1. There was a peak in serum FSH concentrations on Day −4 in both FSH-treated and control ewes ($P > 0.05$), and it was associated with the emergence of follicular wave 1 in both groups (Table 1). The next FSH peak in control ewes occurred on Day 0.5 after treatment (2.8 ± 0.3 ng/ml; Fig. 1). In FSH-treated ewes, there was a peak in FSH concentration on Day 1 after oFSH injection (5.1 ± 0.2 ng/ml). The FSH concentrations in FSH-treated ewes were significantly higher ($P < 0.001$) from Day 0.5 to Day 1.5 after the treatment, compared with control ewes (Fig. 1). As expected, the FSH peak in FSH-treated ewes was approximately twice as high as in control ewes.

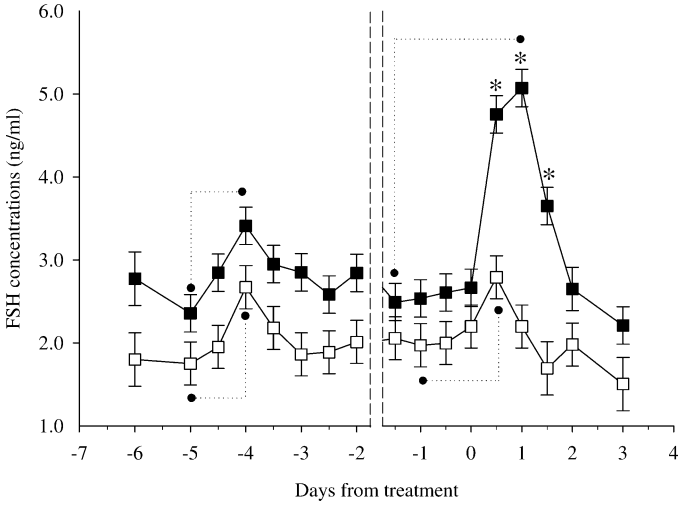

FIG. 1. Mean (± SEM) serum FSH concentrations in FSH-treated (black squares) and control (white squares) ewes (n = 6 per group), from 6 days before to 3 days after treatment (Day 0) with oFSH or vehicle. The ewes were treated 60 h after ultrasonographic detection of the largest follicle of wave 1 at 4 mm in diameter. The treatment was targeted to be administered at the expected time of a peak in endogenous FSH concentrations. FSH concentrations were centralized to the days of FSH peaks, which were Day −4 (left box) and Day 0.5 (right box) in relation to the day of treatment (Day 0), and are shown for Day −6 to Day −2 for the first peak and for Day −1.5 to Day 3 for the second peak. Dotted lines show major statistical trends; the major statistical trends delimit the periods during which there were significant ($P < 0.05$) progressive increases (nadir-to-peak) in mean FSH concentrations. *$P < 0.05$ (between FSH-treated and control ewes).

Experiment 2

Twelve adult, anestrous (May–June) Western White-Face ewes (age, 2 to 3 yr; mean body weight, 86.1 ± 1.8 kg) were used in this experiment.

Treatment, as in experiment 1, involved two s.c. injections of either oFSH (0.5 μg per kg) or vehicle given 8 h apart. Six ewes were injected with oFSH (oFSH-treated group) and six control ewes were injected with vehicle only.

A 4-mm follicle, which grew from the pool of 2- to 3-mm follicles, was detected with ultrasonography, and it was designated as the wave 1 follicle. The first injection of the first treatment with oFSH or vehicle was

TABLE 1. Mean (± SEM) days of wave emergence, the number of follicles per wave and characteristics of the largest follicle of the waves that emerged before (wave 1) or after (waves 2 and 3) treatment, in oFSH-treated and control ewes during anestrus (experiment 1).

Variable	Group[c]	Wave 1	Wave 2	Wave 3
Day of wave emergence[a]	Treatment	−3.4 ± 0.3	0.5 ± 0.0	5.7 ± 1.0
	Control	−3.5 ± 0.0	0.7 ± 0.2	5.8 ± 1.4
Length of growth phase (d)	FSH-treated	2.7 ± 0.2	2.9 ± 0.2	2.7 ± 0.2
	Control	2.5 ± 0.2	2.9 ± 0.2	3.0 ± 0.2
Length of static phase (d)	FSH-treated	3.0 ± 0.4	2.3 ± 0.4	Not determined
	Control	2.5 ± 0.1	2.9 ± 0.1	Not determined
Maximum diameter (mm)	FSH-treated	5.7 ± 0.4	5.9 ± 0.4	5.7 ± 0.4
	Control	5.9 ± 0.5	6.0 ± 0.5	5.5 ± 0.5
Growth rate (mm/d)	FSH-treated	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
	Control	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
Number of follicles per wave[b]	FSH-treated	2.4 ± 0.2	1.9 ± 0.1	2.0 ± 0.1
	Control	2.1 ± 0.2	2.0 ± 0.1	2.2 ± 0.1

[a] Days of wave emergence are expressed relative to the day of treatment (d 0).
[b] Number of follicles that emerged at 2-3 mm in diameter and grew together to reach an ovulatory diameter of ≥ 5 mm.
[c] The ewes (n = 6 per group) were treated with either oFSH or vehicle at the expected time of the peak in serum FSH concentrations, which was associated with the emergence of wave 2.

