Plasma Steroid Profiles Following Follicle-Stimulating Hormone or Equine Chorionic Gonadotropin Injection in Cows Chronically Treated with Gonadotropin-Releasing Hormone Agonist

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ABSTRACT. Plasma steroid profiles following follicle-stimulating hormone (FSH) or equine chorionic gonadotropin (eCG) injection were studied in chronically gonadotropin releasing hormone agonist (GnRH-A)-treated cows. Follicular development and irINH secretion were stimulated by FSH or eCG injection. The plasma concentrations of estradiol-17 β (E₂) and testosterone (T) were markedly increased following eCG injection. However, significant increases of the plasma E₂ and T concentrations were not detected in FSH-treated cows. Ovulation of developed follicles were depended on the hCG injection in both groups. These results show: 1) Follicular response to an exogenous gonadotropin is still remained, 2) Ovulation of developed follicles is induced by hCG injection and 3) FSH and eCG cause disparate plasma steroid profiles, under the influence of repeated GnRH-A treatment.

KEY WORDS: eCG, FSH, GnRH.

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Gonadotropin-releasing hormone (GnRH) is implicated in the control of the reproductive function of many animals, including domestic ruminants [3]. However, a chronic treatment with GnRH or its agonists (GnRH-A) can lead to suppression of the reproductive function that includes pituitary gonadotropin secretion, ovarian follicular growth, steroidogenesis and ovulation [1, 2, 4, 13, 17, 18]. Desensitization of pituitary gonadotropin secretion that induces suppression of follicle-stimulating hormone (FSH) secretion and inhibition of pulsatile release of luteinizing hormone (LH) has been reported as being a result of chronic administration with GnRH-A [14].

The chronic treatment of GnRH-A will supply a model for the study of ovarian responses to exogenous gonadotropin in the insufficiency of endogenous gonadotropic stimulation. However, little is known about the effects of a combined treatment of chronic GnRH-A and gonadotropins on the ovarian functions in the cow.

The objective of this study was to compare the effects of FSH and equine chorionic gonadotropin (eCG) in chronically GnRH-A-treated cows. This paper describes the plasma progesterone (P), estradiol-17 β (E₂), testosterone (T) and immunoreactive inhibin (irINH) profiles following FSH or eCG injection in cows chronically treated with GnRH-A.

Seven Japanese Black cows (aged 4-6 years) with regular estrous cycles were used for this study. Our experimental procedure is shown in Fig. 1. Briefly, animals were subcutaneously injected with 100 μ g of GnRH-A (Fertilerin acetate; Takeda, Osaka, Japan) twice a day from Day 0 (the day of ovulation) to Day 15 of estrous cycle. The injection of

GnRH-A was done at 9 AM and 9 PM. On Day 11, cows were randomly assigned to one of the following two groups: 1) FSH group: 4 cows were twice a day (9 AM and 9 PM) treated subcutaneously with 4 Armour units of porcine FSH (Antrin; Denka, Tokyo, Japan) for consecutive 5 days and 2) eCG group; 3 cows were once treated subcutaneously at 9 AM with 3000 IU of eCG (Peamex; Sankyo, Tokyo, Japan). All cows were given 30 mg of prostaglandin F₂ α (Pancelan Hi. Daiich, Tokyo Japan) on Day 14 (9 AM). The repeated GnRH-A injections were done until Day 15. On day 16, 3 cows in FSH group and a cow in eCG group were subcutaneously injected with 5,000 IU human chorionic gonadotropin (hCG), and others were served as control without hCG. The numbers of developing follicles in both

Fig. 1. The experimental design of the gonadotropin stimulation in cows chronically treated with GnRH agonist.

