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Studies on Cystic Ovarian Disease in Dairy Cattle

I. Adrenocortical Function, Serum Concentratins of Sex steroids and Serum Protein Pattern in Cows with Normal Estrous Cycles and Cows with Cystic Ovaries

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乳牛の卵胞嚢腫に関する研究

I. 卵胞嚢腫牛における副腎皮質機能，血中性ステロイド値並びに血清蛋白像について

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Introduction

Cystic ovarian disease (COD) is one of the commonest and the most serious reproductive disorders in dairy cattle. Cystic degeneration of ovaries is often associated is with either advanced estrous activities or irregular estrus or anestrus, and the recovery is very remote without proper treatment.

Several authors, so far, have described the endocrine changes associated with this disease, postulating their own theories. GARM (1949) carried out the large scale histological examination of major endocrine organs of cows with COD and reported hypertrophy of the adrenal cortex and the pituitary. Later, WAYMAN (1952) observed high levels of total serum protein, especially gamma-globulin and beta-globulin, and suggested possible relationship to adrenocortical function. Recently, MIYAZAWA (1971) suggested declined adrenocortical function in these cows by estimating serum 11-hydroxycorticosteroids (11-OHCS) and its response to exogenous adrenocorticotropic hormone (ACTH). YAMAUCHI (1954) studied pituitary contents of lutenizing hormone (LH) and follicle-stimulating hormone (FSH), and found excess FSH and a lack of LH, thereby concluding that low LH might be contributary to the onset of COD. SHORT (1961) estimated steroid concentrations in ovarian cyst fluids and found no significant correlation between

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steroips and behavioral characteristics of cows with COD. However, SCHJERVEN (1971) suggested that there was a connection between estrous behavior and estrogen concentrations in the cyst fluid by determining estrone and estradiol. GRUNERT (1964) and LUNAAS (1974) demonstrated higher contents of estrogens in the urine of cows with COD, although no correlation was observed between estrogens and estrous behavior by the former author. KITTOK (1972) and GLENCROSS (1974) reported low levels of serum progesterone and high levels of estrogen in cows with COD as compared with those in cycling cows.

As described above there seemed to be no general agreement on the aetiology of COD. And a relatively amall amount of data has been available regarding the relati- of major endocrine disturbance and estrous behavior.

The purposes of the present study were, therefore, to determine adrenocortical function, serum concentrations of progesterone, estrone, estradiol-17 β and estriol, and to describe their relationship to estrous behavior.

Materials and Methods

A total of 112 Holstein Friesian cows including 2 heifers diagnosed as having COD were used between the periods September 1972 – April 1973 and January 1974 – April 1974. These cows from herds belonging to commercial dairy farms around Obihiro Universty of Agriculture and Veterinary Medicine were offered for the present study. Diagnosis of COD was made on the basis of a rectal examination and the estrous behavior of the cow. The animals were divided into 3 groups and each group was classified further into subgroups as follows according to estrous behavior.

Group I: Animals with continuous or intense estrus.
1. Animals exhibiting true nymphomania (5 cases).
2. Animals showing continuous estrus (25 cases).

Group II: Animals with irregular or regular estrus.
1. Animals exhibiting estrus at different intervals (23 cases).
2. Animals showing estrus with normal cycles (15 cases).
3. Animals in first estrus postpartum (5 cases).

Group III. Animals without estrus for more than 45 days.
1. Animals showing anestrus since calving (22 cases).
2. Animals showing anestrus following estrus (17 cases).

Age and days after calving at the time of diagnosis of animals in each group are shown in Table 1.

Fifty- four cows with normal estrous cycles and with no abnormalities of genital organs, from the University dairy, and commercial dairy farms were used as a control.

Blood sampling

Blood samples were collected from the jugular vein of 112 cows with COD between 9 and 12 o'clock in the morning at the time of diagnosis. Sixty cows of these were subjected to a rapid ACTH test, and the corresponding 60 donar cows were subjected to

a blood sampling once again 30 minutes after ACTH injection. Thus the total number of blood samples from cows with COD was 172. Blood was taken also from the jugular vein of 54 control animals during the same period in the morning. Of this number, the blood of 11 cows sampled again after ACTH administration. Special emphasis was placed on the day of sampling regarding the estrous cycles in the case of 15 cows which were sampled on the day of estrus, just before insemination, and the 10th day of estrus.

Serum samples

Blood samples were stood at room temperature for 9 to 12 hours and serum was collected by centrifugation (3,000 rpm for 10 minutes). Serum thus obtained was stored at −20°C until further analysis. The number of serum samples subjected to each examination is given Table 2.

Table 1. Age and days after calving at the time of diagnosis of cows with cystic ovaries classified according to estrous behavior.

Group	Subgroup	Number of cows	Age (years)	Interval after calving (days)
Group I	True nymphomania	5	6.2±1.1	739±1089
	Continuous estrus	25	5.3±2.0	153± 97
	Total	30	5.4±1.9	251± 470
Group II	Irregular estrus	23	6.2±2.4	132± 68
	Regular estrus	15	5.9±2.7	154± 106
	First estrus postpartum	5	5.0±1.7	103± 63
	Total	43	6.0±2.3	133± 80
Group III	Anestrus since calving	22	5.8±1.5	98± 37
	Anestrus after estrus	17	4.8±1.4	238± 105
	Total	39	5.3±1.5	157± 101
Grand total		112	5.7±2.0	151± 104

Table 2. Number of materials from cows with normal estrous cycles (controls) and cows with cystic ovaries subjected to determination of 11-hydroxycorticosteroids (11-OHCS) and its response to ACTH, sex steroids and serum proteins.