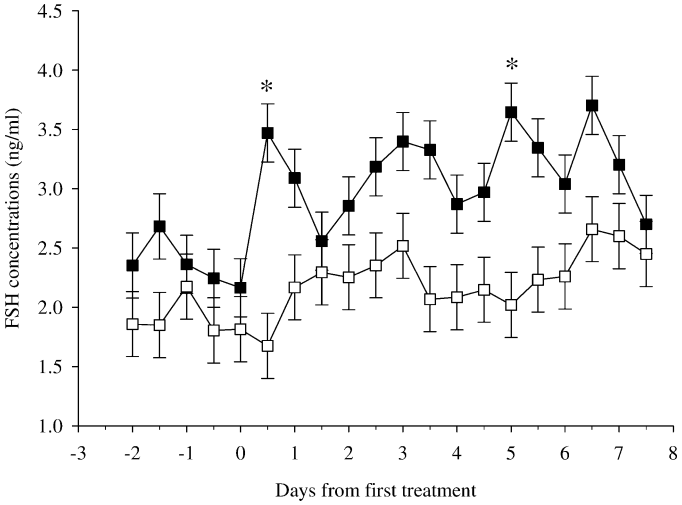

FIG. 2. Mean (± SEM) serum FSH concentrations in FSH-treated (black squares) and control (white squares) ewes (n = 6 per group), from 2 days before to 7.5 days after first treatment (Day 0) with oFSH or vehicle. The ewes were treated 12 h after ultrasonographic detection of the largest follicle of waves 1 and 2 at 4 mm in diameter. The treatments were targeted to be administered at the expected times of 2 consecutive nadirs in endogenous FSH concentrations. Asterisks denote significant ($P < 0.05$) differences between FSH-treated and control ewes.

given 12 h after the detection of the 4-mm follicle of wave 1, but only if this follicle remained at 4 mm or grew further. The second injection was given 8 h later. The follicular wave induced in the FSH-treated ewes by exogenous oFSH was designated wave A. Based on our previous study [12], the following wave, induced by endogenous FSH secretion and designated as wave 2, was expected to emerge at a normal interwave interval of 4 to 5 days after the day of emergence of wave 1. Once the largest follicle of wave 2 was first identified at 4 mm in diameter, the second treatment with oFSH or vehicle was begun, but only if this follicle remained at 4 mm or grew further. The first injection of the second treatment with oFSH or vehicle was given 12 h after the detection of the 4-mm follicle of wave 2 and the second injection was given 8 h later. The follicular wave induced by the second FSH injection was designated as wave B.

The next follicular wave induced by endogenous FSH secretion was designated as wave 3. Timing of the treatments was designed to give oFSH injections during the growth phase of the largest follicle of waves 1 and 2, and at the expected time of the 2 consecutive interwave nadirs (between waves 1 and 2, and waves 2 and 3) in endogenous FSH secretion. This regimen produced 5 peaks in serum FSH concentrations over a time period when only 3 peaks should have occurred.

The mean serum concentrations of FSH from 2 days before to 7.5 days after the first treatment with oFSH or vehicle (Day 0) in FSH-treated and control ewes are given in Figure 2. The first treatment was administered on Day 0 in both the groups of ewes and the second treatment was administered on Day 4.3 ± 0.5 after the first treatment. There were peaks in FSH concentration in FSH-treated ewes on Day 0.5 and Day 5.0 ($P < 0.01$; 3.5 ± 0.3 ng/ml and 3.7 ± 0.3 ng/ml, respectively; Fig. 2) compared with nadirs of FSH concentrations in control ewes on Day 0.5 and Day 5.0 (1.7 ± 0.3 ng/ml and 2.0 ± 0.3 ng/ml, respectively; Fig. 2).

Experiment 3

Cyclic Suffolk (n = 5) and Western White Face (n = 8) ewes were used in this experiment. Blood samples were collected daily for one estrous cycle (October–November) from the 5 Suffolk ewes (period A). Ovariectomy was performed on all ewes on Days 2–3 after ovulation in the following cycle. Blood samples were collected daily from the 5 ovariectomized Suffolk ewes for three separate 17-day periods; first, immediately following ovariectomy (November; period B); second, 2 mo after ovariectomy (January; period C); and third, during the following anestrus period (June; period D). Following ovariectomy, the other group of 8 Western White Face ewes was rested for a 17-day period, without any blood sampling, to allow for the postovariectomy increase and stabilization in serum FSH concentrations. Following the rest period, intravaginal sponges containing medroxyprogesterone acetate (60 mg; Veramix; Upjohn, Orangeville, ON, Canada) were inserted. Four days after sponging, each of the 8 ewes was fitted with a 5-cm silastic rubber implant (Dow Corning, Midland, MI) containing estradiol-17β (10% w/w; Sigma) [21], which remained in place for 10 days. On Day 10, both the sponge and implant were removed from the Western White Face ewes. Blood samples were collected daily from the day of insertion of estradiol implants (Day 0) to 1 day following sponge and implant removal (Day 11; period E).

