Pituitary and Ovarian Hormones in Cows with Ovarian Cysts

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Summary. Gonadotropin profiles as well as sex steroids in the plasma of cows with ovarian cysts or cystic corpus luteum were examined. Bioactive inhibin levels and concentrations of sex steroids in the individual cystic fluid were determined to analyze the type of ovarian cyst. Plasma concentrations of LH in cows with follicular cysts were generally high as compared with basal LH levels in normal cows during the estrous cycle, though high levels of plasma estradiol-17β were noted. LH levels in cows with follicular cysts and corpus luteum, with luteal cyst or with cystic corpus luteum, however, were low similar to the basal levels of LH in the normal estrous cycle, being associated with high levels of plasma progesterone. Plasma concentrations of FSH in cows with any type of ovarian cyst, on the other hand, were within the range of basal levels of the normal estrous cycle, with no correlation to plasma levels of steroids.

These results indicate that elevated levels of LH and basal levels of FSH within the normal range in the estrous cycle are characteristic in cows with follicular cysts. The possibility of involvement of inhibin in lowering FSH to basal levels in these cows with ovarian cysts is discussed.

KEY WORDS: COW, OVARIAN CYSTS, INHIBIN, STEROIDS, GONADOTROPINS.

Ovarian cysts are classified into two groups, follicular cysts and luteal cysts. Follicular cysts are much more common than luteal cysts in dairy cows. Concentrations of progesterone in plasma are low in cows with follicular cysts and high in cows with luteal cysts (Kesler and Garverick, 1982), whereas results of plasma concentrations of estradiol-17β are variable in both types of ovarian cysts (Kittock et al., 1973; 1974; Glencross and Munro, 1974; Cantley et al., 1975; Garverick et al., 1976; Sequin et al., 1976; Kesler et al., 1977; Dobson et al., 1977), as well as the results of luteinizing hormone (LH) (Mori et al., 1974; Cantley et al., 1975; Dobson et al., 1977; Kesler et al., 1980). Less information is available on follicle-stimulating hormone (FSH) secretion in cows with ovarian cysts (Dobson et al., 1977; Mori et al., 1980). In our previous report (Mizumachi et al., 1986), we demonstrated that follicular cysts in dairy cows can be classified into three types (A, B and C) depending on an inhibin activity in cystic fluid. Type A is the cystic follicle with hyper-function of granulosa cells filled with fluid of high amounts of estradiol-17β and inhibin, type B is the cystic follicle with rather degenerating granulosa cells filled with fluid of higher amounts of progesterone, and type C is the cystic follicle with little endocrine function of granulosa cells. While it has been shown that concentrations of steroid hormones and activity of inhibin in cystic fluid are different among three types of follicular cysts, concentrations of LH, FSH and steroid hormones in the plasma of these cystic cows have not been determined.

The present study is to examine gonadotropin profiles as well as plasma levels of steroids in cows with ovarian cysts with reference to concentrations of steroid hormones and inhibin activity in cystic fluid of the same individuals. The possible involvement of inhibin is discussed in relation to the FSH patterns found in cows.
with ovarian cysts.

Materials and Methods

Animals

Adult lactating Holstein cows having ovarian cysts diagnosed by rectal palpation and normal lactating cows used in this study were within the range of 2 to 9 years old. These cows are raised by the farmers in Chiba Prefecture. Clinical diagnosis of follicular cysts was based upon the finding of a single or multiple formation of smooth, fluctuant and rounded structures of more than 25mm in diameter in one or both ovaries. Cystic corpora lutea, which were defined as thick wall and fluid-filled corpora lutea, were also diagnosed by ovarian palpation per rectum. When inner walls of cysts were felt to be rough without a remnant of ovulation by rectal palpation, these cysts were decided as luteal cysts.

Blood samples were collected via jugular vein into heparinized vacutainers after the rectal palpation for clinical diagnosis of ovarian cysts. Follicular or cystic fluid was also collected via vagina using an intrafollicular injector (Kaneda et al., 1980) just after the venous blood collection. Blood samples and cystic fluid were centrifuged at 1700g for 30 min. Plasma or supernatant fraction of cystic fluid was stored at -40°C until assayed.

