# Ovarian Dynamics and Their Associations with Peripheral Concentrations of Gonadotropins, Ovarian Steroids, and Inhibin During the Estrous Cycle in Goats[1]

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## ABSTRACT

Ovarian changes determined by daily transrectal ultrasound and its relationship with FSH, LH, estradiol-17$\beta$, progesterone, and inhibin were investigated in six goats for three consecutive interovulatory intervals. Estrous cycles were synchronized using two injections of prostaglandin $F_{2\alpha}$ analogue 11 days apart. All follicles 3 mm or greater in diameter and corpora lutea were measured daily. A follicular wave was defined as one or more follicles growing to 5 mm or greater in diameter. The day that the follicles reached 3 mm in diameter was defined as the day of wave emergence, and the first wave after ovulation was defined as wave 1. During the interovulatory interval (mean $\pm$ SEM, 21.3 $\pm$ 0.4 days; n = 18), follicular waves emerged at 0.3 $\pm$ 0.5, 6.5 $\pm$ 0.2, and 12.1 $\pm$ 0.4 days for wave 1, wave 2, and wave 3, respectively, in goats with three waves of follicular development and at $-0.6 \pm 0.3$, 4.7 $\pm$ 0.2, 9.4 $\pm$ 0.5, and 13.4 $\pm$ 0.5 days for wave 1, wave 2, wave 3, and wave 4, respectively, in goats with four waves of follicular development (Day 0 = the day of ovulation). The mean diameter of the largest follicle of the ovulatory wave was significantly larger than those of the largest follicles of the other waves. Corpora lutea could be identified ultrasonically at Day 3 postovulation and attained 12.1 $\pm$ 0.3 mm in diameter on Day 8. Transient increases in plasma concentrations of FSH were detected around the day of follicular wave emergence. The level of FSH was negatively correlated with that of inhibin. These results demonstrated that follicular waves occurred in goats and that the predominant follicular wave pattern was four waves with ovulation from wave 4. These results also suggested that the emergence of follicular waves was closely associated with increased secretion of FSH.

*corpus luteum, follicle-stimulating hormone, follicular development, inhibin, progesterone*

## INTRODUCTION

Until recently, the techniques used in studying patterns of follicular development involved measurement, counting, and histological evaluation of the ovaries of animals killed at various times during the estrous cycle or marking of follicles with ink followed by serial laparoscopy. In contrast, the development of ultrasonic probes that can be used intrarectally to visualize ovaries has opened new possibilities for examining the dynamics of follicular growth and regression [1] and provided a means for repeated, direct, noninvasive monitoring and measuring of follicles within the ovary [2].

Studies of ovarian activity during the estrous cycle in goats are limited. Previous ultrasound studies in goats indicated that ovarian follicles reaching ovulatory size throughout the estrous cycle exhibited a wave-like pattern [3, 4]. Some of the factors that affect the number of waves per estrous cycle include dietary intake [5] as well as parity and lactational status [6]. Endocrine events in peripheral blood during the estrous cycle have been studied in detail in ruminants (goats [7], ewes [8], and cattle [9]). However, the temporal relationships between follicular dynamics and hormonal profiles have not yet been clarified throughout the goat estrous cycle. In ewes, a temporal relationship exists between elevations in mean daily serum concentrations of FSH and emergence of successive follicular waves [10, 11]. Surges of FSH were rhythmic and periodic (every 3 or 4 days) [12]. The role of inhibin in regulating the production and secretion of FSH has been documented in sheep [13], cattle [14], and mares [15]. In cattle, a high correlation between ultrasonic assessment of the corpus luteum (CL) and peripheral progesterone levels has been found [16, 17].

In the present study, we investigated the ovarian dynamics and accompanying hormonal profiles during the goat estrous cycle. Also, we investigated the relationships between plasma concentrations of FSH and inhibin.

## MATERIALS AND METHODS

This study was carried out on six Shiba goats (*Capra hircus*) during three consecutive estrous cycles. Their ages ranged from 3 to 5 yr. Animals were housed in a sheltered, outdoor paddock and were fed hay cubes (daily diet, 700 g/head). Clean water and mineralized salt licks were available ad libitum. Estrous cycles were synchronized with two injections of 125 $\mu$g of a synthetic analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Estrumate; Schering Plough Animal Health, Union, NJ) given 11 days apart. Estrous behavior was checked every 6 h with an aproned mature buck. Blood samples were collected daily during the estrous cycle and every 2 h during estrus (to detect LH surge) into heparinized vacutainer tubes (Terumo Venoject II, Tokyo, Japan). Centrifugation was performed at 1200 $\times$ *g* for 15 min, and plasma was separated and stored at $-20°C$ until assayed for hormones.
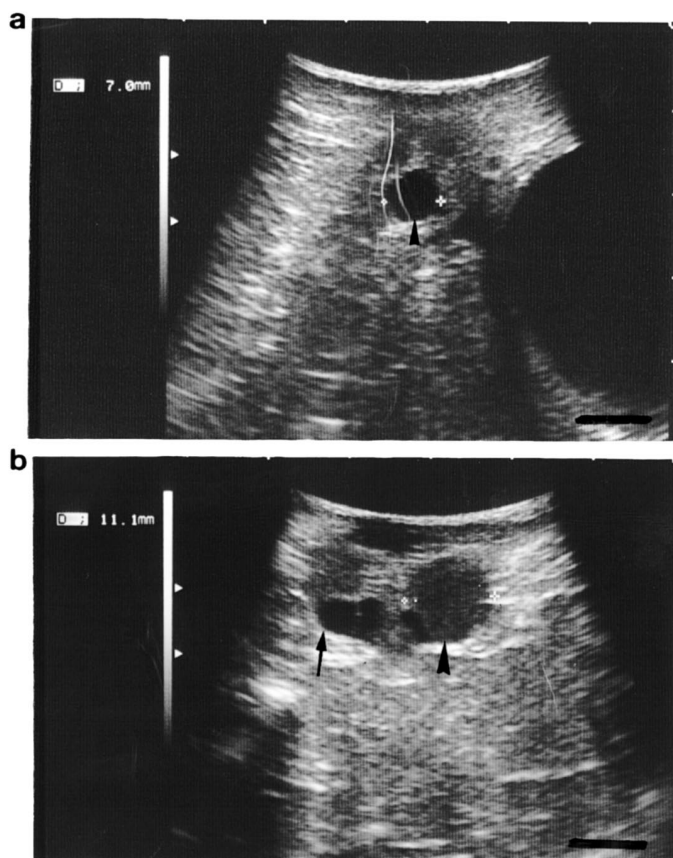
FIG. 1. Ultrasound image of goat ovaries produced using a rigid, transrectal 7.5-MHz transducer. **a**) The arrowhead indicates an antral follicle of 7.0 mm in diameter. **b**) The arrowhead indicates a functional CL of 11.1 mm in diameter, and the arrow indicates a small follicle. Bar = 10 mm.

