Effects of Glucocorticoids and Corticotropin on the Peripheral Blood Levels of 11-Hydroxy corticosteroids and Progesterone and Serum Protein Pattern in Cows with Cystic Ovaries

Toshihiko NAKAO

Department of Veterinary Obstetrics and Gynecology, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro-shi, Hokkaido 080

(Received for publication August 12, 1976)

Abstract. Effects of synthetic glucocorticoids and ACTH on the adrenal cortex were studied in 4 cases of cystic ovarian disease (COD) involving 3 cows. Of 2 cases showing anestrus, one was injected intramuscularly (i.m.) with 20 mg of betamethasone (Bet) daily for 5 days and the other with 10 mg of Bet daily for 4 days. Of the other 2 cases exhibiting symptoms of true nymphomania, one was injected i.m. with 20 mg of Bet daily for 5 days and the other with 10 mg of dexamethasone daily for 4 days. Before and after successive administration with glucocorticoids, 50 IU of ACTH was injected i.m. in the 3 cases. The serum concentrations of 11-hydroxy corticosteroids (11-OHCS), progesterone, and proteins were determined daily throughout the experimental period. The response of serum 11-OHCS to ACTH was examined 50 minutes after injection. Glucocorticoids inhibited the release of adrenal corticosteroids in all the 4 cases. A fall of the serum 11-OHCS level was shown earlier after injection with 20 mg of glucocorticoids than after that with 10 mg. Following the termination of injection with glucocorticoids, the serum 11-OHCS level increased remarkably in the 2 cases showing anestrus, but did not in the 2 cases exhibiting true nymphomania. The response of serum 11-OHCS to ACTH was lower in the cows with COD than in control cows with a normal estrous cycle. The serum progesterone level presented essentially the same pattern of fluctuation in response to glucocorticoids and ACTH as the serum 11-OHCS level. Serum proteins decreased slightly after the 4th day of injection with glucocorticoids. From these results, it is suggested that adrenal dysfunction may be related to the different appearance of estrous abnormalities in cows with COD.

Functional interactions between the adrenal and the gonadal systems have been indicated in the rat [2, 22], lemming [27], turkey [3], pig [24], and cattle [6, 16, 28, 31]. Adrenal hyperactivities causing an increased secretion of corticosteroids and progesterone may inhibit gonadotropin secretion and result in ovulation failures and other reproductive disturbances [2, 8, 16, 22, 28].

Cystic ovarian disease (COD) in cattle is characterized by the cystic degeneration of one or more large anovulatory follicles and abnormalities of estrous behavior. Although the etiology of the disease has not well been elucidated, it is generally believed that cows overfed and producing a high milk yield are very susceptible to COD and that the reduced ability of such cows to adapt themselves to lactational and

* Present address: Department of Veterinary Surgery and Obstetrics, School of Veterinary Medicine, College of Dairying, Rakuno University, 582 Nishinopporo, Ebetsu-shi, Hokkaido 069-01.

Glucocorticoids および ACTH の投与が卵胞翼障の血中 11-hydroxy corticosteroids, progesterone 値並びに 血清蛋白像に及ぼす影響: 中尾敏彦（帯広畜産大学家畜臨床繁殖学教室）
nutritional stress is responsible for the disease.

Since Garm [5] reported hypertrophy of the adrenal cortex and the pituitary of cattle affected with COD, several workers have paid an attention to the syndrome of adrenal virilism in those cattle [4, 26, 32]. Decreased response of the adrenal cortex to exogenous adrenocorticotropic hormone (ACTH) was also pointed out in cows with COD [15, 18]. Besides, a large dose of dexamethasone or betamethasone injected intramuscularly seems to have satisfactory therapeutic effects on COD [17, 19, 25]. Recently, Litrap et al. [11] described that successive injections of ACTH in the follicular phase induced cystic conditions of the ovary in the cow.

In the present study, effects of glucocorticoids and ACTH on the adrenal cortex were investigated in cows with COD. They were compared between cows exhibiting true nymphomaniac and those showing anestrus.

Materials and Methods

Four cases of COD from 3 cows were used for the present experiment. Diagnosis of COD was made on the basis of rectal palpation and observation of estrous behavior.

Case No. 1: Five years old and in the 3rd lactation. Interval between the experiment and the last calving was 160 days. She has been anestrous since the last calving. Betamethasone was injected intramuscularly 20 days before the experiment, but COD was not cured.

Case No. 2: Five years old and in the 2nd lactation. Interval between the experiment and the last calving was 664 days. She was inseminated about 90 days postpartum and did not return to estrus. Nine months after the insemination she was diagnosed as COD and treated with betamethasone. Two weeks after the treatment she came into estrus and was served by artificial insemination. Thereafter, she had not shown any symptom of estrus. Nine months later she again developed COD without showing estrous activities.

