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Emergences of LH Surge Affected by Different Progesterone Levels in Ovariectomized Goats

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Abstract : The purpose of the present study was to determine the priming effects of progesterone that affect the emergence of LH surge mode secretion by three different progesterone levels. In previous studies, we have shown that LH surge occurred in follicular levels of progesterone, whereas there was no surge mode secretion of LH and FSH in either the subluteal or luteal levels of progesterone. In this study, the hypothesis was that the priming effects of progesterone on the timing of the LH surge induced by exogenous estradiol are same between subluteal and luteal levels of progesterone. Long-term ovariectomized Shiba goats that had received implants of estradiol capsules (Day 0) 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. On Day 7, all devices of progesterone packets were removed but estradiol capsules were maintained during the experiment, and blood samples were collected at 1 hr interval for 12 h from the time of progesterone removals to determine peripheral changes of estradiol and progesterone concentration. Then all animals were infused estradiol on the Day 7 after 13 h from the removals of progesterone devices with a peristaltic pump into jugular vein at a rate of 3 μg/h for 36 h. For analysis of peripheral LH and estradiol concentration, blood samples were collected via another jugular vein 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). In all animals of the three groups treated with estradiol infusion, an LH surge was expressed but the peak time of LH surge was different. This time interval from estradiol infusion until the peak of LH surge was gradually and significantly extended by the different levels of progesterone treated before estradiol infusions in the three groups.

Key words **:** luteinizing hormone surge, progesterone, ovariectomized goat.

Introduction

It was demonstrated that estradiol induced LH surge was completely blocked while either subluteal or luteal level of progesterone in the circulation was maintained during estradiol infusion (10). It seems that there is no difference in the progesterone negative feedback action on GnRH surge generator between subluteal and luteal levels. However, some studies strongly suggested that the priming effect of progesterone influence the sensitivity to estradiol in domestic ruminants (6). For example, in the ewe, the first ovulation occurring after seasonal anestrous is not accompanied by a behavioral estrus. For maximal expression of behavioral estrus, progesterone must be present for a certain period of time prior to exposure to estrogen.

Furthermore, it was suggested that subluteal levels of progesterone induced by endogenously or exogenously, such as abnormal corpus luteum or environmental stress, did not suppress the pulsatile frequency of LH secretion and caused a persistent growth of dominant follicle accompanied with increase of estradiol concentration. However, when the progesterone level was decreased to baseline, the interval time to ovulation is shorter than the cases of treatment with functional luteal levels in the bovine (4). These results threw the possibility that the interval from progesterone decrease to the occurrence of LH surge would be different with dose-dependent of progesterone when primed with progesterone. Recent studies showed that the timing of estradiol-induced LH surge, when primed with various progesterone concentrations, is expressed by dose dependent manner and priming progesterone is essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory GnRH surge in the ewe (3,18). The progesterone priming prior to exposure to estradiol would be a critical key to regulate the expression of LH surge during the various conditions and the action of progesterone on the onset of LH surge can be also influenced by prior or concurrent estradiol treatment (2,17).

In this regard, it is necessary to investigate whether preexposure of ovarian steroid hormones exerts an influence on neuronal sensitivity expressing GnRH/LH surge. The purpose of the present study was to determine the priming effects of

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progesterone and estradiol on the timing of estradiol induced LH surge in ovariectomized goats. In this experiment, the hypothesis was that the priming effects of progesterone on the timing of the LH surge induced by exogenous estradiol are same between subluteal and luteal levels of progesterone because the suppression effects of progesterone on the occurrence of gonadotropin surge were not different between subluteal and luteal levels of progesterone treatment in the previous study (10).

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 (8). 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 (19,21), which induced levels similar to luteal levels of estradiol (3-10 pg/ml) (8,21). On the day of implants of estradiol capsules (Day 0), 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) (8,21), 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) (8,14). Daily blood sampling (5 ml) was conducted throughout the experiment to monitor the steroidal level in the circulation. On Day 7, all devices of progesterone packets were removed but estradiol capsules were maintained during the experiment 1, and blood samples were collected at 1 hr interval for 12 h from the time of progesterone removals to determine peripheral changes of estradiol and progesterone concentration. Then all animals were infused estradiol on the Day 7 after 13 h from the removals of progesterone devices with a peristaltic pump into jugular vein at a rate of 3 mg/h for 36 h. 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 (20). 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 (20). For analysis of peripheral LH and estradiol concentration, blood samples were collected via another jugular vein 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).

