pISSN 1598-298X
J Vet Clin 31(1) : 25-30 (2014)

Journal of Veterinary Clinics
www.ksvc.or.kr

Effects of Priming Progesterone on the LH Surge Expressions in Ovariectomized Shiba Goats

Seung-Joon Kim[1]

Department of Veterinary Clinical Reproduction, College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea

(Accepted: Januray 25, 2014)

Abstract : This study tested the hypothesis that the priming effects of progesterone on the timing of the LH surge induced by exogenous estradiol are more potentiated the negative feedback actions of progesterone on LH secretion by the existence of estradiol. In previous studies, the 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. 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 and estradiol 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 3-6 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 was not extended by the different levels of progesterone treated before estradiol infusions and the difference was not significant during this interval between the Low P and the High P groups. Progesterone pretreatment may contribute to regulating the neural system that is responded by estradiol, and estradiol existence potentiates the negative feedback effect of progesterone on GnRH/LH surge-generating system.

Key words **:** estradiol, progesterone, Luteinizing hormone surge, ovariectomized goat.

Introduction

It has been well indicated that high estradiol concentrations induce the preovulatory LH surges by mediating the GnRH discharge in ruminants (1,3,11). These estradiol levels are produced in the late follicular phase, when progesterone levels decreased to basal levels after the regression of corpus luteum and the frequency of LH pulse is high by the absence of progesterone (16). The LH surge induced by a fluctuation of estradiol is connected with ovulation in normal estrous cycle, when dominant ovulatory follicles are existed. On the other hand, it has been demonstrated that functional luteal level of progesterone completely block the estradiol induced LH and GnRH surge in domestic ruminants (7,13). Several lines of evidence suggested that this is due to the suppression effect of progesterone on GnRH surge generator stimulated by estradiol. Therefore, the luteal level of progesterone can suppress both of GnRH pulse generator and GnRH surge generator through the negative feedback action in the hypothalamus.

Several previous evidences have been suggested that progesterone modulate reproductive function, including reproductive failure. For example, the first ovulation occurring after seasonal anestrus is not accompanied by a behavioral estrus (6). For maximal expression of behavioral estrus, progesterone must be present for a certain period of time prior to exposure to estradiol. Moreover, Sanchez *et al*. (15) demonstrated surely about the importance of progesterone concentration for mediating follicular dynamics, namely low levels of progesterone treatment induces high increment of estradiol promoted by prolonged ovarian follicular growth (follicular cyst). Also, these progesterone levels regulating the LH pulses would effect on the follicular dynamics, as previously reported (17,19), showing that low progesterone levels support a persistent growth of dominant follicle but high progesterone levels induce the demise of the dominant follicle and restore the repetition of follicular recruitment. Alternatively, progesterone has been in use as a therapeutic drug for the ovarian disorders in domestic ruminants. For example, progesterone-releasing devices usually had therapeutic effect on ovarian quiescence and ovarian cyst in cows (2,4).

[1]Corresponding author.
E-mail : kjoon00@knu.ac.kr

The various progesterone levels by endogenous factors or exogenous treatments strongly inhibit the LH secretion via negative feedback actions on GnRH. It is worthy to examine the detailed relationship between ovarian steroids and gonadotropin, under the various both progesterone and estradiol levels brought by the normal condition of estrous cycle or artificial treatment. In our laboratory data, the 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.

In this regard, it is necessary to investigate whether pre-exposure 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 progesterone and estradiol on the timing of estradiol induced LH surge in ovariectomized goats and tested the hypothesis that the priming effects of progesterone on the timing of the LH surge induced by exogenous estradiol are more potentiated the negative feedback actions of progesterone on GnRH/LH secretion in the level of hypothalamic and pituitary regions by the existence of estradiol.

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 (20,22), which induced levels similar to luteal levels of estradiol (3-10 pg/ml) (8,22). 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) (8,22), 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 and estradiol packets were removed and blood samples were collected at 1 hr interval for 12 h from the time of progesterone and estradiol removals to determine peripheral changes of estradiol and progesterone concentration. Then, on Day 7, all animals were infused estradiol 13 h after the time of progesterone and estradiol removals. Estradiol was treated at a rate of 6 μg/h during the period of the first 6 h from the start of estradiol infusions, and then the infusion rate again regulated at a rate of 3 μg/h from 6 h to 36 h after the start of estradiol infusions. The reason that estradiol was infused for the first 6 h at a rate of 6 μg/h was that the peripheral estradiol concentration did not increase to the level around 20 pg/ml 2 h after estradiol infusion in our pre-experimental design, when estradiol was infused at a rate of 3 μg/h (pre-experimental result showed that the time to increase the level of 20 pg/ml when the beginning estradiol infusion treated at a rate of 3 pg/ml takes about 10 h). Although the plasma estradiol concentrations range 20-100 pg/ml during estradiol infusion in previous studies (21), the concentration (around 20 pg/ml) after 2 hr from estradiol infusion thought to be an important key to induce the LH surge starting about 10 h after estradiol infusion in ovariectomized goats (21). 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 4°C and centrifuged at × 3000 rpm for 20 min, and then plasma was stored at –20°C until assayed for LH, progesterone, and estradiol.