Follicular Data Analysis

Follicular data were analyzed for experiments 1 and 2. A follicular wave consisted of a follicle or a group of follicles that emerged and grew from 2 or 3 mm in diameter to ≥5 mm (growth phase) and remained at their maximum diameter (static phase) before regressing to 2 or 3 mm in diameter (regression phase) [6]. Follicles emerging within a 24-h period were included in a wave [11]. The following follicular characteristics were analyzed: 1) time of emergence of the follicular waves before and after treatment (expressed in relation to the day of treatment); 2) intervals between emergence of two successive follicular waves (interwave intervals); 3) number of follicles growing from 2 to 3 mm to ≥5 mm diameter per wave; 4) maximum diameter attained by the largest follicle of a follicular wave; and 5) durations of growing, static, and regression phases for the largest follicle of a wave. In addition, daily numbers of small follicles (≥1 mm but ≤3 mm in diameter) were noted for each ewe. The data for small ovarian antral follicles were centralized and analyzed as described for serum FSH concentrations in experiments 1 and 2 (see below for details).

Hormone Data Analysis

Serum FSH concentrations were centralized to the day of treatment (Day 0) and analyzed for the periods from 6 days before to 3 days after the treatment for experiment 1, and from 2 days before to 7.5 days after the first treatment (Day 0) for experiment 2. For experiment 3, daily serum FSH concentrations in ovary-intact ewes (period A) were centralized to the day of ovulation (Day 0), while serum FSH concentrations in ovariectomized ewes were centralized to the first day of blood sampling (Day 0; periods B, C, D, and E). Serum FSH concentrations in experiment 3 were analyzed for the period from Day 0 to Day 17 of blood sampling. Peaks in daily serum concentrations of FSH, in all the 3 experiments, were determined using the cycle-detection program [22]. As expected [21, 23], the overall mean serum concentrations of FSH increased after ovariectomy, reaching concentrations of 7 to 20 ng/ml, depending on the time after ovariectomy.

The cycle-detection program uses the mean FSH concentration for the data set and the CV of the FSH assay to determine peaks in serum FSH concentrations [22]. The CVs of our assays for experiment 3 ranged from 3% to 5%. The basal FSH concentrations postovariectomy had increased by 350% during period B and by 500% during period C compared with ovary-intact ewes during period A of blood sampling. The amplitude of FSH peaks in ewes has been reported to be about 1 to 2 ng/ml [2, 6, 8]. Applied to the data of the present experiment (experiment 3), the cycle-detection program did not identify many apparent peaks in serum FSH concentrations in ovariectomized ewes, while it identified all the peaks in serum FSH concentrations in ovary-intact ewes. To ensure that the program detected peaks in daily serum FSH concentrations of the magnitude seen in normal ovary-intact ewes, but in the face of elevated basal concentrations of FSH postovariectomy, the daily serum concentrations of FSH in all the ewes in experiment 3 (including ovary-intact ewes of period A) were transformed. The transformation of FSH data was intended to correct the high mean FSH values in ovariectomized ewes to those observed in ovary-intact ewes.

To achieve this, a constant (k) value was subtracted from each FSH value in a data set of serum FSH concentrations in each ewe. The constant, k, for each FSH data set was determined by using the formula k = FSH mean − 1.9, where FSH mean is the mean serum FSH concentration for a ewe during a period of blood sampling, and 1.9 is the overall mean FSH concentration for ovary-intact ewes (period A; Fig. 5A). This transformation effectively removed the high basal values for FSH concentrations but retained the variation due to peaks in hormone concentrations. The transformed FSH data set was then subjected to the cycle-detection program. The following variables for serum FSH concentrations were analyzed in experiment 3: 1) overall mean concentrations of FSH (nontransformed data) for each period of blood sampling, 2) number of FSH peaks in the transformed data identified by the cycle-detection program, 3) amplitude of FSH peaks in the transformed data identified by the cycle-detection program, and 4) interpeak intervals in the transformed data for each period of blood sampling. Serum estradiol concentrations in the ovariectomized ewes treated with estradiol-releasing implants were cen-

TABLE 2. Mean days on which peaks in serum FSH concentrations were detected, using the cycle detection computer program, and mean days of emergence of follicular waves in FSH-treated and control ewes (n = 6 per group; experiment 2), in relation to the day of first treatment (d 0).[a]

