ADRENAL SECRETION, ESTRUS, AND OVARIAN ACTIVITY
IN BOVINE TREATED WITH CORTICOSTEROIDS

by
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ABSTRACT

The relationship of plasma corticoids and progestins and their secretion has been studied in Holstein heifers. The heifers were treated with Predef® (9-fluoroprednisolone acetate) following the observation of a normal estrous cycle. The treatment depressed corticoid concentrations without appreciatively altering plasma progestins from their normal cyclic levels. After the initial depression and following estrus, plasma corticoid levels increased at near the same rate as the cyclic increase in plasma progestins showing a positive relationship (p<.01). This indicated that adrenal corticoid secretion was influenced by the plasma progestin concentration.

Predef treatment beginning on the day of estrus tended to lower plasma progestin levels for that cycle.

Heifers treated with Predef did not show psychological signs of estrus.
CHAPTER 1

INTRODUCTION

The cattle industry throughout much of the world suffers from poor reproductive performance due to certain physiological stresses, such as high environmental temperature and poor nutrition. Undoubtedly the adrenals are involved in the stress. But the relationship between the adrenals and reproduction in bovine remains to be determined.

There is evidence that the adrenal-ovary relationship may be involved in regulating reproduction. The hormones secreted by both the adrenals and ovaries are steroids. Both glands are under the tropic control of hormones from the anterior pituitary which in turn is regulated by hypothalamic releasing factors. Research has indicated that adrenal secretions may have feedback control over the release of follicle stimulating hormone (FSH) (43). However, the adrenals appear to be more directly involved in reproduction through the secretion of gonadal steroid hormones, progesterone (14, 20, 33, 38), and estrone (11). The involvement of the adrenals in reproduction does not appear to be a simple one.

This study was undertaken to learn more of adrenal relationship to reproduction. Plasma glucocorticoid and progestin levels were measured and correlated with estrual activity and reproductive performance in Holstein heifers. In addition, adrenal activity was
suppressed by the use of Predef® (9-fluoroprednisolone acetate) to observe its effect on ovarian activity and the estrous cycle.
CHAPTER 2

LITERATURE REVIEW

Both adrenocorticotropic hormone (ACTH) and glucocorticoids (Corticoids) have been shown to affect pituitary gonadotropin release. Selye (31) found an increased secretion of ACTH was accompanied by decreased secretion of gonadotropins in rats. Likewise, Christian, Lloyd, and Davis (4) found that luteinizing hormone (LH) secretion in female mice was inhibited by ACTH. They suggested this was due to increased androgen output by the adrenal which inhibits gonadotropin release.

Hagino, Watanabe, and Goldzieher (13) demonstrated that ACTH is able to block pregnant mare serum-induced ovulation in immature female rats. The effect was shown to be exerted on the hypothalamus and required the presence of the adrenal glands. Observations on human ovarian activity led Selye (32) and Tepperman (41) to conclude that cortisol or its metabolites can inhibit pituitary gonadotropic secretion.

In heifers, Brunner, Donaldson, and Hansel (2) demonstrated an ACTH effect on ovarian activity which required the presence of the uterus. Injections of ACTH from day 2 through 8 of the estrous cycle into intact heifers decreased corpus luteum weight while ACTH injected into hysterectomized heifers had no effect.

Liptrap (18) has shown ACTH and corticoid injections caused changes in estrus, ovulation, and urinary excretion of estrogen in the
sow. He injected ACTH, cortisol, or prednisolone trimethylacetate into different animals daily from 24 hours before estrus or from day 14 of the estrous cycle until the end of estrus. The day 14 to estrus injections delayed the urinary estrogen peak, shortened estrus, and postponed estrus by 1 to 3 days. When injections were started 24 hours before the only noticeable effect was that of shortening estrus. It was also reported that ACTH, but not corticoid, administration interfered with ovulation by causing cystic follicle development on the ovaries. This may be due to the fact that ACTH administration raises and prolongs adrenal secretion while corticoid administration depresses adrenal function and is rapidly metabolized at the same time.

Van Rensburg (43), in describing his research with rabbits, suggested that corticoids may stimulate follicle stimulating hormone (FSH) release. This may explain the results observed following ACTH or corticoid injections in Liptrap's research (18). As mentioned earlier, Brunner et al. (2) observed decreased corpus luteum weight after ACTH injections. These results might be explained by Van Rensburg's suggestion:

1. ACTH would increase corticoid secretion;
2. corticoid secretion then increases FSH release;
3. FSH release raises estrogen output;
4. estrogen brings about luteal regression.

Although Van Rensburg's work (43) offers one explanation of the effect of ACTH on ovarian activity, other work by Selye (31, 32),
Christian et al. (4), and Tepperman (41) indicated that ACTH and corticoids inhibit gonadotropin release in some species.