Blood sampling

Blood samples for analysis of the LH 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 storedat 4ºC and centrifuged at × 3000 rpm for 20 min, and then plasma was stored at –20ºC until assayed for LH, progesterone, and estradiol.

Hormone assays

Plasma concentrations of progesterone and estradiol were assayed by a previously described method (22). 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 LH were measured by a double-antibody radioimmunoassay (13). The following reagents were used: NIADDK-ovine LH-1-3 for radioiodination, NIADDK-ovine LH-25 as a standard, anti-ovine LH rabbit serum (YM No 18) as the first antibody, and goat anti-rabbit immunoglobulin as the second antibody. The sensitivity of the assay was 0.1 ng/ml. The intra-assay and interassay coefficients of variation were 6.77% and 0.12 %, 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 LH surge was defined as the point when a sustained rise (for at least two consecutive points of blood sampling) in the plasma LH concentration exceeded twice the average baseline level during the pretreatment period before the estradiol infusion.

Results

The plasma concentrations of estradiol were increased the

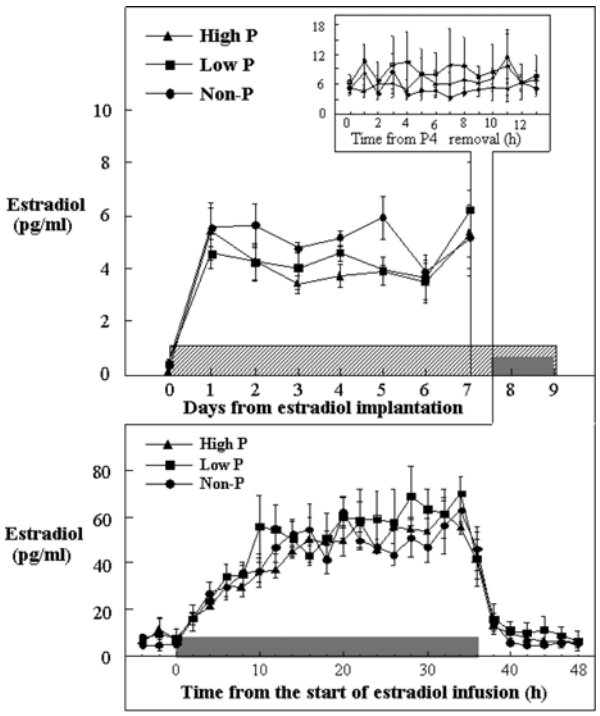

Fig 1. Plasma estradiol concentration (mean ± SEM) during the period of estradiol implantation (upper, dashed rectangle) and the period of progesterone removal on Day 7 (inset in upper panel). Changes in plasma estradiol concentration during estradiol infusion on Day 7 (after 13 h from the removal of progesterone packets) for 36 h (lower; shaded rectangle, estradiol infusion). High P indicates high progesterone group (50 × 70 mm); Low P indicates low progesterone group (25 × 40 mm); Non-P indicates no implant treatment group.

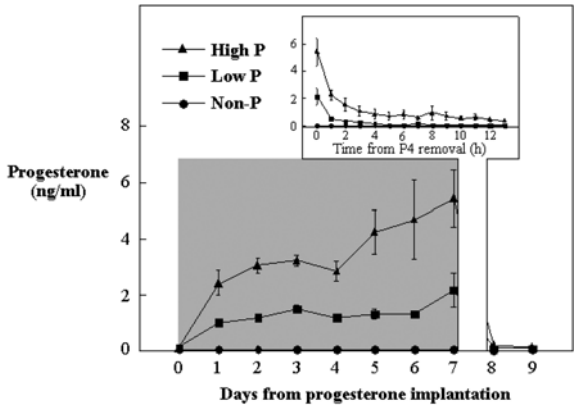

Fig 2. Plasma progesterone concentration (mean ± SEM) during the period of progesterone implantation (shaded rectangle), and the period of 13 h after the removal of progesterone packets on Day 7 (inset in figure), and after the progesterone packets removal. High P indicates high progesterone group (50 × 70 mm); Low P indicates low progesterone group (25 × 40 mm); Non-P indicates no implant treatment group.

