A comparison of adrenal gland function in lactating dairy cows with or without ovarian follicular cysts

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SUMMARY

Two experiments were conducted to determine if adrenal secretion of steroids differed between cows that formed ovarian follicular cysts and normal cycling cows. In experiment 1, lactating Jersey and Holstein cows were diagnosed as having ovarian follicular cysts (follicle diameter ≥20 mm) by rectal palpation. Following diagnosis, ovaries were examined by transrectal ultrasonography three times weekly to detect subsequent ovulation (n=8) or new cyst formation (n=9). Venous blood samples were collected daily to quantify circulating concentrations of cortisol and progesterone. The average concentration of cortisol during the 10-day period prior to ovulation was not different from the concentration prior to the formation of a new cyst. In experiment 2, secretion of cortisol and progesterone was examined in cows with ovarian follicular cysts (n=4) and cyclic, control cows in the follicular phase of the estrous cycle (n=4). An adrenocorticotrophic hormone (ACTH) challenge was administered to cystic cows 4-7 days after new cyst formation

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and to cyclic cows in the follicular phase of the cycle (36 h after induction of luteolysis). Jugular venous blood samples were collected at -60, -30, 0, +10, +20, +30, +60, +90, +120, +180, +240, +300 and +360 minutes relative to ACTH administration. A rapid increase in both cortisol and progesterone was observed immediately following administration of ACTH in each treatment group. Peak concentrations of both steroids were achieved within 60 minutes after administration of ACTH. Concentrations of cortisol and progesterone did not differ between cystic and cyclic cows. In summary, no differences in adrenal function were detected between normal cycling cows and cows with ovarian follicular cysts. Reproductive Biology 2005 5(1): 19-29.

**Key words:** progesterone, cortisol, ovarian follicular cyst, follicle, cow

**INTRODUCTION**

Ovarian follicular cysts are the most common reproductive disorder in dairy cows. Approximately 15% of dairy cows develop ovarian follicular cysts in a given lactation [7, 9, 15]. Each occurrence of ovarian follicular cysts has been estimated to add between 22-64 additional days open [3, 19] and cost $137 in reduced milk production and veterinary expenses [2].

Ovarian follicular cysts are abnormal follicular structures that reach ovulatory size or greater, fail to ovulate, and alter normal ovarian cyclicity. Follicular cysts appear to form due to an inability of estradiol to induce a luteinizing hormone surge at the appropriate time in follicular maturation. Follicles destined to form cysts secrete high concentrations of estradiol [12, 17] yet the cow fails to release a preovulatory surge in luteinizing hormone (LH). Cystic cows also fail to release LH in response to an exogenously administered estradiol challenge [23, 31]. This failure in response to estradiol appears to be exerted at the level of the hypothalamus because GnRH has been shown to be equally effective in stimulating pituitary secretion of LH in cystic and normal cows [30].

Treatments that prevent the preovulatory LH surge in normal cycling cows will result in the formation of persistent follicles, much like cysts [10, 18]. One very effective way to block the LH surge is by administering intermedi-
ate concentrations of progestagens [16]. Cows with ovarian follicular cysts frequently have an abnormal, intermediate concentration of progesterone (0.1-1.0 ng/ml; [13]). Even at these low concentrations of progesterone, the preovulatory surge of LH and subsequent ovulation can be blocked resulting in persistence of ovulatory sized follicles [5, 6]. The source of this progesterone in cystic cows is not known. One possible source is the adrenal gland. Treatment of cows with adrenocorticotropin (ACTH) induced rapid secretion of progesterone from the adrenal gland [1, 11, 27, 28, 29]. Chronic treatment of normal, cyclic cows with ACTH during the follicular phase of the estrous cycle results in an increase in concentrations of progesterone into the intermediate range prevents the LH surge from occurring and leads to the formation of persistent follicles [4, 20, 26]. Interestingly, ovarian follicular cysts are frequently associated with hypertrophy of the adrenal cortex [8]. Based on these observations, there appears to be a link between adrenal hyperactivity and the formation of ovarian follicular cysts. Two experiments were conducted to evaluate and compare adrenal gland activity in normal, cycling cows to cows that form ovarian follicular cysts. The objective of the first experiment was to determine if cows that formed cysts had a higher level of adrenal gland activity than cows that ovulated normally. This was assessed by comparing the concentrations of cortisol in these two groups of cows. The objective of experiment 2 was to determine if the adrenal response to ACTH differed between normal cyclic and cystic cows.

