The role of LH pulse frequency in ACTH-induced ovarian follicular cysts in heifers


Department of Veterinary Obstetrics and Gynaecology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 060-8501, Japan

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Abstract

The aim of the present study was to induce ovarian cysts experimentally in cattle using ACTH and to closely examine the role of LH pulse frequency in ovarian cyst formation. Five regularly cycling Holstein–Friesian heifers (15–18-month-old) were used. Ovaries were scanned daily using an ultrasound scanner with a 7.5 MHz rectal transducer. Daily blood samples were obtained via tail venepuncture for hormone analyses. Additional blood samples (for FSH and LH pulses) were obtained through an indwelling jugular vein catheters every 15 min for 8 h on Days 2 (early luteal phase; ELP), 12 (mid-luteal phase; MLP) and 19 (follicular phase; FP) of control estrous cycle and on alternate days during follicular cyst (FC) formation and persistence. Cysts were induced using subcutaneous injections of ACTH (Cortrosyn® Z; 1 mg) every 12 h for 7 days beginning on Day 15 of the subsequent estrous cycle. Plasma concentrations of progesterone (P₄), estradiol-17β, FSH and LH were determined by double antibody radioimmunoassay while cortisol concentration was determined by enzyme immunoassay (EIA). Ovarian follicular and endocrine dynamics were normal during the control estrous cycles. Ovarian follicular cysts were induced in four of the five heifers. Mean maximum size of cysts was larger ($P < 0.05$) than that of ovulatory follicles ($26.78 \pm 3.65$ versus $14.1 \pm 0.90$ mm), respectively. Cortisol levels were increased during ACTH treatment. High concentrations of estradiol and low progesterone were observed after cyst formation. LH pulse frequency was significantly reduced ($P < 0.05$) during cyst formation and persistence compared to ELP ($7.5 \pm 0.75$) and FP ($6.5 \pm 0.58$), but was not significantly different from MLP ($2.8 \pm 0.29$) pulses. Mean LH pulse amplitude and concentrations were not different. Similarly, the mean pulse frequency, amplitude and concentration of FSH were not different between control study and cystic heifers. These results suggest that the LH pulse frequency observed following

*Corresponding author. Present address: Department of Veterinary Surgery & Reproduction, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria. Tel.: +234-76-342327. E-mail address: ayribadu@infoweb.abs.net (A.Y. Ribadu).

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ACTH treatment may interact with high estradiol concentration to induce ovarian cyst formation in heifers. © 2000 Published by Elsevier Science B.V.

**Keywords:** Cattle-ovary; Luteinising hormone (LH); Pulse frequency; Ovarian cysts; ACTH

1. Introduction

Ovarian follicular cysts are an important reproductive disorder which contribute to extended calving interval in dairy cattle. Many factors such as milk production, season, puerperal stress and nutrition are associated with the development of ovarian follicular cysts, although their exact relationship is not fully understood (Archbald and Thatcher, 1992).

There are several limitations on studies of spontaneous cases of ovarian cysts. It is not feasible to know for how long the cystic structure(s) may have existed before diagnosis. In addition, the circumstances affecting ovarian function at the time of diagnosis may be different to the prevailing conditions at the time of study. It is also difficult to follow clinical cases (once diagnosed) for a long period without treatment as clients request treatment as soon as possible (Ribadu, 1993). It may, therefore, be helpful to induce ovarian cysts experimentally in order to monitor their development closely.

Ovarian cysts have been induced in cattle by a variety of treatments given in late diestrus or proestrus. Such treatments included exogenous estrogens (Wiltbank, 1966; Nadaraja and Hansel, 1976; Carrie’re et al., 1995), a combination of progesterone and estradiol (Erb et al., 1973; Winters et al., 1986; Cook et al., 1990, 1991; Hamilton et al., 1995), antiserum against bovine LH (Nadaraja and Hansel, 1976), testosterone (Fathala et al., 1978) or exogenous adrenocorticotropic hormone (ACTH) (Liptrap and McNally, 1976; Refsal et al., 1987; Kawate et al., 1996).

The association between stress and ovarian cyst formation in cattle has not been clearly elucidated. This is probably because the formation of ovarian cysts is multifactorial, and stress along with several factors enumerated above have been implicated. ACTH caused suppression of baseline LH values during the follicular phase (FP) of the estrous cycle (Stoebel and Moberg, 1982; Li and Wagner, 1983; Ribadu, 1993). Dobson and Smith (1995) have shown that administration of ACTH during the FP results in fewer LH pulses. It has been demonstrated recently that ACTH reduces the self priming effect in vivo and delays the LH surge at least partially, by direct effect at the pituitary gland in ewes (Phogat et al., 1999).

