

## Follicular Turnover and Hormonal Association in Postpartum Goats During Early and Late Lactation

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**Abstract.** To clarify the effect of lactation period on ovarian follicular activity and associated hormonal levels in goats, six goats were monitored daily by ultrasonographic examination with blood sampling during early (Days 5 to 25; Day 0 was the day of kidding) and late (Days 40 to 60) lactation. While the presence of a corpus luteum of pregnancy retarded follicular growth in the ipsilateral ovary until Days 11–13 postpartum, the total follicular number (TFN) and area (TFA) increased during late lactation due to the significant increase in the number of medium- and large-sized follicles and decrease in the number of small follicles. Four goats showed a similar pattern of follicular development during the period studied characterized by the emergence of five and six waves during the early and late lactation, respectively. The largest follicle diameter of the first three waves monitored during early lactation was significantly smaller as compared with the diameter of those existing during late lactation. TFN showed a positive correlation with FSH but showed a negative correlation with immunoreactive (ir-) inhibin and estradiol during the postpartum period. TFA was positively correlated with ir-inhibin, estradiol and PRL and negatively correlated with FSH during the monitored periods. The plasma levels of ir-inhibin and progesterone were significantly higher during late lactation compared with the levels recorded during early lactation. Ir-inhibin levels showed a significant positive correlation with LH and estradiol during early and late lactation but showed a negative correlation with FSH during the whole lactation period. LH was positively correlated with estradiol and PRL during early and late lactation, respectively. These results suggest that the lactation period has a detrimental effect on ovarian activity during the early postpartum period in goats.

**Key words:** Goat, Gonadotropic hormones, Lactation, Ovary, Postpartum

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The understanding of ovarian follicular dynamics and its hormonal control in goats has increased in recent years due to the use of ultrasonography and provides information that is important for establishing improved methods to solve the problem of prolonged lactating anestrus in domestic animals [1]. Previous findings in other ruminants including the wave-like pattern of follicular growth, follicular dominance and the role of progesterone in follicular turnover, have been recently extended to the Caprine species [2]. Nevertheless, information regarding ovarian activity and the mechanisms regulating the postpartum anestrus period in goats is still scarce.

Follicular activity resumed as early as Day 13 in the ovaries

of postpartum goats, and medium- or large-sized follicles were present in the ovaries soon after [3]. Sosa [4] showed that the goat ovarian cyclicity resumed after complete uterine involution at the 5th week postpartum. In ewes, although follicles larger than 2 mm in diameter appeared as early as Day 5 postpartum [5], no follicles larger than 3 mm in diameter were observed in ovaries containing a corpus luteum of pregnancy until Days 21–25 postpartum, and large-sized ( $\geq 5$  mm in diameter) follicles were detected in only two ovaries at 27–28 days postpartum [6]. Even so, ovarian follicular growth and dynamics during the postpartum period still need to be described fully in goats.

In suckled cows, follicular development, emergence of the first follicular wave and formation of a dominant follicle occur early after calving [7, 8], and antral follicles were found at 15, 25 and 35 days postpartum [9]. Additionally, the number of small follicles decreased, though the number of medium [10] and large follicles increased with progression of the postpartum period [11]. The aim of the present study was to evaluate the changes occurring

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in the ovarian follicular population and the associated hormonal modulation during the early and late lactation periods in goats.

## Materials and Methods

### *Animals and experimental design*

Six healthy 2–5 year-old Shiba goats (*Capra hircus*) with average body weights of  $36.85 \pm 3.8$  kg and  $29.52 \pm 4.38$  kg at kidding (end of June to the first week of July) and the time of weaning, respectively, were used in the present study. Each female was kept in a kidding box with its newborn kid(s) under natural daylight and temperature conditions, fed hay cubes (600 g/head/day) and had free access to clean water and mineralized salt licks. Goats were weaned 60 days after kidding (Day 0 = day of kidding).

Blood samples were collected daily from Day 5 to Day 25 (early lactation) and from Day 40 to 60 (late lactation), through jugular vein puncture into heparinized Vacutainer tubes (Terumo Venoject II, Terumo, Tokyo, Japan) and then centrifuged at  $1700 \times g$  for 30 min at 4 °C. The separated plasma was stored at  $-20$  °C for hormonal assay.

### *Ultrasonographic examination and follicular data analysis*

Morphometry of ovarian structures was monitored by transrectal ultrasonography using a real-time B-mode scanner (ECHOPAL ultrasound scanner, Hitachi Medical Corporation, Tokyo, Japan) equipped with a 7.5-MHz ultrasound probe. Measurements of ovarian structures were made using the built-in caliper system on a single frozen image of the apparent maximal area for each structure. Mean follicular diameter was calculated by dividing the sum of two perpendicular diameters by two. The cross-sectional area of each follicle was calculated according to the equation for the area of a circle,  $area = \pi r^2$ , where  $r$ , the radius, was equal to half the calculated mean diameter of the follicle [12]. Follicles  $\geq 2$  mm in diameter were measured, and diagrams of their relative locations were drawn daily on ovarian sketches, allowing individual follicles to be identified for analysis of the follicular waves. The term “follicular wave” referred to one or more antral follicles growing from  $\leq 3$  to  $\geq 5$  mm in diameter before regression [13]. Follicles that were first detected at 4 mm in diameter were assumed to have been 3 mm in diameter on the previous day, which was identified as the day of wave emergence. In the mean time, individual follicles that emerged within a maximum of 48 h were regarded as a single follicular wave [14]. The interwave interval referred to the interval between the onsets of a follicular wave and the subsequent follicular wave. The follicles were classified into small ( $< 2.5$  mm and 2.5–3.4 mm), medium (3.5–4.5 and 4.5–5.5 mm) and large ( $> 5.5$  mm). The average follicular diameter and the number of follicles in individual size classes were determined for each day. Follicular data were collected for both ovaries and combined so that a single observation per goat was used on each day (e.g., the total number of ovarian follicles, the maximal diameter attained by the largest follicle of the wave and the day of wave emergence).

### *Hormonal analysis*

A double antibody radioimmunoassay (RIA) system using  $^{125}I$ -labeled radioligands was applied for estimating the plasma

concentrations of estradiol-17 $\beta$ , progesterone [15], FSH [16], LH [17] and ir-inhibin [18]. For the PRL assay, anti-ovine PRL, NIDDK-anti-oPRL (AFP-C3581069II), and ovine purified PRL, NIDDK-oPRL-I-2 (AFP-7150B), were used for iodination and reference standards. The inter-assay coefficients of variation (CVs) for estradiol, progesterone, FSH, LH, immunoreactive-inhibin and PRL were 3.70, 2.54, 5.28, 8.71, 1.36 and 6.24%, respectively, and the intra-assay CVs were 9.49, 9.52, 13.55, 10.99, 12.43 and 14.54%, respectively. The sensitivities of the estradiol, progesterone, FSH, LH, immunoreactive inhibin and PRL assays were 0.076, 0.1, 8, 0.2, 3 and 30 pg/tube, respectively.

