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Patterns of Pulsatile and Surge Modes of Follicle-Stimulating Hormone Treated with Different Progesterone Levels in Ovariectomized Goats

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Abstract : The objective of the present study was to determine the progesterone levels that effects on the pulsatile and surge modes of FSH secretion. In previous studies we have shown that LH surge occurred in the follicular levels of progesterone, whereas there was no surge mode secretion of LH in either the subluteal or luteal levels of progesterone. LH pulsatile frequencies were high in two groups such as follicular level and subluteal level. But in the luteal level of progesterone the pulsatile pattern of LH were strongly suppressed. Namely, subluteal levels of progesterone, around 1 ng/ml, completely suppressed the LH surge but did not affect the pulsatile frequency of LH secretion. Because of this we hypothesized that the two secretory patterns of FSH are similar to that of LH. Long-term ovariectomized Shiba goats that had received implants of estradiol capsules and three different progesterone silastic packet inducing follicular, subluteal and luteal levels of progesterone were divided into three groups such as non-P, low-P and high-P group. Blood samples were collected daily throughout the experiment for the analysis of gonadal steroid hormone levels and at 10-min intervals for 8 h on Days 0, 3, and 7 (Day 0: just before progesterone treatment) for analysis of the pulsatile frequency of FSH secretion. Then estradiol was infused into the jugular vein of all animals at a rate of 3 μg/h for 16 h on Day 8 to determine whether an FSH surge was induced. Blood samples were collected every 2 h from 4 h before the start of the estradiol infusion until 48 h after the start of the infusion. In each group, the mean ± SEM concentration after progesterone implant treatment was 3.3 ± 0.1 ng/ml for the high P group, 1.1 ± 0.1 ng/ml for the low P group, and < 0.1 ng/ml for the non-P group, concentrations similar to the luteal levels, subluteal levels, and follicular phase levels of the normal estrous cycle, respectively. The FSH pulse frequency was maintained highly in all groups on Day 0, Day 3 and Day 7. An FSH surge was induced in all 4 cases of the Non-P group. In the High P and Low P groups, the plasma concentrations of FSH remained low until 48 h after the start of estradiol infusion, and no occurrence of FSH surge was found in any of the animals. The results of this study not only confirm that the pulsatile patterns of FSH were not inhibited strongly relative to LH, they also suggest that some other mechanism and factor may be controlling the FSH secretion.

Key words : follicle-stimulating hormone, ovary, progesterone.

Introduction

Ovarian steroid hormones, such as estradiol and progesterone have diverse effects upon gonadotropin secretion. It is well known that ovarian steroids can negatively regulate plasma and pituitary concentration of LH (10,26). For examples, increasing levels of progesterone at the luteal phase of estrous cycle, progesterone suppressed pulsatile secretion of LH by negative feedback action (2,14,46). In addition to their negative regulatory effect on LH, estradiol is also capable of increasing the secretion of LH and FSH from the pituitary gland (7,24, 38,45). There are some controversial concepts regarding the mechanism by which estradiol and progesterone regulate LH and FSH. Although it is well known that the patterns of LH secretion are dependent on GnRH patterns (4,42), there are various regulatory factors in FSH secretion, such as steroids (9,37) and proteins (2,5,6,48) at the site of both ovarian and pituitary gland.

Previous observations indicated that the correlation between LH pulse and FSH pulse became loose in the luteal phase and that the pulsatile and surge modes of LH secretion are different in the subluteal levels of progesterone in ovariectomized goats (17). In this regard, it is necessary to examine how FSH secretion is regulated by varying progesterone concentrations and whether secretion of FSH is closely, if not entirely, coupled to peripheral patterns of LH secretion. Also, to develop more effective methods of controlling reproduction, a better understanding of how secretions of FSH are regulated in the similar condition to the period of the estrous cycle is required.

The purpose of the present study was to determine the effects of different progesterone levels similar to follicular phase, subluteal phase, and luteal phase, on secretion patterns of FSH in ovariectomized goats treated with subcutaneous implants of

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progesterone.

Materials and Methods

Animals

Long-term ovariectomized Shiba goats (n = 9) weighing between 20 and 30 kg (mean ± SD, 24.6 ± 1.9 kg) were used. Shiba goats are nonseasonal breeders under natural daylight (25). The goats were ovariectomized at least 3 mo before the start of the experiment and were maintained outdoors with a diet of hay-cubes given twice daily and water given ad libitum. Basically, the animals in this study were divided into three groups in a 3 × 3 Latin square design (finally, n = 4 or 5 for each group), and at least 4 wk were allowed to elapse after each treatment. All animals were housed and loose-tied at the start of the experiment.