Hormone assays

Plasma concentrations of progesterone and estradiol were assayed by a previously described method (23). The sensitivity of the assays for progesterone and estradiol were 0.015 ng/ml and 0.69 pg/ml, respectively. The intra-assay and inter-assay 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 (12). 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 immunoglo-

bulin as the second antibody. The sensitivity of the assay was 0.1 ng/ml. The intra-assay and interassay coefficients of variation were 7.75% and 0.65%, respectively.

Statistical analysis

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 day after treatments with implants of estradiol capsules and were maintained at a basal level (6-12 pg/ml) until Day 7 in 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 (3.1 ± 0.4 ng/ml; range, 2.6-5.9 ng/ml) in the High P group and subluteal phase (1.2 ± 0.1 ng/ml; range, 1.1-1.4 ng/ml) in the Low P group, respectively. There were significant differences among the three groups during the periods after progesterone implantation ($P < 0.05$).

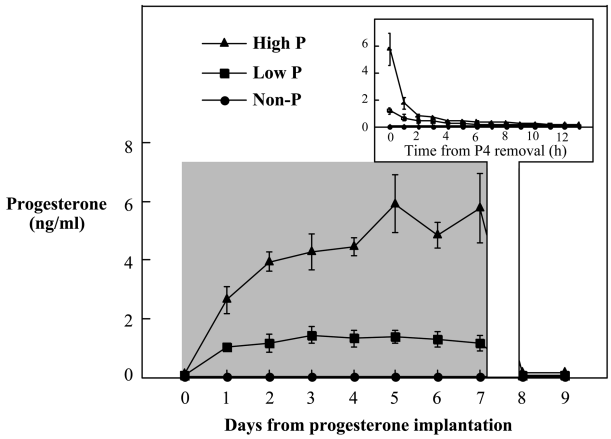

Fig 2. Plasma progesterone (mean ± SEM) concentration during the period of progesterone implantation (shaded rectangle), and the period of 13 h after the removal of progesterone packets on Day 7 (inset in figure), and after the progesterone packets removal. High P indicates high progesterone group (50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

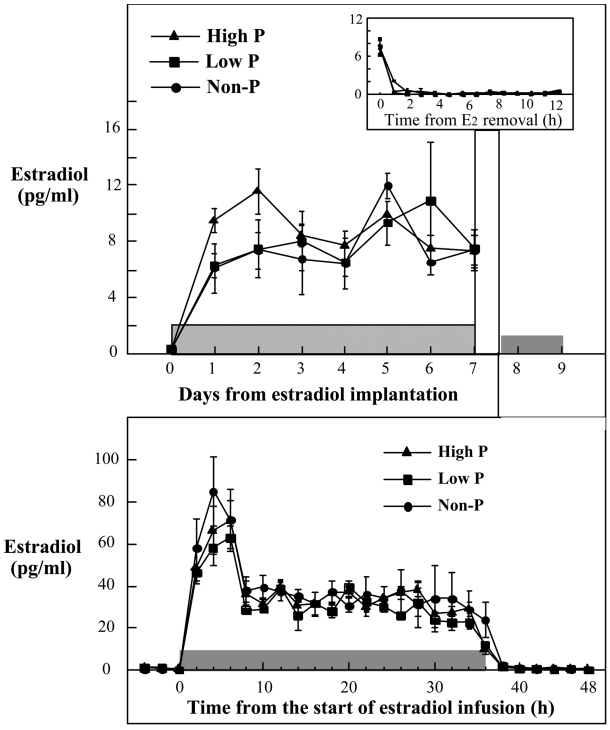

Fig 1. Plasma estradiol concentration (mean ± SEM) during the period of estradiol implantation (upper, dashed rectangle) and the period of estradiol removal on Day 7 (inset in upper panel). Changes in plasma estradiol concentration during estradiol infusion on Day 7 (after 13 h from the removal of estradiol tubes) for 36 h (lower; shaded rectangle, estradiol infusion). During the first 6 h after the start of estradiol infusions, estradiol infused at a rate 6 μg/h (2-folds of infusions in comparison to Exp. 1). 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.

