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SEASONAL CHANGES IN CIRCULATING LEVELS OF GONADAL STEROIDS DURING AN ESTROUS CYCLE OF HOLSTEIN-FRIESIAN COWS[1,2]

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John Yuh-Lin Yu, Jiin-Jia Liaw, Chu-Li Chang, Shan-Nan Lee and Mao-Chiang Chen (1988) Seasonal changes in circulating levels of gonadal steroids during an estrous cycle of Holstein-Friesian Cows. *Bull. Inst. Zool., Academia Sinica* **27**(2): 133-143. Seasonal changes in the circulating levels of various steroid hormones during an estrous cycle of Holstein-Friesian cows were investigated in subtropical Taiwan. Blood samples were collectede very 3 days during estrous cycle from lactating Holstein-Friesian cows (3-7 years of age) by jugular venepuncture. Six cows were used for "winter" group during December, 1984-March, 1985 (temperature, high 18.5°C and low 12.6°C; relative humidity, high 89.6% and low 75.5%); another six cows were used for "summer" group during August-September, 1985 (temperature, high 31.1°C and low 24.4°C; relative humidity, high 83.3% and low 66.4%). Plasma samples were extracted with diethyl ether and concentrations of progesterone, estradiol-17β and androgen were quantified by radioimmunoassays. Progesterone levels were significantly higher in winter than in summer during the luteal phase of the estrous cycle; the difference being 61%. The estradiol-17β levels were, however, remained quite similar between the two seasons. The circulating androgen levels were significantly lower in winter than in summer throughout the entire estrous cycle; the mean difference between the two seasons was 58%. The decreased circulating progesterone levels in summer are likely related to the inefficient reproductive performance of the dairy cows in the hot and humid summer months. The physiological significance of higher androgen levels in summer remains unknown. The seasonal variation of the circulating androgen levels during the estrous cycle of dairy cows was reported here for the first time in the literature.

Key words: Seasonal changes, Bovine estrous cycle, Progesterone, Estradiol-17β, Androgen.

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Ovarian secretions of appropriate levels of both progesterone and estrogen are essential for expression of estrus, for successful fertilization and implantation as well as for maintaining the pregnancy (Hansel & Echternkamp, 1972). The circulating levels of steroid hormones during an estrous cycle of dairy cows have been well documented (Donaldson *et al.*, 1970; Glencross *et al.*, 1973; Shemesh & Hansel, 1974; Saba *et al.*, 1975; Kanchev *et al.*, 1976; Yu & Wang, 1984). It has been well known that the reproductive performances of dairy and beef cows (occurence of estrus, ovulation rate and conception rate) are greatly impaired in hot summer months (Ingraham *et al.*, 1974; Thatcher, 1974; Moberg, 1975; Chesworth & Easdon, 1983). The seasonal variations of circulating levels of progesterone and estrogen during the estrous cycle of dairy cows and buffalo cows have been investigated (Gwazdauskas *et al.*, 1974; Abilay *et al.*, 1975; Rosenberg, 1977; Wolff-Vaught *et al.*, 1977; Gwazdauskas *et al.*, 1981; Rosenberg *et al.*, 1982; Rao & Pandey, 1982, 1983; Chesworth & Easdon, 1983; McNatty *et al.*, 1984). However, the results presented by different laboratories are variable due likely to the diversified environmental conditions (differing temperature and humidity and varying nutritional status, etc.) in their studies.

Androgen has been quantified in estrous cycles of the cows and the sheep (Shemesh & Hansel, 1974; Saba *et al.*, 1975; Kanchev *et al.*, 1976; Herriman *et al.*, 1979). Whether there are seasonal alterations in circulating androgen levels have not been studied by far. A study was thus conducted to compare the seasonal variations (cool winter and hot summer) in circulating levels of progesterone, estradiol, and androgen during the estrous cycle of Holstein-Friesian cows in subtropical Taiwan. The seasonal changes in circulating levels of steroid hormones may provide an explanation of inefficient reproductive performance in hot and humid summer season. The information obtained from the present study may also serve as a basis for practical improvement of the reproductive efficiency of the dairy cows in subtropical Taiwan through the potential application of steroid hormones.