Fig. 2. Changes in the plasma irINH (A), E_2 (B), T (C) and P (D) concentrations (mean $\pm$ SEM) between Day 0 to 16 in FSH ($\blacktriangle$, $n=4$) and the eCG ($\times$, $n=3$) groups. On Day 11, 4 cows were twice a day treated with 4 Armour units of porcine FSH for consecutive 5 days (FSH group), and 3 cows were treated with 3000 IU of eCG once (eCG group). On Day 14, all the cows were treated with 30 mg of $PGF_{2\alpha}$.

groups were examined on Day 16 by using ultrasonography. Estrous detection was done by twice a day observation of standing behavior in paddock between 9 to 9:30 AM and 4:30 to 5 PM.

Peripheral blood was collected daily from Day 0 to 23 via a jugular venipuncture into a heparinized tube. Blood sam-

pling was done between 8:30 to 9 AM. The samples were placed in an ice-cold bath until centrifuged within an hour of collection. The harvested plasma was stored at -20°C until assayed.

The plasma concentrations of P, E_2 and T were measured by radioimmunoassay as previously reported [12, 15]. The plasma irINH concentration was measured by time-resolved fluoroimmunoassay as previously reported [8].

Minimum detectable levels of P, E_2 , T and irINH were: 0.25 ng/ml, 0.5 pg/ml, 2.5 pg/ml and 0.1 ng/ml, respectively. Intra assay coefficients of variation for all assays were less than 10%. Assay values were analyzed with a split plot in time design with repeated measurements [6]. Plasma concentrations of P, E_2 , T and irINH were compared between FSH and eCG groups by using *t*-test and a value of $p < 0.05$ was determined to be statistically significant.

A large numbers of follicles developed, responding to gonadotropin stimulation in both FSH and eCG groups. The numbers of follicles that had developing up to 10 mm in diameter on Day 16 were 16.5 ± 1.3 (mean $\pm$ SEM) in FSH group and 15.7 ± 3.2 in eCG group. On Day 11, prior to gonadotropin treatment, plasma irINH concentrations in FSH and eCG groups were 1.9 ± 0.2 ng/ml and 1.4 ± 0.3 ng/ml, respectively (Fig. 2-A). In both groups, the plasma irINH concentrations significantly increased after gonadotropin treatment. On Day 16, the plasma irINH concentrations in FSH and eCG groups were 5.4 ± 0.9 ng/ml and 4.9 ± 1.2 ng/ml, respectively. No significant difference was observed in the plasma irINH profiles between two groups throughout gonadotropin treatment. All cows exhibited estrus on 2 days after $PGF_{2\alpha}$ injection. The number of ovulation were determined by rectal palpation in all cows treated with hCG in both groups. However, none of the cows ovulated without hCG injection in both groups.

Fig. 3. The changes in the plasma irINH (A), E_2 (B), T (C) and P (D) concentrations between Day 17 to 23. On day 16, cows in FSH group ($\bullet$, $n=3$) and eCG group ($\blacklozenge$, $n=1$) were subcutaneously injected with 5000 IU human chorionic gonadotropin (hCG), and other cow in FSH group ($\blacktriangle$, $n=1$) and eCG group ($\times$, $n=2$) were served as control without hCG.

Estrous signs were already disappeared at the time of ovulation in hCG-treated cows, however cows without hCG treatment exhibited prolonged estrous behavior lasting for days. In both groups irrespective of hCG injection, plasma irINH concentrations further increased after gonadotropin treatment, peaked on Day 18 or 19 and then gradually decreased (Fig. 3-A).