**MATERIALS AND METHODS**

All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

**Experiment 1**

Ovarian follicular cysts were identified in lactating Holstein and Jersey cows during routine reproductive examinations of the University of
Kentucky Dairy Research Herd. Ovaries of all open cows in the herd were palpated per rectum at 9 to 14 day intervals beginning at least 40 days postpartum. Bred cows were also palpated per rectum 50 to 60 days after breeding for pregnancy determination. Ovarian follicular cysts were frequently observed at these exams. When a cyst was diagnosed by palpation, the cystic structure was re-examined by transrectal ultrasonography. For a follicle to be classified as an ovarian follicular cyst, the follicle had to measure at least 20 mm in diameter, be free of tissue that had ecogenic characteristics similar to luteal tissue, and ovaries had to be free of corpora lutea. Ovaries in cows with follicular cysts were then examined via transrectal ultrasonography three times weekly (Monday, Wednesday, Friday) to monitor follicular events. Venous blood samples were collected daily by coccygeal or jugular venipuncture for quantification of cortisol and progesterone. Samples were collected between 0600 and 0800 daily, approximately 2 hours after the morning feeding, so as to avoid fluctuation due to normal diurnal patterns of secretion. Blood samples were collected into tubes containing EDTA as anticoagulant. Plasma was harvested and stored at -20°C until assay. Ovulation (OV; n=8 cows) was defined as the emergence then disappearance a follicle $\geq 10$ mm in diameter and subsequent formation of a corpus luteum (CL) accompanied by an increase in circulating concentrations of progesterone. New cyst formation (NCF; n=9 cows) was defined as a follicle obtaining a diameter $\geq 20$ mm and persisting at that diameter or greater for 6 or more days. Cows that formed cysts were further subdivided into those with low progesterone (mean concentration $< 0.1$ ng/ml; n=4) during the formative period and those with intermediate progesterone during that period (mean concentration 0.1 to 1.0 ng/ml; n=5; [13]). Cows with intermediate progesterone may be expected to have higher concentration of cortisol if the adrenal hyperactivity was contributing to progesterone concentrations. Concentrations of cortisol and progesterone were determined in duplicate samples by radioimmunoassay [14, 22]. For cortisol, sensitivity of the assay was 0.2 µg/dl. Within and between assay CVs were 6% and 6%, respectively. For progesterone, sensitivity of the assay was 0.02 ng/ml. Within and between assay CVs were 9% and 12%, respectively.
The average concentration of cortisol during the 10-day period prior to ovulation or the formation of a new cyst was calculated for each case and then compared between groups by analysis of variance [24]. A 10-day average was chosen to reduce the potential variation due to sampling difficulties or acute environmental stresses.

**Experiment 2**

Control (normal cycling) cows were selected from the herd based on detection of a corpus luteum by rectal palpation and verification by ultrasonography. Cyclic cows received two injections of prostaglandin (PG) F$_{2\alpha}$ (Lutalyse®, Pfizer Animal Health, New York; 25 mg, i.m.), 12 hours apart, to induce luteolysis and initiate the onset of a follicular phase. This was done to eliminate luteal progesterone that could obscure the release of progesterone from the adrenal gland in response to ACTH. Coccygeal venous blood samples were collected daily beginning at the time of corpus luteum detection for quantification of progesterone. These samples were used to confirm that a functional corpus luteum was present and to verify that complete luteolysis was induced by PGF$_{2\alpha}$. Cows with ovarian follicular cysts were identified and ovaries were monitored for the formation of new cysts as described under experiment 1. Coccygeal venous blood samples were collected daily beginning at the time of cyst detection for quantification of progesterone. These samples were used to confirm that the cystic cows had intermediate concentrations of progesterone (0.1 to 1 ng/ml) during the 10-day period prior to administration of ACTH. Adrenal cortisol and progesterone secretion was stimulated with an i.v. injection of adrenocorticotropin hormone (ACTH, Cortrosyn®, Drug Royalty USA, Toronto; 0.06 mg, i.v.). This dose was selected because it elicited a robust, but intermediate, response in previous dose:response challenge studies with cattle [1, 27]. The ACTH challenge was administered to cyclic cows (n=4) 36 hours after the first injection of PGF$_{2\alpha}$ and to cystic cows (n=4) 4-7 days after the formation of a new ovarian follicular cyst. Approximately 12 h prior to the ACTH challenge, indwelling jugular
catheters were inserted to facilitate blood sampling. Jugular venous blood samples were collected at -60, -30, 0, +10, +20, +30, +60, +90, +120, +180, +240, +300 and +360 minutes relative to ACTH administration. ACTH was administered between 0700 and 0900 to avoid variation due to natural diurnal fluctuations. Venous blood samples were analyzed for cortisol and progesterone by radioimmunoassay as described in experiment 1. For cortisol, within and among assay CVs were 11% and 24%, respectively. For progesterone, within and between assay CVs were 10% and 22%, respectively.