### *Statistical analysis*

Data were expressed as means ( $\pm$  SEM), and statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program ver. 14. The total hormonal secretion (expressed by area under the curve) and basal hormonal levels were calculated according to Davies *et al.* [19], and Alvarez and Galindo [20] using a GraphPad program (Ver. 5). The differences between early and late lactation confounded by time effects were evaluated by using one-way ANOVA. A *t*-test was used to compare the overall effect of each lactation period of follicle numbers and hormonal levels. The follicular-hormonal correlations were assessed statistically by using Pearson correlation. A value of  $P < 0.05$  was considered to be significant.

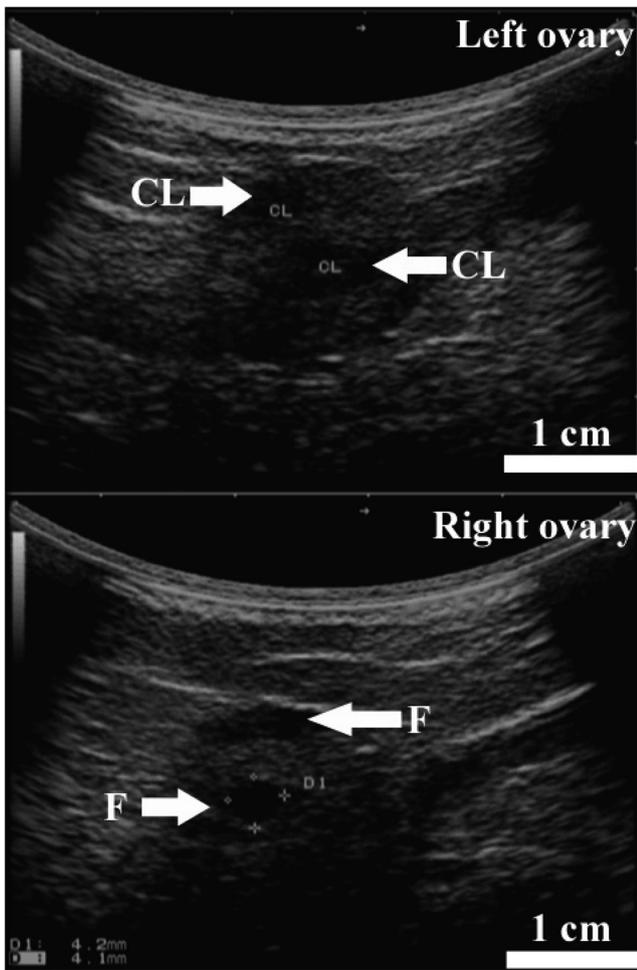
## Results

### *Follicular turnover during early and late lactation*

The daily ultrasonographic monitoring of goat ovaries after kidding revealed that follicular activity was detected by Day 5 postpartum. Follicular growth was delayed in the ovary bearing a corpus luteum of pregnancy that was maintained in the ovary until Days 11–13 postpartum (Fig. 1). None of the examined goats showed ovulation until the end of the experimental period. The effect of lactation period on the overall ovarian follicle population and maximal follicle diameter of the emerged follicular waves is summarized in Table 1. The overall number of small follicles ( $< 3.5$  mm) decreased, while the number of medium- (3.5–5.5 mm) and large-sized ( $> 5.5$  mm) follicles increased significantly ( $P < 0.05$ ) during late lactation (Fig. 2A–E), and this reflected on the increase in the overall total follicular number and area and consequently ovarian activity during this period (Fig. 3A and B).

### *Hormonal changes in goats during early and late lactation periods*

The overall mean levels and hormonal peaks of ir-inhibin and progesterone were significantly higher during late lactation compared with those recorded during early lactation ( $P < 0.05$ ; Fig. 4A and 4F and Table 2). In addition, the basal levels of progesterone were low during early lactation and increased during the late lactation period ( $P < 0.05$ ). The hormonal peak of estradiol was significantly ( $P < 0.05$ ) higher during late lactation as compared with early lactation. Whereas the plasma hormonal concentrations of FSH ( $0.96 \pm 0.05$  vs.  $1.08 \pm 0.04$  mg/ml), LH ( $0.185 \pm 0.012$  vs.  $0.192 \pm 0.024$  ng/ml) and PRL ( $251.60 \pm 15.05$  vs.  $290.60 \pm 15.34$



**Fig. 1.** Ultrasonographic images of a goat ovary on Day 5 postpartum. Note the existence of remnants of a corpus luteum (s) of pregnancy in the left ovary, which appeared as poor echogenic small structures that were difficult to be distinguished from the ovarian stroma, and absence of any follicular growth. Alternatively, the right ovary presented two follicular structures that appeared as echogenic circumscribed areas of medium size with a mean diameter of 4.1 mm. Scale bar=1 cm.