Implantation and infusion of ovarian steroids

Initially, all Shiba goats received implants of silastic capsules (inside diameter, 3.35 mm; outside diameter, 4.65 mm; length, 40 mm; Dow Corning Co., Midland, MI) containing crystalline estradiol (Sigma, St. Louis, MO) subcutaneously as reported previously (39,41), which induced levels similar to luteal levels of estradiol (3-10 pg/ml) (25,41). Seven days later, they were separated into three groups according to the use of an exogenous device for providing progesterone: a high progesterone (high P) group that received a subcutaneous implant of a silastic packet (50 × 70 mm; Dow Corning) containing crystalline progesterone (Wako, Tokyo, Japan), which reproduces the plasma progesterone level in the midluteal phase (3-8 ng/ml) (25,41), and a low progesterone (low P) group that received a subcutaneous implant of a smaller packet (25 × 40 mm; Dow Corning) containing crystalline progesterone, which imitated subluteal levels of progesterone (around 1 ng/ml). The control (non-P) group had no implant progesterone treatment, which paralleled to follicular levels of progesterone (< 0.1 ng/ml) (25,28). Daily blood sampling (5 ml) was conducted throughout the experiment to monitor the steroidal level in the circulation. Blood samples were collected every 10 min for 8 h just before progesterone treatment (Day 0) and 3 days (Day 3) and 7 days (Day 7) after progesterone treatment for analysis of FSH pulses. Then all animals were given estradiol on the day after last repetitive sampling (Day 8) for analysis of the effect on the FSH surge. Estradiol (Sigma) was dissolved in ethanol (100 μg/ml), diluted with sterilized saline to a concentration of 0.3 μg/ml, and infused with a peristaltic mini pump (SJ-1211; Atto, Tokyo, Japan) into the jugular vein at a rate of 3 μg/h for 16 h through one of the catheters fitted bilaterally to the jugular vein, as previously described (40). This treatment is known to be able to induce an LH surge starting approximately 10 h after the start of estradiol infusion in ovariectomized goats, as described previously (40). Blood samples (2 ml) were collected via another jugular catheter at 2-h intervals for 52 h (from 4 h before the start of estradiol infusion to 48 h after the start of estradiol infusion) for analysis of the plasma concentrations of FSH and estradiol.

Blood sampling

Blood samples for analysis of the FSH pulse and FSH surge were collected from the catheterized jugular vein into heparinized tubes. A catheter (18 gauge, 51-mm length; Terumo Co., Tokyo, Japan) was inserted into the jugular vein just before the start of blood sampling. Blood samples were immediately stored at 4°C and centrifuged at × 3000 rpm for 20 min, and then plasma was stored at −20°C until assayed for FSH, progesterone, and estradiol.

Hormone assays

Plasma concentrations of progesterone and estradiol were assayed by a previously described method (43). The sensitivity of the assays for progesterone and estradiol were 0.015 ng/ml and 0.69 pg/ml, respectively. The intra-assay and interassay coefficients of variation were 8.22% and 0.2% for progesterone and 7.37% and 0.19% for estradiol, respectively.

Plasma concentrations of FSH were measured by a double-antibody radioimmunoassay (1). The following reagents were used: NIADDK-ovine FSH-I-1 for radioiodination, NIADDK-ovine FSH-RP-1 as a standard, anti-ovine FSH-1 (AFP-C5288113) as the first antibody, and goat anti-rabbit immunoglobulin as the second antibody. The sensitivity of the assay was 0.08 ng/ml. The intra-assay and interassay coefficients of variation were 9.33% and 10.99%, respectively.

Statistics

Data were analyzed using the Scheffe method of analysis of variance with the StatView computer program (StatView 4.5; Abacus Concepts Inc., Berkeley, CA). During the implantation of steroids, one-way analysis of variance was also used to determine the significance of differences among the mean concentrations of progesterone and estradiol among the three groups. All data are presented as mean ± SEM. The FSH pulse was identified using the Cluster Analysis Program (44). The FSH surge was defined as the point when a sustained rise (for at least two consecutive points of blood sampling) in the plasma FSH concentration exceeded twice the average baseline level during the pretreatment period before the estradiol infusion.