The implant devices of progesterone and estradiol were removed on Day 7 and the plasma progesterone and estradiol concentration was decreased rapidly just after removal of progesterone and estradiol and maintained at the basal levels in the three groups, respectively. The mean concentration of progesterone at 13 h after removal of implants was lower than 0.1 ng/ml in the Low P and High P groups, respectively. The mean concentration of estradiol in all goats at 13 h after removals of implants was lower than 0.5 pg/ml.

The plasma estradiol concentration ranged from 45 pg/ml to 80 pg/ml during the period of the first 6 h after estradiol infusion treated with the rate of 6 μg/h in all goats, and then ranged from 25 pg/ml to 42 pg/ml from 8 h until the period of estradiol infusion after regulation of estradiol infusion at a rate of 3 μg/h. 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 occurred but the appearance time of LH surge was different among the three groups. The mean time interval between estradiol infusion and the peak of LH

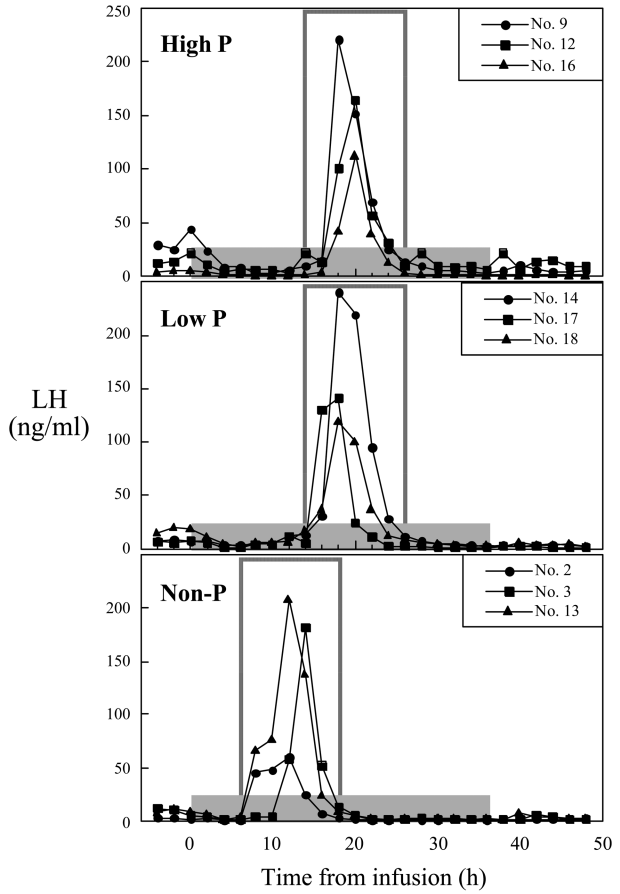

Fig 3. Profiles of luteinizing hormone (LH) concentration after estradiol infusions in the control (Non-P, no treatment group, bottom), low progesterone (Low P, middle) and high progesterone (High P, top) groups. Estradiol was infused at a rate of 6 μg/h from 0 h to 6 h and the estradiol infusion regulated again 3 μg/h from 6 h to 36 h through the catheterized jugular vein, as indicated by the shaded rectangle. On Day 7, both the silastic packet of progesterone and the tube of estradiol were removed at the same time. Each panel shows the LH patterns of all animals in the respective group.

surge was 12.6 ± 0.6 h in the Non-P group, 18.0 ± 0.0 h for Low P and 19.0 ± 1.0 h for High P group, respectively. There was a significant difference in the occurrence of LH surge between Non-P group and progesterone treatment groups, including Low-P and High P group (Non-P vs Low P, $P < 0.05$; Non-P vs High P, $P < 0.05$; respectively; Fig 4). When progesterone and estradiol was removed simultaneously on Day 7, this time interval was not extended by the different levels of progesterone treated before estradiol infusions and the difference was not significant during this interval between the Low P and the High P groups ($P > 0.1$, Fig 4).