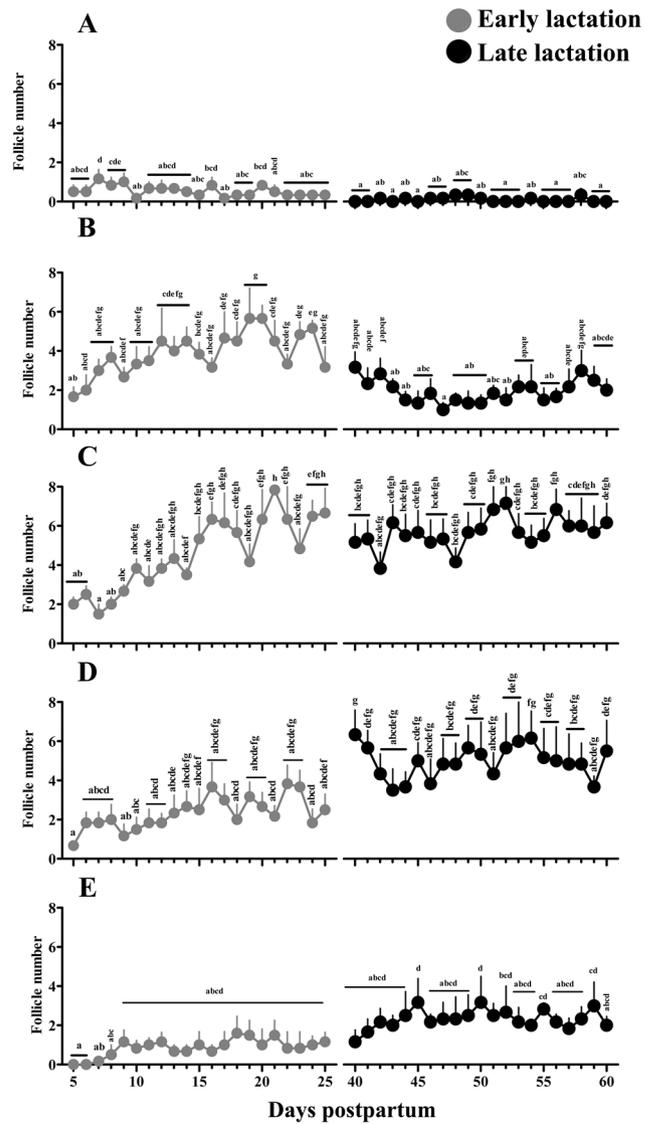
ng/ml) were equivalent during the early and late lactation periods (Fig. 3B, C and D).

*Follicular-hormonal interrelationship in goats during early and late lactation periods*

Total follicle number showed a significant negative relationship with follicular area during the early and late lactation periods ( $r=-0.275$ ,  $P<0.01$ , and  $r=-0.192$ ,  $P<0.05$ , respectively).

The total follicle number showed a positive relationship with FSH ( $r=0.260$ ,  $P<0.01$ , and  $r=0.251$ ,  $P<0.01$ ), but showed a negative correlation with ir-inhibin ( $r=-0.369$ ,  $P<0.001$ , and  $r=-0.370$ ,  $P<0.001$ ) and estradiol ( $r=-0.261$ ,  $P<0.01$ , and  $r=-0.170$ ,  $P<0.05$ ) during the early and late lactation periods, respectively.

The follicular area showed a significant positive correlation with ir-inhibin ( $0.689$ ,  $P<0.0001$  and  $r=0.291$ ,  $P<0.001$ ), estradiol ( $r=0.306$ ,

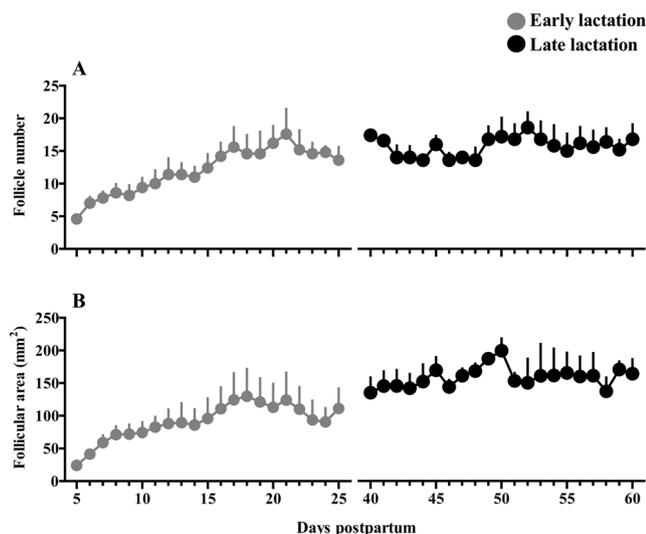


**Fig. 2.** Changes in the number of ovarian follicles populations (A, follicle <2.5 mm; B, follicle 2.5–3.4 mm; C, follicle 3.5–4.4 mm; D, follicle 4.5–5.4 mm; and E, follicle  $\geq 5.5$  mm in diameter) in postpartum goats during early (●) and late (●) stages of lactation. Data of the two ovaries were pooled together. Values are presented as mean  $\pm$  SEM of six animals.

$P<0.001$ , and  $r=0.231$ ,  $P<0.01$ ), PRL ( $r=0.196$ ,  $P<0.05$ , and  $r=0.196$ ,  $P<0.05$ ), but showed a negative correlation with FSH ( $r=-0.209$ ,  $P<0.05$ , and  $r=0.276$ ,  $P<0.001$ ) during the early and late lactation periods, respectively. Furthermore, it was positively correlated with LH during the early lactation period only ( $r=0.301$ ,  $P<0.001$ ).

*Interhormonal relationship in goats during the early and late lactation periods*

While ir-inhibin levels showed a significant positive correlation with LH during early lactation ( $r=0.229$ ,  $P<0.05$ ) and estradiol during early and late lactation ( $r=0.206$ ,  $P<0.05$ , and  $r=0.262$ ,  $P<0.01$ ,



**Fig. 3.** Changes in the total follicle number (A) and mean follicular cross-sectional area per goat (B) during early (●) and late (●) lactation periods. Data of the two ovaries were pooled together. Values (presented as mean  $\pm$  SEM of six animals) with different superscripts are significantly different ( $P < 0.05$ ).

respectively), they showed a negative correlation with FSH during the same monitored periods ( $r = -0.351$ ,  $P < 0.0001$ , and  $r = -0.387$ ,  $P < 0.0001$ ; respectively). LH showed a positive correlation with estradiol ( $r = 0.597$ ,  $P < 0.001$ , and  $r = 0.269$ ,  $P < 0.01$ ) and PRL ( $r = 0.315$ ,  $P < 0.001$ , and  $r = 0.190$ ,  $P < 0.05$ ) during early and late lactation, respectively. Conversely, it was negatively correlated with FSH ( $r = -0.198$ ,  $P < 0.05$ ) during early lactation only.

#### Characterization of follicular waves and associated FSH and immunoreactive inhibin changes during lactation anestrus

In postpartum goats ( $n = 4$ ), follicular development and regression showed the emergence of five and six waves during the early and late lactation periods, respectively (Fig. 5). Two other goats showed a four-wave pattern during the early and late lactation periods (Fig. 6). There was a close association between day of wave emergence and the onset of the FSH peak ( $P < 0.01$ , Table 1). The maximal diameters of the largest follicle of the first three waves monitored during early lactation were significantly smaller when compared with their contemporaries during late lactation (Table 1). The growth and regression rates of the developed follicles during the early and late lactation periods were significantly different ( $0.65 \pm 0.05$  vs.  $0.84 \pm 0.06$  mm/day,  $P < 0.05$ , and  $0.54 \pm 0.04$  vs.  $0.81 \pm 0.05$  mm/day,  $P < 0.001$ ). On the other hand, the interwave interval and interpeak intervals were not significantly different during the early and late lactation periods ( $3.88 \pm 0.33$  days vs.  $3.45 \pm 0.25$  days and  $3.87 \pm 0.31$  days vs.  $3.68 \pm 0.29$  days, respectively).