	No. of cows	11-OHCS	11-OHCS response to ACTH	Progesterone	Estrone	Estradiol -17β	Estriol	Serum proteins
Group I	30	30	20	29	23	14	23	27
Group II	43	41	24	38	28	28	28	32
Group III	39	39	16	36	30	30	30	34
Total	112	110	60	103	81	72	81	93
Controls	54	29	11	18	8	8	8	31

Determination of serum 11-OHCS

Serum concentrations of 11-OHCS were estimated by a modified fluorimetric method as described by USUI, *et al* (1970). Centrifugation was included in the procedure for extraction of corticosteroids by the authors.

Reagents and fluorimetric instruments used were as follows.

a. Methylene chloride (Super Special Grade): Kanto Chemicals.
b. 0.1N Sodium hydroxide
c. 0.1N Sodium hydroxide containing 10 g sodium sulfate (Anhydrous) in 100 ml.
d. Sodium sulfate solution 10 g/dl
e. Fluorescent reagent: Ethanolic-sulfuric acid solution (3 : 7)
   Ethyl alcohol (Super Special Grade): Wako Pure Chemical Industries LTD.
   Sulfuric acid (Super Special Grade): Wako Pure Chemical Industries LTD.
f. Hydrocortisone standard solution (50 μg/dl)
   Hydrocortisone: BDH Chemicals LTD, England.
g. Fluorimetry spectrometer (SHIMAZU KOTAKI-UM-S)
   Primary filter AKA UV-V_2
   Secondary filter AKA UV-V_2

Procedure for determination is outlined in Fig. 1.

Serum concentrations of 11-OHCS were calculated according to the following formula.

$$\text{11-OHCS } (\mu g/dl) = \frac{F - B}{S - B} \times 50$$

F: Fluorescence of sample
S: Fluorescence of hydrocortisone standard
B: Fluorescence of water blank

serum 1.0 ml
- Diluted with 4.0 ml water.
- Added 0.2 ml 0.1N-NaOH.

Extraction
- Added Methylene chloride, 10 ml.
- Stood at −4°C for 15 minutes.
- Shook vigorously for 10 seconds.
- Centrifuged at 3,000 rpm for 10 minutes.
- Discarded upper layer.
- Transfered to another tube.

Washing
- Added 2 ml 0.1N-NaOH solution containing Na_2SO_4 (10 g/dl).
- Shook vigorously for 10 seconds.
- Discarded upper layer.
- Added 2 ml Na_2SO_4 solution (10 g/dl).
- Shook vigorously for 10 seconds.
- Discarded upper layer.
- Transfered 5 ml washed extract to another tube.

Fluorimetry

Added 5 ml Ethanolic sulfuric-acid solution.
Shook vigorously for 10 seconds.
Stood at −4°C for 45 minutes.
Discarded upper layer.
Read fluorescence.

Fig. 1. Procedure for the determination of serum 11-hydroxycorticosteroids.

serum 1.0 ml
Added steroid-^{3}H.
Extraction
Added 10 ml ether.
Shook vigorously.
Discarded serum layer.
Washing
Added 1 ml water.
Shook vigorously.
Transfered ether layer to another tube.
Drying
Microcolumn chromatography (2 g Sephadex LH-20)
Added 0.1 ml Benzen methanol.
Shook.
Applied to microcolumn.
Added 0.1 ml Benzen methanol.
Shook.
Applied to microcolumn.
Chromatography*

*Chromatography
Elution pattern (Fig. 4)

0−0.6 (0.6)	Wash
0.6−1.4 (0.8)	P
1.4−1.6 (0.2)	Wash
1.6−2.4 (0.8)	E_1
2.4−2.8 (0.4)	Wash
2.8−4.2 (1.4)	E_2
4.2−5.2 (1.0)	Wash
5.2−7.4 (2.2)	E_3

1/2 Assay
Added steroid-^{3}H, 10,000 dpm.
Incubated with diluted antiserum.
Added 0.25 ml antiserum.
Shook slowly.
Stood at room temperature for 30 minutes.
Added 0.25 ml $(NH_4)_2SO_4$.
Shook slowly.
Stood at room temperature for 10 minutes.
Centrifuged at 2,000 − 2,500 rpm for 10 minutes.
Transfered upper layer to counting vial tube (0.2 ml).
Added approximately 12 ml dioxan scincillator.
Counted for tritium activities by liquid scincillation counter.

1/2 Recovery
Counted for ^{3}H.

Fig. 2. Procedure for the determination of sex steroids.

Determination of progesterone, estrone, estradiol-17β, and estriol

Serum concentrations of sex steroids were estimated by a radioimmunoassay as described by MAKINO (1973) and also by a simplified radioimmunoassay reported by the same author and others (1973).

serum 0.1 ml
Added progesterone -^{3}H 1,000 dpm.
Extraction
Added 2 ml ether.
Shook vigorously.
Discarded serum layer.
Washing
Added 2 ml water.
Shook vigorously.
Transfered ether layer to another tube.
Assay
Added progesterone-^{3}H 10,000 dpm.
Dried.
Same procedure for assay as shown in Fig. 2.

Fig. 3. Procedure of a simplified radioimmunoassay for serum progesterone.

Reagents and solvents used were as follows.

(1) Non radioactive steroids
a. progesterone: 4-pregnene-3, 20-dione; Merk.
b. estrone: 3-hydroxyestratrien [1, 3, 5 (10)]-on-(7); Merk.
c. estradiol-17β: estradien [1, 3, 5 (10)]-diol (3, 17β); Merk.
d. estriol: estratrient [1, 3, 5 (10)]-triol-(3, 17β); Merk.