## Ultrasound Evaluation

Ovarian images were obtained with a B-mode scanner (ECHOPAL ultrasound scanner; Hitachi Medical Corporation, Tokyo, Japan) equipped with a 7.5-MHz transducer as described by Ginther and Kot [3]. All follicles 3 mm or greater in diameter were recorded, and their diameters were measured. Also, diameter, position, and characteristics of the CL were registered. Each day, ovarian diagrams depicting the relative location of follicles 3 mm or greater in diameter and of CL were made to determine patterns of growth and regression of individual follicles and CL. The beginning of regression of CL (luteolysis) was defined as the first day that the luteal diameter was progressively decreased, as reported for heifers [16].

## Follicle Data Analysis

The total number of follicles 3 mm or greater in diameter was assessed on each day. The term *wave* was defined as one or more antral follicles growing from 3 to ≥5 mm in diameter before regression [4, 10, 18]. The day of emergence of follicles was identified as the day on which the follicle was 3 mm in diameter. Individual follicles emerging within a maximum of 48 h were regarded as a single follicular wave. The following characteristics of follicular waves were determined for each animal: 1) the number of follicular waves, 2) the days of wave emergence, 3) the number of follicles growing to 5 mm or greater in diameter per wave, 4) the maximum diameter attained by the largest follicle of the wave, 5) the number of days between the emergence of sequential follicular waves (interwave intervals), and 6) the growth and regression rates of the largest follicle of the wave. The growing phase of a follicle was defined as the period between its emergence and the day on which it appeared to stop its progressive increase in diameter. The regression phase of a follicle was defined as the period between its progressive decrease in diameter and the day on which it first reached 3 mm in diameter. The static phase of a follicle was defined as the period between the end of the growing phase

and the beginning of the regression phase. The day of ovulation was identified as the first day on which a large follicle disappeared or collapsed [18] and was followed by the development of a CL at that site on the ovary. The ovulatory follicle was considered to be in a growing phase from first detection to ovulation. Ovaries were monitored until at least 7 days after ovulation to confirm ovulation number by the number of CL.

## Hormone Analysis

Plasma concentrations of LH were measured by radioimmunoassay (RIA) system as described by Mori and Kano [19] using anti-ovine LH (YM-18), NIDDK-oLH-I-3 for radio-iodination, and NIDDK-oLH-RP-24 as a reference standard. Plasma concentrations of FSH were measured by RIA as described by Araki et al. [20] using anti-ovine FSH, NIDDK-FSH-I-1 for radio-iodination, and NIDDK-oFSH-RP-1 as a reference standard. The intra- and interassay coefficients of variation were 5.9% and 6.5%, respectively, for LH and 9.8% and 12.6%, respectively, for FSH. Plasma concentration of immunoreactive (ir-) inhibin was measured by double-antibody RIA as described by Hamada et al. [21] using bovine 32-kDa inhibin for radio-iodination and anti-bovine antiserum (TNDH-1). The intra- and interassay coefficients of variation were 3.8% and 11.9%, respectively. Plasma concentrations of estradiol-17β and progesterone were determined by double-antibody RIA using $^{125}I$-labelled radioligands as described previously [22]. Antisera against estradiol-17β (GDN 244) and progesterone (GDN 337) were kindly provided by Dr. G.D. Niswender (Animal Production and Biotechnology, Colorado State University, Fort Collins, CO). The intra- and interassay coefficients of variation were 5.7% and 7.4%, respectively, for estradiol-17β and 8.2% and 9.2%, respectively, for progesterone.

Inhibin A was measured by ELISA as described for use in human plasma [23] and modified for use in sheep plasma [24]. Briefly, the ELISA is based on the use of immobilized monoclonal antibody (E4) against the βA subunit as a capture antibody, a biotinylated monoclonal α C-specific antibody (PPG1/14/6) as a detection antibody, and immunopurified 32-kDa bovine inhibin in ovariectomized sheep plasma as standard in the range of 15.6 to 1000 pg/ml. The samples were denatured by boiling in 6% w/v SDS for 3 min before oxidation with hydrogen peroxide.