### Discussion

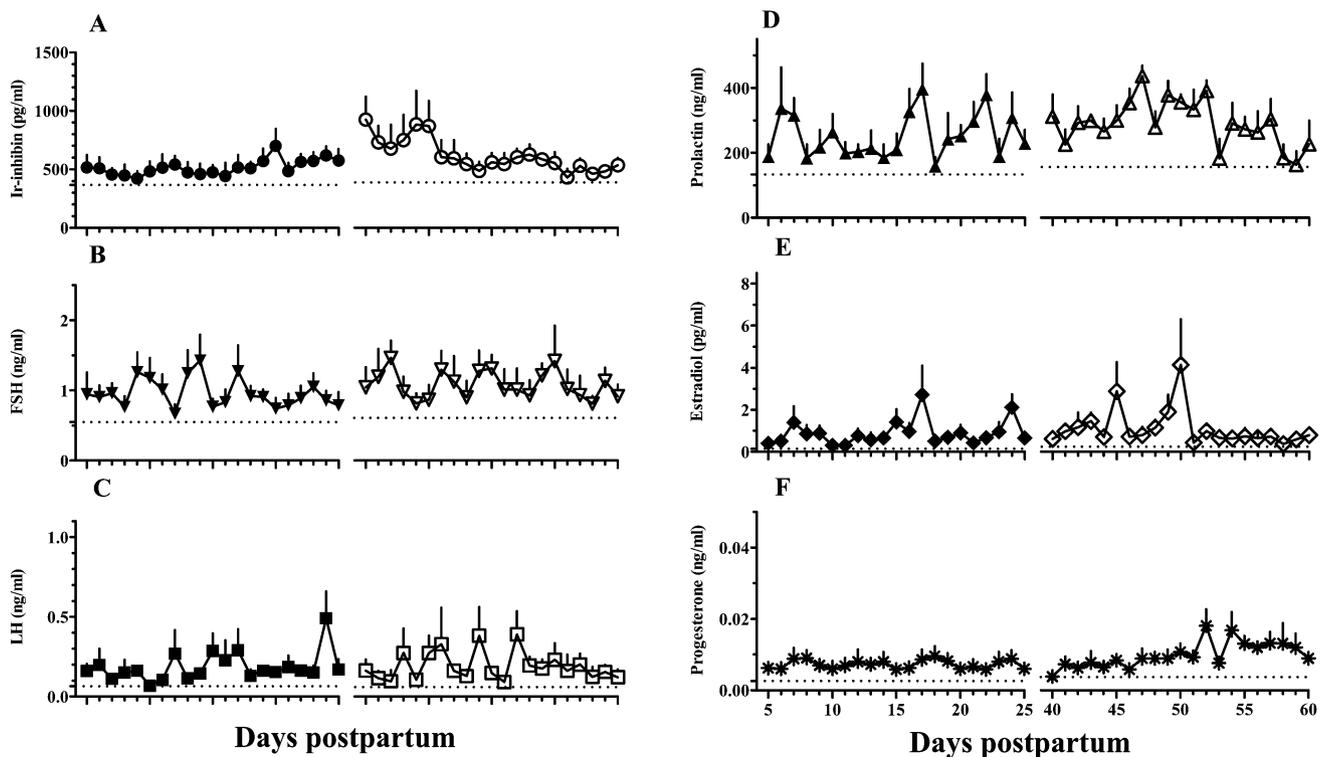
Once follicles develop to the Graafian stage, they begin to progress through stages of recruitment, selection and dominance. The

**Table 1.** The overall mean follicle population numbers, maximum follicle diameter, timing of follicular wave emergence and FSH peaks during the early (5–25 days postpartum) and late (40–60 days postpartum) lactation periods (Day 0 = day of kidding)

	Stage of postpartum period	
	Early lactation	Late lactation
Follicle number/population		
Fol. <2.5 mm	$0.54 \pm 0.06^a$	$0.10 \pm 0.03^b$
Fol. 2.5–3.5 mm	$3.87 \pm 0.24^a$	$1.94 \pm 0.13^b$
Fol. 3.5–4.5 mm	$4.55 \pm 0.25^b$	$5.66 \pm 0.22^a$
Fol. 4.5–5.5 mm	$2.32 \pm 0.17^b$	$4.96 \pm 0.27^a$
Fol. >5.5 mm	$0.94 \pm 0.13^b$	$2.32 \pm 0.17^a$
Max. follicle diameter (mm)/follicular wave		
First wave	$5.6 \pm 0.3^b$	$6.8 \pm 0.7^a$
Second wave	$5.5 \pm 0.2^b$	$6.9 \pm 0.5^a$
Third wave	$5.9 \pm 0.3^b$	$7.0 \pm 0.6^a$
Fourth wave	$6.2 \pm 0.2^a$	$6.9 \pm 0.6^a$
Fifth wave	$6.1 \pm 0.9^a$	$6.4 \pm 0.3^a$
Sixth wave		$6.3 \pm 0.2$
Timing of wave emergence		
First wave	$8.3 \pm 1.0$	$43.3 \pm 0.7$
Second wave	$11.8 \pm 0.8$	$46.8 \pm 1.0$
Third wave	$15.8 \pm 1.0$	$51.3 \pm 0.4$
Fourth wave	$20.3 \pm 0.6$	$54.3 \pm 0.4$
Fifth wave	$23.0 \pm 0.4$	$57.0 \pm 0.4$
Sixth wave		$59.1 \pm 0.5$
Timing of FSH peak		
First peak	$6.3 \pm 0.5$	$42.0 \pm 0.4$
Second peak	$10.0 \pm 0.4$	$45.5 \pm 0.5$
Third peak	$14.0 \pm 0.4$	$49.0 \pm 0.4$
Fourth peak	$17.5 \pm 0.6$	$52.8 \pm 0.6$
Fifth peak	$23.5 \pm 0.5$	$56.0 \pm 0.5$
Sixth peak		$58.3 \pm 0.3$
Growth rate (mm/day)	$0.65 \pm 0.05^b$	$0.84 \pm 0.06^a$
Regression rate (mm/day)	$0.54 \pm 0.04^b$	$0.81 \pm 0.05^a$
Interwave interval (days)	$3.88 \pm 0.33^a$	$3.45 \pm 0.25^a$
Interpeak interval (days)	$3.87 \pm 0.31^a$	$3.68 \pm 0.29^a$