Classification of ovarian cyst

Cows with ovarian cysts were classified into three groups; 1) follicular cyst (n=39), 2) follicular cyst with corpus luteum (n=14) and 3) luteal cyst (n=5). Cows with follicular cysts with or without a corpus luteum were further classified into three types (A, B, and C) according to inhibin activity in cystic fluid as described previously (Mizumachi et al., 1986); inhibin activity of type A, B and C are >1.0 unit, <1.0 unit and less than the sensitivity of the assay, respectively (see “Bioassay of inhibin activity”). In addition, cows with cystic corpus luteum (n=5) were also examined. The assay method of inhibin is described later.

Radioimmunoassay (RIA) of LH, FSH and steroid hormones

Plasma concentrations of LH were measured using USDA-bLH-I-1 for radioiodination, anti-bovine LH serum (DJB-3-1211) and USDA-bLH-B-5 as a reference standard (Echternkamp et al., 1976). Plasma concentrations of FSH were measured using USDA-FSH-BP-3 for radioiodination, anti-bovine FSH β-subunit serum (USDA-5-0122) and USDA-FSH-B-1 as a reference standard (Bolt and Rollins, 1983). The sensitivity of the assay for LH and FSH was 0.25 and 1.2 ng/tube and the intra- and inter-assay coefficients of variation were 6.6 and 11.7% for LH and 4.9 and 5.9% for FSH respectively.

Concentrations of progesterone, testosterone and estradiol-17β in the peripheral plasma and cystic fluid were determined as described previously (Taya et al., 1985) using antisera to progesterone (Gunma OGP-1, Takahashi et al., 1985), testosterone (GDN 250, Gay and Kerlen, 1978) and estradiol-17β (GDN 244, Korenman et al., 1974). The sensitivity of the assay for progesterone, testosterone and estradiol-17β was 0.63, 0.63 and 0.31 pg/tube respectively. The intra- and inter-assay coefficients of variation were 6.1 and 18.5% for progesterone, 8.0 and 14.4% for testosterone and 6.0 and 12.4% for estradiol-17β respectively.

Bioassay of inhibin activity

Individual follicular fluid from normal cyclic cows and individual ovarian cystic fluid from cows with various types of ovarian cysts were assessed for FSH inhibiting activity (inhibin) using the dispersed anterior pituitary cell bioassay system (Kimura et al., 1983), partially modified as described previously (Taya and Sasamoto, 1988) after removal of steroids by charcoal treatment. Measurement of inhibin activity was undertaken as a parallel line assay of the 2×2 point system using the method of Finney (1964). Inhibin activity in 1.0ml follicular or cyst fluid was expressed as relative units to the potency of pooled bovine follicular fluid (reference standard). One unit of inhibin activity was defined as the FSH-suppressing activity of 1.0ml of reference standard. The index of precision was <21% for the assay and 7.5 nl of bovine follicular fluid showed a significant decrease in FSH amounts in the medium of the pituitary cell culture system as compared with controls. Concentrations of FSH in the culture medium were measured using NIADDK radioimmunoassay kit for rat FSH. Iodinated preparation was rat FSH-I-5 and antiserum was anti-rat FSH S-11. Results were expressed in terms of NIADDK FSH-RP-1. The intra- and inter-assay coefficients of variation were 6.8 and 4.8%, respectively.

Statistics

Significance of difference between two means was tested by Student’s t-test; a value of P<0.05 was considered significant.
Table 1. Inhibin activity and concentrations of progesterone, testosterone and estradiol-17β in the follicular fluid of cyclic cows and the cyst fluid of cows with ovarian cysts