(2) Radioactive steroids
a. progesterone-1, 2-^{3}H, 53 Ci/mM; The Radiochemical Center.
b. estrone-6, 7-^{3}H, 40 Ci/mM; New England Nuclear.
c. estradiol-6, 7-^{3}H, 40 Ci/mM; The Radiochemical Center.
d. estriol-6, 7-^{3}H, 42.2 Ci/mM; New England Nuclear.

(3) Antiserum
a. progesterone antiserum (Lot 11-7); Teikoku Hormone Mfg. Co. Ltd.
b. estrone antiserum (Lot 10-6); Teikoku Hormone Mfg. Co. Ltd.
c. estradiol-17β antiserum (Lot 22-7); Teikoku Hormone Mfg. Co. Ltd.
d. estriol antiserum (Lot 24-7); Teikoku Hormone Mfg. Co. Ltd.

(4) Borate buffer

Boric acid	12.405 g		
Potassium chloride	14.912 g	→ 50 ml	
Water	1,000 ml		
1/5N Sodium hydroxide		2.61 ml	pH 8.0
Water		107.39 ml	

(5) Bovine serum albumin crystalline; Nutritional Biochemical Cooperation.
(6) Bovine gamma fraction II; Miles Laboratory Incorporated.
(7) Sephadex LH-20

(8) Dioxan scintillator

POPOP: 1, 4-Bis [2-(5-phenyloxazolyl)] benzene 250 mg

DOP: 2, 5-Diphenyloxazole 10 g

Naphtalene 100 g

Dioxan 1,000 ml

(9) Benzene methanol (85 : 15)

(10) 50 % $(NH_4)_2SO_4$

(11) Liquid scintillation counter

Antiserum was diluted with borate buffer containing 0.06 % bovine serum albumin and 0 05 % bovine serum globulin. Dilution of each steroid antiserum was as follows; 1 : 20,000-40,000 for progesterone, 1 : 200,000-300,0000 for estrone, 1 : 50,000-60,000 for estradiol 17β and 1 : 30,000-40,000 for estriol, respectively.

Procedure for the determination of sex steroids, and a simplified method for progesterone assay are shown in Fig. 2 and 3. Chromatographic pattern of steroids in the sephadex column is given in Fig. 4.

All glassware was rinsed with methanol prior to use in the assay.

Standard displacement curves were obtained by plotting percentage of unbound steroid against pg of each steroid added (Fig. 5). Thereafter concentrations were estimated from the standard calibration curve.

Recovery rates were between 75 % - 100 %. Water blank was 0 - 13 pg.

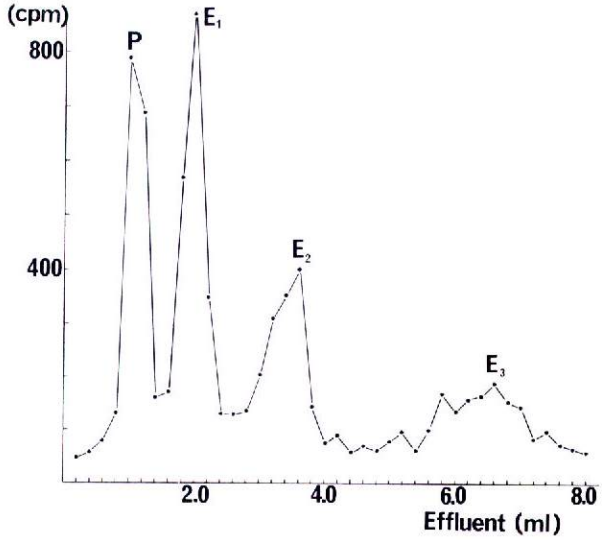

Fig. 4. Chromatographic pattern of steroids in the sephadex column.
Column; Sephadex LH-20, 2 mg
Solvent; Benzene Methanol (85 : 15)

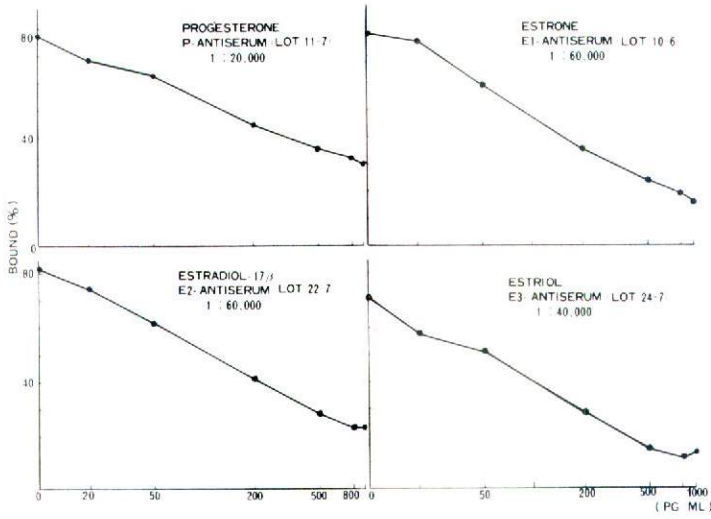

Fig. 5. Standard curves: obtained by plotting percentage of unbound steroid against pg of each steroid added.

Determination of total serum protein and serum protein fractions

Total serum protein concentrations were estimated by a refractometric instrument (Hitachi Hand Refractometer).

In order to fractionate protein, electrophoresis was carried out on cellulose acetate strips Sartrius Membrane Filter; Beckman) using Beckman Microzone System (Beckman, USA) with veronal buffer (pH 8.6, $\mu = 0.07$). Electrophoresis was conditioned by 250 V constant voltage and a 19 minutes running time. The relative densities of the fractions obtained were determined by means of a recording densitometer with an automatic integrator for this system.

