HORMONAL RESPONSES OF DAIRY COWS WITH OVARIAN CYSTS TO GnRH

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SUMMARY

Twenty-four cows, diagnosed via rectal palpation as having ovarian cysts were randomly assigned to one of four groups to receive a single IM injection of either 0, 50, 100 or 250 μg of synthetic gonadotropin-releasing hormone (GnRH). Sixteen lactating dairy cows exhibiting clinically normal estrous cycles were used as controls.

Of the 18 cows receiving 50, 100 or 250 μg GnRH, 13 (72%) responded to treatment and returned to estrus 20.1 ± 1.5 (range 9 to 27) days after treatment as compared to one of six receiving 0 μg GnRH. Clinical changes in most cases were evidenced by increasing firmness of the cystic structure(s) which subsequently had palpable characteristics similar to corpora lutea.

Plasma LH increased significantly (P<.005) on day 0 in cows following injection of GnRH.

INTRODUCTION

Synthetic gonadotropin-releasing hormone (GnRH) is a hypothalamic releasing hormone that acts on the anterior pituitary to initiate a release of pituitary gonadotropins. This release has been reported in pigs (Chakraborty et al., 1973), sheep (Reeves et al., 1971) and in cattle (Kittok et al., 1973; Kaltenbach et al., 1974).

Kittok et al. (1973) induced an LH release with three 100 μg intravenous injections of GnRH into each of five cows with ovarian cysts. All five cows returned to estrus within 24 days following treatment. In a companion paper to this study involving 114 clinical cases of cows with ovarian cysts (Bierschwal et al., 1975), 64 of 86 (74.4%) cows treated with a single intramuscular (IM) injection of either 50, 100 or 250 μg GnRH...
returned to cyclic ovarian activity following treatment. Six of 28 (21%) cows given 0 µg GnRH returned to cyclic ovarian activity.

The objectives of this study were to observe hormonal and clinical responses in cows with ovarian cysts to a single IM injection of either 0, 50, 100 or 250 µg GnRH and to compare hormonal responses between cows responding and not responding to treatment.

**MATERIALS AND METHODS**

Twenty-four dairy cows, 13 Holstein and 11 Guernsey, diagnosed as having ovarian cysts via rectal palpation, and 16 lactating dairy cows (eight Holstein, eight Guernsey) exhibiting clinically normal estrous cycles were used in this study. Clinical diagnosis of ovarian cysts was based upon the finding of a single or multiple formation of smooth, fluctuant, rounded structures of 2.5 cm in diameter or larger on one or both ovaries (Bierschwal et al., 1975). Diagnosis was made during the regular biweekly herd reproductive health examination. All cows were from the University of Missouri-Columbia dairy herds and were 45 to 210 days postpartum when diagnosis was made. During the experimental period all treatment and control cows were housed and fed with the regular milking herd.

At 0700 hr on the day following diagnosis, cows with ovarian cysts were randomly assigned within breed to one of four groups (six cows/group) to receive an intramuscular injection of either 50, 100 or 250 µg of GnRH or a sham injection (0 µg) of the carrier vehicle (sterile H₂O with an alcohol preservative). All groups contained three cows of each breed except for the 0 µg group which contained four Holsteins and two Guernseys. Blood for hormone analysis was taken prior to GnRH injection (time 0) and at .5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 hr after injection (day 0) and on days 1, 5, 9 and 13 post-injection. If the treated cow returned to estrus within 30 days after treatment with GnRH, blood was drawn on the day of estrus (day 0) and on days 1, 5, 9 and 13 post-estrus. Control cows were bled at estrus and on days 1, 5, 9 and 13 post-estrus.

All blood samples collected, except those taken on the day of GnRH administration, were accompanied by rectal palpation to monitor ovarian changes. Blood for hormone analysis was taken via jugular venipuncture and chilled in ice water until centrifugation at 10,000 g for 10 min at 4 C. The plasma was stored at -20 C until assayed.