The normal adrenal cortex may be capable of estrogen formation and secretion. Hopper and Tullner (15) and Engel (10) indicated this to be true in monkeys and humans. However, Barlow (1) reported that this does not appear to be the case in humans. Garverick et al. (11) found, in their study on bovine steroid levels during the estrous cycle, that urinary estrone was positively correlated with plasma corticosterone. Barlow (1) hypothesized that circulating dehydroepiandrosterone from the adrenal might be a significant precursor for estrogen biosynthesis by the ovary in the follicular phase.

Histological changes in the adrenal cortex are sometimes associated with changes in the reproductive system. It was demonstrated (6, 7) that certain reproductive conditions in the bovine, such as nymphomania, lead to a very noted change in adrenal histology. This may be due to malfunctioning of the adrenals, the ovaries, or the pituitary hypothalamic complex causing both the adrenal glands and the ovaries to behave abnormally.

Selinger, Blair, and Mossman (30), using the thirteen lines ground squirrel, found that adrenalectomy caused the hilum of the gonads to differentiate into adrenal-like tissue.

The adrenal gland may be capable of taking in plasma progesterone and converting it into corticoids. Research on the rat (40, 44, 46) demonstrated that plasma progesterone was converted into corticoids in this species. Especially conclusive evidence was given by Vecsei and
Kessler (44) when they reported that radioactive progesterone given intravenously in the rat was converted to corticosterone by the adrenal.

Progesterone is an important hormone in bovine estrous cycles and reproduction. Many researchers in bovine reproductive physiology (8, 9, 12, 17, 23, 36) have shown that plasma progesterone was lowest around estrus and usually highest in the late luteal phase of the estrous cycle after which there was a precipitous drop. Hafs and Armstrong (12) found that the progesterone levels were low in the corpus luteum on the day of ovulation and that it increased along with corpus luteum weight 3 to 6 days after ovulation.

Short (34) has shown that progesterone content in the follicle was far greater than in the blood. However, due to procedure difficulties, this progesterone may well have been contamination from luteal tissue. Rondell (27) emphasized the importance of progesterone for ovulation. Thus, the follicles, as well as any other progesterone source, may aid in ovulation.

Melampy et al. (19) found that high levels of progesterone inhibited estrus, while low levels facilitated estrus in ovarietomized cows pretreated with estradiol. Rothchild (28) suggested that progesterone conditioned the central nervous system mechanisms that control sexual receptivity in such a way that they can respond to estrogen. This may be the reason the first postpartum estrus is normally silent.

According to Hansel and Snook (14), progesterone dominates the events that occur during the estrous cycle since LH rises only after
progesterone has fallen. They also stated that plasma progesterone may be increased by stress which can interfere with reproductive functions. Stott (38) has shown that ovariectomized cows subjected to thermal stress showed an increased plasma progesterone secretion from the adrenals. It was also demonstrated (20) that thermal stress increased adrenal progesterone in heifers. Surgical stress also increased adrenal progesterone secretion levels in bovine (26).

Cupps, Laben, and Huff (5) found that delayed puberty, lowered fertility, slow growth, and response to supplementary cortisol was indicative of a deficiency in synthesis and secretion of cortisol and sex steroids. They suggested that subnormal production of 17α-hydroxyprogesterone led to a lack of cortisol and possibly sex steroids, since this metabolite was a regulating step in the synthesis of these hormones.

This research was undertaken to study the relationship between adrenal function and reproduction in bovine. With improved methods for measuring minute amounts of plasma steroids (16, 21), a specific interest has developed in relating corticoid secretion with ovarian function as determined by plasma progestin levels and ovarian structure change.
CHAPTER 3

EXPERIMENTAL PROCEDURE

Previous laboratory research has indicated a positive relationship between plasma corticoid and progestin levels in bovine. It was initially hypothesized that corticoid levels exerted some control over progestin levels. To test this hypothesis, Predef (9-fluoroprednisolone acetate), a synthetic corticoid produced by Upjohn, was given to heifers to inhibit ACTH release. It was assumed that a lowering of the plasma corticoid levels would lower plasma progestin levels if the initial hypothesis was true.

To test the effects of luteal stage on the results, six Holstein heifers were divided into three groups of two animals each. Each group was injected at a different luteal stage. The first group (heifers 206, 209) began injections on day 0 (estrus) of the estrous cycle. The second (903, 909) and third (906, 908) began injections 8 and 16 days post-estrus, respectively. The injections, 10 ml each (2 mg/ml), were given every other day for 10 days for a total of six injections. Before undergoing injections, each heifer had just completed one normal estrous cycle as determined by plasma progestin measurements, estrus observations, and periodic rectal palpations. Blood samples for hormonal analysis were taken in the late afternoon. Administration of Predef, when given, followed the samplings. After at least 15 days following the last injection, blood samples were taken every third day.
During the course of the experiment it was found that two heifers had no cyclic activity for that estrous period, unrelated to the Predef treatment. Thus it was necessary to repeat this part of the experiment. Two Holstein heifers (262, 263) were selected to complete this part of the experiment. Predef injection dosage for the two heifers was 20 ml instead of 10 ml (injection dosage usually cited in units/kg body weight).