Alterations in basal secretions of LH modulate follicular development during the estrous cycle (Archbald and Thatcher, 1992). Lengthening of the luteal phase with low levels of progesterone (1–2 ng/ml) resulted in prolonged development of follicles which indicated that subtle changes in hormonal milieu can dramatically alter the normal pattern of follicular development in cattle (Sirois and Fortune, 1990). Furthermore, Stock and Fortune (1993) have shown that slight increases in LH pulse frequency promoted follicular growth and dominance which was associated with increased plasma estradiol. Whether a similar endocrine scenario is involved in the formation and persistence of ovarian follicular cysts in cattle is not fully elucidated. Roche and Mihm (1996) have suggested that a possible endocrine cause of ovarian cysts may be an increase in LH pulse frequency, insufficient to cause ovulation but sufficient to cause continued growth of the dominant follicle and high
estrogen production. It could, therefore, be hypothesised that ACTH induces ovarian cysts by decreasing the LH pulse frequency.

The objectives of the present study were to experimentally induce ovarian cysts in heifers using ACTH and to closely monitor cyst formation by ultrasonography and hormone profile with particular reference to the role of LH pulse frequency.

2. Material and method

2.1. Animals and treatment

Five Holstein–Friesian heifers (15–18-month-old) weighing 380–400 kg with normal and regular estrous cycles were used for this study. The heifers were tied in stalls and fed hay, silage, concentrates and minerals licks. Water was provided ad libitum. Animal experimentation was performed in conformity with the University Animal Welfare Committee guidelines.

In order to synchronize estrus (for control estrous cycle study), the heifers were administered 25 mg Dinoprost (Pronalgon F®; Takeda Chemical Co., Ltd., Tokyo, Japan) 11 days apart. Animals were checked for estrous behaviour (restlessness, bellowing, presence of “estrus discharge” and turgidity of the uterus by palpation per rectum) around the time of expected estrus after the second prostaglandin injection. During the control estrous cycle, the heifers did not receive any form of treatment.

Beginning on Day 15 of the subsequent estrous cycle, the heifers were treated with 1 mg ACTH (Cortrosyn® Z; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) subcutaneously every 12 h for 7 days.

2.2. Ultrasonography

Ovaries were scanned using a real-time B-mode ultrasound scanner (Echopal, Hitachi Medical Corporation, Tokyo, Japan) equipped with a 7.5 MHz rectal transducer. All ultrasound examinations were performed by a single operator. The growth and regression of follicles ≥5 mm, corpora lutea and cysts were monitored. Daily ovarian ultrasonography was performed through a complete estrous cycle (control) and from Day 14 (Day 0 = Day of estrus) until Days 35–48 (ACTH treatment group). Representative images were frozen on the ultrasound screen and printed using an on-line thermal printer (EZU-VP5; Hitachi Medical Corporation, Tokyo, Japan).

Cysts detected by ultrasonography were defined as any follicular structure of 20 mm or greater in diameter which was present for 10 days or more without ovulation and CL formation (Cook et al., 1990; Chavette et al., 1993). The first day of cyst formation was the day a follicle attained 20 mm or more in diameter.

2.3. Blood sampling

Daily blood samples were collected by tail venepuncture into heparinised vacutainers throughout the estrous cycle and beginning a day before the initiation of ACTH treatment
(Day 14 of the estrous cycle) until Days 35–48. To assess pulsatile FSH and LH secretion, blood samples were collected through indwelling jugular vein catheters (i.d.: 0.98 mm; o.d.: 1.55 mm; length: 50 cm; Medikit, Tokyo, Japan) every 15 min for 8 h on Days 2 (early luteal phase; ELP), 12 (mid-luteal phase; MLP) and 19 (follicular phase; FP) of the control estrous cycle and on alternate days during cyst formation and persistence (beginning when a dominant follicle has attained a size of 20 mm). In order to avoid clotting inside the catheter, 3 ml heparinised saline solution (20 iu/ml) was flushed into the catheter after each collection. Also, in order to avoid heparinised blood in the sample, 4 ml of blood was drawn and discarded before drawing the next sample.

The blood samples were centrifuged at 1700 \( \times g \) for 20 min. Plasma samples were stored at \(-20^\circ C\) until concentrations of FSH, LH, progesterone, estradiol, and cortisol were determined.