Results

Plasma concentrations of progesterone and estradiol

The plasma concentration is same as described in previous study (17) and used again in this study because blood samples were collected simultaneously to determine the patterns of LH and FSH and steroid hormones. The concentration of estradiol was increased the day after the implantation of estradiol capsules and was maintained at a basal level (4-8 pg/ml) up to Day 7 in all animals (Fig 1). The plasma progesterone concentrations were increased after the treatment with progesterone in the high P and low P groups but not in the non-P group (Fig 1). The mean concentration of progesterone was maintained

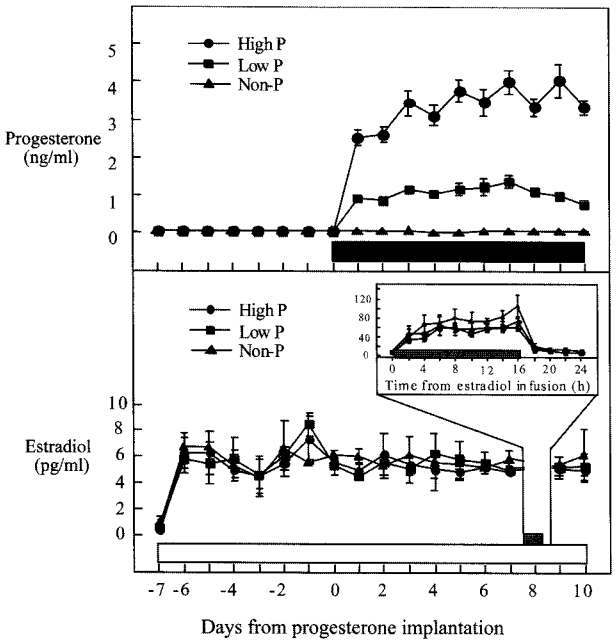

Fig 1. Plasma progesterone (top) and estradiol (bottom) concentration (meanrrrrrSEM) during the period of progesterone/estradiol implantation (black rectangle indicates progesterone implant, blank rectangle indicates estradiol implant). (High P is high progesterone group treated with a large packet (50 × 70 mm), Low P is low progesterone group treated with a small packet (25 × 40 mm); Non-P is no implant treatment). Inset in bottom figure shows the change in plasma estradiol concentration during estradiol infusion on Day 8 (shaded rectangle indicates estradiol infusion).

at the levels of the midluteal phase (range, 2.09-5.49 ng/ml; mean ± SEM, 3.3 ± 0.1 ng/ml) and the subluteal level (range, 0.61-2.59 ng/ml; mean ± SEM, 1.1 ± 0.1 ng/ml) in the high P and low P groups, respectively (17). There were significant differences among the three groups in the mean plasma concentrations of progesterone on all the days examined after the progesterone treatment ($P < 0.05$).

Table 1. The pulsatile frequency of follicle-stimulating hormone (pulses / 8h)[a] after treatment with luteal (High P) and subnormal (Low P) levels of progesterone in ovariectomized goats given a basal level of estradiol

	Day 0	Day 3	Day 7
High P (n = 5)	13.2 ± 0.8	11.0 ± 0.5	9.6 ± 0.7[b]
Low P (n = 5)	11.6 ± 0.4	10.0 ± 0.6	9.4 ± 0.4[b]
Non-P (n = 5)	11.6 ± 0.8	12.0 ± 0.7	10.6 ± 0.5

[a]Data are presented as mean ± SEM
[b]Indicates significant differences from Day 0 ($P < 0.05$)

Patterns of pulsatile FSH secretion

Representative patterns of pulsatile FSH secretion before and after progesterone treatment in the three groups are shown in Fig 2. The number of FSH pulses on Day 0, Day 3 and Day 7 is summarized in Table 1. There was no significant difference in the frequency of FSH pulses among the three groups on Day 0, Day 3 and Day 7, respectively ($P > 0.1$). In the High P and Low P groups, although the patterns of FSH pulses were maintained with high pulsatile frequency, the mean number of pulses was decreased gradually, and there was a significant difference on Day 7 compared to Day 0 ($P < 0.05$). Whereas there was no significant reduction of FSH pulse frequency among the three selected days in non-P groups ($P > 0.1$).