Data are expressed as mean  $\pm$  SEM. Values within the same row with different superscripts are significantly different ( $P < 0.05$ ). Fol.: follicle.

number of ovulatory follicles is determined by the follicles that pass through the last two stages and is markedly influenced by FSH and subsequently LH in all species [21]. Following parturition, the gravid uterus has to return to the nonpregnant state and sexual cyclicity must be resumed to achieve conception. The efficiency of these two critical reproductive processes reflects the significance of the postpartum period as a pivotal time in the production cycle [22]. To the best of our knowledge, the present work is the first report on the antral follicular population and follicular dynamics during lactation anestrus in goats. In the current study, it was possible to confirm that the lactation period has a considerable effect on the follicular dynamic and hormonal parallelism in Shiba goats, as the total follicular area, number of emerged waves and secretion of ir-inhibin, estradiol and progesterone were significantly higher during late lactation.



**Fig. 4.** Changes in plasma concentrations of ir-inhibin (A), FSH (B), LH (C), prolactin (D), estradiol (E) and progesterone (F) during early and late stages of lactation in goats. The dotted line represents the basal hormonal level, which was calculated per goat as the mean of the lowest five measurements during the monitored period. Data are presented as mean  $\pm$  SEM of six goats.

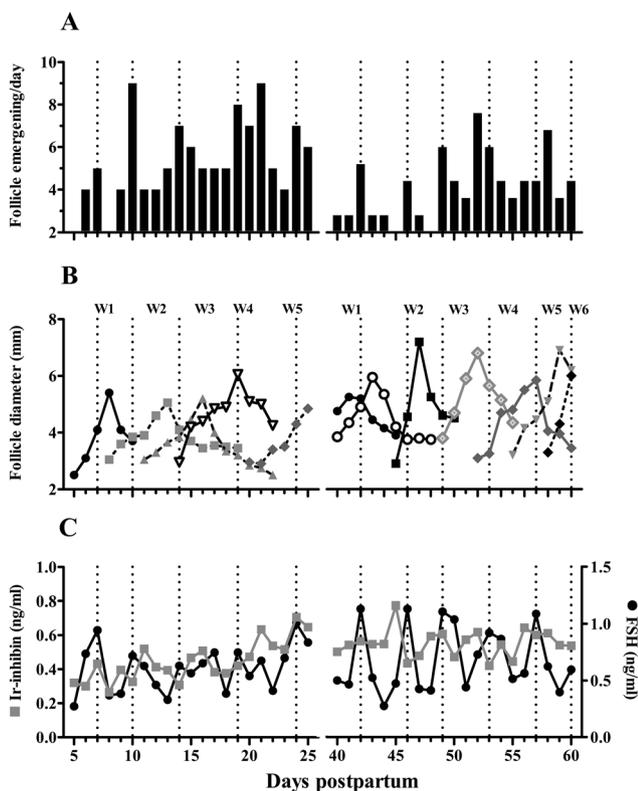
**Table 2.** Changes in the total hormonal secretion, peak hormonal secretion and overall mean hormone level during the early and late lactation periods in goats

	Total hormonal secretion		Hormonal peak		Overall hormone level	
	Early lactation	Late lactation	Early lactation	Late lactation	Early lactation	Late lactation
Ir-inhibin (pg/ml)	10300 $\pm$ 1538 <sup>b</sup>	12260 $\pm$ 1906 <sup>a</sup>	798.5 $\pm$ 122.4 <sup>B</sup>	1267.0 $\pm$ 237.3 <sup>A</sup>	516.3 $\pm$ 14.5 <sup>†</sup>	618.5 $\pm$ 30.6 <sup>‡</sup>
LH (ng/ml)	3.69 $\pm$ 0.69	3.886 $\pm$ 0.6174	0.63 $\pm$ 0.16	0.80 $\pm$ 0.19	0.185 $\pm$ 0.012	0.192 $\pm$ 0.024
Estradiol-17 $\beta$ (ng/ml)	8.28 $\pm$ 2.34	11.54 $\pm$ 3.98	2.83 $\pm$ 0.61 <sup>B</sup>	6.59 $\pm$ 2.05 <sup>A</sup>	0.89 $\pm$ 0.13	1.10 $\pm$ 0.20
Prolactin (ng/ml)	141000 $\pm$ 16110	160200 $\pm$ 12000	610.4 $\pm$ 92.6	468.6 $\pm$ 45.9	251.60 $\pm$ 15.05	290.60 $\pm$ 15.34
FSH (ng/ml)	19.34 $\pm$ 2.51	21.73 $\pm$ 3.75	2.11 $\pm$ 0.26	2.14 $\pm$ 0.40	0.96 $\pm$ 0.05	1.08 $\pm$ 0.04
Progesterone (ng/ml)	0.15 $\pm$ 0.02 <sup>b</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.0166 $\pm$ 0.0018 <sup>A</sup>	0.0263 $\pm$ 0.0022 <sup>B</sup>	0.0073 $\pm$ 0.0003 <sup>†</sup>	0.0096 $\pm$ 0.0008 <sup>‡</sup>

Data are expressed as means  $\pm$  SEM. Values within the same row and category with different superscripts are significantly different ( $P < 0.05$ ).

The current results showed that there was an increase in follicular maturation with advancement of the postpartum period, as indicated by an increase in the number of medium- (3.5–5.5 mm) and large-sized (>5.5 mm) follicles, while the number of small follicles (<3.5 mm) decreased. Such growth of medium and large follicles provides a pool of antral follicles from which ovulatory follicles could be selected [23], with FSH and subsequently LH playing major roles [21]. A similar pattern of follicular development has been observed in the sow [24]; on the other hand, as the lactation progressed there was a gradual shift in the follicles into medium- or large-sized categories, and the rate of atresia declined. This probably resulted from a local residual inhibitory effect of a