Clinical response to treatment was recorded as positive if the animal established a normal estrous cycle or conceived. This response was chosen since some cows conceived at the first estrus following treatment. A negative response was recorded if no change in character, size or location of the cystic structure could be determined via rectal palpation within a 2 to 4 week period (Bierschwal et al., 1975).

**Assay of Hormones.** LH was assayed by double antibody radioimmunoassay as reported by Niswender et al. (1969). Samples were assayed in duplicate in one sample run. Plasma estradiol was assayed as described by Abraham (1969) as modified by Mather et al. (1974) with the exception that the estradiol antiserum used was RDR-8-E. Specificity of this antibody has been previously described (Stellflug et al., 1973; Monk et al., 1975).

Plasma progesterone was assayed using RIA procedures similar to those reported by Thornycroft and Stone (1972). Plasma samples (.2 ml) were extracted in duplicate with 3 ml of anesthesia grade diethyl ether by shaking the mixture for 5 min on a mechanical shaker. The aqueous layer was frozen and the ether layer was decanted into culture tubes. Samples from each cow from each treatment group and one or two cows from each breed of control cows were included in each assay run.

Percent progesterone recovered by ether extraction was determined for each assay by extracting in triplicate 200 µl plasma aliquots containing tracer progesterone. Radioactivity in these aliquots was compared to three 200 µl unextracted aliquots. Unknowns were adjusted to account for procedural loss.

Crystalline progesterone, dissolved in absolute ethanol, was pipetted into culture tubes in amounts of .025, .05, .10, .25, .50, .75, 1.00, 1.25, 1.50 and 2.00 nanograms. Solvents containing standards and extracts of plasma were evaporated to dryness prior to assay.

Two hundred microliters of a 1:1000 dilution of progesterone antisera (RDR-9-P) in the assay buffer (Thornycroft and Stone, 1972) were added to each tube, mixed and allowed to incubate at room temperature. After incubation for .5 hr, 30,000 dpm of 1, 2, 6, 7-³H progesterone in the assay buffer was added to each tube, mixed and the contents incubated at 4 C for 2.5 hours. Following incubation, 1.0 ml of .5% neutral Norit-.125% Dextran solution
(Fisher Scientific Company) in the assay buffer was added to all tubes. The contents were mixed, incubated for 10 min in an ice bath, and then centrifuged at 800 g for 10 min at 4 C. After centrifugation, the supernatant was decanted into scintillation vials containing 10 ml of Beckman Ready-Solve VI scintillation liquid. Radioactivity was measured in a liquid scintillation spectrometer (Beckman LS-335).

The cross reactivity of the progesterone antisera was found to be less than .1% for estradiol-17β and estrone, 10% for testosterone, 1% for cortisol and 10% for corticosterone. To further validate the assay, 54 samples and three pooled samples were extracted as previously described and subjected to column chromatography on Sephadex LH-20 columns (Monk et al., 1975) to separate steroids. Progesterone was eluted in the .5 to 1 ml fraction. The chromatographed samples were then assayed for progesterone and compared to nonchromatographed values previously determined. Mean plasma progesterone in chromatographed vs nonchromatographed samples was 1.11 ± .03 and 1.09 ± .02 ng/ml (r = .93), respectively. The correlation coefficient of the comparison between chromatographed and nonchromatographed progesterone in the pooled plasma was .99.

Differences in physiological responses and hormone parameters were analyzed by analysis of variance.

RESULTS

Clinical Changes

For cows given 50, 100 or 250 µg GnRH, 4/6 (67%), 5/6 (83%) and 4/6 (67%), respectively, returned to clinically normal cyclic ovarian activity in contrast to 1/6 (16%) of the cows given 0 micrograms. Clinical response to treatment was not different between cows given 50, 100 or 250 µg GnRH, but all of these groups were significantly different (P<.01) from the 0 µg group.

For cows that responded to GnRH treatment, the time period to the subsequent estrus was 20.1 ± 1.5 days (range 9 to 27). With the exception of one cow returning to estrus in 9 days and another ovulating (with no estrus observed) in 13 days, all cows returned to estrus or ovulated in 15 to 27 days. Mean services per conception was 1.8 ± .2 with six cows conceiving to the first breeding. Eleven of the 13 cows that responded to treatment became pregnant following treatment.