<table>
<thead>
<tr>
<th></th>
<th>Inhibin Activity (unit/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>Estradiol-17β (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclic</strong></td>
<td></td>
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</tr>
<tr>
<td>Follicular Phase</td>
<td>1.4 ± 0.4 a</td>
<td>218.0 ± 49.2 a</td>
<td>14.0 ± 4.0 a</td>
<td>373.0 ± 121.7</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
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<tr>
<td>Luteal Phase</td>
<td>1.6 ± 0.4 b (8) *</td>
<td>214.1 ± 105.1 bc</td>
<td>2.7 ± 0.6 b</td>
<td>56.0 ± 15.8</td>
</tr>
<tr>
<td>(n=10)</td>
<td>0</td>
<td>(2) *</td>
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<tr>
<td><strong>Follicular cyst</strong></td>
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<tr>
<td>A(n=23)</td>
<td>2.5 ± 0.2 b</td>
<td>210.0 ± 41.2 a</td>
<td>38.9 ± 8.0 c</td>
<td>361.3 ± 76.1</td>
</tr>
<tr>
<td>B(n=7)</td>
<td>0.3 ± 0.1 c d</td>
<td>432.0 ± 101.2 ab</td>
<td>9.6 ± 5.9 abd</td>
<td>124.3 ± 92.1</td>
</tr>
<tr>
<td>C(n=9)</td>
<td>undetectable</td>
<td>694.9 ± 119.2 b</td>
<td>1.5 ± 0.4 b</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td><strong>Follicular cyst</strong> with corpus luteum</td>
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<tr>
<td>A(n=3)</td>
<td>3.3 ± 0.7 b c</td>
<td>46.4 ± 18.1 c</td>
<td>19.5 ± 16.6 abc</td>
<td>148.0 ± 95.0</td>
</tr>
<tr>
<td>B(n=3)</td>
<td>0.5 ± 0.2 a d</td>
<td>517.8 ± 278.3 abcd</td>
<td>48.2 ± 24.1 abcd</td>
<td>1.0 ± 0.9</td>
</tr>
<tr>
<td>C(n=8)</td>
<td>undetectable</td>
<td>1432.9 ± 555.6 abcd</td>
<td>1.1 ± 0.4 c d</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td><strong>Luteal Cyst</strong></td>
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<td></td>
</tr>
<tr>
<td>(n=5)</td>
<td>undetectable</td>
<td>1317.9 ± 563.2 abcd</td>
<td>1.9 ± 0.5 bcd</td>
<td>0.6 ± 0.5</td>
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<tr>
<td><strong>Cystic corpus luteum</strong></td>
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<tr>
<td>(n=5)</td>
<td>undetectable</td>
<td>3121.9 ± 810.4 d</td>
<td>2.7 ± 0.7 bcd</td>
<td>0.1 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± S. E. M.
* Number of cows for each value.
Within rows, values with different superscript letters are significantly different (p<0.05).
The statistical comparisons of mean concentrations of estradiol-17β in the follicular or cystic fluid among each group were not carried out, since variances of these groups showed unequal and values from individual follicles or cysts were variable.

Results

Inhibin activity in normal follicular fluid and cystic fluid (Table 1)
The mean inhibin activity in follicular fluid of the largest follicle at the follicular phase or estrus was not different from that of follicles at the luteal phase of the normal estrous cycle. Inhibin activity in cystic fluid of type A follicular cysts was significantly higher than that in normal estrous follicles and those in cystic fluid of types B and C of follicular cysts. Similar tendency was noted for follicular cyst with corpus luteum. No inhibin activity was noted in cystic fluid of both luteal cysts and cystic corpora lutea.

Concentrations of progesterone, testosterone and estradiol-17β in normal follicular fluid and cystic fluid (Table 1)
There was no significant difference in concentrations of progesterone in normal follicular fluid between the follicular and luteal phase of the normal estrous cycle. In follicular cysts, concentration of progesterone in cystic fluid showed a gradual increase from type A to C. It is also true for follicular cysts with a corpus luteum. Markedly high concentrations of progesterone were noted in cystic fluid of luteal cysts and cystic corpora lutea.
The mean concentrations of testosterone in normal follicular fluid in the follicular phase were significantly higher than those at the luteal phase. Concentrations of testosterone in cystic fluid of type A follicular cysts were significantly higher than those in normal follicles at the follicular phase. The value in cystic fluid of follicular cysts decreased gradually from type A to C. Concentrations of testosterone in cystic fluid of luteal cysts and cystic corpora lutea were relatively low and the values were comparable to those in follicular fluid at the luteal phase in normal estrous cycle.

The statistical comparisons of mean concentrations of estradiol-17β in the follicular or cystic fluid among groups were not carried out, since variances of these groups showed unequal and values from individual follicles or cysts were variable. But the mean concentration of estradiol-17β in normal follicular fluid in the follicular phase and estrus showed a tendency to be high as compared to those found in follicular fluid in the luteal phase in normal estrous cycle. Mean concentration of estradiol-17β in cystic fluid of type A follicular cyst was similar to that in normal follicles in the follicular phase. Concentrations of estradiol-17β in cystic fluid of follicular cyst of type C were very low. It is also true for follicular cysts with corpus luteum. Negligible amounts of estradiol-17β were detected in the cystic fluid of luteal cysts and cystic corpora lutea.