2.4. Hormone analysis

Plasma concentration of FSH were measured by a validated radioimmunoassay (Bolt and Rollins, 1983) using antibovine \( \beta \)-subunit antiserum (USDA-5-pool), \(^{125}\mathrm{I}\)-bFSH (I-2) for radiiodination and USDA-bFSH I-2 as reference standard. Plasma concentrations of LH were measured by a validated RIA (Echternkamp et al., 1976) using anti-ovine LH serum (Ao/LH serum YM #18), USDA-bLH (B-6) for radiiodination and USDA-bLH (B-6) as reference standard. The antiserum to bovine LH was cross-reacted at 0.5% with bovine FSH and 0.4% with bovine TSH. The sensitivities of the assays for FSH and LH were 0.08 and 0.07 ng/ml, respectively. The intra- and inter-assay coefficients of variation at a mean concentration of 0.25 ng/ml were 10 and 16.6% for FSH and 8.1 and 14.4% for LH at a mean concentration of 0.52 ng/ml, respectively. There was no cross reaction between LH and FSH in the assays, as there was no synchrony in the FSH and LH pulses from the same samples analysed.

Plasma concentrations of estradiol and progesterone were determined as described by Taya et al. (1985) using antisera to estradiol-17\( \beta \) (GDN 244) and progesterone (GDN 337) which were kindly provided by Dr. G.D. Niswender. \(^{125}\mathrm{I}\)-labelled estradiol (IM35) and progesterone (IM140) were purchased from Amersham Life Sciences (Tokyo, Japan). For estradiol assay, 1 ml plasma samples, placed in 13 mm \( \times 100 \) mm glass tubes, were extracted with 3 ml anhydrous diethyl ether. The ether phase was decanted into 12 mm \( \times 75 \) mm tubes and evaporated. To remove substances that interfere with the estradiol assay, a solution of a mixture of 1 ml \( n \)-hexane and 0.5 ml acetonitrile was added and the tubes were mixed. After aspiration of the hexane phase, the methanol phase was dried with nitrogen gas and redissolved in 0.1 ml 1% BSA-0.01 M PBS and the solution was used for the estradiol assay. For progesterone assay, 0.02 ml plasma samples were pipetted into 13 mm \( \times 100 \) mm tubes and 0.5 ml of double distilled water (DDW) was added. It was then extracted with 2.5 ml anhydrous diethyl ether. After the ether phase was decanted into 12 mm \( \times 75 \) mm tubes and evaporated, the dried residue was redissolved in 0.1 ml 1% BSA-0.01 M PBS and the solution was used for RIA. The sensitivities of the assays for estradiol and progesterone were 0.28 pg/ml and 0.2 ng/ml, respectively. The intra- and inter-assay coefficients of variation were 6.1 and 13.2% for estradiol (at a mean concentration of 5.0 pg/ml) and 4.2 and 8.3% for progesterone (at a mean concentration of 4.5 ng/ml), respectively.
Plasma cortisol concentrations were measured by enzyme immunoassay (EIA) as described by Nakao et al. (1981). The sensitivity of the assay was 0.2 ng/ml. The intra- and inter-assay coefficients of variation of the assay at a mean concentration of 3.4 ng/ml were 8.6 and 13.8%, respectively.

2.5. Statistical analysis

Luteinising hormone and FSH profiles were characterised similar to the method of Brown et al. (1986). Fluctuations in plasma LH and FSH were determined to be pulses if: (1) the values were at least 40% above the LH or FSH concentration immediately preceding the rise and (2) they were followed by at least two successive values that were declining or represented basal levels. Pulse amplitude was determined by subtracting basal values from the highest value associated with the pulse. Basal gonadotropin concentrations were calculated from values not associated with either the ascending or descending portions of a pulse.

Results were subjected to analysis of variance for repeated measures. For FSH and LH pulse frequency, data was first normalised by square root transformation in order to achieve homogeneity of variance. ANOVA was performed on the transformed data. Student’s t-test was used to compare the difference between two means. A value of \( P < 0.05 \) was considered to be significant.

3. Results

3.1. Control

Daily ultrasound monitoring during a complete estrous cycle revealed normal follicular dynamics in the heifers. Four heifers had two waves of follicular growth per cycle, while one heifer had three waves. The mean length of the estrous cycle was 20.4 ± 0.24. The ovulatory follicle was detected on Day 12.0 ± 0.82 for heifers with two waves per cycle and on Day 15 for the heifer with three waves per cycle. The mean size of the ovulatory follicle (14.1 ± 0.90 mm) was not significantly different (\( P > 0.05 \)) from the mean maximum diameter of the dominant nonovulatory follicle (13.2 ± 0.41). Mean maximal diameter of corpora lutea (CL) on Day 12 of the estrous cycle was 22.5 ± 0.56 mm. Estrous behaviour were noted in all control heifers around Days 19–20 of the estrous cycle.