Definite palpable ovarian changes were detectable by either day 5 or 9 post-injection in the majority of cows which responded positively. In most instances these changes were a definite increase in firmness of the cystic structure(s) which subsequently had palpable characteristics similar to corpora lutea. Three other types of ovarian changes were noted: (1) a great reduction in the size of the ovary on the day following GnRH injection. It is probable that this was due to spontaneous rupture of the cyst due to injection of GnRH (two cows); (2) a gradual reduction of size of the cyst followed by follicular growth and ovulation between days 10 and 13 (two cows). Plasma progesterone was below .5 ng/ml prior to and subsequent to treatment until the subsequent estrus; and (3) development of corpora lutea on the ovary opposite the cystic structure indicating possible induced follicular growth and ovulation. Palpable changes, except for some reduction in size of the cystic structure(s) in a few cows, were not detected in cows which responded negatively during the trial period.

Hormonal Changes

LH. For the time period, 0 to 8 hr following injection of GnRH, plasma LH increased significantly (P<.005) in cows given 50, 100 or 250 µg GnRH, but did not change significantly for cows receiving 0 µg (figure 1). Differences in LH response among the 50, 100 or 250 µg

![Figure 1. Plasma LH concentrations in cows with ovarian cysts following treatment (0 to 8 hr) with GnRH.](image-url)
GnRH groups of cows were not significant; however, response of cows in these groups was greater (P<.005) than cows given 0 μg GnRH. Mean peak concentrations for LH for cows receiving 50, 100 and 250 μg GnRH were 9.6 ± 3.4 ng/ml, 12.5 ± 5.1 ng/ml and 21.0 ± 6.5 ng/ml, respectively, and occurred 1.5 to 2.5 hr post-injection.

There was no pattern of differentiating cows that clinically responded to treatment (7.9 ± 2.1 ng/ml) as compared to those that did not (5.6 ± 1.6 ng/ml) on the basis of LH response during the time, 0 to 8 hr post-injection (P>.05). Of the five cows given 50, 100 or 250 μg GnRH that did not respond to treatment, two had the lowest LH response, two had a LH response near the mean and one had the highest LH response for their respective groups.

For the time period, days 1 to 13 post-injection, differences in plasma LH among treatment groups were not significant. Therefore, the groups given 50, 100 or 250 μg GnRH were combined and differences between cows responding to treatment and those which did not were compared (table 1). In this comparison and in all subsequent comparisons where differences in hormonal concentrations in cows responding vs not responding to treatment are made, cows given 0 μg GnRH were not included in the analysis. However, hormonal changes following the sham treatment are included in tables 1 through 3. Plasma LH concentrations on days 1 to 13 post-injection for cows responding positively to treatment were lower as compared to cows not responding to treatment (P<.01). Differences among days were not significant.

For comparisons of plasma LH involving subsequent estrous cycles of treated cows or estrous cycles of control cows, the day of estrus was omitted from the analysis since the LH surge was missed in some cases due to once a day sampling. During days 1 to 13 post-estrus, plasma LH concentrations in cows that responded to treatment were not different from controls (table 1). Similarly, differences in plasma LH on days 1 to 13 post-injection, were not significant in cows responding to treatment when compared to LH concentrations in either treated or control cows on days 1 to 13 of the post-estrus period.

**Progesterone.** Prior to injection of GnRH or the carrier vehicle, plasma concentrations of progesterone varied widely from less than .1...
ng/ml to 4.0 ng/ml in cows with ovarian cysts. Eighteen of the 24 cows had progesterone concentrations of less than .8 ng/ml; concentrations in the remaining six ranged from 1.4 to 4.0 ng/ml. During the time period, 0 to 8 hr post-injection, progesterone concentrations did not change across time, but differences among treatment groups were significant (P<.01). Progesterone concentrations on day 0 in cows receiving 50 µg GnRH (X = .41 ± .07 ng/ml) were lower (P<.01) when compared to mean concentrations of 1.29 ± 28, 1.10 ± .19 and .89 ± .17 ng/ml for cows receiving 0, 100 or 250 µg GnRH, respectively. Since progesterone concentrations did not change across time, the differences were probably due to chance assignment of cows. Differences between the 50 µg group as compared to the others was due largely to one or two cows in each of the other groups which has progesterone concentrations above 1.4 ng/ml on the day of injection.