A rapid ACTH test

Animals were kept under quiet and restful conditions. Blood samples were taken in the morning, and synthetic ACTH, β^{1-24} corticotropin (Tetracosactide) (Cortrosin Injection, DAIICHI Pharmachemical Co. Ltd.), 0.5 mg, equivalent to 50 IU natural ACTH, was injected intramuscularly in 5 ml physiological saline solution, soon after. Thirty minutes after the ACTH injection blood was collected again.

Results

1. Serum 11-OHCS and the response to ACTH.

(1) Serum concentrations of 11-OHCS in cows with normal estrous cycles and cows with COD.

Mean values of serum 11-OHCS in cows with normal estrous cycles and cows with COD are shown in Table 3. No significant differences were observed between controls

and cows with COD or among the groups and subgroups divided by estrous behavior.

(2) Response of serum 11-OHCS to ACTH.

Serum concentrations of 11-OHCS prior to and 30 minutes subsequent to ACTH administration in cows with normal estrous cycles and cows with COD are presented in Fig. 6 and summarized in Table 4.

A significant rise in 11-OHCS was evident in all control cows following intramuscular injection of ACTH. Mean values of serum 11-OHCS increased (following ACTH injection) more than two fold of those before ACTH injection. Significant differences were observed in serum concentrations of 11-OHCS prior and 30 minutes subsequent to ACTH injection ($P \leq 0.01$).

In cows with COD serum 11-OHCS response to ACTH was lower than in control cows. Mean serum 11-OHCS increments following ACTH injection in cows with COD were significantly lower ($P \leq 0.05$) than in the controls. However response to ACTH

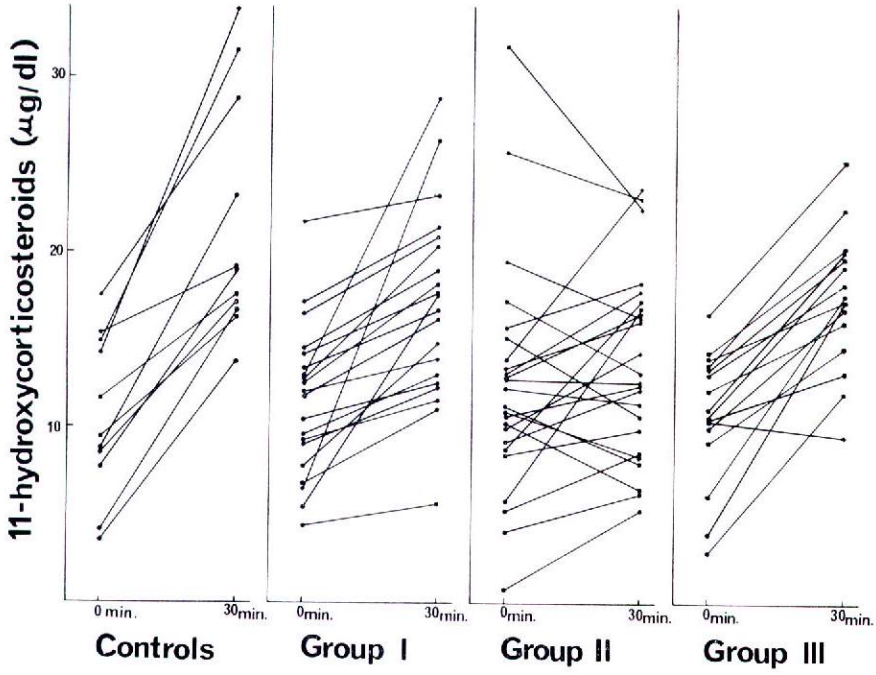

Fig. 6. Response of serum 11-OHCS to ACTH (β^{1-24} corticotropin 0.5 mg) injected intramuscularly in cows with normal estrous cycles and cows with cystic ovaries.

Group I: Cows with cystic ovaries exhibiting continuous or intense estrus.

Group II: Cows with cystic ovaries showing irregular or regular estrus.

Group III: Cows showing anestrus.

differed among cows having different patterns of estrous behavior.

Group I : Continuous or intense estrus

With the exception of 3 cows whose response was as high as the controls, all cows belonging to this group showed lowered response to ACTH as compared to the controls. No significant differences were observed in response to ACTH among cows exhibiting true nymphomania and cows showing continuous estrus. There seems to be a trend where serum 11-OHCS response to ACTH of cows with continuous or intense estrus is higher than that of cows with an irregular or regular estrus, and lower than that of cows showing anestrus.

Group II : Irregular or regular estrus

Along with 9 cows, where serum 11-OHCS did not respond to ACTH and decreased following ACTH injection, cows with irregular estrus showed significantly lower response ($P \leq 0.05$) to ACTH than the controls. 11-OHCS response to ACTH in these cows was considerably lower than in continuous or intense estrous cows and significantly lower than in anestrous cows ($P \leq 0.05$).

Group III : Anestrus

With the exception of one cow which showed a decrease in serum 11 OHCS following ACTH injection, cows showing anestrus had higher 11 OHCS response to ACTH than cows with continuous estrus or irregular estrus. The response of anestrouscows was not found in a remarkable difference from those of the controls, although being a little lower than in the controls.

Table 3. Serum concentrations of 11-hydroxycorticosteroids (11-OHCS) in cows with normal estrous cycles and cows with cystic ovaries.