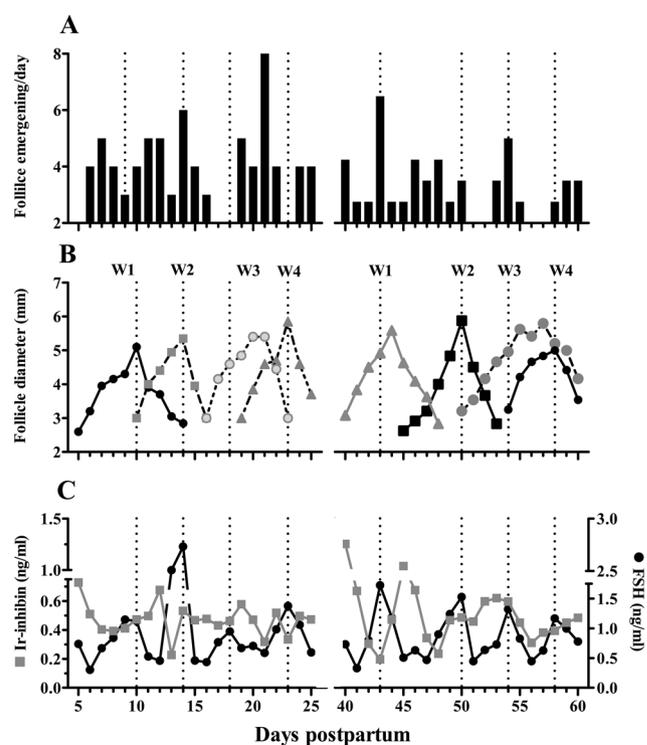
CL of pregnancy [6], low diet and negative energy balance [25], strong suckling stimulus [26], which is greater in highly stressed animals [27], or physiological production stress, which decreases serum IGF-1 [28], during the first half of lactation. Studies in ewes [6] indicated that residual local inhibition of follicular development by a CL of pregnancy extends to the postpartum period. Moreover, the number of small antral follicles (which constitute more than half of the total follicular population) was lowest on day 17 and highest on day 32, while the number of large follicles was highest on day 24 and slightly lower on day 32 postpartum [29]; it also seemed to be influenced by diet and can be increased by administration of GnRH [25]. In postpartum rats, suckling inhibits GnRH/LH-pulses, reduces



**Fig. 5.** Changes in daily follicle emergence (A) and follicle diameter profiles (mm) (B) for a representative individual goat having 5 and 6 waves were examined for 20 days during the postpartum anestrus period (first and second periods: 5–25 days and 40–60 days postpartum, respectively) and accompanying hormonal changes (C) in ir-inhibin (■) and FSH (●). The dotted line indicates the days of FSH peaks, which were defined as the days on which the plasma FSH level increased to > 30% more than the previous nadir level. Follicles were included in a wave if they emerged within the 48-h period of wave emergence.

follicular development and delays the resumption of ovulation [30]. Intracerebroventricular administration of corticotropin-releasing factor or beta-endorphin caused a prolonged inhibition of LH and FSH secretion in the lactating rat [31], suggesting that the stress of suckling alone is able to suppress the secretion of both LH and FSH. In cows under extensive management, follicles during the first 6 months postpartum grew to less than 6 mm in diameter [32] due to production stress during early lactation. On the other hand, mid lactation was associated with potential differences in follicle measurements and an increase in serum IGF-1 [28].

Understanding the pattern of follicle development is increasingly important for designing improved methods to manipulate reproduction in animals. The results of the present study showed that the number of emerged follicular waves changed with the lactation period, since five and six waves of follicle development were monitored during the early and late lactation periods, respectively. The study of follicular waves in postpartum animals of different levels of fertility and during different periods of activity may provide an



**Fig. 6.** Changes in daily follicle emergence (A) and follicle diameter profiles (mm) (B) for a representative individual goat having 4 waves were examined for 20 days at two different times during the postpartum anestrus period (first and second periods: 5–25 and 40–60 days postpartum, respectively) and accompanying hormonal changes (C) in ir-inhibin (■) and FSH (●). The dotted line indicates the days of FSH peaks, which were defined as the days on which the plasma FSH level increased to > 30% more than the previous nadir level. Follicles were included in a wave if they emerged within the 48-h period of wave emergence.

objective tool for selection of animals and periods with potentially higher fertility [33]; nevertheless, the dynamics of follicle wave development in goats have received less attention than in cattle or sheep. In cows [34] and ewes [21], follicles of various sizes were present throughout postpartum period, and folliculogenesis occurs in a wave-like pattern. In cows, follicular waves resumed generally 10 days after parturition [35] and became more regular from 7 to 12 months postpartum [32]. The high metabolic demands of lactation were found to markedly alter the follicular dynamics during the postpartum period [36].

In the current results, the plasma FSH levels fluctuated during the postpartum period, with the incidence of FSH peaks every 3–6 days, and the timing of FSH peaks was closely correlated with the timing of wave emergence. In ewes, FSH secretion followed a quite clear wave-like pattern from the day after lambing that suggested a 4–6-day rhythm [37]. These findings are concomitant with earlier findings in cyclic goats [38] and indicated that the follicular waves occur regularly under conditions of basal LH and FSH during the postpartum period in the goat.

In regard to the hormonal secretion in postpartum goats, the

overall mean plasma levels of FSH, LH, PRL and estradiol hormones did not show differences between the two lactation periods. On the other hand, the overall mean levels of ir-inhibin and progesterone and the hormonal peaks of estradiol were significantly higher during the late lactation period. Earlier work in dairy cows [39] demonstrated that the pituitary FSH is greatest at parturition and decreases during the early postpartum period but that plasma FSH during days 21 to 48 postpartum does not differ from the levels on days 0 to 20. Plasma LH concentrations during the first 30 days postpartum are usually low in suckled cows [40]. A gradual increase in LH concentration after parturition did not appear in postpartum ewes in good body condition [41]. Kann and Martinet [42] demonstrated a large release of PRL in nursing ewes, and this reflex secretion of PRL decreases as lactation progresses, whereas the hypothalamic mechanism mediating suckling-induced PRL release becomes refractory to the suckling stimulus during the preweaning period [43]. In ewes, plasma estrogen concentrations decrease rapidly during the day after parturition and remain low until follicular growth occurs [41]. Significantly higher estradiol-17 $\beta$  levels were found in late lactating (20 days postpartum) compared with early lactating (10 days postpartum) rats [44]. The observed increase in ir-inhibin and peaks of estradiol during late lactation might be due to the high number of the recruited large-sized follicles during this period. Inhibins are gonadal glycoprotein hormones produced mainly by all ovarian follicles with a higher production from the large antral follicles and are used as a potential indicator of follicular development [45], whilst estradiol is produced largely from large matured follicles [46]. In cows, increases in plasma inhibin A levels were associated with increases in plasma estradiol levels during most follicular waves; however, there was no increase in plasma estradiol level and no relationship between patterns of estradiol and FSH during follicular waves observed during the early postpartum period [47]. In nursing rats, the concentrations of ir-inhibin in the peripheral plasma were always low on days 3 and 5 of lactation and became high on days 17 and 20 of lactation [48].

