ADRENAL-GONAD INTERACTIONS IN CATTLE. CORPUS LUTEUM FUNCTION IN INTACT AND ADRENALECTOMIZED HEIFERS1, 2

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Summary

To determine the effect of the hyperadrenal state on corpus luteum (CL) function, we treated intact and adrenalectomized (ADRX) heifers with adrenocorticotropin (ACTH) and hydrocortisone. ACTH treatment of intact heifers by infusion of 1 mg of ACTH/24 hr from day 2 to 25 of an estrous cycle increased progesterone concentrations in plasma to 4 ± .52 ng/ml plasma on days 3 to 4. Thereafter, progesterone concentrations declined, indicating severe suppression of CL function as compared to that of control heifers. Similar infusions of ACTH to ADRX heifers did not alter CL function as reflected by progesterone concentrations in jugular plasma. Infusion of ADRX animals with hydrocortisone succinate (100 mg/24 hr) decreased progesterone in plasma during the cycle, with maximum values averaging <4 ng/ml, compared to concentrations of 6 to 7 ng/ml in control ADRX heifers. Chronic administration of ACTH or hydrocortisone to heifers caused a decreased progesterone secretion during the luteal phase of the estrous cycle. On the basis of these results, we suggest that stress, as indicated by the hyperadrenal state, could inhibit progesterone secretion by the corpus luteum.

(Key Words: Adrenal, Corpus Luteum, Cortisol, Progesterone, Bovine.)

Introduction

Many studies have shown a close relationship between the adrenal and reproductive functions in various species. Stress, adrenocorticotropin (ACTH) and corticosteroids interfere with ovarian function in laboratory animals (Christian, 1971) and wild populations of small rodents (Christian et al., 1965; Andrews, 1970). In cattle, ACTH treatment results in small corpora lutea (CL) and decreased concentrations of progesterone in peripheral plasma (Brunner et al., 1969; Wagner et al., 1972). Stress increases ACTH secretion, which in turn stimulates adrenal production of hormones such as progesterone (Wagner et al., 1972), estrogen (Lunaas, 1970) and androgen (Christian, 1971), which may influence development and(or) function of CL.

Tomasgard (1976) treated heifers with prednisolone during an estrous cycle and inhibited the increase in progesterone in peripheral plasma normally associated with metestrus and diestrus. Wagner et al. (1977) found that perfusion of the ovary with hydrocortisone or ACTH did not decrease progesterone content in plasma, although these two hormones, when infused via a carotid artery or jugular vein, decreased progesterone concentrations in plasma. Thibier and Rolland (1976) reported decreased luteinizing hormone (LH) and testosterone in plasma of bulls treated with dexamethasone, while Chantaraprateep and Thibier (1978) reported that dexamethasone decreases the LHRH-induced LH release in bulls. The primary purpose of this investigation was to study the effect of ACTH or hydrocortisone upon CL function in heifers. Concentrations of corticoids, progesterone and estrogen in plasma of intact and adrenalectomized virgin heifers were measured.

Materials and Methods

General. Twenty-five virgin heifers exhibi-
ting normal estrous cycles were used. Heifers were observed for estrus twice daily. Heifers were housed in stanchions in a heated building and fed a diet of alfalfa hay ad libitum plus a 14% protein concentrate mix (1.8 kg/day); a mineral supplement was also provided free choice in the exercise lot. The heifers were randomly assigned to one of two groups, either intact or adrenalectomized (ADRX), then further randomly assigned to one of five treatment groups of five animals each: intact-saline, intact-ACTH, ADRX-saline, ADRX-ACTH and ADRX-hydrocortisone succinate. After adrenalectomy, all heifers were allowed a minimum of one normal estrous cycle before being placed on the experiment.

All treatments were administered via continuous infusion into a jugular vein with a Harvard peristaltic pump. The infusion period was from 0900 hr on day 2 of an estrous cycle (day 0 was day of estrus) until the next estrus or day 25, whichever occurred first. Solutions for infusion were made up in .9% saline containing 1% gelatin and 45 U of heparin/ml and held at 5 C during the infusion period. ACTH was infused at the rate of 1 mg of α 1-24 ACTH6/24 hr. Hydrocortisone succinate7 was infused at the rate of 100 mg/24 hr. All solutions were infused at the rate of 10 ml/hr. Blood was collected from a jugular vein and carotid artery three times daily (0800, 1200 and 1600 hr), from day 1 of the cycle until the end of the infusion period.