Both dosage levels of Predef, 10 ml and 20 ml, were apparently over the threshold for inhibiting adrenal secretions. A preliminary test indicated that a 10 ml or 20 ml injection of Predef would give minimal adrenal cortical secretion for up to four days.

In another part of the experiment, five different Holstein heifers were tested using a modified injection schedule. Three heifers (250, 251, 257) were given two Predef injections one day apart. Heifer 250 began injections on day 16 post-estrus. Heifer 251 began injections on day 2. Heifer 257 began on day 3. The two remaining heifers (254, 258) received a total of three Predef injections, each being a day apart. Heifer 254 began Predef injections on day 8. Heifer 258 began on day 12. Each injection volume was 20 ml Predef for these five heifers. Blood samples were taken every third day.

An intravenous injection of 100 I.U. of ACTH was given two heifers (254, 258) following the last Predef injection. The ACTH injections were given to measure adrenal response and the possible effect of luteal phase on that response. Another ACTH injection was given to the two heifers in a later estrous cycle to determine if Predef had inhibited the previous adrenal response to ACTH injection.
Five to ten ml of blood were collected from each heifer in heparinized tubes by puncture of the middle coccygeal vein or the middle coccygeal artery in the tail. The blood was centrifuged and the plasma removed and frozen until used for corticoid and progestin analysis.

Modifications of the competitive protein binding method for corticoids by Murphy (21) and of the rapid progesterone method of Johannson, Neill, and Knobil (16) were used for bovine plasma analysis of corticoids and progestins. The modifications for the progestin procedure follows:

1. 40 mg Florosil was used instead of 80 mg;
2. dog serum (2.0 ml) was used instead of dog plasma (2.5 ml);
3. n-hexane (3 ml) was used for extraction two times without centrifuging in place of one extraction of petroleum ether (5 ml) after centrifuging;
4. 0.1 M phosphate buffer was employed in place of distilled water as the solvent for the corticoid binding globulin (CBG) and labeled corticosterone.

The corticoid procedure employed was similar to the progestin procedure except 0.05 ml of plasma was extracted twice with 0.5 ml of ethanol after centrifuging.

External controls were included in both the corticoid and progestin procedures. These measured the variation in the analysis from one series of samples to the next. To do this a large amount of pooled plasma was divided into many smaller amounts, each of which had
the same steroid level, to give a standard control for each series. Two different standardized controls were used for each series. One control contained high steroid levels, and the other contained low steroid levels.

Since it appeared that the plasma corticoid and progestin concentrations were related to each other, it was deemed necessary to determine if the procedure was responsible for the apparent observed interaction.

Willet and Erb (49) report in their research that hexane extracted $85\pm3\%$ of the progesterone and less than $2\%$ of the cortisol and corticosterone present. This indicated that only corticoid levels above $50$ ng/ml would be of major importance in interfering with progestin measurements. Therefore, it would appear the progestin procedure used in the present experiment was not noticeably affected by normal corticoid levels.

To test the corticoid procedure specificity, plasma samples, which previously were analyzed by the method, were extracted with n-hexane to rid the corticoid procedure of any progestin effect. The plasma was then extracted with ethanol for corticoids. No significant difference was found in the corticoid levels obtained by these two procedures. Thus, the corticoid procedure appeared unaffected by the progestin levels present. As previously mentioned, it was thought that plasma corticoid levels had some control over plasma progestin levels. Observations of the hormonal levels under Predef treatment and aneestrous conditions indicated the opposite, that progestin levels were a controlling factor of corticoid levels.
Regression analysis was used to measure the relationship between plasma levels of corticoids and progestins in the original six Holstein heifers. The regression analysis was broken down into four components (Table 2). The first component, A, consisted of all corticoid and progestin levels for each of four heifers (206, 209, 903, 909) before Predef injections and for each of two heifers (906, 908) before anestrus. The second component, B, consisted of the base plasma corticoid levels, which will be explained shortly, matched with the progestin levels for those days. The base levels used for each of the four heifers were those before injections and for each of the two heifers those before anestrus. Pooling the A's of all heifers gave the component C, which measures the normal relationship between plasma corticoid and progestin levels in this group of six heifers. Pooling the B's of all heifers gave the component D which measures the relationship between plasma corticoid and progestin base levels for the group.

The base levels were composed of corticoid levels which were lower than adjoining levels. For example, a base level day occurred where the corticoid level of that day is lower than the corticoid levels of either adjacent day. The progestin level of that day was matched with the corticoid level for regression analysis. This arbitrary method for base levels was chosen because it was quick and would eliminate most high corticoid levels due to excitation. Significant differences in corticoid and progestin levels from the estrous cycle with injections versus the pre-injected cycles were determined by analysis of variance.
CHAPTER 4

RESULTS AND DISCUSSION

Plasma Progestin Concentrations
of Normal Estrous Cycles

In Table 1, the mean maximum progestin concentration of the six original heifers for estrous cycles before experimental treatment (Z and Y cycles of Table 1) is 9.4 ng/ml, with a range of 5.9 to 12.7 ng/ml. These estrous cycles, which were considered normal, had the maximum progestin concentration occurring anywhere from day 9 to day 18 of the cycle. Of interest was a high level progestin peak which occurred in most estrous cycles just before the precipitous drop of progestins at the end of the luteal phase (Figures 1-6). This may be a "last gasp" effort of the corpus luteum. Whatever causes the demise of the corpus luteum may also be responsible for the last peak output of progestins.