## Statistical Analysis

Mean values (± SEM) were calculated and analyzed using two-way ANOVA. The Duncan multiple-range test was used for detection of significant differences using the SAS computer package [25]. The follicles were combined for the two ovaries, and the analysis of data began on Day −2 rather than on Day 0 (day of ovulation). The hypothesis that waves of follicles emerged at periodic intervals was tested by ANOVA for sequential data to evaluate day effects averaged over 18 interovulatory intervals. A significant ($P < 0.05$) day effect was followed by the Duncan multiple-range test to detect significant nadirs and peaks. The association between emergence of follicular waves and occurrence of identified FSH peaks was studied by paired Student $t$-test to compare the number of waves with the number of peaks per interovulatory interval and the interwave and interpeak intervals. In addition, the Wilks lambda correlation was made between the number of follicular waves and the number of FSH peaks and among the interwave and interpeak intervals. A peak in hormone concentration was identified by a significant difference in mean values between the peak and each of the encompassing nadirs according to the method described by Ross et al. [26].

## RESULTS

### Estrus Detection and Estrous Cycle Duration

After injection of PGF$_{2\alpha}$, all animals exhibited estrous behavior. The mean length of the estrous cycle was 21.6 ± 0.4 days (n = 18). The mean interovulatory interval was 21.3 ± 0.4 days (n = 18), and the interval from PGF$_{2\alpha}$ to estrus was 55.7 ± 3 h (n = 18).

### Follicular Dynamics and Characteristics of Follicular Waves

The images of ovarian structures recorded in the present study are shown in Figure 1. The follicles were detected as echo-free black circles, and the CL were detected as a gray

TABLE 1. Characteristics of follicular waves during the estrous cycle in goats.

| | Goats with four waves of follicle development (n = 9) | | | | Goats with three waves of follicle development (n = 5) | | |
|---|---|---|---|---|---|---|---|
| | Wave 1 | Wave 2 | Wave 3 | Wave 4 | Wave 1 | Wave 2 | Wave 3 |
| Mean day of wave emergence | −0.6 ± 0.3 | 4.7 ± 0.2 | 9.4 ± 0.5 | 13.4 ± 0.5 | 0.3 ± 0.5 | 6.5 ± 0.2 | 12.1 ± 0.4 |
| Largest follicle | | | | | | | |
|   Maximum diameter (mm) | 6.7 ± 0.1[b] | 6.2 ± 0.2[c] | 6.3 ± 0.1[cb] | 7.8 ± 0.2[a] | 6.6 ± 0.1[cb] | 6.2 ± 0.1[c] | 8.0 ± 0.1[a] |
|   Growth rate (mm/day) | 0.9 ± 0.1[a] | 1.0 ± 0.1[a] | 0.9 ± 0.1[a] | 0.9 ± 0.1[a] | 0.9 ± 0.1[a] | 0.9 ± 0.1[a] | 0.8 ± 0.1[a] |
|   Regressing rate (mm/day) | 0.8 ± 0.1[a] | 0.8 ± 0.1[a] | 0.9 ± 0.1[a] | — | 0.9 ± 0.1[a] | 0.8 ± 0.1[a] | — |
| No. follicles/wave | 2.6 ± 0.2[a] | 2.4 ± 0.2[a] | 2.2 ± 0.2[a] | 2.8 ± 0.2[a] | 3.0 ± 0.5[a] | 2.4 ± 0.2[a] | 2.6 ± 0.2[a] |

[a,b,c] Within rows, values with no common superscripts are significantly different ($P < 0.05$).
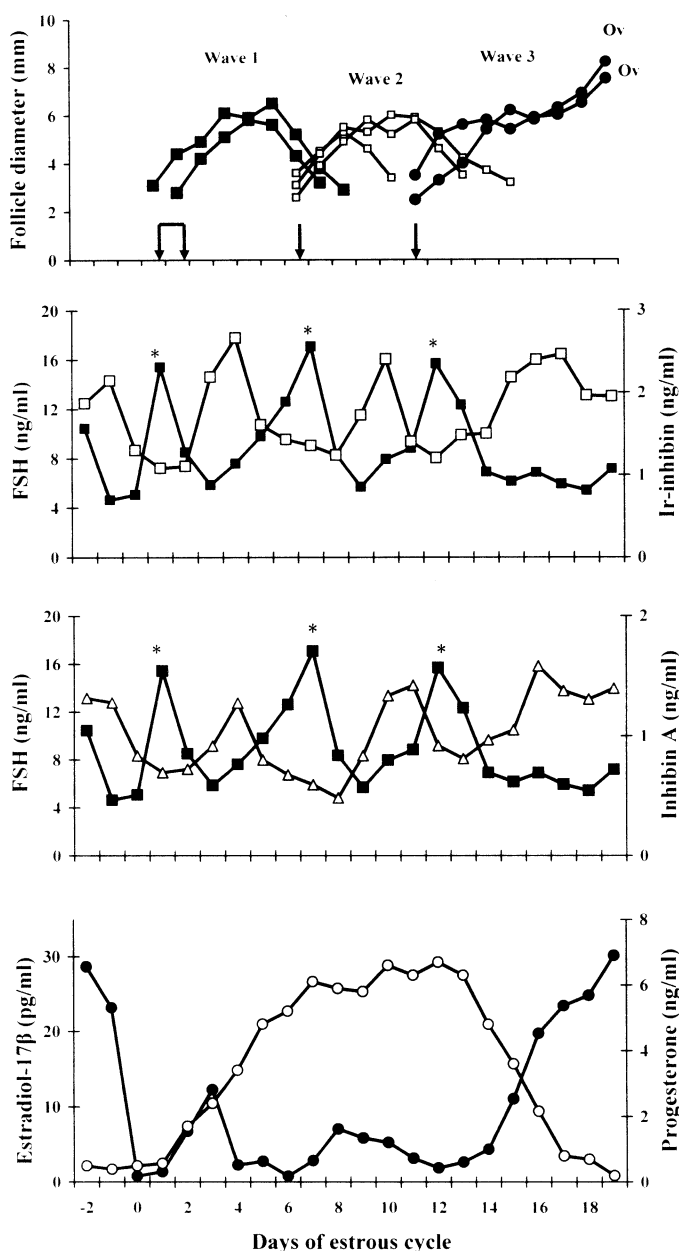