Group	Subgroup	Number of cows	Serum 11-OHCS (μg/dl)
Controls		29	11.56±5.01
Group I	True nymphomania	5	11.48±6.41
	Continuous estrus	25	10.64±3.66
	Total	30	10.78±4.10
Group II	Irregular estrus	22	11.99±5.19
	Regular estrus	15	12.13±6.94
	First estrus postpartum	4	12.50±3.85
	Total	41	12.09±5.67
Group III	Anestrus since calving	22	10.90±3.25
	Anestrus after estrus	17	11.24±4.19
	Total	39	11.05±3.64
Grand total		110	11.36±4.61

(Mean ± S.D.)

Table 4. Serum concentrations of 11-hydroxycorticosteroids and the response to ACTH in cows with normal estrous cycles and cows with cystic ovaries.

	Number of cows	Serum 11-hydroxycorticosteroids (μg/dl)		
		0 minute	30 minutes	increment
Controls	11	10.58±4.82	21.64±6.86	11.06±4.71
Group I				
True nymphomania	5	11.48±6.41	16.48±4.26	5.00±4.87
Continuous estrus	15	11.35±3.62	17.27±6.14	5.93±5.20
Total	20	11.38±4.28	17.08±5.63	5.70±5.01
Group II				
Irregular estrus	11	12.30±6.50	15.31±5.96	3.02±4.52
Regular estrus	11	12.73±7.96	13.09±4.73	3.05±4.52
First estrus postpartum	2	13.15	9.50	−3.65
Total	24	12.57±6.81	13.81±5.32	2.48±4.62
Group III				
Anestrus since calving	4	12.53±2.81	19.33±5.26	6.80±3.28
Anestrus after estrus	12	10.52±4.00	17.28±3.59	6.76±3.83
Total	16	11.02±3.75	17.79±3.98	6.77±3.59
Grand total	60	11.76±5.29	15.96±5.34	4.70±4.82*

* Significantly lower than in the control ($P \leq 0.05$). (Mean ± S.D.)

2. Serum concentrations of progesterone, estrone, estradiol 17β and estriol.

Concentrations of sex steroids in the peripheral blood of cows with normal estrous cycles and cows with COD are summarized in Fig. 7. Mean values of progesterone in normal cows at the luteal phase were significantly higher ($P \leq 0.01$) than those of the cows at the follicular phase. However there was no significant difference in estrone, estradiol-17β, or estriol between luteal phase and follicular phase, although these estrogens tended to be a little higher at follicular phase than at luteal phase.

In the case of sex steroid concentrations in the peripheral blood of cows with COD, the values differed among groups and subgroups divided by estrous behavior.

Group I : Continuous or intense estrus

In 4 cows exhibiting true nymphomania, serum progesterone was much lower than in the controls at the follicular phase and in the 2 cases where determined estrogens, estrone and estradiol-17β were remarkably higher than those of the controls at the follicular phase. Estriol concentrations, however, were lower than those of the controls at the follicular phase. In 25 cows with continuous estrus, progesterone concentrations did not differ markedly from the values of the controls at the follicular phase, while estrone concentrations were remarkably higher and estradiol-17β concentrations were significantly higher than those of the controls at the follicular phase ($P \leq 0.01$).

Group II : Irregular or regular estrus

Out of 38 cows with irregular or regular estrus, 18 cows examined during estrus

had lower concentrations of progesterone and higher concentrations of estradiol-17β than the 20 cows examined during diestrus. As compared with those of normal cycling cows at the follicular phase, estradiol-17β concentrations were considerably higher, whereas progesterone concentrations were almost equal in those cows examined during estrus. In contrast to these findings, cows examined during diestrus showed remarkably higher concentrations of progesterone and lower concentrations of estrone and estriol than the control cows at the follicular phase. When the steroid concentrations in cows belonging to this group were compared to the values of those having continuous or intense estrus, it was observed that in the case of progesterone, the concentrations were lower in cows examined during estrus and higher in cows examined during diestrus than those of cows with continuous estrus. Significant differences were observed in estrone, estradiol-17β and estriol among cows showing continuous estrus and those exhibiting irregular or regular estrus ($P \leq 0.05$).

Group III : Anestrus

In anestrous cows with COD, progesterone concentrations were significantly higher

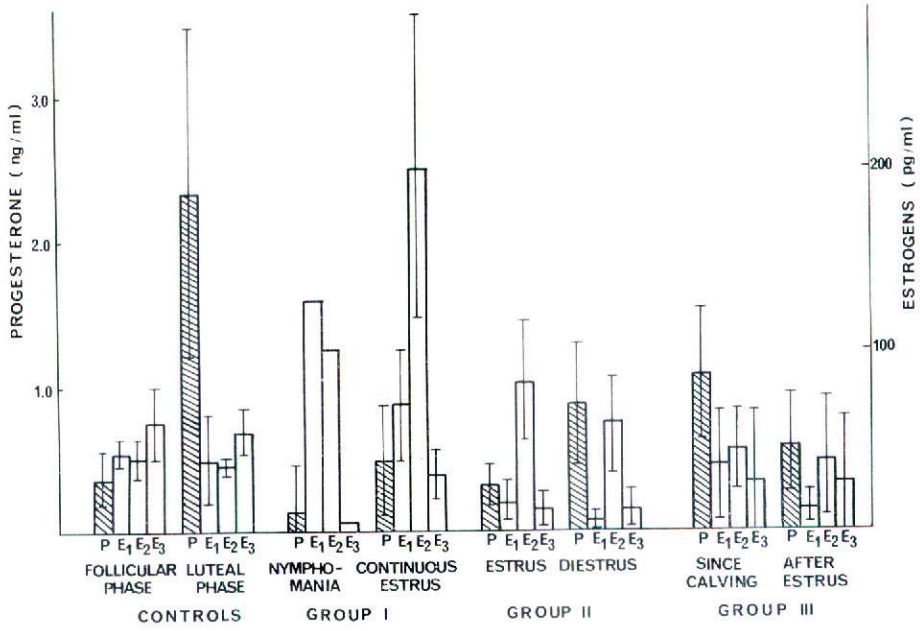

Fig. 7. Serum concentrations of progesterone (P), estrone (E_1), estradiol-17β (E_2) and estriol (E_3) in cows with normal estrous cycles and cows with cystic ovaries at the time of diagnosis.