The baseline concentration of progesterone and cortisol for each cow was determined by taking the mean concentration of samples collected at -60, -30 and 0 h relative to ACTH administration. The peak concentration (above baseline), the area under the response curve (above baseline) and the time of the peak relative to the ACTH administration were calculated for each cow. The effects of reproductive status (cystic versus cyclic) on the peak concentrations, area under the response curve and the time of the peak were determined by analysis of variance [24].

RESULTS

In experiment 1, the average cortisol concentrations during the 10-day period prior to ovulation (0.61±0.09 μg/dl) were not different from the concentrations prior to the formation of new cysts associated with either low progesterone (0.35 ± 0.13 μg/dl) or intermediate progesterone (0.64±0.12 μg/dl; p=0.24).

In experiment 2, administration of ACTH was associated with an immediate increase in both cortisol and progesterone in both control and cystic cows. The patterns of adrenal secretion of cortisol and progesterone were similar in the two groups (p>0.05; fig. 1). The time to peak concentration, peak concentration, and area under the response curves were not different between groups for either hormone (tab. 1).
DISCUSSION

In experiment 1, concentrations of cortisol were used as an index for basal adrenal activity. No differences were detected in cortisol concentrations between cows that formed ovarian follicular cysts versus cows that ovulated normally. Concentrations of cortisol did not differ between cystic cows with intermediate progesterone and cystic cows with low progesterone either. Therefore, there was no evidence to indicate that
Table 1. A comparison of the cortisol and progesterone responses to an ACTH challenge in cyclic and cystic cows (experiment 2)

<table>
<thead>
<tr>
<th></th>
<th>hormone</th>
<th>cyclic</th>
<th>cystic</th>
<th>sem</th>
<th>significance</th>
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</thead>
<tbody>
<tr>
<td><strong>Peak Concentration</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cortisol (μg/dl)</td>
<td>8.4</td>
<td>8.0</td>
<td>1.6</td>
<td>p=0.85</td>
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<td>progesterone (ng/ml)</td>
<td>3.8</td>
<td>2.6</td>
<td>0.6</td>
<td>p=0.21</td>
<td></td>
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<td><strong>Area Under Curve</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>cortisol (μg·min/dl)</td>
<td>760</td>
<td>591</td>
<td>119</td>
<td>p=0.85</td>
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<tr>
<td>progesterone (ng·min/ml)</td>
<td>217</td>
<td>155</td>
<td>35</td>
<td>p=0.26</td>
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<td><strong>Time to Peak</strong></td>
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<tr>
<td>cortisol (min)</td>
<td>38</td>
<td>38</td>
<td>8</td>
<td>p=1.00</td>
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</tr>
<tr>
<td>progesterone (min)</td>
<td>43</td>
<td>25</td>
<td>8</td>
<td>p=0.15</td>
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</table>

basal adrenal activity differed between cystic and normal cows. Although we made every effort to collect blood samples at the same time each day, it must be recognized that the concentrations of cortisol exhibit diurnal fluctuations and that these fluctuations can mask significant differences. In experiment 2, adrenal secretion of cortisol and progesterone following administration of ACTH was not different between cystic and normal, cyclic cows. The magnitude of the response was similar to previous reports for normal, cycling cows [1, 29]. If the adrenal gland was the source of the intermediate concentration of progesterone that is frequently associated with the formation of ovarian follicular cysts, then an enhanced secretion of progesterone in response to ACTH might be anticipated. This was clearly not the case. Thus, there was no evidence that cyclic cows and cystic cows differed in adrenal function when stimulated acutely with ACTH. In previous experiments, chronic administration of ACTH has been shown to induce a cyst-like condition in normal cows [4, 22, 28].
However, the results from the experiments reported here clearly indicate that the naturally-occurring cystic condition is not associated with abnormalities in adrenal secretion of either cortisol or progesterone.

Despite these results, adrenal involvement in the formation of ovarian follicular cysts cannot be dismissed completely. The bovine adrenal gland secretes a variety of steroids, including androgens [25]. Adrenal androgens have been implicated in the etiology of polycystic ovarian disease in women [21]. Adrenal androgens were not examined in this experiment. The effects of androgens on the LH surge have not been thoroughly examined in cows.

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