FIG. 2. Representative pattern of growth and regression of individual follicles during estrous cycles in a goat with three waves of follicular development and accompanying plasma concentrations of FSH (■), ir-inhibin (□), inhibin A (△), estradiol-17β (●), and progesterone (○). Arrows indicate the emergence of waves. Asterisks indicate FSH peaks. Ov, Ovulation.

echogenic structure with marked boundaries. Two interovulatory intervals (11.1%) had two follicular waves, five (27.8%) had three waves, nine (50.0%) had four waves, and two (11.1%) had five waves. In all animals, the last follicular wave of the interovulatory interval contained ovulatory follicle(s), and the ovulation rate was 1.8. The characteristics of follicular waves in those animals that had three of four follicular waves are shown in Table 1. Growth rates for the largest follicles of the successive waves were not different. Meanwhile, the maximum diameter of the ovulatory follicles were significantly ($P < 0.05$) larger than the maximum diameter of the largest follicles of the other waves. For goats with three or four waves of follicular growth per cycle, the number of 3-mm follicles emerging per day that subsequently grew to 5 mm or greater in diameter differed by day ($P < 0.05$). Follicles growing to 5 mm or greater in diameter emerged at 0.3 ± 0.5, 6.5 ± 0.2, and 12.1 ± 0.4 days for wave 1, wave 2, and wave 3, respectively, in goats with three waves of follicular development and at −0.6 ± 0.3, 4.7 ± 0.2, 9.4 ± 0.5, and 13.4 ± 0.5 days for wave 1, wave 2, wave 3, and wave 4, respectively, in goats with four waves of follicular development. In goats with three follicular waves, the number of 3-mm follicles peaked on Days 0, 7, and 11, whereas in goats with four follicular waves, the number of 3-mm follicles peaked on Days −1, 5, 11, and 15. Individual follicle profiles (follicle growing from 3 to ≥5 mm in diameter) for representative animals and accompanying concentrations of FSH, ir-inhibin, inhibin A, estradiol-17β, and progesterone are depicted in Figures 2 and 3.

### Estradiol-17β and Follicles

Plasma estradiol-17β concentrations increased from the day of ovulation (1.9 ± 0.1 pg/ml) to Day 4 and then decreased to the basal level. Estradiol-17β levels remained low for the rest of the luteal phase, apart from some isolated fluctuations, and increased in coincidence with progesterone declination, reaching a peak (30.9 ± 1.0 pg/ml) 2 days before ovulation. The fluctuation in estradiol-17β level during the luteal phase was associated with the growth of the largest follicles of the follicular waves (Figs. 2 and 3). The large follicles of the first and last wave secreted more estradiol-17β compared to the other midluteal waves.

### Progesterone and CL

The CL could be detected ultrasonically on Day 3 postovulation. The newly forming CL was less echogenic than at later stages. The mature CL was observed as a gray, echogenic structure with marked boundaries. The CL attained a maximum diameter of 12.1 ± 0.3 mm on Day 8 postovulation. During the early luteal phase, the mean diameter of CL increased in parallel with the mean plasma
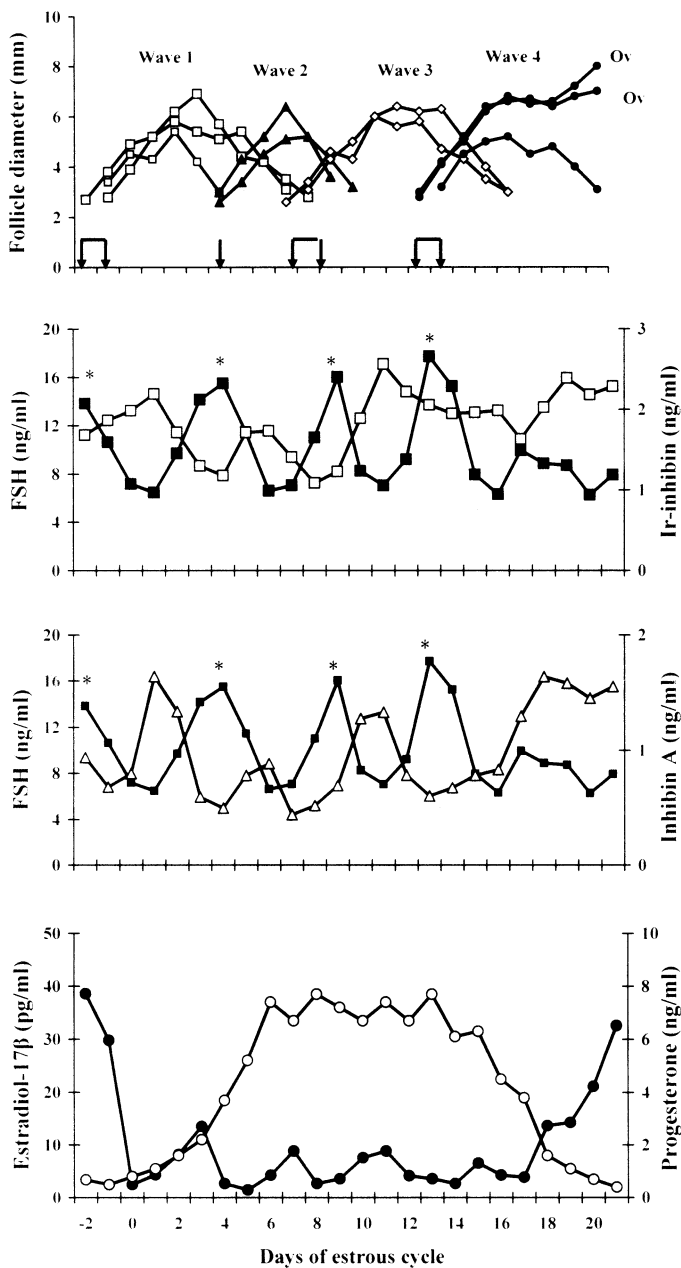
FIG. 3. Representative pattern of growth and regression of individual follicles during estrous cycles in a goat with four waves of follicular development and accompanying plasma concentrations of FSH (■), ir-inhibin (□), inhibin A (△), estradiol-17β (●), and progesterone (○). Arrows indicate the emergence of waves. Asterisks indicate FSH peaks. Ov, Ovulation.