Group I: Cows with cystic ovaries exhibiting continuous or intense estrus.

Group II: Cows with cystic ovaries showing irregular or regular estrus.

Group III: Cows with cystic ovaries showing anestrus.

($P \leq 0.05$) when compared to the controls at the follicular phase, while the concentrations were remarkably lower when compared to the luteal phase. The concentrations of estrone and estriol in anestrous cows were considerably lower than the controls both at the follicular phase and the luteal phase, though estradiol 17β concentrations did not differ.

When compared these values in anestrous cows to those in cows with continuous estrus, progesterone concentrations were remarkably higher and estrone, estradiol-17β and estriol concentrations were much lower in anestrous cows. A significant difference ($P \leq 0.01$) was observed in estradiol-17β concentrations between continuous estrous cows and anestrous cows. Compared to steroid concentrations of cows showing irregular or regular estrus which were examined during estrus, progesterone concentrations were significantly higher ($P \leq 0.05$), but estradiol-17β concentrations were considerably lower in anestrous cows. When compared with cows examined during diestrus, it was found that progesterone concentrations were almost equivalent and estradiol-17β concentrations were slightly lower in anestrous cows.

3. Total serum protein and serum protein fractions.

Serum protein values of cows with normal estrous cycles and those with COD are shown in Table 5 both as an absolute value and as a percentage of total protein. Total serum protein values of cows with COD were significantly higher ($P \leq 0.05$) than those of control cows. Among the protein fractions, γ-globulin, both as an absolute value and as a percentage, was higher in cows with COD than in control cows. There was a significant difference in this value, when taken as an absolute value, between cows with COD and control cows ($P \leq 0.05$). Absolute values of albumin in cows with COD were higher than in the controls, while no difference was observed in these values as a percentage.

In cows showing true nymphomania, the total serum protein values and γ-globulin as an absolute value were significantly higher ($P \leq 0.05$) than in the controls. In the case of cows with anestrus since calving, total protein values were significantly higher ($P \leq 0.05$) than in the controls. However γ-globulin values were not significantly higher, and albumin values, as an absolute value, were significantly higher ($P \leq 0.05$) as compared with those of the controls. In the comparison of protein values of cows with true nymphomania and cows showing anestrus since calving, albumin values, both as an absolute value and as a percentage were remarkably higher, while γ-globulin values were considerably lower in anestrous cows than in nymphomaniac cows. No other significant differences in protein values were detected among cows having different patterns of estrous behavior.

Values of total serum protein and γ-globulin, as an absolute value and a percentage of total protein increased with age, while albumin values as a percentage and albumin globulin ratios decreased with age in cows with COD. Significant correlations were

observed between age and total protein values ($n=93$, $r=0.47$, $P\leqq0.01$), between age and γ-globulin values, both as an absolute value ($n=93$, $r=0.46$, $P\leqq0.01$), and a percentage ($n=93$, $r=0.41$, $P\leqq0.01$) between age and albumin values as a percentage ($n=93$, $r=-0.35$, $P\leqq0.01$),and betweem age and albumin globulin ratio ($n=93$, $r=-0.32$, $P\leqq0.01$), respectively.

Table 5. Total serum protein and serum protein fraction values in cows with normal estrous cycles and cows with cystic ovaries.

	Number of cows		Total protein	Albumin	Globulin			A/G
					α-globulin	β-globulin	γ-globulin	
Controls	31	g/dl	7.0±0.6	3.57±0.34	0.91±0.20	0.66±0.15	1.91±0.29	1.06
		%		50.8± 2.6	13.0± 2.3	9.3± 1.8	27.0± 3.5	±0.18
Group I								
True nymphomania	4	g/dl	7.8±0.4*	3.46±0.29	0.92±0.09	0.72±0.08	2.65±0.45*	0.82
		%		44.7± 3.8	11.9± 1.4	9.3± 0.7	34.1± 5.0	±0.12
Continuous estrus	23	g/dl	7.6±0.7	3.77±0.46	0.87±0.13	0.69±0.10	2.26±0.50	1.01
		%		49.8± 5.3	11.6± 1.8	9.2± 1.4	29.6± 5.0	±0.22
Total	27	g/dl	7.6±0.7	3.72±0.45	0.88±0.12	0.70±0.12	2.32±0.50	0.99
		%		49.1± 5.3	11.6± 1.7	9.2± 1.3	30.3± 5.2	±0.22
Group II								
Irregular estrus	20	g/dl	7.3±0.5	3.61±0.37	0.87±0.16	0.65±0.14	2.15±0.58	1.01
		%		49.7± 5.8	12.0± 2.0	8.9± 1.8	29.3± 6.4	±0.22
Regular estrus	10	g/dl	7.5±0.7	3.58±0.43	0.96±0.20	0.64±0.10	2.45±0.80	0.97
		%		47.9± 8.0	12.7± 2.1	8.6± 1.5	30.9± 8.6	±0.32
First estrus postpartum	2	g/dl	7.3	3.67	0.80	0.51	2.32	1.03
		%		50.4	11.0	7.0	31.7	
Total	32	g/dl	7.4±0.6	3.60±0.37	0.90±0.17	0.64±0.12	2.26±0.64	1.00
		%		49.2± 6.4	12.2± 2.0	8.7± 1.7	29.9± 6.9	±0.25
Group III								
Anestrus since calving	20	g/dl	7.7±0.5*	3.91±0.38*	0.90±0.19	0.67±0.19	2.24±0.61	1.07
		%		50.8± 6.1	11.7± 2.5	8.7± 2.3	28.7± 5.9	±0.27
Anestrus after estrus	14	g/dl	7.3±0.5	3.62±0.30	0.90±0.11	0.69±0.13	2.07±0.55	1.01
		%		49.8± 4.9	12.5± 1.5	9.6± 1.9	28.2± 6.0	±0.19
Total	34	g/dl	7.5±0.6	3.79±0.38	0.90±0.16	0.68±0.17	2.17±0.58	1.04
		%		50.4± 5.6	12.0± 2.2	9.1± 2.2	28.5± 5.9	±0.24
Grand total	93	g/dl	7.5±0.6	3.71±0.40	0.89±0.15	0.67±0.14	2.24±0.68*	1.01
		%		49.6± 5.8	12.0± 2.0	9.0± 1.8	29.5± 6.1	±0.24