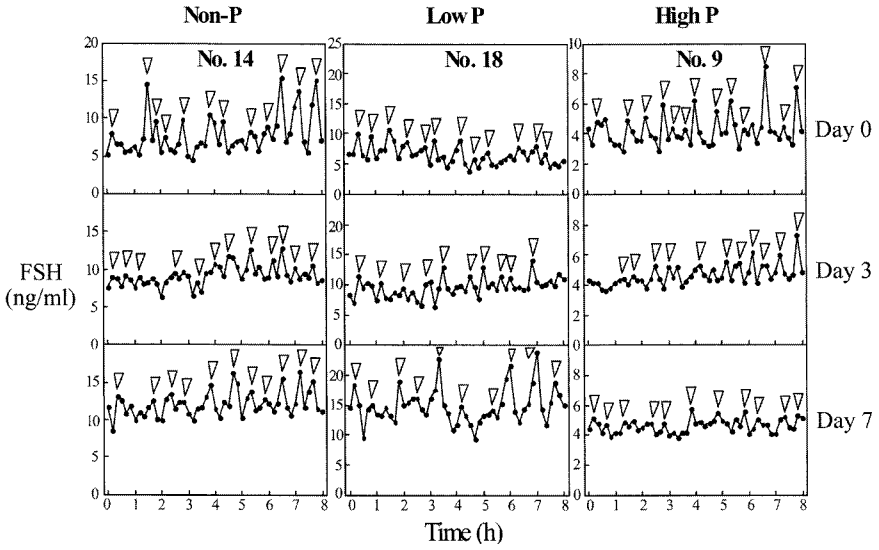

Fig 2. Patterns of pulsatile follicle-stimulating hormone (FSH) secretion in representative animals from the control (Non-P) group (No. 14, left panels), low progesterone (Low P) group (No. 18, middle panels) and high progesterone (High P) group (No. 9, right panels) on the day just before the progesterone treatment (Day 0, upper), and on the third day (Day 3, middle) and seventh day (Day 7, bottom) of progesterone treatment. Arrowheads indicate the FSH pulses.

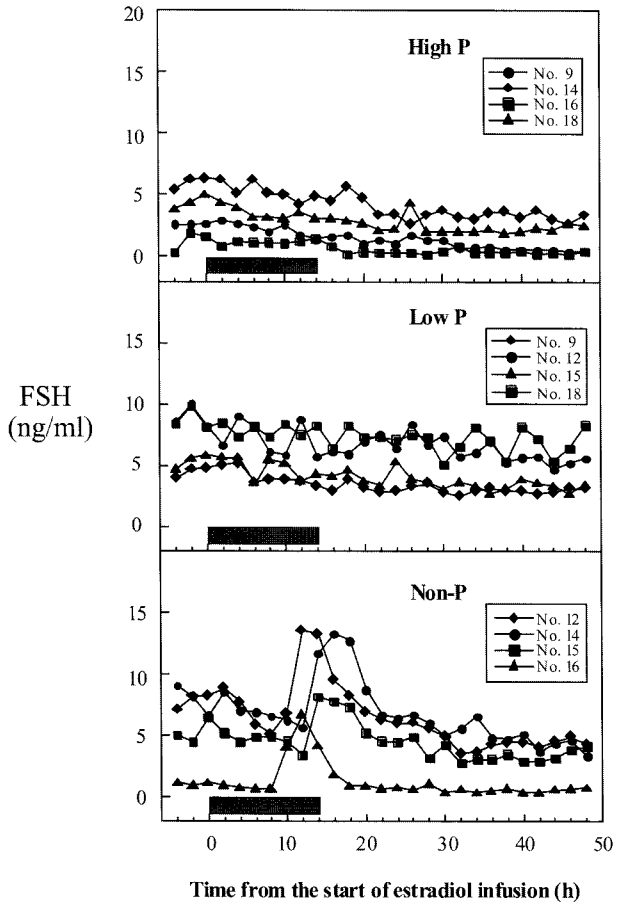

Fig 3. Profiles of follicle-stimulating hormone (FSH) concentration after the start of estradiol infusion in the high progesterone group (High P, top), low progesterone (Low P, middle) and control (Non-P, bottom) groups. Estradiol was infused at the rate of 3 μg/h through the jugular vein for 16 h, as indicated by the shaded rectangle. Each panel shows the FSH patterns of all animals in the respective group.

Occurrence of FSH Surges

The changes in the FSH concentration after estradiol infusion in all the animals examined are shown in Fig 3. An FSH surge was induced in all 4 cases of the Non-P group. The mean level of the peak of the FSH surge reached 10.4 ± 1.7 ng/ml and the time interval from estradiol infusion until the peak of FSH surge was 13.0 ± 0.9 h. In the High P and Low P groups, the plasma concentrations of FSH remained low (4.12 ± 0.2 ng/ml) until 48 h after the start of estradiol infusion, and no occurrence of FSH surge was found in any of the animals.