concentration of progesterone, whereas during the late luteal phase, the plasma concentration of progesterone decreased more rapidly than the CL regression (Fig. 4). A positive correlation ($r = 0.9$, $P < 0.001$) was found between CL diameter and progesterone concentration during the estrous cycle.

*Relationship Between Follicular Wave Development, Plasma FSH, Inhibin, and LH Concentrations*

Goats with three and four waves of follicular emergence per cycle were included in this analysis. Plasma FSH concentration was highest at the emergence of each wave and decreased ($P < 0.05$) as the follicle grew to 5 mm in di-
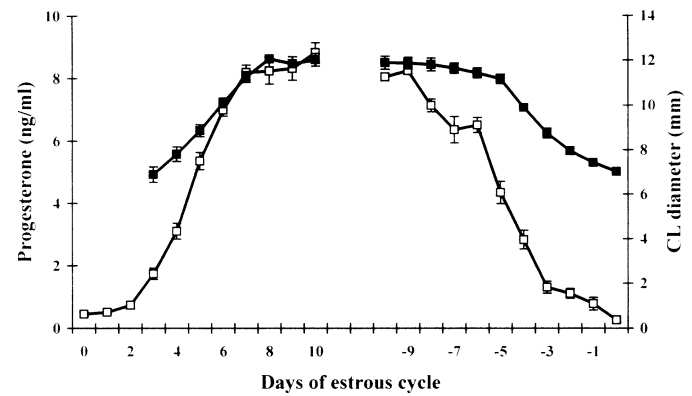


FIG. 4. Diameter of CL (■) and progesterone concentrations (□) during the estrous cycle in goats. Data were normalized to the day of ovulation. Values are the mean ± SEM (n = 18).

ameter; meanwhile, plasma levels of ir-inhibin and of inhibin A were low during follicular wave emergence and increased with the growth of follicles at the time of declining FSH. The number of emerging follicular waves and of identified FSH peak values per goat did not differ (3.6 ± 0.2 and 3.9 ± 0.2). The duration of the interval between adjacent days of wave emergence (interwave intervals) was positively correlated with the duration of the interpeak interval for FSH fluctuations ($r = 0.8$, $P < 0.001$). The length of the interval between emergence of waves did not differ significantly from the length of the intervals between FSH peak values (5.6 ± 0.3 and 5.2 ± 0.2). The number of waves and the number of peaks were positively and significantly correlated ($r = 0.8$, $P < 0.001$). In contrast to FSH, the plasma concentration of LH remained at low levels during the estrous cycle, except during the preovulatory LH surge.

*Relationship Between FSH and Inhibin*

Daily plasma FSH and inhibin concentrations were normalized to the day of ovulation (Day 0). Three distinct FSH peaks could be seen in goats with three waves of follicular development and four peaks in goats with four waves. A significant ($P < 0.05$) decrease in plasma inhibin concentration was noticed to coincide with FSH peaks (Figs. 5 and 6). A negative correlation was found between FSH and both ir-inhibin ($r = -0.59$, $P < 0.0001$) and inhibin A ($r = -0.47$, $P < 0.001$).