Prior and subsequent to injection of GnRH on day 0, plasma progesterone concentrations (.90 ± .35 ng/ml) in cows responding to treatment were higher (P<.01) as compared to cows not responding to treatment (.33 ± .07; table 2). Three cows responding to treatment had pre-injection progesterone concentrations of between 1.4 and 4.0 ng/ml. The remaining 10 cows responding to treatment and the five cows not responding to treatment had pre-injection progesterone concentrations of less than .8 ng/ml. Differences in plasma progesterone for the time period, days 1 to 13 post-injection, were not significant among treatments or across times. Therefore, the groups were combined and differences between cows responding or not responding to treatment were compared (table 2). Plasma progesterone in cows responding to treatment (1.44 ± .19 ng/ml) was higher (P<.01) as compared to that of cows not responding to treatment (.85 ± .19 ng/ml). Progesterone concentrations increased in cows responding to treatment (P<.01), but not in cows failing to respond to treatment.

Progesterone concentrations at estrus and on days 1, 5, 9 and 13 post-estrus in controls and in cows responding to treatment were not different, but differences among days were highly significant (P<.005; table 2). Similarly, plasma progesterone concentrations in controls and treated cows post-estrus as compared to days 1 to 13 post-injection in cows responding to treatment were not different.

**Estradiol.** Mean estradiol concentrations...
prior to treatment (26.5 ± 4.8 pg/ml) and for
the time period 0 to 8 hr post-injection were
not different among treatment groups. Similarly,
estriadiol concentrations were not different
across time. Therefore, the groups were
combined and differences between cows re-
sponding to treatment and those that did not
were compared (table 3). Mean estradiol con-
centrations in cows responding to treatment
were higher (P<.01) than those not responding
to treatment both prior to injection and on day
0.

There was considerable variation in plasma
estriadiol concentrations on the day of GnRH
administration. Nine cows had mean striadiol
concentrations less than 11 pg/ml while the
remaining 15 cows had striadiol concentrations
between 27 and 65 pg/ml. Of the nine cows
with mean striadiol concentrations of less than
11 pg/ml on day 0, five were given 50, 100 or
250 μg GnRH; the remaining four were given 0
micrograms. Of the five given GnRH, three
responded to treatment. Ten of 13 cows with
striadiol concentrations on day 0 exceeding 27
pg/ml responded to treatment. Also of interest
was the rapid decline of estradiol concentra-
tions from an average of 54.8 pg/ml on day 0 to
less than 11 pg/ml on day 1 post-treatment in
three cows responding to treatment. Only two
of 24 cows in this study could be classified as
nymphomaniac (Bierschwal et al., 1975). Pre-
injection plasma estradiol concentrations in
these cows were 42 and 45 pg/ml.

Mean plasma estradiol concentrations were
not different among treatments or days for the
time period, days 1 to 13 post-injection. Simi-
larly, there was no difference in cows which
responded to treatment as compared to those
not responding (table 3).

In cows responding to treatment, plasma
estradiol concentrations were higher (P<.005)
on days 1 to 13 post-injection (22.0 ± 3.1
pg/ml) as compared to days 1 to 13 following
the subsequent estrus (9.1 ± 1.4 pg/ml) or in
controls (9.1 ± 0.6 pg/ml) on days 1 to 13 of
the estrous cycle. Differences on days 1 to 13
post-estrus in cows responding to treatment as
compared to controls were not significant.