The statistical comparisons of mean concentrations of estradiol-17β in normal follicular fluid in the follicular phase and estrus showed a tendency to be high as compared to those found in follicular fluid in the luteal phase in normal estrous cycle. Mean concentration of estradiol-17β in cystic fluid of type A follicular cyst was similar to that in normal follicles in the follicular phase. Concentrations of estradiol-17β in cystic fluid of follicular cyst of type C were very low. It is also true for follicular cysts with corpus luteum. Negligible amounts of estradiol-17β were detected in the cystic fluid of luteal cysts and cystic corpora lutea.

Table 2. Plasma concentrations of LH, FSH, progesterone, testosterone and estradiol-17β in cyclic cows and cows with ovarian cyst

<table>
<thead>
<tr>
<th></th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Testosterone (pg/ml)</th>
<th>Estradiol-17β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclic</strong></td>
<td></td>
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</tr>
<tr>
<td>Follicular phase</td>
<td>0.7±0.2ac</td>
<td>21.3±1.7*a</td>
<td>0.6±0.1ac</td>
<td>29.8±2.6*a</td>
<td>13.7±3.0*a</td>
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<tr>
<td>(n=5)</td>
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<tr>
<td>Luteal phase</td>
<td>0.6±0.1ac</td>
<td>20.8±1.5*a</td>
<td>6.3±1.1b</td>
<td>18.8±2.4ac</td>
<td>4.1±0.9b</td>
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<tr>
<td>(n=5)</td>
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<tr>
<td><strong>Follicular cyst</strong></td>
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<tr>
<td>without corpus luteum</td>
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<tr>
<td>A (n=20)</td>
<td>2.1±0.5b</td>
<td>23.8±1.8*a</td>
<td>0.8±0.2ac</td>
<td>19.6±1.4b</td>
<td>26.2±1.9ab</td>
</tr>
<tr>
<td>B (n=6)</td>
<td>4.1±1.2b</td>
<td>23.9±3.7*a</td>
<td>0.8±0.3a</td>
<td>16.1±3.1bc</td>
<td>9.7±3.4ab</td>
</tr>
<tr>
<td>C (n=6)</td>
<td>1.0±0.2ab</td>
<td>25.2±4.1*a</td>
<td>0.8±0.3a</td>
<td>18.6±1.9b</td>
<td>7.6±1.1ac</td>
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<tr>
<td><strong>Follicular cyst</strong></td>
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<tr>
<td>with corpus luteum</td>
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<tr>
<td>A (n=3)</td>
<td>0.6±0.2ac</td>
<td>22.0±3.9*a</td>
<td>3.5±0.9bc</td>
<td>17.7±1.5b</td>
<td>5.9±1.4ab</td>
</tr>
<tr>
<td>B (n=3)</td>
<td>0.3±0.1c</td>
<td>18.4±1.8*a</td>
<td>10.6±2.9abc</td>
<td>17.4±3.8bc</td>
<td>3.5±0.5b</td>
</tr>
<tr>
<td>C (n=6)</td>
<td>0.7±0.2a</td>
<td>32.4±3.9*a</td>
<td>5.4±1.1b</td>
<td>17.9±1.9bc</td>
<td>8.4±2.9ab</td>
</tr>
<tr>
<td><strong>Luteal cyst</strong></td>
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</tr>
<tr>
<td>(n=5)</td>
<td>0.9±0.2a</td>
<td>44.5±19.5*a</td>
<td>3.5±0.9bc</td>
<td>13.1±1.1c</td>
<td>4.0±0.8b</td>
</tr>
<tr>
<td><strong>Cystic corpus luteum</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=5)</td>
<td>1.4±0.5abc</td>
<td>25.2±7.0*a</td>
<td>14.1±2.6d</td>
<td>19.0±1.7b</td>
<td>4.2±1.0bc</td>
</tr>
</tbody>
</table>

Values are means ± S. E. M.  
Within rows, values with different superscript letters are significantly different (p<0.05).

* 4 cows were used.

Plasma concentrations of LH and FSH (Table 2)

In normal cows, plasma concentrations of LH and FSH were maintained at basal levels during the follicular and luteal phases of the estrous cycle. There was no
significant difference in plasma levels of LH between the follicular and luteal phase. The mean plasma LH levels in cows with types A and B follicular cyst were significantly higher than those in normal cows, whereas the levels in cows with type C follicular cysts showed non-significant high values as compared with those of normal cows. The mean plasma concentrations of LH in cows with both follicular cysts and corpus luteum, with luteal cysts or with cystic corpora lutea were within basal levels of normal cows. Mean plasma concentrations of FSH in cows with various types of ovarian cysts, however, were similar to those in normal cows.