day after treatments with implants of estradiol capsules and were maintained at a basal level (3-7 pg/ml) until Day 7 in

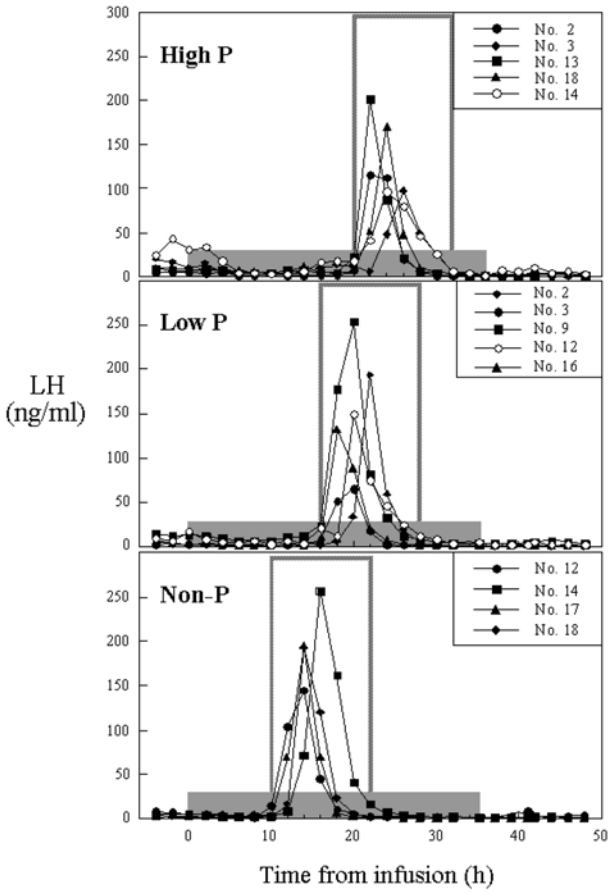

Fig 3. Profiles of luteinizing hormone (LH) concentration after estradiol infusions in the control (Non-P, no treatment group, bottom), low progesterone (Low P, middle) and high progesterone (High P, top) groups. Estradiol was infused at a rate of 3 μg/h through the catheterized jugular vein for 36 h, as indicated by the shaded rectangle. On the Day 7, the silastic packet of progesterone was removed but the tube of estradiol was remained during the experiment. Each panel shows the LH patterns of all animals in the respective group.

all animals (Fig 1). The progesterone concentrations were increased after implantation of progesterone silastic packets in the Low P and High P groups but not in Non-P group treated with no progesterone implants (Fig 2). The mean concentration of progesterone was maintained at the levels of functional luteal phase (3.6 ± 0.4 ng/ml; range, 2.4-5.4 ng/ml) in the High P group and subluteal phase (1.3 ± 0.1 ng/ml; range, 0.9-2.1 ng/ml) in the Low P group, respectively. There were significant differences among the three groups during the periods after progesterone implantation ($P < 0.05$).

The implants devices of progesterone were removed on Day 7 and the plasma progesterone concentration was decreased rapidly just after the removal of progesterone and maintained at a basal levels in the Low P and High P groups, respectively. The mean concentration of progesterone at the 13 h after removal of implants was 0.09 ng/ml (range, 0.04-0.19 ng/ml) and 0.4 ng/ml (range, 0.13-0.62 ng/ml), in the Low