Surgery. Before the adrenalectomy, all feed was withheld from the heifers for 24 to 48 hours. The heifers were given atropine (8 mg) SC and chlorpromazine (35 mg) IV 30 min before surgery. Just before surgery, heifers were given 2.5 mg of flumethasone, 6.0 million U penicillin and 10 g streptomycin. Thiopental sodium was given IV to induce anesthesia and allow endotracheal intubation. Heifers were maintained under anesthesia with halothane. Both adrenals were removed through a single incision in the upper right flank without invasion of the peritoneal cavity. The incision was made from the caudal border of the last rib and extended parallel and 3.5 cm ventral to the transverse processes of the lumbar vertebrae, to a point near the tuber coxa. A dorsal portion of the last rib on the right side was always removed. The adrenals were removed through blunt dissection with the fingers. Following surgery and daily throughout the study, all ADRX heifers were given 10 mg deoxycorticosterone acetate (DOCA) and 25 mg of cortisone acetate IM as a maintenance treatment.

Cannulation. Two silastic8 catheters (ID 1.016 mm, OD 2.032 mm) were placed in one jugular vein (designated as proximal and distal), and one catheter was placed in the ipsilateral carotid artery. Catheterization was done under general anesthesia (halothane). All catheters were passed subcutaneously and exteriorized at the top of the shoulder. When not being used, the catheters were kept filled with sterile .9% saline containing 400 U of heparin/ml.

Progesterone Pulse Dose. To test the possibility that adrenalectomy or the treatments altered clearance rates of progesterone from plasma, we gave a progesterone pulse treatment to each heifer on the day designated as the last sampling day (day of estrus or day 25 of cycle). While the animals were still being infused with their respective treatments, 5 mg of progesterone in 5 ml of 50% ethanol (v/v) were administered via the proximal jugular vein catheter. All blood samples were collected from the distal jugular catheter. Progesterone concentrations were monitored in plasma collected as follows: 10 and 15 min before progesterone was given; at the time progesterone was given, and at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min after progesterone. Blood samples were immediately centrifuged, and plasma was removed and stored at −20 C until assayed.

Hormone Assay. Corticoids were assayed in a competitive binding system as described by Wagner et al. (1977). Progesterone was assayed in a standard radioimmunoassay procedure consisting of petroleum ether extraction, evaporation of the ether, reconstitution with 1.6 ml methanol and assay of .5-, .3- and .15-ml aliquots of the methanol solution after evaporation. The antibody used (Prog-S3-AK1) was supplied by Dr. B. Hoffmann, Berlin, West Germany (Hoffmann, 1977). The antiserum was raised against progesterone conjugated at the 11 position with hemisuccinate bovine serum albumin.

Cross-reactivity of this antiserum with other steroids is low. The three steroids showing the

6 Synacthen, supplied by the Ciba-Geigy Company, author please give location.
7 SoluCortef, supplied by the Upjohn Company, Kalamazoo, MI.
8 Dow Corning Co., Midland, MI.
TABLE 1. ANALYSIS OF VARIANCE FOR PROGESTERONE, CORTICOID AND TOTAL ESTROGEN CONCENTRATIONS IN JUGULAR PLASMA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Corticoids df</th>
<th>MS</th>
<th>Progesterone df</th>
<th>MS</th>
<th>Estrogen df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>307,934**</td>
<td>4</td>
<td>77.1*</td>
<td>4</td>
<td>1,389**</td>
</tr>
<tr>
<td>Animal (treatment)</td>
<td>20</td>
<td>5,920</td>
<td>20</td>
<td>18.8</td>
<td>19</td>
<td>334</td>
</tr>
<tr>
<td>Day</td>
<td>19</td>
<td>1,460**</td>
<td>19</td>
<td>56.7**</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Day X treatment</td>
<td>76</td>
<td>1,445**</td>
<td>76</td>
<td>7.3**</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>Residual</td>
<td>352</td>
<td>287</td>
<td>362</td>
<td>3.9</td>
<td>333</td>
<td>12</td>
</tr>
</tbody>
</table>

*P<.05.
**P<.01.
***P<.001.

The greatest cross-reactivity were 17α-OH-progesterone (3.1%), 20β-OH-progesterone (4.2%) and pregnenolone (4.5%). All others were 1.0 to 2.0% or less.