3.2. ACTH treatment

Ovarian follicular cysts were induced in four of the five heifers. Mean ± S.E.M. day of initial cyst detection was 24.8 ± 1.89. Each follicle that became cystic in these four heifers was present at the initiation of ACTH treatment. The cysts diameter were present at >20 mm for 10.8 ± 0.48 days before regression commenced. Mean maximum size of cysts was 26.78 ± 3.65 mm in diameter. This was larger (\( P < 0.05 \)) than the mean maximum diameter (14.1 ± 0.90 mm) of ovulatory follicles. The cysts appeared ultrasonographically as uniformly nonechogenic ovarian structures ≥20 mm in diameter with a wall thickness of <3 mm. The heifer which failed to develop an ovarian cyst subsequently showed estrous
signs and ovulated a dominant follicle (20 mm) on Day 24 from last ovulation. The control estrous cycle length and ovulatory follicle size for this heifer was 20 days and 14.8 mm, respectively. None of the four heifers in which cysts developed exhibited estrous behaviour or nymphomania during ACTH treatment and cyst formation.

After completion of ACTH treatment on Day 21; progesterone concentrations were maintained at subluteal levels (1.16–2.14 ng/ml) between Days 21 and 23. The hormone profile during the last week of the control estrous cycle and during ACTH treatment in heifers in which cysts developed is presented in Table 1. Plasma progesterone concentration during the last week of the estrous cycle (i.e. Days 15–21) was not significantly different \((P > 0.05)\) from similar period after ACTH treatment. However, both estradiol-17\(\beta\) and cortisol concentrations were significantly higher \((P < 0.05)\) during ACTH treatment (Days 15–21) compared to the similar time scale during the control estrous cycle. Estradiol-17\(\beta\) concentrations fluctuated greatly both within and between heifers. Two of the cystic heifers showed high concentrations with peak amounts of 62.56 ± 15.80 pg/ml, while the remaining two heifers showed a peak of 8.04 ± 0.53 pg/ml. It was also noted that sharp decline in estradiol preceded the initiation of morphological regression of the cysts by about 4 days.

The mean LH pulse frequency, amplitude and concentration during the ELP, MLP and FP of estrous cycle and during follicular cyst formation (FC) is depicted in Table 2. There were

<table>
<thead>
<tr>
<th>Day of estrous cycle</th>
<th>Progesterone (ng/ml) Control</th>
<th>Progesterone (ng/ml) ACTH</th>
<th>Estradiol (pg/ml) Control</th>
<th>Estradiol (pg/ml) ACTH</th>
<th>Cortisol (ng/ml) Control</th>
<th>Cortisol (ng/ml) ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>9.0 ± 0.82 a</td>
<td>6.5 ± 1.30 a</td>
<td>3.5 ± 1.04 b</td>
<td>8.7 ± 1.23 c</td>
<td>3.1 ± 1.66 d</td>
<td>4.9 ± 1.79 e</td>
</tr>
<tr>
<td>16</td>
<td>6.2 ± 1.08 a</td>
<td>6.6 ± 0.54 a</td>
<td>3.4 ± 0.89 b</td>
<td>8.2 ± 1.91 c</td>
<td>1.7 ± 0.35 d</td>
<td>11.4 ± 2.26 e</td>
</tr>
<tr>
<td>17</td>
<td>2.2 ± 0.65 a</td>
<td>6.1 ± 0.72 f</td>
<td>3.6 ± 1.07 b</td>
<td>6.6 ± 1.68 c</td>
<td>1.0 ± 0.40 d</td>
<td>14.6 ± 1.93 e</td>
</tr>
<tr>
<td>18</td>
<td>0.9 ± 0.44 a</td>
<td>4.5 ± 1.39 f</td>
<td>4.9 ± 1.61 b</td>
<td>5.5 ± 1.52 b</td>
<td>0.6 ± 0.08 d</td>
<td>9.6 ± 1.24 e</td>
</tr>
<tr>
<td>19</td>
<td>0.8 ± 0.40 a</td>
<td>4.8 ± 0.20 f</td>
<td>8.8 ± 1.95 b</td>
<td>9.1 ± 1.52 b</td>
<td>1.9 ± 0.50 d</td>
<td>5.6 ± 0.81 e</td>
</tr>
<tr>
<td>20</td>
<td>0.6 ± 0.17 a</td>
<td>4.4 ± 0.80 f</td>
<td>7.1 ± 1.39 b</td>
<td>6.5 ± 1.74 b</td>
<td>2.4 ± 0.80 d</td>
<td>10.1 ± 1.94 e</td>
</tr>
<tr>
<td>21</td>
<td>0.4 ± 0.16 a</td>
<td>1.4 ± 0.25 a</td>
<td>2.2 ± 0.27 b</td>
<td>6.5 ± 1.94 c</td>
<td>4.9 ± 1.73 d</td>
<td>8.1 ± 1.95 e</td>
</tr>
</tbody>
</table>