Heifer 206 had the highest average maximum progestin concentration for the Z and Y estrous cycles (11.4 ng/ml). Heifer 909 had the lowest (7.12 ng/ml). Upon examination of the controls for both series of samples, it definitely appeared that the variation was due to a true difference among the heifers, and not to procedural error.

Research data is limited in measuring maximum plasma progestin levels of normal estrous cycles in heifers as was done in this research. The literature reports some work, however, in measuring maximum
Table 1. Plasma progestin and corticoid concentration means and ranges for estrous cycles of six Holstein heifers.

<table>
<thead>
<tr>
<th>Heifer</th>
<th>Cycle</th>
<th>Progestin Mean ng/ml</th>
<th>Progestin Range (Low - High) ng/ml</th>
<th>Corticoid Mean ng/ml</th>
<th>Corticoid Range (Low - High) ng/ml</th>
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<td>Z</td>
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<td>4.2 - 19.0</td>
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<tr>
<td></td>
<td>Y</td>
<td>6.4</td>
<td>0.9 - 12.7</td>
<td>10.3</td>
<td>4.1 - 18.3</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>4.2</td>
<td>0.4 - 9.6</td>
<td>3.2</td>
<td>0.3 - 11.0</td>
</tr>
<tr>
<td>209</td>
<td>Z</td>
<td>5.4</td>
<td>1.6 - 10.7</td>
<td>10.1</td>
<td>4.7 - 20.8</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>4.0</td>
<td>0.8 - 7.7</td>
<td>11.2</td>
<td>3.2 - 27.0</td>
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<td>X</td>
<td>3.5</td>
<td>0.0 - 8.5</td>
<td>3.8</td>
<td>1.5 - 16.0</td>
</tr>
<tr>
<td>909</td>
<td>Z</td>
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<td>1.4 - 5.9</td>
<td>9.0</td>
<td>5.4 - 15.8</td>
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<td>--------</td>
</tr>
<tr>
<td>908</td>
<td>Z</td>
<td>4.4</td>
<td>0.8 - 8.6</td>
<td>9.2</td>
<td>4.4 - 19.6</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>5.1</td>
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<td>12.5</td>
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</tr>
<tr>
<td></td>
<td>X</td>
<td>---</td>
<td>--------</td>
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</table>

| ALL 6 Total Z & Y | 4.9 | 1.1 - 9.4 | 9.7 | 3.7 - 21.2 |
| ALL 6 Total Z, Y & X | 4.8 | 1.0 - 9.4 | 8.3 | 2.9 - 20.3 |

Z = 1st pretreatment estrous cycle
Y = 2nd pretreatment estrous cycle
X = estrous cycle with Predef treatment
Figure 1. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 206.
Figure 2. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 209.
Figure 3. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 909.
Figure 4. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 903.
Figure 5. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 906.
Figure 6. Relationship of plasma corticoid and progestin concentrations during estrous cycles of heifer 908.
progesterone levels in cows by various methods. It is of interest and value to compare the progesterone concentrations in the present research with that of other data from bovine.

Dobrowalski, Stupnicka, and Domanski (8), using the 2,4-dinitrophenylhydrazone method, with blood taken from the bovine ovarian vein, obtained a maximum progesterone level of 18.0 ng/ml for either day 14 or 15 of the estrous cycle. Plotka et al. (22), using the double isotope derivative method for progesterone measurements, obtained a peak progesterone level of 25.8±3.9 ng/ml for day 14 of the bovine estrous cycle. The peak progesterone levels of these reports (8, 22) were higher than those found in this research and most other current reports, although the high concentrations found by Dobrowalski et al. (8) might be expected since the source of blood was the ovarian vein.

Pope, Gupta, and Munro (23), using gas-liquid chromatography (GLC), found the mean maximum progesterone level for 22 bovine estrous cycles (about 13 days after ovulation) to be 9.0 ng/mg. They also noted the most rapid fall of progesterone occurred four days before ovulation. Stabenfeldt, Ewing, and McDonald (36) found the mean progesterone level for six cows through 7 complete estrous cycles to be 6.6 ng/ml (6.1 to 10.2) at peak luteal phase by GLC. Stabenfeldt et al. (37) obtained similar results in a different experiment. Donaldson, Bassett, and Thorburn (9), using a protein binding method, found a mean progesterone concentration on day 14 of a 21 day cycle to be 6.8 ng/ml for eighteen cows with 11 complete estrous cycles and
parts of 15 estrous cycles measured. These peak progesterone values, especially those of Pope et al. (23), are similar to those obtained in this research.