In our laboratory, two prepared standard bovine plasma pools were used in all assays. The .5 ng standard plasma pool gave an average concentration of .57 ng (95% CI = .46 to .67). The 3.0-ng standard pool gave an average concentration of 2.95 ng (95% CI = 2.41 to 3.50). The interassay coefficient of variation was 21% for the .5-ng sample and 24.0% for the 3.0-ng sample. The intraassay coefficient of variation was 2.39%. Corrections for procedural losses were made through the addition of 14C progesterone to the original sample before extraction.

Estrogens were measured as total estrogens with an antibody against 17β-estradiol-17-Hemisuccinate bovine serum albumin. This antisemum gave slightly better binding to estrone than to 17β-estradiol. At 50% relative binding for estrone, 17α-estradiol and 17β-estradiol gave a binding result approximately 80% of estrone (Hoffmann, 1977). All progestogens and glucocorticoids gave less than 1% cross-reaction.

Plasma pools were prepared by the addition

![Figure 1. Jugular plasma corticoid concentrations in intact heifers treated with saline or ACTH (41.7 µg/hr) infusion beginning on cycle day 2.](image-url)
of estrone to essentially negative plasma from an adrenalectomized-gonadectomized heifer at concentrations of 5 and 20 pg/ml. In six assays, these two pools gave mean concentrations and interassay coefficients of variation of 13.10 pg, 6.3% and 30.48 pg, 30.5% for the 5- and 20-pg pools, respectively. The intraassay coefficient of variation (n = 53) was 2.1%. Two milliliters of plasma were extracted with diethyl ether. The ether extract was dried, methanol was added and two aliquots were assayed in separate tubes. After drying, 1,2,6,7-3H-estrone and antibody were added and incubated for 18 hours. Charcoal-dextran was added and centrifuged, and .5 ml of supernatant were transferred to a scintillation vial. Scintillation fluid was added and radioactivity was determined in a Beckman LS-230 counter. Corrections were made for sample and assay aliquots in order to express results as picograms per milliliter of plasma.

**Statistical Analysis.** The experiments were established as a randomized factorial design. Animals were randomly assigned to treatment on the basis of occurrence of estrus. Hormone treatments, day of estrous cycle and their interactions were considered as sources of variation. An analysis of variance was used that would allow for unequal subclass numbers (Steel and Torrie, 1960).

**Results**

The analysis of variance for all hormone measurements is given in table 1. Intact heifers treated with ACTH (intact-ACTH) had a mean concentration of corticoid in plasma of 130 to 160 ng/ml throughout the cycle (figure 1), which was greater (P<.001) than the comparable mean (15 ng/ml) for intact heifers given saline.

Among ADRX heifers, there were no significant differences in corticoid concentrations between the ACTH- and saline-treated groups; however, the ADRX heifers treated with hydrocortisone (ADRX-hydrocortisone) had a higher (P<.01) mean corticoid concentration in plasma (figure 2).

In intact heifers given saline, mean progesterone in jugular plasma increased steadily from .3 ± .05 ng/ml on day 1 to a peak of 8.0 ± 1.6 ng/ml on day 12, and then declined to 1.2 ± .8 ng/ml by day 20. In intact heifers given ACTH, mean progesterone concentrations in plasma were very low (.2 ± .5 ng/ml) during the first 2 days of the cycle, then increased to 3.5 ± .7

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**Figure 2.** Jugular plasma corticoid concentrations in ADRX heifers treated with constant infusion of saline, hydrocortisone succinate (4.17 μg/hr), or ACTH (41.7 μg/hr) beginning on cycle day 2.
ng/ml on days 3 to 6 of the cycle, but decreased to 2.0 ± .5 ng/ml at midcycle (figure 3).

Comparisons between intact groups of heifers showed higher (P<.001) plasma progesterone concentrations for heifers given saline than for those in the ACTH group. Mean progesterone in plasma at midcycle (days 11 to 12) was higher (P<.05) for heifers given saline than for heifers given ACTH. Comparison between heifers in the ADRX-saline and ADRX-ACTH groups did not show any significant difference; however, heifers in the ADRX-saline group had a higher (P<.025) progesterone concentration in the plasma than did heifers in the ADRX-hydrocortisone group (figure 4).

Estrogen concentrations in plasma were higher (P<.05) in intact heifers given ACTH than in intact heifers given saline (20.74 vs 10.82 ng/ml) when averaged over the entire cycle. Similarly, the intact heifers given ACTH also had higher (P<.01) estrogen concentrations than the ADRX heifers given ACTH. These results suggest a role for the adrenal gland as an estrogen source when stimulated by ACTH.