\(a\) Different letters within a row indicate significant differences \((P < 0.05)\).

Table 2
Mean LH pulse frequency (number of pulses/8 h), pulse amplitude and concentration (ng/ml) during early luteal phase (ELP), mid-luteal phase (MLP) and follicular phase (FP) of control estrous cycle \((n = 5)\) and after cyst formation \((n = 4)\)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control ((n = 5))</th>
<th>Cystic ((n = 4))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELP</td>
<td>MLP</td>
</tr>
<tr>
<td>LH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (pulses/8 h)</td>
<td>7.5 ± 0.75 a</td>
<td>2.8 ± 0.29 b</td>
</tr>
<tr>
<td>Amplitude (ng/ml)</td>
<td>0.21 ± 0.09</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>0.46 ± 0.11</td>
<td>0.33 ± 0.11</td>
</tr>
</tbody>
</table>

\(a\) Different letters within a same row indicate significant difference \((P < 0.05)\); mean ± S.E.M.
Table 3
Mean FSH frequency (number of pulses/8 h), pulse amplitude and concentration (ng/ml) during early luteal phase (ELP), mid-luteal phase (MLP) and follicular phase (FP) of control estrous cycle \((n = 5)\) and after cyst formation \((n = 4)\)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control ((n = 5))</th>
<th>MLP</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (pulses/8 h)</td>
<td>3.5 ± 0.47(^a)</td>
<td>4.5 ± 0.33</td>
<td>4.8 ± 0.29</td>
</tr>
<tr>
<td>Amplitude (ng/ml)</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± S.E.M.

no significant differences \((P > 0.05)\) in LH pulse frequency, amplitude and concentration across the days during cyst formation and persistence; hence only a single mean (FC) column is shown in Table 2. LH pulse frequency was significantly reduced \((P < 0.05)\) during cyst formation and persistence compared to ELP and FP. It was not significantly \((P = 0.23)\) different from MLP pulse frequency \((3.72 ± 0.20\) versus \(2.75 ± 0.29\), respectively. The mean LH amplitude and concentration were not different among the different phases of the estrous cycle and during cyst formation.

The mean FSH pulse frequency, amplitude and concentration during the ELP, MLP, FP of the estrous cycle is shown in Table 3. There was no significant difference \((P > 0.05)\) in the FSH pulse frequency, amplitude and concentration across the days during cyst formation and persistence, hence only a single mean (FC) column is shown in Table 3. Mean pulse frequency, amplitude and concentration of FSH were not different \((P > 0.05)\) between control and cystic heifers.

In two of the heifers with cysts, cyst regression was followed by the emergence of a follicle that subsequently ovulated and formed a CL. In one heifer, the cyst was maintained at >20 mm despite the occurrence of ovulation and CL formation. In the fourth heifer, however, follicular development leading to ovulation was not observed despite complete cyst regression (<5 mm) at the time of termination of the experiment on Day 48.

4. Discussion

The LH pulse frequency during cyst formation was significantly lower than control ELP and FP pulses but not significantly different from midluteal phase pulses. The LH pulse frequency observed which was intermediate between midluteal phase pulses and FP pulses may interact with high estradiol concentration resulting in ovarian follicular cyst formation. The results of the current study shows for the first time that significant reduction in LH pulses may be involved in follicular cyst formation and persistence. They support the hypothesis that ovarian follicular cysts formation may be associated with a reduction in LH pulse frequency. Roche and Mihm (1996) have proposed that ovarian follicular cyst formation may be associated with an LH pulse frequency that is sufficient to cause continued follicular/cyst growth but insufficient to cause ovulation. During the estrous cycle, decreases in LH pulse
frequency are associated with decreased estradiol production and the demise of the dominant follicle (Ireland and Roche, 1983; Savio et al., 1993; Stock and Fortune, 1993), since dominant follicles are critically dependent on LH for survival (Campbell et al., 1995). Conversely, an increase in LH pulse frequency is required for successful ovulation (Rahe et al., 1980; Roche and Mihm, 1996).