**DISCUSSION**

The present study demonstrates the relationship between follicular dynamics and hormonal profiles during the estrous cycle of goats. The results of daily ultrasonography indicate that the interovulatory interval in goats is characterized by a wave-like pattern of follicular development. This confirms previous observations reported in goats [3, 4]. Ginther and Kot [3] found a predominant wave pattern of four waves, emerging around Days −1, 4, 8, and 13 of the interovulatory interval. In the present study, the wave pattern found ranged between two and five follicular waves. The day of emergence of waves for goats that have four waves was between the range reported by Ginther and Kot [3]. In goats with three follicular waves, the number of 3-mm follicles peaked on Days 0, 7, and 11, whereas in goats with four follicular waves, 3-mm follicles peaked on Days −1, 5, 11, and 15. This demonstrates a progressive growth of follicles in a wave-like fashion.
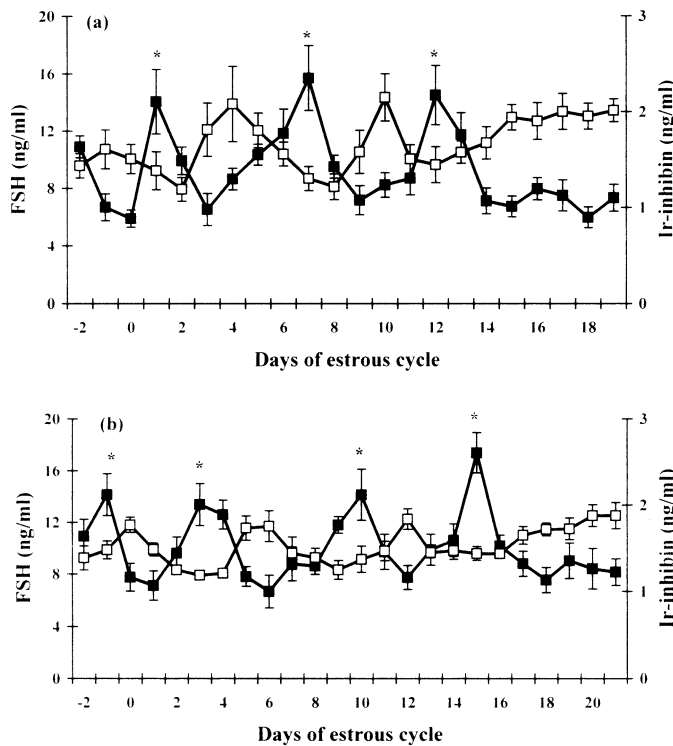
FIG. 5. Plasma FSH (■) and ir-inhibin (□) concentrations during the estrous cycle in goats with three (**a**; n = 5) or four (**b**; n = 9) waves of follicular growth. Values are means ± SEM. Asterisks indicate FSH peaks.
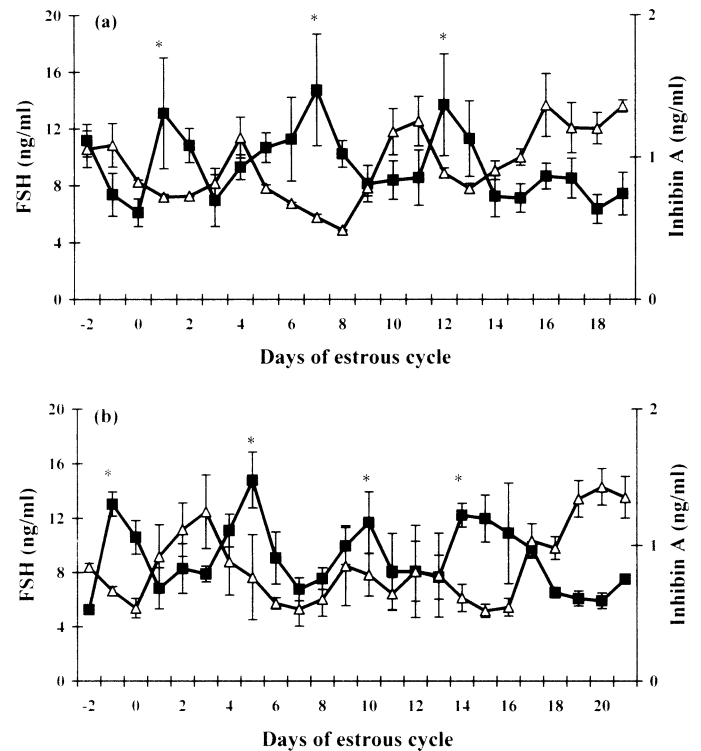
FIG. 6. Plasma FSH (■) and inhibin A (△) concentrations during the estrous cycle in goats with three (**a**; n = 3) or four (**b**; n = 3) waves of follicular growth. Values are means ± SEM. Asterisks indicate FSH peaks.

The estradiol-17β profiles in the present study are characterized by gradual increase from the day of ovulation to Day 4 then decreased to the basal level. Estradiol-17β levels remained low during the rest of the luteal phase, apart from some isolated fluctuations then increased, reaching a peak 2 days before ovulation. Early work in cows [27] and sheep [13] showed that estrogens are mainly produced by the dominant follicle of a wave and that subordinate follicles contribute less than 10% of the ovarian estradiol-17β production. In the present study, although estradiol-17β was produced by the large follicles of each follicular wave, the first and last waves secreted more estradiol-17β compared with other midluteal waves. This might be attributed to decreased LH release during the midluteal phase [28, 29]. Previous reports showed that the production of estradiol was stopped before the time when the follicle attained its maximum diameter [30]. Another study showed that estradiol concentrations were greatest during the growing phase of the first dominant follicle and significantly reduced when the dominant follicle attained its large diameter [31]. The present study demonstrated that the diameter of the CL was positively correlated with plasma progesterone concentrations. This is in agreement with the findings of previous reports [32, 33]. This strong correlation between CL diameter and progesterone concentration indicates that CL diameter might be used as an index of the peripheral progesterone level in goats.