**Progesterone Pulse.** There was no significant difference in the rate of clearance of progesterone from blood between heifers in the various groups after administration of an intravenous progesterone pulse dose of 5 milligrams.

Among intact heifers, there was a small but nonsignificant difference in progesterone clearance rate between animals that received saline and those that received ACTH (see figure 5).

**Discussion**

Decreased progesterone concentrations in plasma during the luteal phase were seen in only two groups of heifers (ACTH-intact and ADRX-hydrocortisone). On the basis of hormone analyses, increased concentrations of glucocorticoids were present in both groups of heifers, and progesterone concentration was also increased early in the ACTH treatment period. Previous studies have shown that ACTH can stimulate adrenocortical secretion of progesterone in cattle (Wagner et al., 1972; Gwazdauskas et al., 1973).

No alternations in progesterone concentration occurred in the plasma of the ADRX heifers given ACTH or saline, confirming that ACTH requires the adrenal glands to exert its effect and that adrenalectomy itself has no effect on ovarian function when heifers are adequately maintained with exogenous corticoids. Confirmation of completeness of the adrenalectomy procedure is provided by the lack of glucocorticoid response to ACTH treatment in the ADRX heifers.
Among heifers in the ADRX groups, mean progesterone concentrations in plasma did not differ during midcycle between the heifers that were given ACTH and those that were given saline, although concentrations were lower in the ADRX heifers given hydrocortisone than they were in the other two ADRX groups. These findings suggest that the suppressive effect of ACTH upon luteal progesterone secretion may be due partly to secretion of steroids such as cortisol from the adrenal cortex. ADRX heifers infused with ACTH were not able to respond to the ACTH stimulus by initially increasing their progesterone and corticoid concentrations; the decrease in luteal progesterone secretion seen in intact animals was not observed. Our data are supported by those of Wagner et al. (1977), who found that ACTH, locally applied to the bovine ovary for 1 hr, caused no decrease in progesterone concentrations in plasma, although the effect was seen when ACTH or hydrocortisone were used as a continuous systemic infusion.

Tomasgard (1976) treated intact heifers with prednisolone during an estrous cycle and determined peripheral plasma concentrations of progesterone. The main findings were inhibition of the increase of plasma progesterone normally associated with metestrus and lower plasma progesterone at diestrus. These findings were said to be caused by a direct effect of prednisolone on the development of the CL, thus resulting in small CL and decreased progesterone secretion. Results from Thibier and Rolland (1976) with bulls indicate, however, that exogenous synthetic corticoids (dexamethasone) decrease LH secretion from the pituitary and subsequently result in decreased testosterone concentrations in the plasma.

The higher (P<.01) total estrogen concentrations in plasma of the intact heifers given ACTH than in the ADRX heifers given ACTH suggest that the adrenal gland produces estrogens or substances that crossreact with the antiserum used for these assays. No attempt was made to characterize this material further, although the intact heifers given ACTH also had higher total estrogen concentrations than the intact heifers given saline.

The results from the progesterone pulse dose administration provide evidence that low plasma progesterone concentrations at midcycle in heifers in the intact-ACTH and ADRX-hydrocortisone groups were due to a decrease in CL secretion and(or) release rather than to an increase in progesterone metabolism or
excretion. ACTH treatment of intact heifers suppressed or altered CL function and thus decreased peripheral plasma concentrations of progesterone, but this effect probably occurred through the adrenal cortex secretion of steroids such as cortisol, which was found to exert a depressive effect on progesterone in plasma of ADRX heifers. It is also possible that the increased progesterone concentrations in plasma observed early in the ACTH treatment period provided additional suppression of CL function, because Woody et al. (1967) have demonstrated that progesterone administration could cause early luteolysis.

The studies reported by Chantaraprateep and Thibier (1978) shed further light on the possible mechanisms involved. Treatment of bulls with dexamethasone reduced LH secretion in response to 250 μg LHRH, which suggests that glucocorticoids can interfere with pituitary responsiveness. Whether this is also true for natural glucocorticoids such as cortisol must be examined. Also, the possibility of effects occurring at the hypothalamic level cannot be ignored.

**Figure 5.** Progesterone concentration in jugular plasma following a bolus IV injection of 5.0 mg progesterone. Part A represents the data for intact heifers and Part B the ADRX heifers.

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**Literature Cited**