Variable	Group[b]	Peak 1	Peak A	Peak 2	Peak B	Peak 3
Day of FSH peak	FSH-treated	−1.5 ± 0.1	0.5 ± 0.0	3.0 ± 0.2 (−1.2 ± 0.2)	5.0 ± 0.0 (0.5 ± 0.0)	7.4 ± 0.1 (3.0 ± 0.3)
	Control	−1.5 ± 0.2	No peak A	2.9 ± 0.2 (−1.5 ± 0.2)	No peak B	7.3 ± 0.5 (2.5 ± 0.6)
		Wave 1	Wave A	Wave 2	Wave B	Wave 3
Day of wave emergence	FSH-treated	−1.3 ± 0.1	0.6 ± 0.1	2.9 ± 0.2 (−1.4 ± 0.2)	4.8 ± 0.2 (0.7 ± 0.1)	7.4 ± 0.1 (3.0 ± 0.3)
	Control	−1.4 ± 0.2	No wave A	2.9 ± 0.1 (−1.4 ± 0.1)	No wave B	7.3 ± 0.5 (2.8 ± 0.6)

[a] All values are mean ± SEM. Values in parentheses are the mean days in relation to the day of the second treatment.
[b] The anestrous ewes were treated with oFSH or vehicle during the growth phase of the largest follicle in waves 1 and 2.

tralized to the day of implant insertion and analyzed for the period of the day of implant insertion (Day 0) to Day 11.

Statistical Analyses

Statistical differences were assessed by one-way or two-way analysis of variance (ANOVA) (SigmaStat Statistical Software, version 2.0 for Windows 95, NT and 3.1, 1997; Chicago, IL). Repeated-measures ANOVA was used when data were collected repeatedly over a period of time. Multiple comparisons were made with the Fisher least significant difference method. Results are reported as least square means and SEM. Statistical significance was defined as $P < 0.05$.

RESULTS

Experiment 1

Emergence of follicular waves. The mean days of emergence of follicular waves (waves 1, 2, and 3) in relation to the day of treatment (Day 0) with oFSH or vehicle (Table 1) did not differ significantly between the groups ($P > 0.05$). The interwave interval between waves 1 and 2 did not differ between FSH-treated and control ewes ($P > 0.05$; 4.2 ± 0.3 days vs. 4.5 ± 0.2 days, respectively). Likewise, the interwave interval between wave 2 that emerged immediately after treatment and the following wave 3 did not differ between the two groups of ewes ($P > 0.05$; 5.2 ± 1.0 day in FSH-treated ewes vs. 5.3 ± 2.0 days in control ewes). The mean daily numbers of small follicles (≥1 mm and ≤3 mm in diameter) did not vary ($P > 0.05$) with time, from 6 days before to 3 days after treatment, and did not differ between the two groups of ewes studied ($P > 0.05$). The overall mean numbers of small follicles for the period of analysis (which encompassed the emergence of waves 1 and 2; Table 1) were 13.9 ± 1.5 (range, 11 to 16) and 14.1 ± 1.6 (range, 11 to 16) in FSH-treated and control ewes, respectively.

Characteristics of follicular waves. The growth characteristics of the largest follicles of waves 1, 2, and 3 are given in Table 1. There was no difference ($P > 0.05$) between FSH-treated and control ewes in the duration of the growth and static phases, maximum diameter, and growth rate of the largest follicle in waves 1, 2, and 3. Likewise, the number of follicles that grew together from 2 to 3 mm to ≥5 mm to constitute a follicular wave did not differ ($P > 0.05$) between the two groups of ewes nor between follicular waves within each group of ewes (Table 1).

Experiment 2

Days of FSH peaks and the emergence of follicular waves. The mean days on which peaks in FSH concentrations were detected using the cycle-detection program and mean days of follicular wave emergence are given in Table 2. The mean interval between days of emergence of successive follicular waves (waves 1, A, 2, B, and 3 in FSH-treated ewes and waves 1, 2, and 3 in control ewes) was significantly shorter in FSH-treated ewes (2.2 ± 0.1 day; $P < 0.001$) than in control ewes (4.1 ± 0.2 day). However, if only the waves associated with endogenous FSH peaks (waves 1, 2, and 3) were considered in both the groups of ewes, then the interwave interval did not differ ($P > 0.05$) between FSH-treated and control ewes. The mean interwave intervals between waves 1 and 2, and waves 2 and 3 were 4.4 ± 0.5 day and 4.5 ± 0.5 day, and 4.4 ± 0.3 day and 4.2 ± 0.3 day in FSH-treated and control ewes, respectively. The mean daily numbers of small follicles (≥1 mm and ≤3 mm in diameter) did not vary ($P > 0.05$) with time from 2 days before to 7.5 days after the first treatment, and did not differ between the two groups of ewes studied ($P > 0.05$). The overall mean numbers of small follicles for the period of analysis (which encompassed the emergence of waves 1, A, 2, B, and 3 in FSH-treated ewes and waves 1, 2, and 3 in control ewes; Table 2) were 14.9 ± 2.5 (range, 12 to 18) and 15.6 ± 1.9 (range, 12 to 17) in FSH-treated and control ewes, respectively.