Although peak progestin levels are usually reported in research, the mean progestin level for the entire cycle, where samples were taken each day of the cycle, gave a better picture of the daily secretion of progestin. In Table 1, the mean progestin levels for Z and Y estrous cycles for the six experimental heifers are illustrated. Heifer 909 had the lowest progestin mean (3.4 ng/ml), whereas heifer 206 had the highest mean (6.4 ng/ml). Though there were wide differences among the heifers, cycles within the same animal (Z and Y) were within a narrow range.

It can be seen that progestin concentrations at estrus were comparatively low (Figures 1-6). Since these concentrations seem unaffected by Predef treatment, it was decided to pool all the concentrations at estrus to obtain a mean. By doing this, 24 observed days of estrus in which blood samples were taken from the original six heifers can be used. The mean progestin level then was 1.3 ng/ml (.15 to 2.5). This agrees rather well with other reports (8, 9, 17, 23, 36, 37), but is much lower than the mean value of 10.1±1.1 ng/ml obtained by Plotka et al. (22).

Plasma Corticoid Concentrations of Normal Estrous Cycles

The mean corticoid level for days of estrus occurring before Predef injections was 12.0 ng/ml in the six experimental animals. The
mean daily corticoid concentration for the estrous cycles before injections was 9.7 ng/ml. This data agrees with Sprague et al. (35) in finding plasma corticoid levels higher at estrus. However, it should be noted that corticoid levels (Figures 1-4) near the time of estrus, excepting estrus were usually low. It may be that some physiological mechanism or possibly excitation at estrus raised the corticoid levels.

Venkataseshu and Estergreen (45), using isotope dilution and spectrophotometry, measured a cortisol value in Holstein cow blood of $9.3 \pm 2.4 \, \mu g/100 \, ml$ (93 ng/ml), which is about ten times greater than the levels obtained in the present research. Most other research has obtained corticoid levels comparable, although somewhat lower, to the levels in the present research. Saba (29) reported a mean corticoid level of 0.5 \, \mu g/100 \, ml (5 ng/ml) in bovine by using a soda fluorescence reaction on paper. Protein binding methods have yielded a mean cortisol level of $0.4 \pm 0.3 \, \mu g/100 \, ml$ (4 ng/ml) for twenty Holstein-Friesian cows that were pregnant and nonlactating (48), a mean corticoid level of 4.5 ng/ml for three nonlactating cows and 6.8 ng/ml for three milking cows (47), and a corticoid level which varied between 6.0 and 13.0 ng/ml for the bovine estrous cycle (3).

Of 11 estrous cycles before Predef injections in the present study, all but 4 had the highest corticoid level between day 12 and day 18 of the estrous cycle, ranging from 0 to 5 days before the end of the luteal phase. Of the four remaining high corticoid levels, two occurred at estrus. The peak corticoid levels during the latter part
of the luteal phase could indicate that heifers are more excitable at this time or that some physiological mechanism tends to bring about higher corticoid levels for this period. This mechanism may involve plasma progestin concentrations influencing adrenal corticoid secretion. Regression analysis (Table 2) indicated this is of some importance.

**Corticoid and Progestin Relationships during Normal Estrous Cycles**

The components involved in the regression analysis in Table 2 have been explained in the experimental procedure. For each component a regression \((R^2)\) has been determined. The \(R^2\) is a measure of influence, which can vary from 0 (no influence) to 1 (complete influence) that plasma progestin levels have on plasma corticoid levels.

Component A consists of all the plasma corticoid and progestin concentrations for each heifer prior to Predef treatment. Three heifers were not significant in the \(R^2\) values for A, two were significant \((p \leq .05)\), and one was highly significant \((p \leq .01)\). The \(R^2\) range varied from .0061 to .1864.

Pooling the A components of the six heifers (C) gave a highly significant \(R^2\) value of .0515. This value indicated that 5.15% of the variation in corticoid concentration is due to influence of progestin, with a probability of less than 1% of having no influence.

It appeared possible that varying degrees of progestin control of plasma corticoid concentrations might occur from heifer to heifer. It was also possible that some of the difference in \(R^2\) values for each A is due to differential excitability among the heifers causing increased corticoid concentrations.
Table 2. Summary of regression analysis for corticoids and progestins on Holstein heifers.

<table>
<thead>
<tr>
<th>Heifer</th>
<th>Components</th>
<th>$R^2$</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>206</td>
<td>A</td>
<td>.0474</td>
<td>.247</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.5312**</td>
<td>.443</td>
</tr>
<tr>
<td>209</td>
<td>A</td>
<td>.0061</td>
<td>-.141</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.4077*</td>
<td>.429</td>
</tr>
<tr>
<td>909</td>
<td>A</td>
<td>.0565</td>
<td>-.451</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.2548</td>
<td>.488</td>
</tr>
<tr>
<td>903</td>
<td>A</td>
<td>.0931*</td>
<td>.592</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.1644</td>
<td>.666</td>
</tr>
<tr>
<td>906</td>
<td>A</td>
<td>.1864**</td>
<td>.590</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.1737</td>
<td>.393</td>
</tr>
<tr>
<td>908</td>
<td>A</td>
<td>.0989*</td>
<td>.643</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>.2659*</td>
<td>.467</td>
</tr>
<tr>
<td>ALL 6</td>
<td>C</td>
<td>.0515**</td>
<td>.376</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>.2396**</td>
<td>.460</td>
</tr>
</tbody>
</table>

Components A, B, C, and D found in Experimental Procedure.