Case No. 3: This case was obtained from the same animal as used in case No. 2. She was 6 years old and in the 2nd lactation. Interval between the experiment and the last calving was 826 days. Approximately 1 month after the end of the experiment mentioned in No. 2, she showed mounting to other cows in estrus in the same herd, but did not accept mounting by any other cow. The anatomical changes associated with bovine nymphomania, i.e., raising of the tail head, relaxation of the sacrosciatic ligament, hypertrophy of the clitoris, and edema of the vulva, were observed.

Case No. 4: Eight years old and in the 1st lactation. Interval between the experiment and the last calving was about 6 years. Although details of the previous treatment were not clearly known, she had developed COD showing an irregular estrus since the early stage of lactation. This condition lasted for a few years. She had not responded to any treatment employing gonadotropic hormones. When she was admitted to the University Veterinary Hospital, the same anatomical changes of the tail head, sacro-sciatic ligament, clitoris and vulva as observed in case No. 3 were noticed.

Glucocorticoids test: All the procedures, including blood sampling and injection of glucocorticoids, were performed between 10:00 and 11:30 in the morning. Nos. 1 and 3 were injected intramuscularly with 20 mg of betamethasone (Betsolan, Glaxo) daily for 5 days. Blood was taken from the jugular vein just before the injection or at a definite time over a period from 1 day before the first injection to 2 days after the last injection. No. 2 was given daily intramuscular injection of 10 mg of betamethasone for 4 days. Blood samples were collected in the same manner as in No. 1 over a period from the day of the first injection to 2 days and also 21 and 30 days after the last injection. No. 4 was injected intramuscularly with 10 mg of dexamethasone daily for 4 days. Blood was taken from it by the same method as from the cases mentioned above over a period from the first day of injection to 4 days after the last injection.

ACTH test: All the procedures of the ACTH test were conducted between 10:00 and 11:30 in the morning. Eleven cows were used as controls for the ACTH test on the cows with COD. They had shown normal estrous cycles and were free from any abnormality of the genital organs. Average age and interval between the experiment and the last calving were $4.2 \pm 2.2$ years and $177.2 \pm 67.9$ days, respectively. Four of the 11 control cows were injected intramuscularly with 0.5 mg of synthetic ACTH, $\beta^{24}$ corticotropin (tetracosactide) (Cortrosin, Daiichi Pharmaceutical Co., Ltd.), equivalent to 50 IU of natural ACTH. Blood samples were collected from the jugular vein prior to and 30
ADRENAL FUNCTION IN COWS WITH CYSTIC OVARIIES

and 60 minutes subsequent to the ACTH injection. The serum level of 11-hydroxycorticosteroids (11-OHCS) determined by fluorometry rose significantly \((P \leq 0.01)\) 30 minutes after the ACTH injection. Almost the same level as this was maintained for 30 minutes. Blood was taken from the remaining 7 control cows before and only 30 minutes after the ACTH injection. The ACTH test thus established was applied to the 3 cases of COD before and after the glucocorticoids test. Blood samples were stored at room temperature for 9 to 12 hours and centrifuged at 3,000 rpm for 10 minutes to collect serum.

Serum 11-OHCS was determined by a modification of the fluorometric method described by Ustl et al. [29] and progesterone by radioimmunoassay reported by Makino [14]. Total serum protein was determined by a refractometric instrument (Hitachi hand refractometer). Protein fraction values were obtained by electrophoresis using cellulose acetate strips. The procedures used for analysis were reported previously [18].

Results

1. Glucocorticoids test

The peripheral blood levels of 11-OHCS

![Fig. 1. Serum 11-hydroxycorticosteroids and progesterone levels in 2 cows with cystic ovaries showing anestrus after injection with glucocorticoids (Case No. 1, 20 mg/day; Case No. 2, 10 mg/day)](image)

![Fig. 2. Serum 11-hydroxycorticosteroids and progesterone levels in 2 cows with cystic ovaries exhibiting symptoms of nymphomania after injection with glucocorticoids (Case No. 3, 20 mg/day; Case No. 4, 10 mg/day)](image)

and progesterone after the glucocorticoids injection are shown in Figs. 1 and 2.

In case No. 1 the serum 11-OHCS level decreased gradually after the first injection of glucocorticoids and reached a minimum on the fourth day of injection. Then it increased to the pretreatment level 1 day after the last injection (Fig. 1). The serum progesterone level started to rise gradually on the fourth day of injection and remained high up to 2 days after the last injection.