Characteristics of follicular waves. The growth characteristics of the largest follicles of waves 1, A, 2, B, and 3 in FSH-treated ewes and of waves 1, 2, and 3 in control ewes are given in Table 3. Diameter profiles of the largest follicles during the growth phase of waves are shown in Figure 3. There was no difference ($P > 0.05$) in the duration of the growth and static phases, maximum diameter and growth rate of the largest follicle in waves 1, 2, and 3 between FSH-treated and control ewes, and no difference ($P > 0.05$) between endogenous (waves 1, 2, and 3) and induced (waves A and B) follicular waves in FSH-treated ewes. Likewise, the number of follicles that grew together from 2 to 3 mm to ≥5 mm and that constituted a follicular wave did not differ ($P > 0.05$) between the two groups of ewes nor among follicular waves within each group of ewes (Table 3).

Experiment 3

Transformed values for daily serum FSH concentrations in representative ewes for each period of blood sampling are shown in Figure 4. Mean serum estradiol concentrations during the period of treatment with estradiol-releasing implants in ovariectomized ewes are overlaid on daily serum FSH concentrations in a representative ewe (Fig. 4E). The overall mean concentrations of FSH during each period of blood sampling were significantly higher ($P < 0.05$) in

TABLE 3. The number of follicles per wave and characteristics of the largest follicle of the waves that emerged before or after treatment in oFSH-treated and control ewes during anestrus (experiment 2).[a]

Variable	Group[c]	Wave 1	Wave A	Wave 2	Wave B	Wave 3
Duration of growth phase (d)	FSH-treated	2.6 ± 0.2	2.9 ± 0.1	2.8 ± 0.1	2.6 ± 0.2	2.7 ± 0.2
	Control	2.8 ± 0.2	No wave A	3.1 ± 0.1	No wave B	2.5 ± 0.2
Duration of static phase (d)	FSH-treated	3.1 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	3.2 ± 0.4	Not determined
	Control	3.0 ± 0.1	No wave A	3.0 ± 0.1	No wave B	Not determined
Maximum diameter (mm)	FSH-treated	5.5 ± 0.4	5.9 ± 0.4	5.2 ± 0.4	5.6 ± 0.4	5.7 ± 0.4
	Control	5.6 ± 0.5	No wave A	5.7 ± 0.5	No wave B	5.9 ± 0.5
Growth rate (mm/d)	FSH-treated	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
	Control	1.2 ± 0.1	No wave A	1.2 ± 0.1	No wave B	1.2 ± 0.1
Number of follicles per wave[b]	FSH-treated	2.4 ± 0.2	1.9 ± 0.1	2.9 ± 0.1	1.6 ± 0.1	1.9 ± 0.3
	Control	2.1 ± 0.2	No wave A	2.7 ± 0.1	No wave B	1.3 ± 0.3

[a] All values are mean ± SEM.
[b] Number of follicles that emerged at 2-3 mm diameter and grew together to reach an ovulatory diameter of ≥ 5 mm.
[c] The anestrous ewes were treated with oFSH or vehicle during the growth phase of the largest follicle in waves 1 and 2.

ovariectomized ewes compared with ovary-intact ewes (Fig. 5A). Mean FSH concentration increased with time after ovariectomy as indicated by the significant difference ($P < 0.05$) between the control period (period A) and a period immediately following ovariectomy (period B), 2 mo after ovariectomy (period C), during the following anestrus (period D), and during the treatment with estradiol-releasing implants (period E). There were no significant differences ($P > 0.05$) in the number of FSH peaks detected (Fig. 5B), the amplitude of FSH peaks (Fig. 5C), or interpeak intervals (Fig. 5D) among the periods of blood sampling.

DISCUSSION

In a previous study from our laboratory [12] we demonstrated the possibility of inducing the emergence of a new follicular wave by exogenous oFSH in both cyclic and anestrous ewes. Previously, it was shown that the patterns of peaks in serum FSH concentrations and associated follicular waves are remarkably similar between cyclic and anestrous ewes [9, 10]. In the present study (experiments 1 and 2), the use of anestrous ewes was justified, as their endocrine milieu is not complicated by changes in progesterone concentrations and LH secretory pattern.

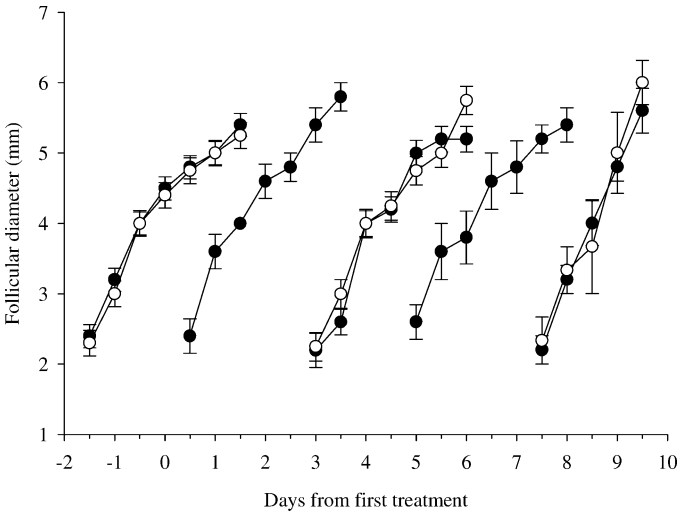

FIG. 3. Diameter profiles (means ± SEM), during the growth phases, of the largest follicles of waves 1, A, 2, B, and 3 in FSH-treated ewes (black circles; n = 6) and of waves 1, 2, and 3 in control ewes (white circles; n = 6). The diameter profiles were centralized to the mean day of emergence of individual waves.