The hormonal interrelationships during the postpartum period were not studied in postpartum goats until now. The present study was the first study to elucidate the positive ir-inhibin-estradiol, LH-estradiol and LH-PRL correlations and the negative ir-inhibin-FSH correlation in postpartum goats, but some correlations were previously reported in cyclic goats [38] and cows [47]. The positive correlation between LH and ir-inhibin during early lactation probably resulted from low pituitary production of LH [40] and low follicle maturation that reflected on low inhibin production. Conversely, PRL was negatively correlated with FSH during early lactation due to high release of PRL in nursing animals [42] and depletion of pituitary PRL stores in late lactation [44], indicating that the strong suckling stimulus after parturition might interfere with the resumption of cyclic ovarian activity [49] through the hypothalamic-pituitary-adrenal axis [49–51]. Ewes nursing lambs had a large release of PRL and long postpartum anestrus period, 60–80 days [42].

In summary, data presented in this study showed that the late lactation period was characterized by an increase in the population of medium- and large-sized follicles and the emerged follicular waves, as well as the changes in the ovarian hormones and hormonal

interrelationship. Such changes indicated an improvement in ovarian function, which might help in designing improved methods to manipulate goat reproduction and ending lactation anestrus in the goat. The negative correlation between PRL and FSH during early lactation indicated that the strong suckling stimulus after kidding might interfere with the resumption of cyclic ovarian activity through the hypothalamic-pituitary-adrenal axis in Caprine species.

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## References

1. Evans AC. Characteristics of ovarian follicle development in domestic animals. *Reprod Domest Anim* 2003; **38**: 240–246. [Medline] [CrossRef]
2. Rubianes E, Menchaca A. The pattern and manipulation of ovarian follicular growth in goats. *Anim Reprod Sci* 2003; **78**: 271–287. [Medline] [CrossRef]
3. Ababneh MM, Degefa T. Bacteriological findings and hormonal profiles in the postpartum Balady Goats. *Reprod Domest Anim* 2006; **41**: 12–16. [Medline] [CrossRef]
4. Sosa GAM. Ultrasonographic and hormonal studies on normal pregnant and pregnant-toxicemic Balady goats during late gestation and postpartum period. *In: Proc The 1st Inter Conf Vet Div, NRC, 2004; Cairo, Egypt, 7–18.*
5. Al-Gubory KH, Martinet J. Effect of the corpus luteum on ovarian follicular population and growth in ewe. *Anim Reprod Sci* 1987; **13**: 269–281. [CrossRef]
6. Bartlewski PM, Beard AP, Rawlings NC. Ultrasonographic study of ovarian function during early pregnancy and after parturition in the ewe. *Theriogenology* 2000; **53**: 673–689. [Medline] [CrossRef]
7. Bellin ME, Hinshelwood MM, Hauser ER, Ax RL. Influence of lactation and side of corpus luteum of pregnancy on folliculogenesis in postpartum cows. *Biol Reprod* 1984; **31**: 849–855. [CrossRef]
8. Murphy MG, Boland MP, Roche JF. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. *J Reprod Fertil* 1990; **90**: 523–533. [Medline] [CrossRef]
9. Dufour JJ, Roy GL. Distribution of ovarian follicular populations in the dairy cows within 35 days after parturition. *J Reprod Fertil* 1985; **73**: 229–235. [Medline] [CrossRef]
10. Spicer LJ, Leung K, Convey EM, Gunther J, Short RE, Tucker HA. Anovulation in postpartum suckled beef cows. I. Associations among size and numbers of ovarian follicles, uterine involution, and hormones in serum and follicular fluid. *J Anim Sci* 1986; **62**: 734–741. [Medline]
11. Lucy MC, Staples CR, Michel FM, Thatcher WW. Energy balance and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. *J Dairy Sci* 1991; **74**: 473–482. [Medline] [CrossRef]
12. Griffin J, Emery BR, Huang I, Peterson CM, Carrell DT. Comparative analysis of follicle morphology and oocyte diameter in four mammalian species (mouse, hamster, pig, and human). *J Exp Clin Assist Reprod* 2006; **3**: 2. [Medline] [CrossRef]
13. Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. *Theriogenology* 1994; **42**: 987–1001. [Medline] [CrossRef]
14. Bartlewski PM, Beard AP, Rawlings NC. An ultrasound-aided study of the temporal relationships between the patterns of LH/FSH secretion, development of ovulatory-sized antral follicles and formation of corpora lutea in ewes. *Theriogenology* 2000; **54**: 229–245. [Medline] [CrossRef]
15. Taya K, Watanabe G, Sasamoto S. Radioimmunoassay for Progesterone, testosterone-