The LH pulse frequency during the control estrous cycle (ELP, MLP and FP) was consistent with previous reports (Rahe et al., 1980; Schallenberger et al., 1985; Stock and Fortune, 1993; Cupp et al., 1995). The mean LH pulse frequency in heifers with cysts in this study (3.72 ± 0.20 pulses/8 h) was lower than the frequency (5–7 pulses/8 h) reported in postpartum cows with cystic follicles by Savio et al. (1990). A higher mean LH pulse frequency, amplitude and concentration were reported during the FP in cows that developed follicular cysts following progesterone/estradiol treatment compared to the cows which failed to develop cysts and controls (Cook et al., 1991). ACTH treatment seemed to modify the pulsatile release of LH either directly at the level of the hypothalamus or indirectly through the suppressive effect of cortisol on LH (Stoebel and Moberg, 1982; Li and Wagner, 1983) and/or adrenal progesterone production (Watson and Munro, 1984).

In the present study, the mean FSH pulse frequency, amplitude and concentration during the control estrous cycle (ELP, MLP and FP) were not different with cystic heifers. These findings supplements earlier reports (Cook et al., 1990; Hamilton et al., 1995) and indicates that changes in the secretory pattern and concentrations of FSH may not be a factor in the etiology of ovarian cysts.

During the control estrous cycle, ovarian follicular development was characterised by two waves per cycle in four heifers and three waves per cycle in the fifth heifer. The wave pattern of follicular growth was consistent with previous reports (Siros and Fortune, 1988; Savio et al., 1988; Knopf et al., 1989). In the present study, administration of ACTH resulted in alteration in follicular dynamics with subsequent ovarian follicular cyst formation in four out of five heifers. In previous studies, Refsal et al. (1987) reported experimental induction of cysts in two out of five heifers while Liptrap and McNally (1976) obtained cysts in five out of five cows. Kawate et al. (1996) similarly induced ovarian cysts using ACTH in four out of four cows. The growth rate of ovulatory follicles during the control estrous cycle were similar to cystic follicles. Similar findings were reported by Hamilton et al. (1995).

Ovarian cysts are often classified as follicular or luteal based on wall thickness and progesterone concentration (Roberts, 1986). In this study, all the cysts were classified as follicular as the cyst walls were thin and the plasma progesterone concentration during the period of cyst existence were also low (<0.5 ng/ml). In addition, the ultrasound appearance were consistent with that of follicular cysts (i.e. uniformly nonechogenic antrum >20 mm with a wall thickness of less than 3 mm) (Carroll et al., 1990; Farin et al., 1992; Ribadu et al., 1994). No evidence of cyst luteinization was observed in this study. A similar observation was made by Ribadu et al. (1993) in seven cows with spontaneous ovarian follicular cysts monitored (by alternate day ultrasonography and progesterone profile) over a 2-week period.

The peak concentration of estradiol observed in this study (62.56 ± 15.80) after cyst formation was higher than the peak concentration during the estrous cycle. Savio et al. (1990) and Hamilton et al. (1995), similarly, observed greater concentrations of estradiol in cows with ovarian cysts. In the present study, the high concentrations of estradiol (presumably from cysts) declined about 4 days before initiation of cyst regression, thereby
allowing the emergence of a wave of follicular growth culminating in ovulation. The size
of the cyst as observed by ultrasonography was >20 mm at the time of decline in estradiol
concentration. This indicated that morphological dominance of the cyst (as observed by
ultrasonography) may not be equated with functional dominance. A similar observation
was made by Savio et al. (1990) who noted that estradiol returned to basal levels 10–15
days before first ovulation, despite the fact that morphologically the cyst was still detectable
on ultrasound examination. The increased cortisol concentrations observed following the
initiation of ACTH treatment concurred with previous studies (Nadaraja and Hansel, 1976;
Refsal et al., 1987).

In conclusion, the results of the present study has shown that LH pulse frequency (but
not features of FSH secretion) was reduced during cyst formation and persistence which
suggests that alterations in the pulsatile release of LH may interact with high estradiol
concentration to cause ovarian follicular cyst formation in heifers.

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