In case No. 2 the serum 11-OHCS level did not show a decrease till the third day of injection and reached a minimum 1 day after the last injection. The changing pattern of the serum progesterone level in response to injection with glucocorticoids was similar to that of the serum 11-OHCS.
Fig. 3. Serum protein levels in 2 cows with cystic ovaries showing anestrus after injection with glucocorticoids (Case No. 1, 20 mg/day; Case No. 2, 10 mg/day)

Case No. 1  
Betamethasone  
Total protein  
-  8  
-  6  
-  4  
-  2  
0  1  2  3  4  5  6
Serum protein level (g/dl)
Time in days after the first treatment

Case No. 2  
Betamethasone  
Total protein  
-  8  
-  6  
-  4  
-  2  
0  1  2  3  4  5  6
Serum protein level (g/dl)
Time in days after the first treatment

Fig. 4. Serum protein levels in 2 cows with cystic ovaries exhibiting symptoms of nymphomania after injection with glucocorticoids (Case No. 3, 20 mg/day; Case No. 4, 10 mg/day)

Case No. 3  
Betamethasone  
Total protein  
-  8  
-  6  
-  4  
-  2  
0  1  2  3  4  5  6
Serum protein level (g/dl)
Time in days after the first treatment

Case No. 4  
Dexamethasone  
Total protein  
-  8  
-  6  
-  4  
-  2  
0  1  2  3  4  5  6
Serum protein level (g/dl)
Time in days after the first treatment

level (Fig. 1).

In case No. 3 a gradual fall of the serum 11-OHCS level was seen following the first injection with glucocorticoids. It was not followed by such an increase of the level as observed in Nos. 1 and 2 after the termination of the injection (Fig. 2). The serum progesterone remained quite low and showed no response to the injection throughout the experiment.

In case No. 4 the serum 11-OHCS level decreased in response to injection with glucocorticoids and reached a minimum on the last day of the injection (Fig. 2).

Changes of the level after the last injection were similar to those in No. 3. The level increased a little 4 days after the last injection. Although the serum progesterone level remained low, the fluctuation pattern of this level after injection with glucocorticoids was a little similar to that of the serum 11-OHCS level.

Figs. 3 and 4 show serum protein level in the 4 cases of COD after injection with glucocorticoids. In all the 4 cases the total serum protein level increased gradually following the first injection and decreased after four consecutive injections. Gamma-globulin was considered to contribute mainly to changes in the total serum protein, as most clearly shown in case No. 3 (Fig. 4).

2. ACTH test

In 4 control cows the serum 11-OHCS level prior to and 30 and 60 minutes subsequent to an intramuscular injection with 50 IU of ACTH was 6.4±2.9, 16.4±1.7, and 15.3±3.6 µg/dl, respectively. In the other 7 control cows this level prior to and 30 minutes subsequent to the ACTH injection was 13.0±5.5 and 24.6±6.2 µg/dl, respectively. Therefore, in the 11 control cows
ADRENAL FUNCTION IN COWS WITH CYSTIC OVARIIES

Fig. 5. Serum 11-hydroxycorticosteroids and progesterone levels prior to and subsequent to an intramuscular injection with 50 IU of ACTH in 3 cows with cystic ovaries (Case No. 1, anestrus; Cases No. 3 and 4, nymphomania).

the serum 11-OHCS level increased from 10.6±4.8 μg/dl to 21.6±6.9 μg/dl 30 minutes after the ACTH injection (Fig. 5). Fig. 5 presents the results of the ACTH test in the cows with COD.

Case No. 1 had a higher serum 11-OHCS level than the controls and well responded to ACTH injection, indicating a hyperfunction of the adrenal cortex. Although the serum 11-OHCS level decreased slightly after administration with glucocorticoids, it was still higher than in the controls. Two cases, Nos. 3 and 4, had almost the same serum 11-OHCS level as the controls, whereas they did not so well respond to the ACTH injection as the controls. After completion of successive administration with glucocorticoids the serum 11-OHCS level decreased and the response to the ACTH injection did not increase. An increase of the serum progesterone level in response to the ACTH injection was observed in No. 1, but not in any other case.

Discussion

Diurnal variation in the peripheral blood levels of adrenal corticosteroids has been demonstrated in man [10, 23] and the rat [7], but not so clearly in cattle [9, 12, 21, 30]. In the present study the time of blood sampling was set between 10:00 and 11:30 in the morning, since Wagner et al. [30] suggested that there might be a difference in corticosteroid concentration between samples collected in the morning and those in the afternoon.