$R^2 = \text{coefficient of determination (effect of progestins on corticoids based on maximum effect of 1.0000)}$

* = $p<.05$

** = $p<.01$

b = regression coefficient (change of corticoids with each progestin rise of 1.000)
In Table 2, it can be seen that the regression coefficient \( b \) for A is positive in five of the six heifers. The \( b \) indicates whether the relationship between corticoids and progestins is negative or positive. The one heifer having the negative \( b \) also had the lowest \( R^2 \) indicating that there was almost a zero relationship between plasma corticoids and progestins. Therefore, whether the \( b \) was negative or positive was of little consequence.

To accomplish the objective of determining if the plasma progestin concentrations might influence the level of plasma corticoids, it became evident that high corticoid concentrations due to excitation had to be eliminated before a regression analysis would facilitate the interpretation of the data. One way to accomplish this would be to arbitrarily set a certain corticoid concentration above which all corticoid values would be eliminated from use in the regression analysis. This method for choosing corticoid values seemed too subjective. An alternate method, which was less subjective, was selected. It was decided to use daily corticoid concentrations which were lower than the concentrations of the preceding or the following day. This method termed the base level method, would eliminate many of the corticoid values due to acute excitation from the regression analysis and hopefully include mainly those that would represent base levels of normal secretion. The chosen plasma corticoid concentrations were matched with the plasma progestin concentrations of the same day and used in computing the regression analysis.
The $R^2$ for the base level of each heifer (B) (Table 2) was higher (.1644 to .5312) than each corresponding A in all heifers but one (906). Even though this was the case, the number of heifers having significant $R^2$ in B remained the same as in A. This was due to fewer values of B than A for analysis. There were three heifers not significant in $R^2$ for B, two significant, and one highly significant. It is interesting to note that only one heifer (908) had a significant $R^2$ for both A and B. Heifer 909 did not have a significant $R^2$ for either A or B. In all cases for B, there is a positive b (Table 2).

By pooling B for all heifers (D), a highly significant $R^2$ of .2396 results. This is about four times the $R^2$ of C, which also is highly significant. Component D is probably a more reliable indicator of adrenal corticoid secretion in the normal heifer than C.

There are a few facts about the base level method that should be noted. The first is that some of the corticoid values eliminated by using the base level method would be normal corticoid levels, not levels due to excitation. Another point is that the base level method is biased toward the lower corticoid values. Considering these facts, it is evident that the $R^2$ for B calculated a lower relationship than actually exists.

From the evidence just presented, it is hypothesized that the bovine adrenal takes up plasma progesterin and converts it to corticoids. However, it should be noted that Garverick et al. (11) could show no statistical relationship between either cortisol and corticosterone and progesterone in an experimental group that included both heifers and cows.
Observations supporting the above hypothesis come from research in rats. Telegdy et al. (40) found that corticosterone secretion in the rat can be increased significantly following the administration of large doses of progesterone. Volk (46) has noted that rats treated with progesterone have histological changes in the adrenal cortex similar to those known to occur when the adrenal is hyperactive. The most convincing evidence for the hypothesis comes from the research of Vecsei and Kessler (44), which has shown that radioactive progesterone given intravenously in the rat is converted to corticosterone and aldosterone by the adrenal.

**Corticoid and Progestin Relationships during Anestrus**

Corticoid and progestin concentrations during anestrus, as well as those under Predef treatment, have been studied by regression analysis, but are not included in Table 2. It was felt relating corticoid and progestin concentrations under these conditions and by this procedure was not valid because of the following:

Plasma progestin concentrations become stable and constant during anestrus (Figures 5 and 6). This is similar to plasma corticoid concentrations becoming stable and constant under Predef treatment (Figures 1-6). Slight fluctuations which may only be due to procedural error, have a significant effect on the regression in these cases.

As can be seen in Figures 5 and 6, a positive relationship appears to exist between plasma corticoids and progestins during anestrus
for the two heifers (906, 908). In heifer 906, the mean plasma corticoid concentration during anestrus (7.0 ng/ml) was somewhat lower than plasma corticoid concentrations of previous estrous cycles noted in Table 1. It would appear that the fall in plasma progestin concentrations in this heifer influenced the lowering of plasma corticoids immediately, although not dramatically.

In heifer 908, a mean corticoid level of 12.5 ng/ml is found during the anestrous phase before Predef injections. The same level occurred in the previous normal estrous cycle (12.5 ng/ml). Two points should be noted, the animal was very excitable and quite importantly the mean corticoid level for the last 5 of the 17 anestrous days was only 5.9 ng/ml. As can be seen in Figure 6, the drop in corticoid levels for this part of anestrus was very great.

