Changes in Plasma Concentrations of Gonadotropins and Steroid Hormones during the Formation of Bovine Follicular Cysts Induced by the Administration of ACTH

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ABSTRACT. Bovine follicular cysts were induced by treatments with ACTH (3 mg, im) daily for 14 days beginning in the late luteal phase. Cortisol concentrations in plasma significantly increased after ACTH treatments. During the formation of follicular cysts induced by the injections of ACTH, mean plasma concentrations of progesterone were significantly higher than those in the untreated preovulatory period, while mean plasma concentrations of estradiol-17β were significantly lower. During the treatment period, mean plasma concentrations of LH and FSH remained low, and the preovulatory surges of LH and FSH did not occur. Suppressed concentrations of LH and FSH might be caused by the increases in secretions of cortisol and progesterone, and by the decrease in secretion of estradiol-17β.
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Follicular cysts are a serious cause of reproductive failure in cattle, because they occur frequently and prolong the calving interval [13]. It has been proposed that stress might be one of the causes of bovine follicular cysts, as a number of cows with cysts have also shown an enlargement of the adrenal cortex [17]. As a mechanism for the effect of stress in the formation of follicular cysts, it is supposed that some hormones, increased by stress, may exert inhibition on some sites of the hypothalamus-pituitary-ovarian axis which controls ovulation [11, 12, 15] but the exact mechanism is still vague. It is emphasized that most endocrine data from cows with follicular cysts has been collected at unknown intervals after cyst formation [22], and it cannot be elucidated whether the abnormalities are the cause or the result of cyst formation. Some researchers, using methods for inducing follicular cysts by the administration of ACTH, examined the endocrine profiles during cyst formation [16, 18, 22], but the mechanisms whereby follicular cysts are formed through exogenous ACTH are not fully elucidated. ACTH treatment blocked the preovulatory surge of LH in synchronized cows [22], probably through a cortisol-mediated inhibition of LH release [15]. Although basal levels of gonadotrophins are required for follicular recruitment [25], the influence of cortisol on the basal release of gonadotropins has not been clearly documented. Furthermore, the involvement of other adrenal steroids such as progesterone in the inhibition of gonadotropin release is also unknown. Recently, we have demonstrated that progesterone inhibits the release of GnRH from the perfused pituitary stalk-median eminence of cows [12].

The objective of this study was 1) to determine the secretory patterns of gonadotropins and steroid hormones associated with the development of cysts in dairy cattle, and 2) to document how ACTH affects the hypothalamic-pituitary-ovarian axis.

Four multiparous Holstein cows showing normal estrous cycles were used. The day of ovulation (Day 0) was determined by palpation per rectum and confirmed by a real-time linear array ultra-sound scanner (EUB-25M, Hitachi Co., Ltd., Tokyo) with a 5-MHz rectal transducer, as described previously [6]. The cows were treated with synthetic ACTH (3 mg/day, im; Cortrosyn Z, Daiichi Pharmaceutical Co., Ltd., Tokyo) twice daily for 14 consecutive days beginning at Day 14. The examination of the ovaries was performed by palpation per rectum or visualization using the ultrasound scanner daily from Day 20 to Day 43. The follicular cysts were defined as follicular structures of 25 mm or more in diameter that were present in the ovary for 10 days without occurrence of ovulation and CL development [1].

In order to gain control data for the changes of hormonal concentrations around the normal estrous period, blood samples were taken every 2 hr on Day -3, every 1 hr on Day -2 and -1, every 2 hr on Day 0, every 4 hr on Day 1, once a day from Day 2 to 4, and thereafter every 2 days until Day 14. The untreated period, therefore, extended from Day -3 to Day 13. To examine changes of hormonal concentrations during formation of follicular cysts (treated period, Days 14 to 28), blood samples were taken once a day from Day 14 to 17, every 2 hr on day 18, every 1 hr on day 19 and 20, every 2 hr on day 21, every 4 hr on Day 22, and thereafter once a day until Day 28. The blood was collected by jugular venepuncture using a heparinized disposable syringe and immediately centrifuged at 800 × g for 30 min at 4°C. The plasma was frozen and kept at −20°C until assayed.

The concentrations of LH and FSH in plasma were measured by double antibody RIA as described previously [19, 20]. The lactoperoxidase method was used to radioiodinate highly purified bovine LH (LER-1072) and FSH. NIH-LH B10 and NIH-FSH-B1 were used as the standards. The concentrations of progesterone, cortisol and

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estradiol-17β were measured by validated RIA as described previously [5, 7]. The sensitivities of the assays were determined to be 50, 50, 10, 25 and 0.2 *pg*/tube for LH, FSH, progesterone, cortisol and estradiol-17β, respectively. The intra-assay CV each hormone measured were less than 10%. Each hormone in all samples was quantified within an assay.