Discussion

During the estrous cycle of ruminant animals, progesterone is an important factor regulating the pulsatile pattern of LH secretion for the development of a dominant follicle, and secretion of estradiol from the dominant follicle (8,38). Until now, it is well known that the existence and absence of progesterone modulate the pattern of LH and FSH with differential effects (34,35,45,46). To identify the direct effects of ovarian steroid hormones in the modulation of FSH secretion patterns, the present study is conducted with long-term ovariectomized goats implanted with progesterone and estradiol.

The results of the present study indicate that progesterone above subluteal levels (around 1 ng/ml) suppressed the pulsatile secretion of FSH by a little difference in comparison to Day 0 with a manner of time dependence. However, the suppression patterns of pulsatile FSH secretion showed absolutely different from those of pulsatile LH secretion, by means of concordance of LH and FSH pulse, at the selected day in the three groups. Also, the entire pulsatile patterns of FSH showed a high and irregular pulsatile frequency during the treatment period of progesterone and estradiol as previously reported (33,34). These results are in agreement with, in part, the previous report that the slight reduction of FSH pulse frequency during the functional luteal phase could be a result of negative feedback inhibition by progesterone on GnRH generating system (35). The surge pattern of FSH induced by estradiol treatment occurred and was similar to natural follicular phase in the Non-P group but not in the subluteal and luteal levels of progesterone, moreover, the timing of FSH surge corresponded with expression of LH surge. It would be likely that subluteal and luteal levels of progesterone can completely suppress the surge pattern of both LH and FSH regulated by GnRH secretion.

It is also well established that progesterone regulates the GnRH neurosecretory system by the negative feedback action (15,22,36). However, a number of studies suggested that pulsatile FSH secretion is independent on GnRH (known as regulating the LH and FSH secretion) (9,12,31,47). For examples, GnRH pulsatile pattern regulates completely the LH pulse in the pituitary and peripheral levels (16) but not FSH pulse (30), suggesting that FSH pulsatility may be regulated by hypothalamic FSH-releasing factor different from LH-releasing hormones (LHRH) or the other factors including inhibin, activin and follistatin (2,32,48). Furthermore, previous studies demonstrating selective regulation of FSH release after ablation (20), deafferentation (19), or destruction (21) of the dorsal anterior hypothalamic area or electrochemical stimulation into hypothalamic regions apart from those of regulating LH secretion (12) strongly support the existence of a specific site of control for FSH release. Evidence supporting the existence of FSH-releasing factor was first suggested by Igarashi and McCann (13). Although considerable anatomical and biochemical evidence supports this possibility (29) and partial separation of a separate FSH-releasing activity has been achieved (23), no specific FSH-releasing factor has been isolated or found to be released into hypophyseal portal blood. However, the results of the present study showed that pulsatile FSH secretion was not inhibited abruptly by the administration of both subluteal and luteal levels of progesterone, showing slight and significant suppression in non-agreement with those of LH. Furthermore, the difference of pulsatile secretion between LH and FSH is disappeared in the course of gonadotropin

surge, suggesting that the surge mode of LH and FSH is simultaneously modulated by GnRH surge-generating mechanism in the normal and ovariectomized. This result also indicates that subluteal levels of progesterone also can suppress both pulsatile and surge modes of FSH, in disagreement with those of LH (17). In this regard, the surge mode of FSH secretion induced by estradiol may be regulated directly by neural generator of GnRH secretion, i.e., GnRH surge generator in hypothalamus (40), suggesting that GnRH surge generator would activate the secretion of GnRH.