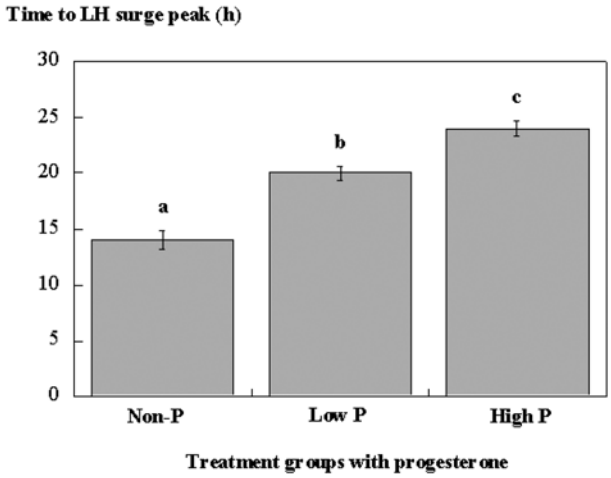

Fig 4. Mean time (± SEM) from the estradiol infusions to the peak of luteinizing hormone (LH) concentration in the three groups (Non-P indicates no treatment group, Low P indicates low progesterone group, High P indicates high progesterone group). Different superscripts indicate significant difference statistically (a vs b, $P < 0.01$; b vs c, $P < 0.01$; a vs c, $P < 0.001$).

P and High P groups, respectively, and there was no significant difference between two groups ($P > 0.05$). However, the plasma estradiol concentration during the period after removal of progesterone was maintained estradiol concentration similar to that before removal of progesterone.

The plasma estradiol concentration ranged from 18 pg/ml to 70 pg/ml after estradiol infusion in all goats and there was no significant difference among the three groups (Fig 1). The changes in the LH concentration before and after estradiol infusions in all goats are shown in Fig 3. In all animals of the three groups treated with estradiol infusion, an LH surge was expressed but the peak time of LH surge was different. The time interval between estradiol infusion and the peak of LH surge was 14.0 ± 0.8 h in the Non-P group, 20.0 ± 0.6 h for Low P and 24.0 ± 0.6 h for High P group, respectively. There was a significant difference in the occurrence of LH surge among the three groups (Non-P vs Low P, $P < 0.05$; Low P vs High P, $P < 0.05$; Non-P vs High P, $P < 0.001$; respectively; Fig 4). This time interval from estradiol infusion until the peak of LH surge was gradually and significantly extended by the different levels of progesterone treated before estradiol infusions in the three groups.

Discussion

The present study demonstrates that pre-exposures of progesterone influence the expression of LH surges induced by estradiol treatment, when progesterone concentrations are decreased by removal of progesterone implants to the basal levels. These results do not support the hypothesis, namely that the expression of LH surge delayed in the primed progesterone group with high concentration of progesterone compared to those groups with low progesterone. These are in agreement with some previous reports that treatments primed with progesterone regulated the estradiol inducing LH surge-generating system with a manner of dose-dependent (3,18). It would appear that estradiol is essential for potentiating the negative feedback action of progesterone on LH surge generating system.

Estradiol plays a pivotal role to activate the LH surge by positive feedback action during the follicular phase of normal estrous cycle (13) and artificially induced models (9), when a rise of circulating estradiol concentration sufficient for surge induction is happened. It has been well discussed that estradiol and progesterone regulate the GnRH neuronal mechanism for generating the surge pattern of LH secretion by positive and negative feedback mechanism. For example, Richter and colleagues (15) suggested that progesterone might prevent the expression of GnRH surge by preventing the stimulation of GnRH neurons by estradiol-responsive systems. Furthermore, progesterone could inhibit the LH surge either by preventing the activation stage or by altering the activity of neurons (transmission stage) of GnRH surge induction system during the three stages such as activation, transmission and hormone surge (release) of GnRH. In this regard, progesterone and estradiol would modulate the GnRH neurons inducing LH surge in the hypothalamus.

On the other hand, a number of cells contained progesterone receptor are increased by estradiol treatment through the hypothalamus in some species (1,12,16), suggesting the hypothesis that combined treatment of progesterone and estradiol may enhance the negative feedback action on GnRH secretion rather than progesterone alone treatment. The finding in the present study that the combined treatment with both estradiol and progesterone showed a significant delayed expression of LH in the High P groups may support the view that the priming actions of progesterone on the timing of LH surge gradually suppressed with dose-dependent inhibition manner when estradiol combined. However, in the condition of estradiol blank, those effects of dose-dependent inhibition on the expression of LH surge is lost. In this respect, this data provide a new information as to the synergistic mechanism of two steroid hormones regulating the GnRH/LH secretion.