In previous studies using ultrasonography and blood sampling once daily, large antral follicles (attaining ≥5 mm in diameter) grew in waves across the estrous cycle of ewes, and around the time of wave emergence (growth from 3-mm pool follicles), a transient elevation (2–3 days) occurred in plasma concentrations of FSH [10, 11]. In the present study, we found this pattern of FSH secretion, in which plasma FSH concentrations were high coincident with follicular wave emergence, decreased after emergence, and remained low during the growing phase of follicles. These results suggest that the fluctuation of the circulating FSH levels is involved in the recruitment and selection of follicles. Within the present study, the number of follicular waves and the number of FSH peaks did not differ, nor did the length of the intervals between waves versus FSH peaks. The positive correlation between the numbers of the two events within interovulatory intervals was both high and significant. It is well established that the secretion of FSH during the estrous cycle is regulated by both estradiol and inhibin [13], and fluctuations in the pattern of secretion of these two hormones would be expected to be responsible for the fluctuations in FSH that are associated with follicular waves. The results of the present study suggest that changes in the secretion of inhibin control FSH during the estrous cycle in goats. In the present study, looking at the patterns of plasma FSH concentrations in the alignments to follicular wave strongly suggested a link between FSH secretion and follicular growth patterns. Mean plasma FSH started to increase from approximately the time of the end of the growth phase/onset of the static phase of the follicular wave, suggesting that secretion of follicular inhibitor of FSH release declined at that time. New follicular waves emerged within 1 or 2 days of the onset of the static phase of the previous wave, suggesting that the changed secretory activity of follicles in the static phase permits the increase in FSH secretion that heralds the next follicular wave. This would mirror the concepts that have been proposed for cattle [34], except that follicular dominance is less apparent in goats, because several follicles can emerge and grow to a similar final stage in a single wave. This experiment showed that the pattern of secretion of dimeric inhibin A is related to the presence of large follicles and is negatively correlated with FSH concentration, suggesting that inhibin

A is a product of differentiated follicles and has an important role in controlling FSH secretion. Similarly, in rats, inhibin A is at its highest concentration during proestrus, concomitant with the selection of large follicles [35]. Previous studies demonstrated that whereas estrogenic large follicles are a major source, both small and large nonestrogenic follicles contribute significantly to the ovarian secretion of ir-inhibin [13, 36]. Evidence from passive immunoneutralization studies supports a functional role for inhibin in regulating FSH secretion. The relationship between the pattern of inhibin release, FSH, and follicular growth is similar to that reported by Kaneko et al. [37] in cycling cows. Also, inhibin A is inversely correlated with FSH concentration, which is similar to that recorded in cattle [38]. This inverse relationship confirms the hypothesis that inhibin A contributes to the inhibition of FSH secretion.

In conclusion, ultrasonography can be reliably used in goats to study ovarian follicular dynamics, and the growth of ovarian follicles exhibits a wave-like pattern. The CL diameter was correlated with plasma progesterone concentrations; therefore, CL can be used as an index of peripheral progesterone levels in goats. The secretion of ir-inhibin and dimeric inhibin A is related to the presence of large antral follicles and is negatively correlated with FSH. The mean FSH secretion peaks at or around antral follicle emergence (follicles growing from 3 to $\geq 5$ mm in diameter).