* Significantly higher than in the control ($P\leqq0.05$). (Mean±S.D.)

Discussion

Adrenocortical function;

Only limited data has been available on adrenocortical function in cattle. Since the developement of quantitative analytical methods of adrenocorticoids in blood, it has become possible to examine adrenocortical function by means of the adrenocorticotropin stimulation test besed on serum concentrations of adrenocorticoids. SHAW (1962, 1963) demonstrated a rapid rise of 17-hydroxycorticosteroids following 200 IU ACTH intramuscular injection in cattle. Later, VENKATASESHU, *et al* (1970) observed a significant

increase of cortisol, 60 minutes subsequent to ACTH 200 IU intramuscular injection. GWAZDAUSKAS, *et al* (1971) also described a remarkable rise of cortisol and a slight rise of corticosterone following intravenous injection of ACTH 200 IU. The response of serum 11 OHCS to ACTH in cattle has also been studied and reported by SASAKI *et al* (1971), MIYAZAWA (1971) and IINO (1973). In this study, the normal range of serum 11-OHCS between 9 A.M. and 12 noon was established as be tween 9.66 μg/dl and 13.46 μg/dl according to 95 % confidence limits of the mean value in cows with normal estrous cycles. And the intramuscular injection of 0.5 mg synthetic ACTH (equivalent to 50 IU natural ACTH) more than doubled the serum concentrations of 11-OHCS of control cows half an hour later. These results corresponded to the findings of VENKATASESHU, *et al* (1970), MIYAZAWA (1971) and IINO (1973).

MIYAZAWA reported a lowered response of serum 11-OHCS to ACTH injected intramuscularly in cows with COD as compared with that in cows with normal estrous cycles, but did not demonstrate the statistical significance of the change. In the present study, a significant difference ($P \leqq 0.05$) in serum 11-OHCS increments 30 minutes subsequent to ACTH injection was detected between cows with COD and cows with normal estrous cycles. From this findings it is understood that the cows with COD appear to have declined adrenocortical function as compared with cows having normal estrous cycles.

Since GARM (1949) reported higher values for adrenal weights in cows showing continuous or frequent estrus than in those which had regular, infrequent or ceased estrus, in this study, serum 11-OHCS response to ACTH was compared among groups classified according to estrous behavior. Anestrous cows had the highest serum 11-OHCS response to ACTH and cows showing irregular or regular estrus had the lowest serum 11-OHCS response, while in cows exhibiting continuous or intense estrus serum 11-OHCS response was higher than in irregular or regular estrous cows and lower than in anestrous cows. These findings indicated a possible adrenal contribution to estrous behavior in cows with COD. Relationship between adrenocortical function and serum concentrations of sex steroids will be discussed on the other paper which is to be published later.

Sex steroids;

Numerous data is available on sex steroid concentrations in the peripheral blood of cattle during the normal estrous cycle. Progesterone concentrations have been observed to increase progressively following ovulation showing a peak between the 11th - 15th day of estrous cycle and then declined sharply 2-4 days before the onset of the next estrus (GARVERICK, *et al* 1971, HENRICKS, *et al* 1972, WAGNER, *et al* 1972, ERB, *et al* 1972, EDGERTON, *et al* 1973, DOBSON, *et al* 1973, DOMEKI, *et al* 1974). In contrast to the change in progesterone concentrations, estrogen concentrations have been known to increase gradually during the 3 days preceeding estrus and to show a sharp peak round about 4 hours before the onset of estrus. Thereafter, estrogen concentrations begin to

decline by the time of onset of estrus and reach minimum levels just before ovulation. Later, another peak of estrogens is shown on the 9 th - 11 th day of estrous cycle (SHEMESH, *et al* 1972, HENRICKS, *et al* 1972, MASON, *et al* 1972). Values of progesterone in cows with normal estrous cycles at follicular phase and luteal phase were in close agreement with the data presented by the above workers. The authors, however, could not demonstrate a significant increase of estrogens at the follicular phase. This was probably due to the time of blood sampling at the follicular phase. In this study, blood sampling was performed at about 12 hours after the onset of estrus, just before insemination, when estrogen concentrations had declined already as low as minimum levels as described by SHEMESH, *et al* (1972).