It is well known that the ability of progesterone to enhance the estradiol-induced LH surge may be responsible for the increased magnitude of the LH surge in the rat (7,11). It may be supported by previous report that GnRH receptors in ewes pretreated with progesterone and estradiol were increased more than alone treatment with estradiol (24), suggesting that pretreatment of progesterone may strengthen the estrus behavior. However, prolonged estradiol treatment suppressed the surge pattern of LH by decreasing activation of GnRH neurons in rat causing the anestrus (23).

It is interesting to discuss that amounts of GnRH receptor mRNA, as an important indicator of activation for LH secretion, were not changed by treatment with different steroid levels mimic to physiological levels of the estrous cycle or late

pregnancy in intact ewes (24). However, the GnRH receptor increased in the cases with high estradiol concentration in ovariectomized ewes. Furthermore, although treatment of high progesterone levels of luteal phase combined with estradiol for 16 h increased the expression of GnRH receptor mRNA by a several-folds in comparison with either alone treatment, the receptor mRNA decreased more than follicular condition of estrous cycle in ovariectomized ewes. Those results support hypothesis of the present study that the inhibitory actions of progesterone on LH surge generating system are more enhanced by combination of estradiol and may be regulated with dose-dependent of progesterone. Our data are also in agreement with those of some reports (2,5) that the ability of progesterone to suppress LH secretion was enhanced by estradiol and prolonged the onset of LH surge by the synergistic effects of two steroid hormones.

In conclusion, the results of this study demonstrate that treatments primed with different progesterone levels delayed the onset of LH surge with a dose dependent manner, in agreement with previous report (18). Thus, the latent effects of estradiol on the negative feedback actions of progesterone may have a pivotal role by synergistic effects with progesterone to control of the reproductive mechanism. Also, plasma progesterone concentration during the pre-exposure (luteal phase of female estrous cycles) may be important for regulating the timing of ovulation in follicular phase of the estrous cycle.

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난소제거된 염소에서 Progesterone 농도의 영향에 의한 LH surge 분비에 미치는 영향

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요 약 : 본 연구의 목적은 LH surge 형태의 분비가 선행적으로 투여되는 각각 다른 Progesterone (P) 농도에 의해 어떻게 조절되는지에 대한 탐구이다. 선행된 연구에서 LH surge는 난포기(follicular phase)에서 보이는 P 농도에서만 발현되었고 황체기(luteal phase)와 아황체기(subluteal phase) P 농도에서는 완전히 억제되었다. 이런 결과를 토대로 본 연구의 가설은 아황체기 P농도(스트레스, 황체형성부전)와 황체기 P 농도를 선행 처치 후 외인성 estradiol에 의한 LH surge 발현은 동시에 발생할 것으로 예상한다. 이 가설을 증명하기 위해 난소가 제거된 Shiba 염소를 이용하여 인위적으로 난포기(non-P), 아황체기(low-P), 기능성황체기(high-P)의 P 농도 및 Estradiol (E) 농도를 유도하였다 (Day 0: P와 E 이식일). 스테로이드 호르몬의 농도를 알아보기 위해 매일 혈액을 채혈하였으며, Day 7에 각 그룹의 P 농도를 유지하기 위한 packet를 제거하고 E 처치용 capsule은 그대로 유지시켰다. 또한 Day 7에 P와 E의 농도변화를 세밀하게 조사하기 위해 12시간 동안 1시간 간격으로 채혈 후 검사하였다. LH surge 분비패턴은 P packet 제거 후 13시간부터 E를 3 μg/h 농도로 36시간 동안 주입하고, 52시간 동안(E 주입 4시간 전부터 주입 후 48시간) 2시간 간격으로 채혈을 실시했다. 그 결과, 모든 3그룹의 염소는 LH surge가 발현되었지만 그 발현시간은 각 그룹마다 유의성있는 발현시간 차를 나타내었다. 즉, 각각의 선행된 P 농도 (non-P, low-P, high-p) 그룹은 외인성 Estradiol에 의한 LH surge 발현시간에 농도의존성으로 영향을 미치는 것으로 판단되었다.

주요어 : Luteinizing hormone surge, 프로제스테론, ovariectomized goat