It would have been interesting to have followed these naturally lowered corticoid levels through anestrus. However, the Predef injections were initiated without knowing the altered cycle had occurred.

**Predef Effects on Plasma Corticoid and Progestin Concentrations**

The corticoid concentrations for each heifer were dramatically decreased by Predef administration (Tables 1, 3, and Figures 1-6). This probably works through blockage of pituitary secretion of ACTH, thus blocking corticoid output by the adrenal. A similar reaction would be expected by treatment with any corticoid synthetic or natural.
Table 3. The effect of Predef treatment on plasma progestin and corticoid concentrations.

<table>
<thead>
<tr>
<th>Heifer</th>
<th>Hormone</th>
<th>Estrous Cycle Comparison</th>
<th>Days of Cycle Compared</th>
</tr>
</thead>
<tbody>
<tr>
<td>206</td>
<td>P</td>
<td>X vs $\frac{Y+Z}{2}$ **</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>206</td>
<td>P</td>
<td>Y vs Z</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>206</td>
<td>C</td>
<td>X vs $\frac{Y+Z}{2}$ **</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>206</td>
<td>C</td>
<td>Y vs Z *</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>209</td>
<td>P</td>
<td>X vs $\frac{Y+Z}{2}$ **</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>209</td>
<td>P</td>
<td>Y vs Z **</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>209</td>
<td>C</td>
<td>X vs $\frac{Y+Z}{2}$ **</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>209</td>
<td>C</td>
<td>Y vs Z</td>
<td>1-15, -3, -2, -1</td>
</tr>
<tr>
<td>909</td>
<td>P</td>
<td>X vs $\frac{Y+Z}{2}$</td>
<td>9-14, -3, -2, -1</td>
</tr>
<tr>
<td>909</td>
<td>P</td>
<td>Y vs Z</td>
<td>9-14, -3, -2, -1</td>
</tr>
<tr>
<td>909</td>
<td>C</td>
<td>X vs $\frac{Y+Z}{2}$ **</td>
<td>9-14, -3, -2, -1</td>
</tr>
<tr>
<td>909</td>
<td>C</td>
<td>Y vs Z</td>
<td>9-14, -3, -2, -1</td>
</tr>
<tr>
<td>903</td>
<td>P</td>
<td>X vs Y</td>
<td>9-15, -3, -2, -1</td>
</tr>
<tr>
<td>903</td>
<td>C</td>
<td>X vs Y **</td>
<td>9-15, -3, -2, -1</td>
</tr>
</tbody>
</table>

Z = 1st pretreatment estrous cycle  
Y = 2nd pretreatment estrous cycle  
X = estrous cycle with Predef treatment  
* = p .05  
** = p .01  
P = progestins  
C = corticoids  
-3, -2, -1 = the 3 days before onset of estrus
Four heifers of the original six had estrous cycles under Predef treatment (the x estrous cycles of Table 1). The other two entered into a state of anestrus a short while before Predef treatment. Considering only the estrous cycles of the four heifers at the time of Predef administration, a mean maximum progestin level of 9.3 ng/ml (7.7 to 11.4) was found which is almost identical with the 9.4 ng/ml mentioned previously for all Z and Y (normal) estrous cycles. It seems apparent that Predef lowering of plasma corticoid does not affect maximum progestin levels of the estrous cycles. However, in the two heifers (206, 209) beginning Predef treatment at estrus, progestin concentrations were significantly reduced throughout the treated cycle (Table 3).

Interestingly, in each heifer of the original six, the estrous cycle following Predef administrations had a concomitant rise of corticoids with progestins. The corticoids increased at the same rate as progestins from the beginning of the luteal phase until the progestins had reached the peak of the luteal phase (Figures 1-6). This appears to be more than a coincidental recovery of corticoid levels from Predef injections. It suggests that the plasma progestin concentrations are influencing the secretion of corticoids into the blood during adrenal recovery from Predef treatment.

In heifer 209 (Figure 2), there is a somewhat higher corticoid level at the estrus following Predef injections. This indicates that the adrenal does have some ability to respond before the progestin rise. However, it should be noted that corticoid levels do tend to be higher at estrus as mentioned before. It may be that the release of
gonadotropins at estrus is accompanied by pituitary ACTH secretion. Stott and Robinson (39) have suggested plasma corticoids may be indicators of gonadotropin secretion in bovine.

**Lowered Corticoid Effects on Estrus**

Thomas (42) has noted that sub-maintenance rations will reduce corticoid concentrations and signs of estrus. Therefore, it was thought that a lowering of corticoid levels through Predef injections might similarly be accompanied by diminished estrual signs. This indeed was the case. In not one of the six original heifers was riding or mounting observed during any part of the first follicular phase following Predef injections. Mucous was only slight or not at all. The approximate time of ovulation was determined from the previous estrus and by familiarity with the characteristic action of the six heifers. Diminished estrual signs are usually associated with lowered estrogen secretion.