**Plasma concentrations of progesterone, testosterone and estradiol-17β** (Table 2)

The mean plasma concentrations of progesterone in the luteal phase of normal cows were significantly higher than that in follicular phase. Plasma levels of progesterone in cows with any types of follicular cysts were low and comparable to that in the follicular phase of normal cows. However, in cows with both follicular cysts and corpus luteum, with luteal cysts or with cystic corpora lutea, plasma progesterone levels were significantly higher than those observed in cows with follicular cysts but comparable to those found in the luteal phase of normal cows.

The mean plasma concentrations of testosterone in the follicular phase of normal cows were significantly higher than in the luteal phase. Plasma concentrations of testosterone were similar among cows with any type of ovarian cysts, but the levels were significantly lower than those found in the normal follicular phase.

The mean plasma concentrations of estradiol-17β in normal cows were significantly higher in the follicular phase than the luteal phase. Plasma concentrations of estradiol-17β in cows with type A follicular cyst showed high values with a wide range of variation, whereas the values in cows with luteal cysts or cystic corpus luteum were low and similar to those found at the luteal phase in normal cows.

**Discussion**

The present results indicate that plasma concentrations of FSH in cows with ovarian cysts were not high but almost similar to the basal levels during the normal estrous cycle of the cow. LH levels in cows with follicular cysts, on the other hand, showed higher values than those in normal cows during follicular and luteal phases, and further an inverse relationship between plasma concentrations of LH and progesterone was noted.

Concentrations of progesterone in the plasma are generally low in cows with follicular cysts and higher in cows with luteal cysts due to partial luteinization of the cyst (Kesler and Garverick, 1982). Concentrations of estradiol-17β and LH in the plasma are variable in cows with ovarian cysts, depending on the type of the cyst (Kittok et al., 1973; 1974; Glencross and Munro, 1974; Mori et al., 1974; Cantley et al., 1975; Garverick et al., 1976; Sequin et al., 1976; Kesler et al., 1977; Dobson et al., 1977; Kesler et al., 1980). With regard to FSH, Mori et al. (1980) showed the higher levels of FSH in cows with ovarian cysts than those in normal cycling cows except the time of gonadotropin surge, though Dobson et al. (1977) found similar levels of plasma FSH in cows with follicular or luteal cysts as those in the normal estrous cycle.

In the present study, as shown in Table 1, inhibin activity was noted in the follicular fluid of cystic follicles except type C, suggesting that granulosa cells of cystic follicles still have the ability to secrete inhibin. Therefore, ovarian inhibin from these cystic follicles may be involved in the maintenance of FSH secretion at the basal level. Though no inhibin activity was detected in cystic fluid of type C follicular cysts, luteal cysts or cystic corpora lutea, FSH levels in the plasma remained low. In some of these cows with ovarian cysts, follicles were detected by rectal palpation. These follicles may be a source of inhibin to suppress FSH secretion at the basal level. Resolution of this problem awaits measurement of inhibin levels in the peripheral plasma in these cows. Granulosa cells of the cow have been shown to be a source of inhibin (Henderson and Franchimont, 1981; Rogers et al., 1989) as in other kinds of animals (de Jong, 1988). Luteinization of granulosa cells in culture was accompanied by a reduction in their inhibin production and an inverse relationship existed between inhibin and progesterone production by these culture cells. In addition, bovine corpus luteum cells in culture failed to produce detectable amounts of inhibin (Henderson and Franchimont, 1981). Inhibin activity and concentrations of progesterone in the cystic fluid of the present study agreed with these previous findings. Bovine corpus luteum may have no function to secrete an enough amount of inhibin to play a role in regulation of FSH secretion, though corpus luteum of the macaques (Fraser et al., 1989) and human (Davis et al., 1988) may be another source of inhibin.
Kesler et al. (1980) reported that ovarian cysts are not static structures and that luteinization of follicular cysts occurs in some untreated cows. In the present results, follicular cysts originally classified as type A, in which granulosa cells showed high activity to secrete inhibin as well as estradiol-17β, may be subsequently transformed into type B of lower activity of granulosa cells, with local luteinization by relatively high levels of LH stimulation. Cystic follicles classified as type C with little endocrine function may be rather unusual, and most of this type of cystic follicles with no inhibin activity may contain fluid with high levels of progesterone due to luteinization of the theca. But the degree of luteinization of these cystic follicles may not be sufficient to increase plasma levels of progesterone similar to the level of the luteal phase in normal cows.