Our regimen of oFSH administration in experiment 1, as expected, doubled the amplitude of the endogenous FSH peak preceding a follicular wave in anestrous ewes (Fig. 1). However, none of the growth characteristics of the largest follicles of the FSH-induced wave (FSH-treated ewes)

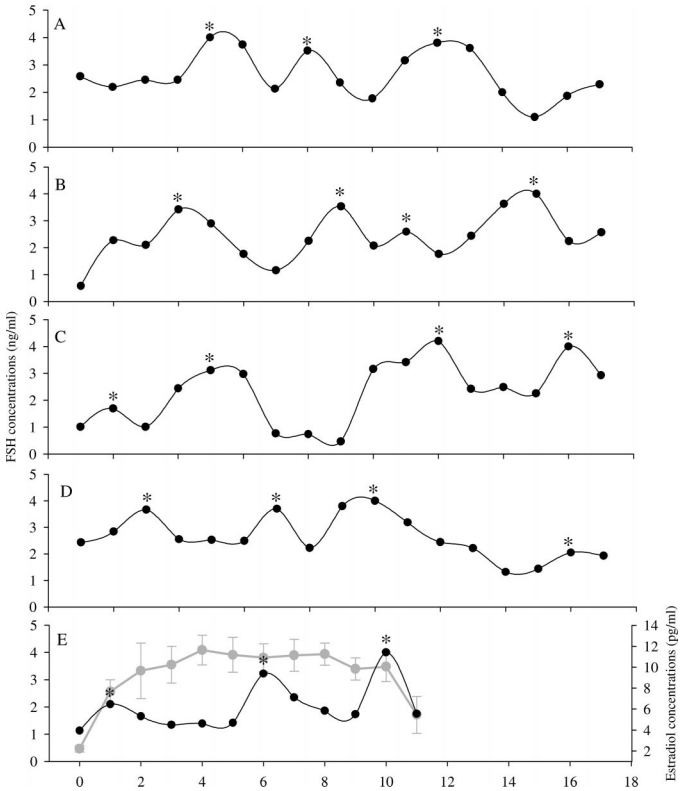

FIG. 4. Daily serum FSH concentrations in representative ewes from all periods of bleeding including the control period (ovary-intact ewes; period A), immediately after ovariectomy (period B), 2 mo after ovariectomy (period C), the following anestrus (period D), and a period of treatment with estradiol-releasing implants (separate group of ewes; period E). Asterisks denote successive FSH peaks as determined with the cycle-detection program [22]. To ensure that the cycle-detection program detected peaks in daily serum FSH concentrations, of the magnitude seen in normal intact ewes but in the face of elevated basal concentrations of FSH, the daily serum concentrations of FSH in all the ewes in experiment 3 (including ovary-intact ewes of period A) were transformed. The transformation of FSH data were intended to correct the high mean FSH values in ovariectomized ewes to those seen in ovary-intact ewes (see text for details). Mean (± SEM) daily concentrations of estradiol in the group of ewes treated with estradiol-releasing implants are overlaid on the daily FSH concentrations in a representative ewe (E).

differed in comparison to the largest follicles of the corresponding wave in vehicle treated ewes (Table 1).

Suppression of the secondary surge in FSH secretion, after the preovulatory gonadotropin surge, blocks the emergence of the first follicular wave of the cycle in heifers [24]. Injection of a superovulatory dose of FSH before the selection of a dominant follicle in heifers increases the diameter of the subordinate follicles while it decreases the diameter of the dominant follicle [25]. A superovulatory dose of FSH stimulates the growth of multiple ovulatory-sized follicles in sheep [16–19]. However, the effects of superovulatory doses of FSH are not physiological. There is a great deal of evidence that FSH is the key regulator of recruitment of follicles into waves in most species, and in the ewe, there is a threshold of FSH concentration below which recruitment cannot proceed [15]. This threshold appears to vary among ewes [26] and among follicles within a specific ewe [27]. In the present study (experiment 1), doubling the concentrations of FSH in the peaks that precede follicular waves did not affect either the number or the growth characteristics of small, nonrecruited follicles or FSH-dependent follicles recruited into a wave. Thus, it is logical to argue that serum FSH concentrations within a physiological range do not necessarily correlate with the number of ovulatory-sized follicles growing in a wave. Several lines of evidence further support this argument. No study, as yet, has shown a correlation between serum FSH concentrations and the number of follicles recruited into a wave [15, 28]. Previous studies [27, 29–31] have reported no differences either in mean circulating FSH concentrations or in the concentration and duration of the FSH peaks that precede follicular waves [5, 6] between prolific and nonprolific breeds of sheep. In homozygous Booroola *FECB* gene carrier ewes, the serum FSH concentrations have been shown to be higher than in noncarrier ewes [32–34]. However, it has been suggested that the *FECB* gene exerts its effects at the ovarian level by increasing the follicular sensitivity to FSH rather than by increasing pituitary FSH secretion [35, 36]. It would be of interest to examine the effects of the *FECB* gene on the characteristics of peaks in serum FSH concentrations that precede follicular wave emergence.