- one and estradiol 17 $\beta$  using <sup>125</sup>I-iodohistamine radioligands. *Jpn J Anim Reprod* 1985; **31**: 186–197.
16. **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. [Medline]
  17. **Mori Y, Kano Y.** Changes in plasma concentration 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. [Medline] [CrossRef]
  18. **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. [Medline]
  19. **Davies JS, Carter DA, Wells T.** Photic stimulation inhibits growth hormone secretion in rats: a hypothalamic mechanism for transient entrainment. *Endocrinology* 2004; **145**: 2950–2958. [Medline] [CrossRef]
  20. **Alvarez L, Galindo F.** Daily cortisol profile in lactating and non-lactating dairy goats. *Res J Anim Sci* 2008; **2**: 72–77.
  21. **Hunter MG, Robinson RS, Mann GE, Webb R.** Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci* 2004; **82–83**: 461–477. [Medline] [CrossRef]
  22. **Abeygunawardena H, Dematawewa CMB.** Pre-pubertal and postpartum anoestrus in tropical Zebu cattle. *Anim Reprod Sci* 2004; **82–83**: 373–387. [Medline] [CrossRef]
  23. **Villeneuve P, Dufour JJ, Guilbault LA.** Influence of infusion of prostaglandin F<sub>2a</sub> (PGF<sub>2a</sub>) and weaning on surface and histologic populations of ovarian follicles in early postpartum beef cows. *J Anim Sci* 1988; **66**: 3174–3184. [Medline]
  24. **Britt JH, Armstrong JD, Cox NM, Ebsenshade KL.** Control of follicular development during and after lactation in sows. *J Reprod Fertil Suppl* 1985; **33**: 37–54. [Medline]
  25. **Mitchell LM, Ranilla MJ, Quintans G, King ME, Gebbie FE, Robinson JJ.** Effect of diet and GnRH administration on post-partum ovarian cyclicity in autumn-lambing ewes. *Anim Reprod Sci* 2003; **76**: 67–79. [Medline] [CrossRef]
  26. **Taya K, Greenwald GS.** Mechanisms of suppression of ovarian follicular development during lactation in the rat. *Biol Reprod* 1982; **27**: 1090–1101. [Medline] [CrossRef]
  27. **Asai S, Ohta R, Shirota M, Tohei A, Watanabe G, Taya K.** Endocrinological responses during suckling in Hatano high- and low-avoidance rats. *J Endocrinol* 2004; **182**: 267–272. [Medline] [CrossRef]
  28. **Walters AH, Pryora AW, Baileyb TL, Pearsona RE, Gwazdauskas FC.** Milk yield, energy balance, hormone, follicular and oocyte measures in early and mid-lactation Holstein cows. *Theriogenology* 2002; **57**: 949–961. [Medline] [CrossRef]
  29. **Vlčková R, Valocký I, Lazar G, Sopková D, Maraček I.** Histological and ultrasonographic monitoring of folliculogenesis in puerperal ewes after spring lambing. *Acta Vet Brno* 2008; **77**: 65–72. [CrossRef]
  30. **Williams GL, Gazal OS, Guzmán Vega GA, Stanko RL.** Mechanisms regulating suckling-mediated anovulation in the cow. *Anim Reprod Sci* 1996; **42**: 289–297. [CrossRef]
  31. **Taya K, Sasamoto S.** Inhibitory effects of corticotrophin-releasing factor and  $\beta$ -endorphin on LH and FSH secretion in the lactating rat. *J Endocrinol* 1989; **120**: 509–515. [Medline] [CrossRef]
  32. **Ruis-Cortés ZT, Olivera-Angel M.** Ovarian follicular dynamics in suckled zebu (*Bos indicus*) cows monitored by real time ultrasonography. *Anim Reprod Sci* 1999; **54**: 211–220. [Medline] [CrossRef]
  33. **Oliveira JF, Neves JP, Moraes JC, Gonçalves PB, Bahr JM, Hernandez AG, Costa LF.** Follicular development and steroid concentrations in cows with different levels of fertility raised under nutritional stress. *Anim Reprod Sci* 2002; **73**: 1–10. [Medline] [CrossRef]
  34. **Savio JD, Boland MP, Hynes N, Roche JF.** Resumption of follicular activity in the early post-partum period of dairy cows. *J Reprod Fertil* 1990; **88**: 569–579. [Medline] [CrossRef]
  35. **Driancourt MA.** Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. *Theriogenology* 2001; **55**: 1211–1239. [Medline] [CrossRef]
  36. **Thatcher WW, de la Sota RL, Schmitt EJ, Diaz TC, Badinga L, Simmen FA, Staples CR, Drost M.** Control and management of ovarian follicles in cattle to optimize fertility. *Reprod Fertil Dev* 1996; **8**: 203–217. [Medline] [CrossRef]
  37. **Schirar A, Cognié Y, Louault F, Poulin N, Meusnier C, Levasseur MC, Martinet J.** Resumption of gonadotrophin release during the post-partum period in suckling and non-suckling ewes. *J Reprod Fertil* 1990; **88**: 593–604. [Medline] [CrossRef]
  38. **Medan MS, Watanabe G, Sasaki K, Sharawy S, Groome NP, Taya K.** Ovarian dynamics and their associations with peripheral concentrations of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. *Biol Reprod* 2003; **69**: 57–63. [Medline] [CrossRef]
  39. **Dobson MS.** Radioimmunoassay of FSH in the plasma of post-partum dairy cows. *J Reprod Fertil* 1978; **52**: 45–49. [Medline] [CrossRef]
  40. **Radford HM, Nancarrow CD, Mattner PE.** Ovarian function in suckling and non-suckling beef cows post partum. *J Reprod Fertil* 1978; **54**: 49–56. [Medline] [CrossRef]
  41. **Gonzalez A, Murphy BD, de Alba J, Manns JG.** Endocrinology of the postpartum period in the Pelibuey ewe. *J Anim Sci* 1987; **64**: 1717–1724. [Medline]
  42. **Kann G, Martinet J.** Prolactin levels and duration of postpartum anoestrus in lactating ewes. *Nature* 1975; **257**: 63–64. [Medline] [CrossRef]
  43. **Joke T.** Prolactin release in response to milking stimulus in the cow and goat estimated by radioimmunoassay. *Endocrinol Jpn* 1969; **16**: 179–185. [Medline] [CrossRef]
  44. **Selmanoff M, Selmanoff C.** Pup age, estradiol-17 $\beta$  and pituitary responsiveness in the differences in the suckling-induced prolactin response during early and late lactation. *Biol Reprod* 1983; **29**: 400–411. [Medline] [CrossRef]
  45. **Nambo Y, Tanaka Y, Nagata S, Sato F, Hasegawa T, Nakai R, Oki H, Watanabe G, Taya K.** Inhibin as a Possible Indicator of Follicular Development during Perinatal Period in Mares. *J Reprod Dev* 2002; **48**: 265–270. [CrossRef]
  46. **Glister C, Groome NP, Knight PG.** Bovine follicle development is associated with divergent changes in activin-A, inhibin-A and follistatin and the relative abundance of different follistatin isoforms in follicular fluid. *J Endocrinol* 2006; **188**: 215–225. [Medline]
  47. **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. [Medline] [CrossRef]
  48. **Taya K, Sasamoto S.** Mechanisms responsible for suppression of FSH and LH during lactation in the rat. *J Endocrinol* 1991; **129**: 119–130. [Medline] [CrossRef]
  49. **Taya K, Sasamoto S.** Difference in the response of follicular maturation and ovulation between early and late lactating rats after removal of the litter. *J Endocrinol* 1987; **113**: 271–276. [Medline] [CrossRef]
  50. **Taya K, Sasamoto S.** Involvement of the adrenal gland in the suckling-induced decrease in LH and FSH secretion and the concomitant increase in prolactin secretion in the rat. *J Endocrinol* 1990; **125**: 279–285. [Medline] [CrossRef]
  51. **Hinshelwood MM, Dierschke DJ, Hauser ER.** Effect of lactation on the hypothalamic-pituitary axis in postpartum beef cows, independent of ovarian secretions. *Biol Reprod* 1985; **32**: 290–300. [Medline] [CrossRef]