Preovulatory surges of LH and FSH were defined as peaks with magnitudes of at least 10 and 100 *ng*/m*l*, respectively, lasting for 8 or more consecutive 1-hr intervals [22]. The differences in mean plasma hormone levels between groups were evaluated by ANOVA, followed by Fisher's least significant difference post-hoc analysis, using the Stat View^R computer program. The level of significance was set at $P<0.05$.

The occurrence of ovulation determined by the disappearance of a large follicle 16 to 19 mm in diameter was detected at the normal estrous period in each cow. These cows exhibited estrous behaviour on Day -1 and showed evidence of luteal tissue, detected by ultrasonography, on approximately Day 3. CL regression occurred after beginning the daily treatment with ACTH on Day 14, followed by the appearance of a single follicle with 14 to 16 mm diameter on Day 20 in each animal. These follicles increased to 25 to 32 mm in diameter on Day 23 to 25 and were diagnosed as follicular cysts. The cysts remained for at least 10 days. None of the cows which developed cysts exhibited estrous behaviour.

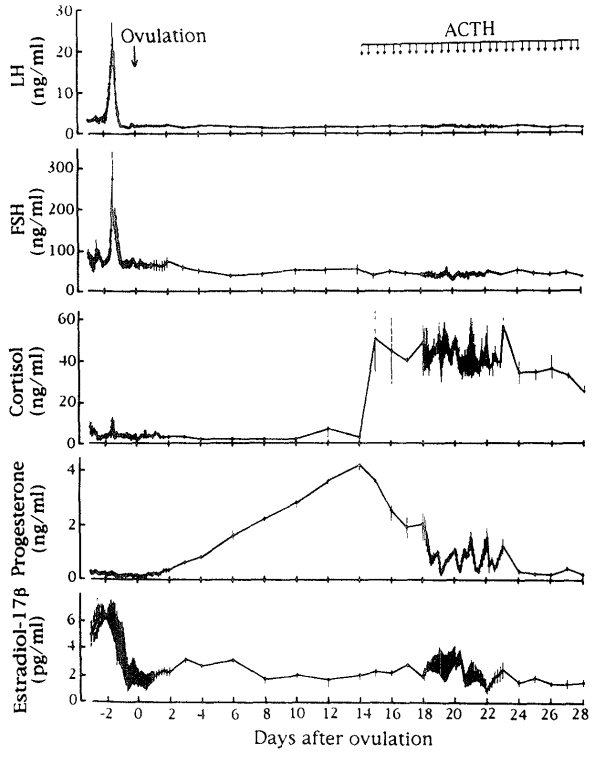

Fig. 1. Changes in LH, FSH, cortisol, progesterone and estradiol-17β concentrations in plasma of cows during a normal estrous cycle and the formation of follicular cysts induced by treatments with ACTH. Data are the mean ± SEM of 4 cows.

The changes in concentrations of LH, FSH, cortisol, progesterone and estradiol-17β in plasma of four cows are shown in Fig. 1 and Table 1. The cortisol concentrations in plasma were low before treatment with ACTH, although episodic releases were observed during the serial blood sampling at 1 to 4 hr intervals in each cow. After beginning the daily treatments with ACTH, cortisol concentrations, compared with those of the untreated period, significantly increased ($P<0.01$) and remained high for 14 days.

Progesterone concentrations in plasma were low in the untreated preovulatory period, then gradually increased after the ovulation, reaching maximal levels on Day 12. After progesterone concentrations declined with regression of CL, numbers of small peaks for 4–5 hr were observed after each treatment with ACTH and the mean concentrations of progesterone during this period were, therefore, significantly higher than those in the untreated period ($P<0.05$).

Estradiol-17β concentrations in plasma increased prior to the preovulatory surges of LH and FSH and decreased during the surges of gonadotropins in the untreated period. During the formation of follicular cysts, induced by treatments with ACTH, estradiol-17β concentrations slightly increased after the decrease of progesterone concentrations, although the levels were significantly lower than those in the untreated period ($P<0.05$).

Preovulatory surges of LH and FSH, which continued for approximately 10 hr, were observed in the untreated period. During the treatment period, mean plasma concentrations of LH and FSH remained low, and the preovulatory surges of LH and FSH did not occur in any of the four cows.

In the present study, we tried to induce bovine follicular cysts by daily treatments with ACTH for 14 days beginning at Day 14 to Day 28. It was shown that ovulation was suppressed and follicular cysts were induced in all 4 cows by the treatments. It has been reported that ACTH treatment is not a 100% efficient way to experimentally produce cysts in synchronized cows [22]. It seems possible that the ability of exogenous ACTH to produce cysts may depend on the day of the estrous cycle when treated, or the dosage and duration of treatment.