## ACKNOWLEDGMENTS

## REFERENCES

1. Fortune JE, Sirois J, Turzillo AM, Lavoir M. Follicle selection in domestic ruminants. J Reprod Fertil Suppl 1991; 43:187–198.
2. Griffin PG, Ginther OJ. Research applications of ultrasonic imaging in reproductive biology. J Anim Sci 1992; 70:953–972.
3. Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. Theriogenology 1994; 42:987–1001.
4. De Castro T, Rubianes E, Menchaca A, Rivero A. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology 1999; 52:399–411.
5. Murphy MG, Enright WJ, Crowe MA, McConnel K, Spicer LJ, Poland MP, Roche JF. Effect of dietary intake on pattern of growth of dominant follicles during the estrous cycle in beef heifers. J Reprod Fertil 1991; 92:333–338.
6. Lucy MC, Savio JD, Badinga L, Delasota RL, Thatcher WW. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 1992; 70: 3615–3626.
7. Chemineau P, Gauthier D, Poirier JC, Saumande J. Plasma levels of LH, FSH, prolactin, estradiol-17β, and progesterone during natural and induced estrus in the dairy goat. Theriogenology 1982; 17:313–323.
8. Campbell BK, Mann GE, McNeilly AS, Baird DT. The pattern of ovarian inhibin, estradiol and androstenedione secretion during the estrous cycle in the ewe. Endocrinology 1990; 127:227–235.
9. Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127:916–924.
10. Ginther OJ, Kot K, Wiltbank MC. Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrous cycle in ewes. Theriogenology 1995; 43:689–703.
11. Bartlewski PM, Beard AP, Cook SJ, Chandolia RK, Honaramooz A, Rawlings NC. Ovarian antral follicular dynamics and their relationships with endocrine variables in two breeds of sheep differing in prolificacy. J Reprod Fertil 1999; 115:111–124.
12. Gibbons JR, Kot K, Thomas DL, Wiltbank MC, Ginther OJ. Follicular and FSH dynamics in ewes with a history of high and low ovulation rates. Theriogenology 1999; 52:1005–1020.
13. Mann GE, McNeilly AS, Baird DT. Hormone production in vivo and in vitro from follicles at different stages of estrous cycle in sheep. J Endocrinol 1992; 132:225–234.
14. Glencross RG, Bleach ECL, Wood SC, Knight PG. Active immunization of heifers against inhibin: effects on plasma concentrations of gonadotrophins, steroids and ovarian follicular dynamics during prostaglandin-synchronized cycles. J Reprod Fertil 1994; 100:599–605.
15. Nambo Y, Kaneko H, Nagat S, Oikawa M, Yoshihara T, Nagamine N, Watanabe G, Taya K. Effect of passive immunization against inhibin on FSH secretion, folliculogenesis and ovulation rate during the follicular phase of estrous cycle in mares. Theriogenology 1998; 50: 545–557.
16. Kastelic JP, Bergfelt DR, Ginther OJ. Relationship between ultrasonic assessment of corpus luteum and plasma progesterone concentration in heifers. Theriogenology 1990; 33:1269–1278.
17. Singh J, Pierson RA, Adams GP. Ultrasound image attributes of bovine corpus luteum: structural and functional correlates. J Reprod Fertil 1997; 109:35–44.
18. Bartlewski PM, Beard AP, Rawlings NC. An ultrasound-aided study of temporal relationships between the patterns of LH/FSH secretion, development of ovulatory-sized antral follicles and formation of corporal lutea in ewes. Theriogenology 2000; 54:229–245.
19. Mori Y, Kano Y. Changes in plasma concentrations of LH, progesterone and estradiol in relation to the occurrence of luteolysis, estrus and time of ovulation in Shiba goats (*Capra hircus*). J Reprod Fertil 1984; 72:223–230.
20. Araki K, Arai K, Watanabe G, Taya K. Involvement of inhibin in the regulation of follicle-stimulating hormone secretion in the young adult male Shiba goat. J Androl 2000; 21:558–565.
21. Hamada T, Watanabe G, Kokuho T, Taya K, Sasamoto S, Hasegawa Y, Miyamoto K, Igarashi M. Radioimmunoassay of inhibin in various mammals. J Endocrinol 1989; 122:697–704.
22. Taya K, Watanabe G, Sasamoto S. Radioimmunoassay for progesterone, testosterone and oestradiol-17β using $^{125}$I-iodohistamine radioligands. Jpn J Anim Reprod 1985; 31:186–197.
23. Groome N, Illingworth P, O'Brien M, Pai R, Mather J, McNeilly AS. Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 1996; 81:1400–1405.
24. Knight PG, Feist SA, Tannetta DS, Bleach ECL, Fowler PA, O'Brien M, Groome NP. Measurement of inhibin-A (αBA dimer) during the estrous cycle after manipulation of ovarian activity and during pregnancy in ewes. J Reprod Fertil 1998; 113:159–166.
25. SAS Statistics, version 6.11. Cary, NC: SAS Institute, Inc.; 1987.
26. Ross JL, Barnes KM, Brody S, Merriam GR, Loriaux DL, Cutler GB Jr. A comparison of two methods for detecting hormone peaks: the effect of sampling interval on gonadotropin peak frequency. J Clin Endocrinol Metab 1984; 59:1159–1163.
27. Badinga L, Driancourt MA, Savio JD, Wolfenson D, Drost M, de la Sota RL, Thatcher WW. Endocrine and ovarian responses associates with the first-wave dominant follicle in cattle. Biol Reprod 1992; 47: 871–883.
28. Walters DL, Schams D, Schallenberger E. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the estrous cycle in the cow. J Reprod Fertil 1984; 71:479–491.
29. Kawate N, Morita N, Tsuji M, Tamada H, Inaba T, Sawada T. Roles of pulsatile release of LH in the development and maintenance of corpus luteum function in the goat. Theriogenology 2000; 54:1133–1143.
30. Kaneko H, Terada T, Taya K, Watanabe G, Sasamoto S, Hasegawa Y, Igarashi M. Ovarian follicular dynamics and concentrations of oestradiol-17β, progesterone, luteinizing hormone and follicle stimulating hormone during the periovulatory phase of the oestrous cycle in the cow. Reprod Fertil Dev 1991; 3:529–535.
31. Rhodes FM, Fitzpatrick LA, Entwistle KW, Kinder JE. Hormone concentrations in the caudal vena cava during the first ovarian follicular wave of the estrous cycle in heifers. J Reprod Fertil 1995; 104:33–39.
32. Samartzi F, Belibasaki S, Vainas E, Boscos C. Plasma progesterone concentration in relation to ovulation rate and embryo yield in Chios ewes superovulated with PMSG. Anim Reprod Sci 1995; 39:11–21.
33. Amiridis GS, Rekkas CA, Fthenakis GC, Vainas E, Lymberopoulos A, Christodoulou V, Belibasaki S. Progesterone concentration as an

indicator of ovarian response to superovulation in Chios ewes. Theriogenology 2002; 57:1143–1150.

34. Ginther OJ, Wiltbank MC, Fricke PM, Gibbson JR, Kot K. Selection of dominant follicle in cattle: minireview. Biol Reprod 1996; 55: 1187–1194.

35. Woodruff TK, Besecke LM, Groome N, Draper B, Schwartz NB, Weiss J. Inhibin A and inhibin B are inversely correlated to follicle-stimulating hormone, yet are discordant during the follicular phase of the rat estrous cycle, and inhibin A is expressed in a sexually dimorphic manner. Endocrinol 1996; 137:5463–5464.

36. Campbell BK, Scaramuzzi RJ, Evans G, Downing JA. Increased ovulation rate in androstenedione-immune ewes is not due to elevated plasma concentrations of FSH. J Reprod Fertil 1991; 91:655–666.

37. Kaneko H, Kishi H, Watanabe G, Taya K, Sasamoto S, Hasegawa Y. Changes in plasma concentrations of immunoreactive inhibin, estradiol and FSH associated with follicular waves during the estrus cycle of the cow. J Reprod Dev 1995; 41:311–320.

38. Kaneko H, Noguchi J, Kikuchi K, Todoroki J, Hasegawa Y. Alterations in peripheral concentrations of inhibin A in cattle studied using a time-resolved immunofluorometric assay: relationship with estradiol and follicle-stimulating hormone in various reproductive conditions. Biol Reprod 2002; 67:38–45.