In cattle, cauterization of the dominant follicle of the first wave advances the emergence of the second wave of the cycle [37]. This indicates that, in cattle, the lifespan of the dominant follicle influences the time of occurrence of the next endogenous FSH peak [38]. Such a concept has also been suggested for sheep [39–41]. However, this concept was not supported in the sheep by a recent study [12], wherein the largest follicle (apparent dominant follicle) of a wave induced by exogenous oFSH did not postpone the next endogenous FSH peak and emergence of a follicular wave. In the present study (experiment 2), the emergence of waves A and B (induced waves) did not alter the time of occurrence of the endogenous FSH peaks that preceded the emergence of waves 2 and 3 (endogenous waves; Fig. 2 and Table 2). Waves 2 and 3 clearly emerged during the growth phases of waves A and B. The results of experiment 2 strongly support the notion that serum FSH concentrations in sheep may not be under the stringent control of growing antral follicles [12].

In studies on superovulation in sheep, the presence of a large follicle may [42] or may not [43, 44] affect the ewe's response to a superovulatory treatment. Similarly, in cattle, there are contradictory reports [45] that support [46–49] or fail to support [50–52] the inhibitory effects of the presence

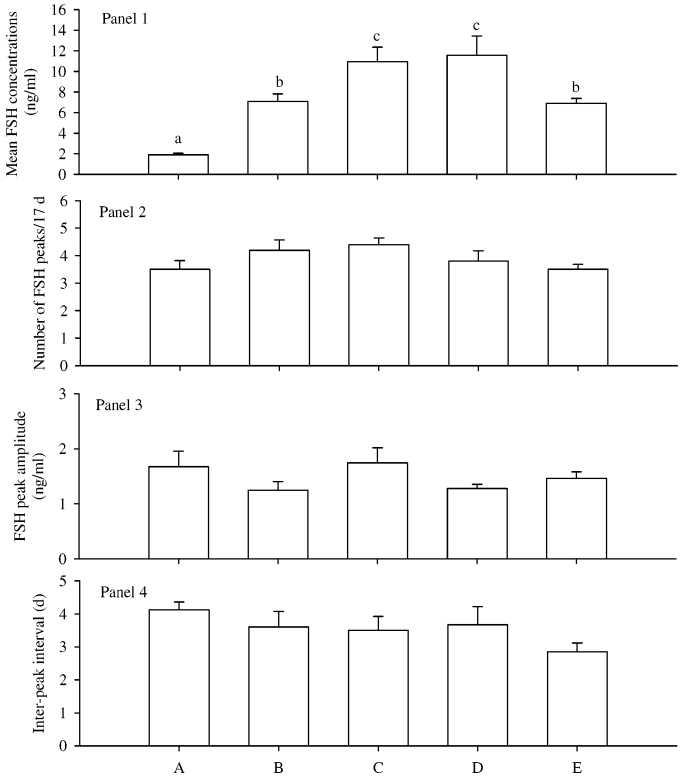

FIG. 5. Overall mean concentration of FSH (**1**), number of FSH peaks (**2**), FSH peak amplitude (**3**), and interpeak interval (**4**) for the 17-day periods of blood sampling (periods A, B, C, D, and E; n = 5–8 ewes). All values are means ± SEM. Daily blood samples were collected from a group of 5 ewes for one estrous cycle and these ewes were ovariectomized on Day 2–3 after ovulation in the following cycle. Daily blood samples were also collected during the three 17-day periods: immediately after ovariectomy (period B), 2 mo after ovariectomy (period C), and during the following anestrus (period D). A separate group of 8 ovariectomized ewes was treated with estradiol-releasing implants and daily blood samples were collected for a 10-day period (period E). [a–c]Means denoted by different letters differ ($P < 0.05$).