On Day 11, at the commencement of gonadotropin stimulation, the plasma E_2 , T and P concentrations in FSH group vs. eCG group were E_2 : 1.0 ± 0.2 pg/ml vs. 1.1 ± 0.1 pg/ml, T: 5.8 ± 2.9 pg/ml vs 5.7 ± 1.7 pg/ml and P: 3.9 ± 0.2 ng/ml vs. 5.1 ± 1.1 ng/ml (Fig. 2-B, C and D). The plasma E_2 concentrations in FSH group increased slightly during gonadotropin treatment and reaching to the value of 5.1 ± 0.4 pg/ml on Day 16 (Fig. 2-B). In contrast, plasma E_2 concentrations in eCG group significantly increased after eCG injection, reaching the value of 118 ± 49.0 pg/ml on Day 16. After Day 16, plasma E_2 concentrations in cows without hCG injection in eCG group still increased and reached to the value of more than 200 pg/ml on Day 19, whereas in other cows the values were relatively low and consistent in both groups (Fig. 3-B). The plasma T level in FSH group was consistent throughout gonadotropin treatment and the value was 8.3 ± 1.2 pg/ml on Day 16 (Fig. 2-C). In contrast, plasma T concentrations in eCG group significantly increased after eCG injection, reaching to the value of 32.6 ± 12.6 pg/ml on Day 16. Following Day 17, plasma T concentrations further increased in cows without hCG injection in both groups, whereas in cows treated with hCG, the values were relatively lower and consistent (Fig. 3-C). The plasma P concentration in FSH group was consistent during first 3 days of gonadotropin treatment (Fig. 2-D). By contrast, plasma P concentration in eCG group increased during first 2 days after the eCG injection, reaching to the peak of 7.3 ± 0.6 ng/ml on Day 13. In both groups, the plasma P values rapidly decreased following PGF₂ α injection. Plasma P concentrations increased following hCG injection in both groups corresponding to luteal development, however in cows without hCG injection, plasma P concentrations still remained less than 1 ng/ml (Fig. 3-D).

The repeated treatment with GnRH-A has been reported to suppress pulsatile secretion of LH and block the development of dominant follicles [7]. These resulted in the prevention of preovulatory LH surge and ovulation in heifers [7]. In the absence of pulsatile LH secretion, FSH stimulates the growth of large and estrogenic follicles which, when stimulated, ovulate to produce viable corpora lutea in the ewe [16]. In this study, cows were treated with either FSH or eCG which was similar to practical superovulation. In both groups, gonadotropin treatment resulted in the development of many follicles grown up to 10 mm in diameter on Day 16. Developed follicles were similar in number to those induced by eCG without the chronic GnRH-A treatment [11]. Results from the present study clearly show that ovaries could respond to exogenous gonadotropins and a large number of antral follicles (> 10 mm in diameter) grew in both groups.

Previous studies indicate that bovine superovulation regimen using either FSH [10] or eCG [11] induces a higher concentrations of plasma E_2 and INH during follicular development without the repeated GnRH-A injections. Thus, it is generally accepted that large follicles which induced to develop by an exogenous gonadotropin are the main sources of plasma E_2 and INH [11].

However, in our study, FSH failed to increase plasma E_2 and T concentrations in chronically GnRH-A-treated cows, although it induced successful follicular development and irINH secretion. In the synthesis of estrogen, a unique cascade (the so-called two-cell two-gonadotropin mechanism) has been demonstrated in the ovary [5]. By contrast, in cows treated repeatedly with GnRH-A, the activity of pituitary LH to stimulate thecal androgen synthesis might be almost ceased as previously reported in the ewe [14]. Therefore, injected FSH could not stimulate E_2 production because of the lack of substrate for aromatization in granulosa cells, although it exhibited FSH-like activity in granulosa cells to stimulate follicular growth and irINH secretion. In contrast, the cows in the eCG group showed a significant increase in plasma E_2 and T concentrations. It is well known that eCG exerts LH-like activity in the cow as well as FSH-like one [9]. In eCG group, it is likely that eCG stimulates androgen synthesis in the theca cells as well as aromatization in the granulosa cells. In this study, different steroidogenic profiles in FSH and eCG groups might depend on the difference of LH-like activity in each hormone preparations.

To conclude, results in the present study suggest that the repeated treatment of GnRH-A provides an experimental model for the study of ovarian responses to exogenous gonadotropin. The present study shows that: 1) The ovarian response to exogenous gonadotropins for follicular development is still remained regardless of repeated GnRH-A treatment, 2) Ovulation of developed follicles is induced by hCG injection and 3) FSH and eCG cause disparate steroidogenic profiles.

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