In women with polycystic ovarian diseases, many papers have documented elevated serum levels of LH while serum FSH is usually within or below the normal range in the menstrual cycle (Yen et al., 1970; Gambrell et al., 1973; Aono et al., 1974; De Vane et al., 1975; Kletzky et al., 1975; Rebar et al., 1976). Using frequent blood sampling for gonadotropin measurement several investigators have found increased LH pulse amplitude and frequency in women with polycystic ovarian disease (Santen and Bardin, 1973; Rebar et al., 1976; Scaglia et al., 1976; Wentz et al., 1976; Baird et al., 1977; Laatikainen et al., 1983; Burger et al., 1985; Kazer et al., 1987; Dunai et al., 1988; Waldstreicher et al., 1988). And further, polycystic follicles contain normal inhibin activity in their follicular fluid in spite of reduced numbers of granulosa cells (Tanabe et al., 1983).

Endocrine profiles of cows with ovarian follicular cysts in the present results were similar to those found in women with polycystic ovarian disease, though high concentrations of testosterone in the plasma were not observed in these cows.

Although several different hypotheses have been suggested as possible causes of ovarian cysts formation, Kesler and Garverick (1982) suggested that ovarian cysts developed when hypothalamus and pituitary appeared to be less responsive in releasing LH under the influence of estradiol. Nadaraja and Hansel (1976) suggested that premature LH release or an insufficiency of LH at the time of ovulation may result in ovarian cystic diseases in the cow. In the rhesus monkey Ferin et al. (1984) demonstrated that the reduction in luteinizing hormone releasing hormone (LH-RH) pulse frequency was associated with a progressive decline in follicular development and anovulation. Increasing the LH-RH pulse frequency also resulted in the delay of the next ovulation for a long time. Ferin et al. (1984) have suggested that a reduction in the frequency of the LH-RH oscillator during the luteal phase is necessary for normal gonadotropin secretion for cyclicity in the rhesus monkey. Appropriate changes in LH-RH secretion by its oscillator may also be necessary for the recurrence of regular estrous cycles in the cow, and patterns of LH-RH release may not be altered as a result of the cystic conditions (De Silva and Reeves, 1988).

In conclusion, cows with follicular cysts showed an increase in LH secretion and a normal range of FSH levels as compared with those in normal cycling cows during the period of basal gonadotropin levels of the estrous cycle. The presence of inhibin activity in the fluid of cystic follicles suggests the involvement of ovarian inhibin in the suppression of FSH secretion in cows with ovarian cysts. Bovine corpus luteum seems to be not an active site of inhibin secretion.

Acknowledgments

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卵巣囊腫牛の下垂体および卵巣機能について

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臨床症状が明らかである乳牛の個体別囊腫内貯液中インヒビン、プロジェステロン、テストステロンおよびエストラジオール-17β濃度および同一牛の血液中のFSH、LH、ステロイドホルモンを測定し、正常に発情周期を示す乳牛の値と比較検討した。牛卵胞囊腫は前報の如く顆粒層細胞の機能によって3つの型、即ち、卵胞機能亢進型（A）、卵胞機能退行型（B）及び内分泌性喪失型（C）に分類した。その結果、次の事実が判明した。

1. 血中 LH 濃度は、卵胞囊腫の型により若干の差はみられるが、いずれの囊腫型においても発情周期中の基底レベルよりも高く、黄体囊腫および囊腫様黄体では基底レベルと差のない値を示した。血中 LH 濃度は、血中プロジェステロン濃度と負の相関関係を示した。

2. 血中 FSH 濃度は、全ての卵胞囊腫、黄体囊腫、囊腫様囊腫の場合で発情周期中の基底レベルと差のない値を示した。

3. 囊腫卵胞液中のインヒビン活性の高い牛のみならず、囊腫卵胞液中にインヒビン活性を認められないものでも FSH 濃度が基底レベルに抑制されていた。これは囊腫卵胞以外に存在する卵胞からのインヒビン分泌が関与するものと推察された。

4. インヒビン活性を欠くC型卵胞囊腫内貯液にはプロジェステロンレベルの高いものが多いて、内分泌機能を失うものは稀であった。従って、C型卵胞囊腫は顆粒層細胞機能異常型と表現する方が適正であると判断された。