In the second experiment, three of the seven heifers had no observed estrus for the cycle following injections. The two heifers (254, 258) which had a total of three Predef injections, both failed to show estrus, as did the heifer (250) which had a total of two Predef injections, beginning treatment on day 16. Normal cycles were observed following the quiet estrus in each of the three heifers.

**Response to ACTH Administration**

To determine if Predef had any direct inhibitory effect on adrenal secretion, 100 I.U. of ACTH was injected intravenously into two
heifers (254, 258) the day following the last Predef injection. During a later estrous cycle, 100 I.U. of ACTH was again given to test normal adrenal response. Administration of ACTH was on the same day of the estrous cycle in both the Predef treatment cycle and the normal cycle to control any possible ovarian effect on adrenal response.

Heifer 254 was administered ACTH on day 13 of the Predef treated cycle. The corticoid levels were raised from 4.3 ng/ml to 21.6 ng/ml 1/2 hour after administration and to 51.6 ng/ml after 1 hour. The next ACTH injection was given three estrous cycles later on the same day of the estrous cycle. The corticoid levels increased from 6.7 ng/ml to more than 80.0 ng/ml for both 1/2 hour and 1 hour post-injection periods. The progestin level for the second ACTH injection increased in this heifer from 2.5 ng/ml to 5.5 ng/ml after 1 hour. Part of the progestin increase may be due to a high plasma corticoid level interference. If 100 ng/ml of corticoids was present, up to 2 ng/ml of this amount could be present due to procedure in the progestin measurement (49). However, if the plasma progestin increase is real, it would seem most likely that the increase is due to adrenal progestin release. It has been shown (38) that the bovine adrenal is capable of secreting high levels of progesterone. Other explanations of the observed results include stimulation of ovarian progestin secretion by either ACTH or corticoids and increased pituitary LH release due to either ACTH or corticoids. In this heifer, the adrenal response was greater in the normal estrous cycle than the Predef treated cycle.
Heifer 258 was given ACTH injections two estrous cycles apart on day 17 of the estrous cycle each time. The first time ACTH was injected the corticoids increased from 5.0 ng/ml to 37.2 ng/ml after 1/2 hour, but then fell to 23.5 ng/ml after 1 hour. The second ACTH injection raised the corticoid levels from 9.4 ng/ml to 41.1 ng/ml after 1/2 hour, which then fell to 26.1 ng/ml after 1 hour. The adrenal response remained the same for both the Predef treated and normal cycles in this heifer. There was no plasma progestin increase observed after either administration.

It should be noted that the responses of these two heifers were quite different to ACTH administration. This could be due to either physiological response difference between the two heifers or effect of luteal stage on adrenal response. It is hypothesized that the latter is of importance, since plasma progestin may be used in "priming" the pathways for synthesizing adrenal corticoids; thus, the importance of luteal stage. However, it is evident that further research in this area needs to be undertaken.

Ovarian Changes

Rectal palpation of ovarian anatomy is not always in agreement with the observed progestin picture. The discrepancies in the six original heifers reported below involved only abnormal cycling. In heifer 903 a large corpus luteum was found 13 days after the first estrus noted in Figure 4. By the progestin picture shown, there was uncertainty as to whether the heifer was really cycling. In heifer 906 (Figure 5), a large corpus luteum was found on the right ovary during
the middle of the anestrous period. In heifer 908 (Figure 6), a
follicle on the right ovary, which burst when palpated, was found near
the middle of the anestrous period. Apparently, ovarian anatomy might
continue to go through cyclical changes even though plasma progestin
concentrations indicate ovarian inactivity. Aside from these three
discrepancies, the progestin picture agreed with the results found
from ovarian palpation in the six original heifers (Figures 1-6).

**Fertility in Predef Treated Heifers**

Four heifers (206, 209, 903, 906) of the original six were
bred as soon as estrus was thought to occur following the Predef treat-
ment. Due to the diminished signs of estrus after Predef treatment,
it was questionable as to whether the breeding of these heifers was
done at the proper time. Only the two heifers beginning Predef injec-
tions on day 0 became pregnant on the first breeding. The other heifers
took two to three breedings before conceiving.

The diminished signs of estrus included relatively little mucus
and lack of swollen vulva. These two signs are both associated with
the amount of estrogen output. Apparently the estrogen output was
decreased on the day of estrus by Predef treatment. Some reports
(24, 25) have emphasized that an estrogen peak on day 0 of the estrous
cycle is important for conception. It is possible chronic stress,
which is somewhat analogous to Predef treatment, may modify estrogen
secretion causing lower conception rates.
The following can be concluded:

1. Corticoid concentrations tend to be greatest in the late luteal phase and at estrus.

2. Plasma progestin levels have a positive effect on plasma corticoid levels.

3. Predef injections begun at estrus can significantly reduce mean plasma progestin levels for that estrous cycle.

4. Predef injections greatly diminish the signs and behavior associated with estrus, probably through affecting estrogen secretion.

5. Corticoid secretion by the adrenal in response to ACTH may be affected by the day of the estrous cycle in which ACTH is given.