of a dominant follicle on the superovulatory response. In experiment 2, it could be argued that the induction of additional follicular waves (waves A and B) by exogenous oFSH overrode the dominance of the largest follicles of the noninduced (endogenous) follicular waves (waves 1, 2, and 3). However, the noninduced follicular waves (waves 2 and 3) in FSH-treated ewes emerged during the growth phase of the largest follicles of waves A and B (Fig. 3). The growth characteristics of the largest follicles of the induced follicular waves in the present study did not differ from those of the largest follicles of the noninduced follicular waves in both FSH-treated and control ewes (Table 3). Previously, we have shown that the largest follicles of oFSH-induced follicular waves were associated with normal peaks in estradiol concentrations as compared with endogenous follicular waves in both FSH-treated and control ewes [12]. These observations indicate that the largest estrogen-producing (presumptive dominant) follicles of waves A and B were not effective in postponing the occurrence of the next endogenous FSH peaks and follicular waves in experiment 2. The uninterrupted emergence of 5 follicular waves in FSH-treated ewes in the time frame of the emergence of 3 follicular waves in control ewes suggested that small FSH-sensitive follicles are available on a daily basis to enter a wave in response to a physiological FSH stimulus. This supposition is supported by our previous observation that

the pool of small follicles (1 to 3 mm in diameter) remains constant, except during the periovulatory period, despite the rhythmic emergence of 3 to 4 follicular waves in each ovine estrous cycle [11]. The concept of follicular dominance does not appear to be as convincing in the ewe as it is in cattle [11, 15, 27, 44, 53]; at least in breeds of sheep, which are strictly monovulatory [40, 41]. Taken together, it appears that the large ovulatory-sized follicles in a wave in sheep do not exert dominance to the extent observed in cattle.

Several studies have demonstrated that FSH secretion is regulated by factors produced by growing antral follicles such as estradiol [21, 54–57] and inhibin [23, 58]. The studies showing negative effects of estradiol treatment on pituitary FSH secretion in ovary-intact [57] or ovariectomized [21] ewes have largely used high (supraphysiological) doses of estradiol. At physiological levels, an inverse temporal relationship between serum concentrations of estradiol and FSH in association with the growth of follicular waves has been demonstrated during the breeding season [6, 59], but not during anestrus [9, 10, 60, 61]. However, peaks in FSH concentrations and associated follicular waves in sheep have been observed during both the breeding and nonbreeding seasons [1, 9, 10, 60–62]. The studies showing a negative effect of inhibin on FSH secretion have used various preparations such as follicular fluid (crude inhibin) [63] or human recombinant inhibin A [64], or passive immunization against inhibin [23]. It appears there are no negative relationships between circulating concentrations of inhibin and FSH in physiologically uncompromised ewes [3, 10]. In addition, there have been reports that additional follicular factors, other than estradiol and inhibin, can suppress FSH and follicular growth [65, 66]. A steroid-depleted fraction of follicular fluid suppressed follicle development in cattle even though the injected follicular fluid was >95% free of inhibin [65]. In one other study [66] in heifers, follicular fluid suppressed both FSH secretion and follicular growth despite the injection of an inhibin antiserum with the follicular fluid.

In a previous study in cyclic ewes, a 10-day treatment with estradiol-releasing implants designed to raise the serum estradiol concentrations to 2.4-fold that in control ewes resulted in the absence of follicular wave emergence; nevertheless, there were rhythmic but truncated peaks in serum FSH concentrations during the period of treatment compared with that of the control ewes (Barrett, Bartlewski, and Rawlings, unpublished data). A 28-day intravaginal treatment of cyclic ewes with oral contraceptives (each pill containing 150 μg of levonorgestrel or 180 μg of norgestomet and 30 μg of ethinyl estradiol) resulted in the absence of follicular waves and follicular growth beyond 2 mm in diameter in most ewes; however, there were rhythmic peaks in serum FSH concentrations similar to those in the control ewes that received inert pills (Bartlewski, Duggavathi, and Rawlings, unpublished data). Based on the observations above, and from the present results of experiments 1 and 2, we hypothesized that there was an endogenous rhythm of peaks in daily serum concentrations of FSH which was, at least in part, independent of regulation by the ovarian follicular growth pattern. This hypothesis is supported by the results of experiment 3, in which we observed peaks in serum FSH concentrations in serum samples collected daily in ovariectomized ewes (Fig. 4). These peaks were similar to those in ovary-intact ewes in terms of peak amplitude and interpeak intervals, even in the face of elevated basal FSH concentrations postovariectomy (Fig. 5).

In summary, doubling the amplitude of the FSH peak that precedes the emergence of a follicular wave did not alter the characteristics of the resulting follicular wave in anestrous sheep. Creation of physiological peaks of serum FSH concentrations every 2–2.5 days stimulated the emergence of additional follicular waves but did not alter the rhythmic occurrence of FSH peaks and follicular wave emergence. Follicular waves resulting from endogenous peaks in FSH secretion emerged and grew in the presence of the growing largest follicle of the follicular waves induced by exogenous oFSH, bringing the concept of follicular dominance in the ewe into question. In sexually mature, nonpregnant sheep, the ovary appears to contain small follicles (2 to 3 mm in diameter) capable of responding to a physiological increase in FSH secretion to produce a follicular wave on a daily basis. Finally, it seems that there is an endogenous rhythm of peaks in daily serum FSH concentrations, which is, at least in part, independent of regulation by ovarian follicular growth patterns.

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