Discussion

The present study demonstrates the exclusive evidence that estradiol is essential to strengthening the negative feedback

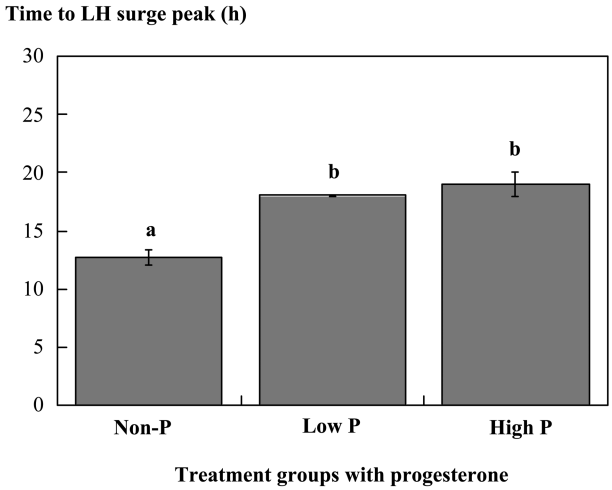

Fig 4. Mean time (± SEM) from the estradiol infusions to the peak of luteinizing hormone (LH) concentration in the three groups (Non-P indicates no treatment group, Low P indicates low progesterone group, High P indicates high progesterone group). Different superscripts indicate significant difference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

action of progesterone on LH surge discharges induced by estradiol infusion. The removal of two ovarian steroid hormones at the same time caused the simultaneous occurrence of LH surge in two groups such as subluteal and luteal levels of progesterone. It would appear that estradiol is essential for potentiating the negative feedback action of progesterone on LH surge generating system.

It has been well established by previous report that the increase of estradiol by endogenous or exogenous manners causes the synchronous surges of LH and FSH in normal estrous cycle of domestic ruminant (5,12) and ovariectomized animals (9,21). it demonstrated the LH and FSH surges were completely blocked by various level of progesterone, when progesterone levels are above about 1 ng/ml (range, 0.6-2 ng/ml), as previously reported (7,10,13). Interestingly, the low (or subluteal) levels of progesterone regulated the pulsatile and surge patterns of LH differently, that endocrinological threshold levels of progesterone that induce negative feedback effects on the pulsatile and surge modes of LH secretion are different (10). Subnormal levels of progesterone induced by physiological and environmental stresses may inhibit the GnRH surge generation activity that regulates the LH and FSH surges. It is concluded that the threshold level of progesterone for suppression of GnRH surge generator is less than that for suppression of pulse generator. This theory may be supported by previous reports that subluteal levels of progesterone caused a prolonged development of dominant follicle, by increased pulsatile frequencies of LH, and induce estradiol secretion, without LH surge and ovulation (17,19). In this respect, it is likely that low level of progesterone has a role in inducing the follicular growth and estradiol secretion and in blocking the ovulation in domestic ruminants. LH

surge was induced earlier in the absence of progesterone pretreatment compared to progesterone priming groups, as previously reported by Skinner *et al*. (18). In general, it is known that the silent ovulation (or silent estrus) observed at the onset of the breeding season in ewe is thought to be due to the absence of a prior luteal phase, resulting in LH surge and ovulation but not estrous behavior (6).

In conclusion, this study clearly demonstrated the hypothesis that the negative feedback actions of progesterone on GnRH/LH secretion in hypothalamic and pituitary levels would be potentiated more intensively by the existence of estradiol. The result is very interesting as a viewpoint of synergistic interactions between estradiol and progesterone affecting to the LH surge-generating system responsible for estradiol. In this regard, progesterone pretreatment may contribute to regulating the neural system responded by estradiol, and estradiol existence potentiates the negative feedback effect of progesterone on GnRH/LH surge-generating system.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-313-C00818). I want to thank Drs. Tomomi Tanaka, Hideo Kamomae, Gen Watanabe, Tokyo University of Agriculture and Technology for the help and support.