As shown in Fig. 7, sex steroid concentrations in the peripheral blood of cows with COD differed among groups divided according to estrous behavior. In cows exhibiting true nymphomania associated with obvious anatomical changes such as raising of the tail head and relaxation of the sacro-sciatic ligaments with permanent edema of the vulva, progesterone concentrations were considerably lower, and estrone and estradiol-17β were markedly higher than those in control cows at estrus. Lower serum concentrations of progesterone in cows with COD showing intense estrus were also reported by GLENCROSS (1974) and GRUNERT, *et al* (1974). Higher concentrations of estradiol-17β were evident in cows showing continuous estrus. In contrast to these changes in cows with intense or continuous estrus, relatively higher concentrations of progesterone and lower concentrations of estrogens were observed in anestrous cows. As for cows showing irregular or regular estrus, sex steroid concentrations varied between those examined during estrus and those during diestrus. Thus cows with COD showing estrous symptoms appeared to have higher concentrations of estrogens and almost equivalent or lower concentrations of progesterone as compared with those of control cows at estrus or cows with COD showing no symptoms of estrus. On the other hand, cows with COD showing anestrus, including cows with irregular or regular estrus which were examined during diestrus, had higher concentrations of progesterone and lower concentrations of estrogens in comparison either with cows at normal estrus or cows exhibiting intense or continuous estrus and also with cows with irregular or regular estrus which were examined during estrus. These findings indicate that serum concentrations of progesterone and estrogens, particularly estradiol-17β, are responsible for estrous behavior of cows with COD.

Serum protein;

The increase of total serum protein, particularly of γ-globulin in cows with COD was consistent with the observation of WAYMAN, *et al* (1952). The cause of this increase of serum protein has not been well elucidated. This could be due to high levels of protein intake, although the author could not demonstrate evidence to support this view in the present study. The increase of serum protein may be partly explained by the relationship with adrenocortical function and sex steroid levels in the blood that

will be discussed on the other paper.

Conclusions

1. One hudred and twelve cows with COD were classified into the following groups and subgroups according to estrous behavior; Group I: True nymphomaina (5), Continuous estrus (25), Group II: Irregular estrus (23), Regular estrus (15), First estrus postpartum (5), Group III: Anestrus since calving (22), Anestrus after estrus or insemination (17).

2. Cows with COD had apparently declined adrenocortical function, since serum 11-OHCS increments following intramuscular injection of ACTH in such cows were significantly lower than in cows with normal estrous cycles ($P \leq 0.05$).

3. There were considerable differences in adrenocortical function among the groups classified according to estrous behavior. Serum 11 OHCS response to ACTH was highest in Group III and lowest in Group II. The difference in serum 11-OHCS response between cows belonging to Group III and those belonging to Group II was significant ($P \leq 0.05$).

4. It appeared that serum concentrations of progesterone and estrogens are closely associated with estrous behavior of cows with COD. Progesterone concentrations were relatively higher and estrogens were lower in Group III than in Group I. Cows in Group II examined during estrus had lower concentrations of both progesterone and estrogens than continuous estrous cows in Group I, while cows examined during diestrus showed almost equivalent concentrations of both progesterone and estrogens as cows in Group III. Cows exhibiting symptoms of true nymphomania showed markedly lower concentrations of progesterone and higher estrogens as compared with cows having normal estrous cycles at estrus.

5. Serum concentrations of total protein and γ-globulin were significantly higher ($P \leq 0.05$) in cows with COD than in cows with normal estrous cycles.

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摘　　要

卵胞嚢腫牛の副腎皮質機能，血中性ステロイド値並びに血清蛋白像を明らかにするため，臨床上卵胞嚢腫と診断された 112 例と正常性周期を回帰している健康牛 54 頭について，血中 11-hydroxycorticosteroids (11-OHCS), progesterone (P), estrone (E_1), estradiol-17β (E_2), estriol (E_3) 並びに血清蛋白質の測定を行うとともに ACTH 負荷試験を実施した。

1. 卵胞嚢腫牛は外部徴候により，下記の 3 型 7 群に区分された。

I　思牡狂および持続性発情型
　i）定型的思牡狂群　　(5 頭)
　ii）持続性発情群　　(25 頭)
II　不規則発情型
　i）不規則発情群　　(23 頭)
　ii）規則発情群　　(15 頭)
　iii）分娩後初回発情群　　(5 頭)
III　無発情型
　i）分娩後無発情群　　(22 頭)
　ii）発情・授精後無発情群　(17 頭)

2. 血中 11-OHCS の ACTH に対する反応性（副腎皮質予備能）は，正常性周期の対照牛に比らべ卵胞嚢腫牛では低下していた ($P \leqq 0.05$)。また副腎皮質予備能は 3 型間で異なり，無発情型で最も高く，思牡狂および持続性発情型がこれにつぎ，不規則発情型では最低であった ($P \leqq 0.05$)。

3. 血中性ステロイド値も 3 型間で異なっていた。すなわち，P 値は，対照牛の発情期に比らべ，定型的思牡狂群では低かったが，持続性発情型および不規則発情型の発情期のものではほとんど差がなく，不規則発情型の非発情期のものおよび無発情型では高かった。E_2 値は対照牛の発情期に比らべ，思牡狂および持続性発情型並びに不規則発情型の発情期のもので高かったが，不規則発情型の非発情期のものおよび無発情型ではほとんど差がなかった。これらの結果から，思牡狂症状および持続性発情は高 E_2，低 P 値，無発情は低 E_2，高 P 値，そし

て不規則発情は低 P，高 E_2 値と相対的な高 P，低 E_2 値の繰り返しであることがわかった。

4. 血清総蛋白および γ-globulin 値は対照牛に比べ高かった ($P \leq 0.05$) が，3 型間に差はなかった。