In the respect of the amplitude of both LH and FSH surge, interestingly, it also may be discussed that FSH is regulated directly by scant strength of GnRH in comparison with LH. The results of the present studies showed that there has been a several folds (range, 5-10 folds) lower in the amplitude of FSH than LH, when estradiol treated exogenously in ovariectomized goats (17). Therefore, it may suggest that GnRH is a central factor to regulate directly and strongly the pulsatile and surge modes of LH. Furthermore, the results of previous study (30,37) may suggest that GnRH have influence on FSH secretion with an indirect and scant quantity in the hypothalamic levels. In this respect, it is also possible to consider that the pulsatile frequency of FSH secretion would be controlled by the different activator from GnRH pulse generator, known as that induce concomitantly LH pulse (42), because some studies suggested that the neural generator of GnRH pulse only regulate the GnRH secretion and LH secretion (18,27). Furthermore, the pulsatile pattern of FSH secretion in the present study did not agree with the modes of LH pulse, indicating that the GnRH pulse generator only regulate the LH pulse directly. In this regard, it can be considered that FSH pulse may be controlled by the other factors (23,29,32) in the hypothalamic and pituitary levels. These characteristics of FSH secretion pattern has more clearly established by Padmanabhan *et al.* (30) that pulsatile pattern of FSH may be controlled by two hypothalamic factors, i.e., GnRH-associated pulse regulation (concurrent with GnRH pulses) and non-GnRH-associated pulse (not concurrent with GnRH pulse). Namely, the non-GnRH-associated FSH pulses may be also regulated by the other factors in the ovarian and pituitary levels, including inhibin, follistatin, activin and follicular fluids.

In conclusion, the results of the present study demonstrated that the subluteal and luteal levels of progesterone suppressed completely the surge pattern of FSH and slightly, but significantly, the pulsatile pattern of FSH with a time-dependent manner. Also, because this result showed a differential expression between LH (17) and FSH in pulsatile pattern in the peripheral levels, it is able to consider that the hypothalamic regions for regulation of FSH pulse may differ from the sites of pulsatile regulation of LH. Insights into that reduction of FSH pulsatile frequency, but with high pulsatile frequency, is induced by treatments with low level and high concentration of progesterone to mimic subluteal and luteal levels of estrous cycle in the present study may suggest that the FSH pulse generator exist in the hypothalamic level different from GnRH pulse generator regulating LH pulsatile secretion. However, it is very ambiguous in that pulsatile FSH secretion (or basal concentration of FSH) can be regulated and altered by various factors, such as steroid hormones and peptides in the ovarian and pituitary levels.

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난소제거된 Shiba 염소에서 다른 농도의 Progesterone 처치에 의한 FSH Surge 및 Pulse 분비에 미치는 영향

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요 약 : 본 연구의 목적은 FSH 분비 조절에 있어서 각각 다른 progesterone (P) 농도가 Pulse 또는 Surge 형태의 분비 패턴에 어떤 영향을 미치는가를 탐구한다. 선행된 연구에서 LH surge는 난포기 (follicular phase)에서 보이는 P 농도에서만 발현되었고 황체기(luteal phase) 또는 아황체기(subluteal phase) P 농도에서는 완전히 억제되었다. 또한 LH pulse 분비는 황체기 P농도에서만 시간 의존적으로 감소되었으나 난포기 또는 아황체기 농도에서는 높은 빈도로 유지되었다. 즉 아황체기 P농도 (스트레스, 황체형성부전에서 발생)는 LH surge는 억제시키나 LH pulse 조절에는 아무런 영향을 미치지 않았다. 이 결과를 바탕으로 FSH surge 및 pulse 분비패턴을 알아보기 위해 난소가 제거된 Shiba 염소를 이용하여 인위적으로 난포기, 아황체기, 기능성황체기의 P 농도 및 estradiol(E) 농도를 유도하였다. 스테로이드 호르몬 농도의 변화를 알아보기 위해 매일 혈액을 채취하였으며, LH pulse 용 혈액은 10분 간격으로 8시간 동안 Day 0, Day 3, Day 7 (Day 0: P packet 이식 직전)에 연속채혈을 실시하고, LH surge 분비패턴은 Day 8에 16시간 estradiol를 3 μg/h 농도로 주사하고, 52시간 동안 2시간 간격으로 채혈을 실시했다. 그 결과 유도된 P 농도는 염소의 정상적 발정주기에서 볼 수 있는 난포기(< 0.1 ng/ml), 아황체기(1.1 ± 0.1 ng/ml), 황체기(3.3 ± 0.1 ng/ml) 수준의 농도로 유도되었다. FSH pulse 분비는 3그룹에서 모두 높은 빈도로 유지되었고, FSH surge는 LH surge와 같은 시간대에서 난포기 그룹에서만 확인되었다. 본 연구의 결과는 FSH pulse는 LH pulse에서 보이는 progesterone에 의한 억제 효과와 다르게 나타났으며, 이러한 결과는 FSH 분비 조절에 있어서 LH 분비와 다른 기전 및 요소들이 관여하는 것으로 판단되었다.

주요어 : 난포자극호르몬, 난소, progesterone