The increased secretion of cortisol, during cyst formation, observed in this study confirms earlier findings on cows treated with ACTH [21, 22]. Plasma concentrations of

Table 1. Comparison (mean ± SEM) of plasma hormone concentrations in four cows during a normal preovulatory period (from Day -3 to -1) and the formation of follicular cysts (from Day 18 to 20) induced by treatments with ACTH

Hormone	Day -3 to -1	Day 18 to 20
LH (*ng*/m*l*)	5.0 ± 0.6	1.5 ± 0.2**
FSH (*ng*/m*l*)	89 ± 8	37 ± 6**
Cortisol (*ng*/m*l*)	4.4 ± 0.6	43.9 ± 3.2**
Progesterone (*ng*/m*l*)	0.18 ± 0.02	0.91 ± 0.21*
Estradiol-17β (*pg*/m*l*)	4.8 ± 0.5	2.6 ± 0.6*

* $P<0.05$, ** $P<0.01$.

cortisol after ACTH treatment were comparable to those seen during various psychological stresses [26]. *In vitro* studies with bovine pituitary cells showed that ACTH had no effect on GnRH responsiveness, but incubation of the cells with cortisol strongly reduced this property [1]. Perphaps ACTH treatment blocked the preovulatory surges of gonadotropins through a cortisol-mediated inhibition of gonadotropin release. However, it has been observed that infusion with cortisol succinate to adrenalectomized heifers, did not alter pituitary responsiveness to GnRH [4]. Matteri and Moberg [18] also found that ACTH treatment was more effective than cortisol in suppressing LH secretion. So, the exact site of action of ACTH has not yet been fully defined.

The present study shows increased secretion of progesterone during cyst formation. In our study the relative contribution of the adrenal gland and ovary as sources of circulating progesterone was unknown. The present study supports a previous hypothesis that a prolonged development of the dominant ovarian follicle resulted when suprabasal concentrations of progesterone persisted in circulation [2, 14]. In the normal estrous cycle the sharp decrease in progesterone concentrations after luteolysis is followed by an increase in the frequency of LH pulses, which in turn stimulate the follicle to grow [2, 24]. Although the blood sampling scheme used in the present study was not designed to investigate LH pulsatility, it is presumable that the slow decrease in progesterone concentrations after luteolysis induced an increased LH pulse frequency which supported the continuous growth of the ovulatory follicle. Even though basal LH concentrations were not significantly increased during cyst formation, previous reports have demonstrated that an increase in frequency of LH pulses is not necessarily accompanied by higher LH basal concentrations [2, 8, 23]. These suprabasal concentrations of progesterone might suppress the release of GnRH from the hypothalamus and might, therefore, suppress the preovulatory release of LH and FSH from the pituitary, as proposed by Kawate *et al.* [12].

In the present study, the rise in plasma estradiol-17β that accompanies cyst development was not observed. One of the reasons for the lack of estradiol-17β secretion in the developing cysts might be that the sustained increase of cortisol directly inhibits the secretion of estradiol-17β in the preovulatory follicle. It has been suggested previously that high concentrations of cortisol decrease the secretion of estradiol-17β in cultured bovine granulosa cells [11]. The reduced synthesis of estradiol-17β and the reduction of total numbers of LH and FSH receptors in the follicular cysts has also been observed [9, 10]. This lowered concentration of estradiol-17β might fail to exert a positive feedback on the hypothalamic pituitary axis, and consequently the preovulatory surges of LH and FSH did not occur.

Erb and co-workers [3, 4] hypothesized that an excess of FSH, overstimulating follicular development, and a failure of the mechanism controlling release of LH are possible causes of spontaneously occurring bovine ovarian cysts. Regarding the possibility of the latter cause for the cysts, it has been already indicated that ovulation is suppressed and follicular cysts are induced by the administration of antiserum to LH in cows during estrus [21], but the possibility that an excess of FSH secretion causes cysts has not yet been elucidated. In the present study, we examined changes of FSH concentrations in blood, in addition to those of LH, during formation of bovine follicular cysts induced by treatments with ACTH. It is evident that the basal and preovulatory release of LH and FSH are suppressed during the formation of the cysts, and the possibility that an excess of FSH secretion causes cysts is therefore denied in the case of the ACTH-induced bovine follicular cysts in the present study.

In conclusion, the present study demonstrates that the preovulatory release of LH and FSH is blocked during the formation of bovine follicular cysts induced by treatments with ACTH. The suppressed release of FSH and LH might be caused by increases in secretions of cortisol and progesterone, and by the decrease in secretion of estradiol-17β.

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