Discussion

A single IM injection of 50, 100 or 250 μg
GnRH in cows with ovarian cysts resulted in a
release of LH and a return to normal cyclic
ovarian activity in most cases. Thirteen of 18

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<th>Day</th>
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<tr>
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<td>7.8 ± 2.2</td>
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<td>9</td>
<td>9.2 ± 2.4</td>
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<td>Controls (estrus cycle)</td>
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<td>28.3 ± 5.7</td>
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*Days = days following treatment or estrus. Day 0 is the day of estrus or treatment.

b Cows with ovarian cysts were given a sham injection of the carrier vehicle for GnRH.

c Cows with ovarian cysts were given a single IM injection of either 50, 100 or 250 μg GnRH.
(72%) cows in this study receiving GnRH returned to estrus within 27 days following treatment. Similarly, Bierschwal et al. (1975) reported 64 of 86 (74%) cows receiving either 50, 100 or 250 μg GnRH responded to treatment and Kittok et al. (1973) reported that five of five cows treated with three intravenous injections of 100 μg GnRH at 2-hr intervals returned to estrus within 24 days.

Plasma LH concentrations were elevated within 30 min following injection of GnRH at each of the three dose levels used in this study and remained elevated for 4 hr, which agrees with another study (Kaltenbach et al., 1974) in which beef heifers were given 250 μg GnRH 36 hr following the removal of a progestogen implant. The length of time for elevation of LH reported in this study is similar to that reported by Schams and Karg (1969) for the pre-ovulatory surge of LH. However, others (Henricks et al., 1970; Snook et al., 1971; Swanson and Hafs, 1971) have reported LH concentrations during the pre-ovulatory LH surge to be elevated for at least 8 hours. LH response increased with increasing doses of GnRH; however, differences were not significant. Similar findings have been reported by Kaltenbach et al. (1974), using higher levels of GnRH, and by Zolman et al. (1974). Peak LH response in cows with ovarian cysts in this study occurred 1.5 to 2.5 hr post-treatment, which agrees with the findings of Kittok et al. (1973).

Plasma LH concentrations in cows responding to treatment on days 1 to 13 post-treatment were similar to those found during the subsequent estrous cycle or as compared to normal cycling cows (controls). However, LH concentrations were lower on days 1 to 13 post-treatment than in those cows not responding to treatment. These findings are understandable since plasma progesterone during this same period was higher in cows responding to treatment than in cows not responding. Garverick et al. (1971) reported a negative relationship between LH and progesterone during the luteal phase of the estrous cycle. Similarly, Tillson et al. (1970) reported negative correlations between LH and progesterone on days 0 to 12 post-estrus in nonpregnant and pregnant sows.

Plasma progesterone concentrations in cows responding to treatment increased significantly (P<.01) but not in cows not responding. The failure of progesterone production by some cows, presumably from the cystic structure, is not understood since plasma LH response in cows responding or not responding to treatment was not different. However, mean pre-injection plasma concentrations of progesterone and estradiol were higher in cows responding than in cows not responding to treatment. Increased steroidogenesis may indicate an increased sensitivity to LH at the cellular level.

Estradiol concentrations prior to treatment with GnRH were similar to estradiol concentrations during estrus in cycling cows and higher (P<.005) than concentrations observed during the luteal phase of the estrous cycle. This is in contrast to results of Kittok et al. (1973) who reported concentrations of plasma estradiol and estrone in cows with ovarian follicular cysts to be similar to concentrations in cows during the luteal phase of the estrous cycle. The results of our study, however, agree with an earlier report by Kittok et al. (1972), who observed elevated plasma estrogen concentrations in cows with cystic ovarian follicles.

Results of our study and those of Kittok et al. (1973) demonstrate that cows with ovarian cysts are able to release LH in response to GnRH. Palpable changes in cows responding to treatment in this study were usually detectable within 5 to 9 days following treatment with GnRH as evidenced by increased firmness of the cystic structure and increased plasma progesterone. The increased plasma progesterone following treatment may be due to luteinization of the cystic structure as a result of ovarian response to GnRH induced LH release.

LITERATURE CITED