The secretion of corticosteroids from the adrenal cortex is influenced by ACTH originated from the anterior pituitary which is under the control of corticotropin-releasing hormone derived from the hypothalamus. Both endogenous and exogenous corticosteroids were known to inhibit the output of ACTH to decrease the secretion of corticosteroids from the adrenal cortex by suppressing the activity of corticotropin-releasing hormone at the pituitary level [1]. In this study intramuscular injection with synthetic glucocorticoids was observed to inhibit the release of corticosteroids from the adrenal cortex in cows with COD. The serum 11-OHCS level decreased earlier after injection with 20 mg of glucocorticoids than after injection with 10 mg. Daily administration with a high dose of glucocorticoids for several weeks may cause ad-
renal atrophy and a decreased secretion of adrenocortical hormones. Such a high daily dose for 4 days or less may not seriously affect the pituitary adrenal axis [13].

In the present study there was a difference in adrenocortical response to the termination of injection with glucocorticoids between the cases of COD showing anestrus and those exhibiting true nymphomaniac symptoms. The serum concentration of 11-OHCS increased remarkably 1 or 2 days after the last injection with glucocorticoids in the 2 cases of COD showing anestrus. On the contrary, in 2 cases exhibiting nymphomania the serum 11-OHCS level did not increase 2 or 3 days after the termination of glucocorticoids. Since the serum concentration of ACTH was not determined, it could not be proved whether the difference in serum 11-OHCS level after the termination of glucocorticoids between the cases showing anestrus and those exhibiting nymphomania was due to the reduction of ACTH release from the pituitary or to the decrease of the adrenocortical response to ACTH.

Results of the ACTH test performed prior to and subsequent to the glucocorticoids test revealed that adrenocortical response to ACTH was quite distinct in the two cases, Nos. 3 and 4, exhibiting nymphomania. This indicates that in the nymphomaniac cases daily injection with glucocorticoids for 4 or 5 days suppressed the release of ACTH from the pituitary and that the suppression of ACTH lasted even 2–3 days after the termination of injection with glucocorticoids. In the anestrous cases, however, the suppression of ACTH by glucocorticoids was removed within 1 or 2 days after the last injection and followed by a rise in the secretion of adrenal corticosteroids in response to ACTH. The cows with COD had a lower adrenal response to ACTH than the control cows with a normal estrous cycle. These results agree with those previously reported [15, 18].

The fluctuation pattern of the serum progesterone level in response to injection with glucocorticoids and ACTH were similar to those of the serum 11-OHCS level. During the experiment no distinct changes of the ovaries, including luteinization of the follicular cyst, were found by rectal palpation. Since the adrenal cortex is known to produce a significant amount of progesterone in cattle [31], the increase in the serum progesterone level responding to injection with glucocorticoids and ACTH in the cows with COD may be adrenal origin.

Previously, Nakao et al. [18] carried out the ACTH test on 60 cows with COD and found that there was a difference in adrenocortical response to ACTH between cows showing anestrus and those exhibiting nymphomania, continuous estrus, and irregular estrus. The response of the adrenal cortex to ACTH was higher in the anestrous cows than in the nymphomaniac cows. The serum concentration of progesterone was significantly higher (P ≤ 0.05) in the former cows than in the latter. No role of adrenal progesterone has been clarified in an incidence of COD or no appearance of abnormal estrous activities interpreted as yet. The results of the authors' previous and present studies indicate that the adrenal dysfunction is one of the main causes of the formation of follicular cysts and is involved in the variation of estrous abnormalities associated with COD in the cow.

Glucocorticoids cause a catabolism of proteins and inhibition of antibody production. The previous study of Nakao et al. [20] indicates that there was a significant negative correlation (P ≤ 0.05) between the
ADRENAL FUNCTION IN COWS WITH CYSTIC OVARIES

serum concentrations of 11-OHCS and total protein, was as gamma-globulin, in cows with COD. The results of this study show that 10 or 20 mg of synthetic glucocorticoids had an indistinct effect on the serum proteins, though these proteins, especially gamma-globulin, decreased slightly after the fourth injection with glucocorticoids. A large dose of glucocorticoids given for a longer period may induce a more apparent drop of the serum protein level.

Acknowledgments: The author expresses his hearty gratitude to Dr. M. Miyake, professor of Veterinary Obstetrics and Gynecology at the Obihiro University of Agriculture and Veterinary Medicine for his encouragement to this study. Thanks are also due to Drs. H. Ono, associate professor, and K. Sato, lecturer, of Dr. Miyake's department for their advice and criticism. The assistance of Drs. S. Sasaki and H. Kitagawa, who were then veterinary students at the same university, in carrying out the experiment is acknowledged sincerely.

The outline of this study was read before the 80th Meeting of the Japanese Society of Veterinary Science in Osaka on November 5, 1975.

References