Reference

1. Caraty A, Locatelli A, Martin GB. Biphasic response in the secretion of gonadotropin-releasing hormone in ovariectomized ewes injected with oestradiol. J Endocrinol 1989; 123: 375-382.
2. Cavalieri J, Kinder JE, Fitzpatrick LA. Effect of acute treatment with progesterone on the timing and synchrony of ovulation in Bos indicus heifers treated with a norgestomet implant for 17 days. J Reprod Fertil 1998; 112: 249-258.
3. Clarke IJ. Gonadotrophin-releasing hormone secretion (GnRH) in anoestrous ewes and the induction of GnRH surges by oestrogen. J Endocrinol 1988; 117: 355-360.
4. Dailey RA, Inskeep EK, Washburn SP, Price JC. Use of prostaglandin F2 alpha or gonadotropin releasing hormone in treating problem breeding cows. J Dairy Sci 1983; 66: 1721-1727.
5. Dobson H. Plasma gonadotropins and oestradiol during oestrus in the cow. J Reprod Fertil 1978; 52: 51-53.
6. Fabre-Nys C, Martin GB. Roles of progesterone and estradiol in determining the temporal sequence and quantitative expression of sexual receptivity and the preovulatory LH surge in the ewe. J Endocrinol 1991; 130: 367-379.
7. Kasa-Vubu JA, Dahl GE, Evans NP, Thrun LA, Moenter SM, Padmanabhan V, Karsch FJ. Progesterone blocks the estradiol-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 1992; 131: 208-212.
8. Kano Y, Mori Y. The Shiba goat. In: Mammalian Reproductive Physiology: Aspects of its Animal Experimentation [in Japanese]. Tokyo:Soft Science. 1982: 367-379.
9. Kesner JS, Padmanabhan V, Convey EM. Estradiol induces and progesterone inhibits the preovulatory surges of luteinizing hormone and follicle-stimulating hormone in heifers. Biol Reprod 1982; 26: 571-578.
10. Kim S, Tanaka T, Kamomae H. Different effects of subnormal levels of progesterone on the pulsatile and surge mode secretion of luteinizing hormone in ovariectomized goats. Biol Reprod 2003; 69: 141-145.
11. Moenter S, Caraty A, Karsch FJ. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990; 127: 1375-1384.
12. Mori Y, Kano Y. Changes in plasma concentrations of LH, progesterone and oestradiol in relation to the occurrence of luteolysis, oestrous and time of ovulation in the Shiba goat (Capra hircus). J Reprod Fertil 1984; 72: 223-230.
13. Nanda AS, Ward WR Dobson H. Effect of endogenous and exogenous progesterone on the oestradiol-induced LH surge in dairy cows. J Reprod Fertil 1988; 84: 367-371.
14. Orita J, Tanaka T, Kamomae H, Kaneda Y. Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. J Reprod Dev 2000; 46: 31-37.
15. Sanchez T, Wehrman M E, Kojima FN, Cupp AS, Bergfeld EG, Peters KE, Mariscal V, Kittok RJ, Kinder JE. Dosage of the synthetic progestin, norgestomet, influences luteinizing hormone pulse frequency and endogenous secretion of 17 beta-estradiol in heifers. Biol Reprod 1995; 46: 464-469.
16. Schams D, Schallenberger E, Hoffmann B, Karg H. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. Acta Endocrinol (Copenh) 1977; 86: 180-192.
17. Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127: 916-925.
18. Skinner DC, Harris TG, Evans NP. Duration and amplitude of luteal phase progesterone increment times the estradiol-induced luteinizing hormone surges in ewes. Biol Reprod 2000; 63: 1135-1142.
19. Stock AE, Fortune JE. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 1993; 132: 1108-1114.
20. Tanaka T, Akaboshi N, Inoue Y, Kamomae H, Kaneda Y. Fasting-induced suppression of pulsatile luteinizing hormone secretion is related to body energy status in ovariectomized goats. Anim Reprod Sci 2002; 72: 185-196.
21. Tanaka T, Mori Y, Hoshino K. Hypothalamic GnRH pulse generator activity during the estradiol-induced LH surge in ovariectomized goats. Neuroendocrinology 1992; 56: 641-645.
22. Tanaka T, Mori Y, Hoshino K. Long-term recording of hypothalamic GnRH pulse generator activity during programmed administration of progesterone and estradiol in the ovariectomized goat. J Reprod Fertil 1994; 40: 133-138.
23. Taya K, Watanabe G, Sasamoto S. Radioimmunoassay for progesterone, testosterone and estradiol-17 beta using ^{125}I-iodohistamine radioligands [in Japanese]. Jpn J Anim Reprod 1985; 31: 186-196.

LH surge 발현에 대한 서로 다른 Progesterone 농도의 효과

김승준[1]

경북대학교 수의과대학 수의임상번식학교실

요 약 : 본 연구의 목적은 외인성 Estradiol(E)에 의해 발현되는 LH surge가 다른 농도의 Progesterone (P)에 억제되고, 그 기전이 E의 존재에 의해 더욱 강화된다는 것을 증명하는 것이다. 선행된 연구에서 E의 존재하에 LH surge는 P의 농도에 의해 LH surge 발현 시간은 점진적/유의성 있게 억제된다는 것을 확인했다. 이런 결과를 토대로 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 packet과 E 처치용 capsule를 동시에 제거하였다. 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 3-6 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 발현 시간은 Low-P와 High-P 그룹에서 유의성 없는 발현시간 차를 나타내었다. 즉, 각각의 선행된 P 농도 (Non-P, Low-P, High-p) 그룹은 외인성 Estradiol에 의한 LH surge 발현을 조절하는데 있어서 E의 존재가 중요하며, E는 GnRH/LH surge-generating 시스템에 작용하는 P의 억제적 효과를 더욱 강화시키는 것으로 생각되었다.

주요어 : 에스트라디올, 프로제스테론, Luteinizing hormone surge ovariectomized goat