MATERIALS AND METHODS

Animals

Lactating Holstein-Friesian cows (3–7 years of age) were used in this study. The cows were grazing on the pangola pasture during summer and were fed ad libitum with pangola hay during winter. In addition, the cows were supplemented daily with concentrate mixture that contained 17% of crude protein, 71% of total digestible nutrient, 0.75% of calcium and 0.64% of phosphorus. The amount of concentrate fed to the cows depended on the milk yield, body weight and lactation number

Sample Collection and Storage

Blood samples were collected every 3 days during the estrous cycle of the cows by jugular venepuncture, with heparinized vaccum syringe. Six cows were used for "winter" group during December, 1984–March, 1985 (temperature: high, 18.5±0.4°C; low, 12.6 ±0.3°C; mean, 14.8±0.3°C; and relative humidity: high, 89.6±1.4%; low, 75.5±0.6; mean, 83.8±1.2%); another six cows were used for "summer" group during August-September, 1985 (temperature: high, 31.1± 0.2°C; low, 24.4±0.2°C; mean, 28.8±0.2°C; and relative humidity: high, 83.3±1.1%; low, 66.4±1.6%; mean, 75.7±1.5%). The occurence of estrus was checked two times a day by means of behavioral symptoms. The blood samples were kept in ice immediately after collection. Plasmas were obtained by centrifugation at 5°C and stored at -20°C until assays of steroids.

Radioimmunoassays of Steroids

The blood samples were extracted with diethyl ether and allowed to freeze in dry ice-ethanol medium. The ether was decanted into another tube and dried under ventilation at 38°C. The dried residue was dissolved in

0.01 M PBS (pH 7.40) containing 0.1% gelatin and incubated at room temperature for one hour. Aliquots of the PBS-gelatin dissolved steroids were used for radioimmunoassays without further chromatographic separation of the steroids.

A. *Progesterone*

The radioimmunoassay procedure for progesterone was described previously (Yu & Wang, 1984). In a preliminary experiment the progesterone determinations were compared with and without celite chromatographic separation following diethyl ether extraction of steroids from the blood plasma of cyclic cows. The results revealed that the progesterone values were very much similar between them. Consequently, the celite chromatographic separation procedure was omitted in analysis of progesterone in this study. The antibody against progesterone was produced in rabbits by immunization with progesterone-11-HS:BSA (Steraloids Co.). The lower detection limit was 5 pg/assay tube. The cross reactions of the antiserum were as follows: progesterone, 100%; 11α-hydroxyprogesterone, 62%; 11β-hydroxyprogesterone, 17%; 17α-hydroxyprogesterone, 3%; 5α-pregnane-3, 20-dione, 3%; corticosterone, 0.6%. The cross reactions for other steroids were less than 0.01%. The coefficients of variation of intra- and inter-assay were 6.7% and 12.3%, respectively (N=8).

B. *Estradiol-17β*

The radioimmunoassay of estradiol-17β was described previously (Yu *et al.*, 1985). The antiserum was produced in rabbits by immunization with estradiol-6-CMO:BSA (Steraloids Co.). The antibody was highly specific for estradiol-17β. The percent cross reactions relative to estradiol-17β (=100%) were: estrone, 2.9%; estriol, 0.6%; estradiol -17α, 0.2%; and other steroids had negligible cross reactions with this antiserum. The lower detection limit was 1 pg/assay tube. The coefficients of variation of intra-and inter-assay were 5.5% and 12.2%, respectively (N=8).

C. *Androgen*

The antiserum was produced in rabbits by immunization with testosterone-3-CMO: BSA (Steraloids Co.). The percent cross reactions of the antiserum were: testosterone, 100%, 5α-dihydrotestosterone, 74%, androstenedione, 1.2%, 11-ketotestosterone, 0.4% and androstenediol, 0.6%. The lower detection limit was 5 pg/assay tube. Since a chromatographic separation procedure of steroids following disthyl ether extraction was omitted, the concentration of androgen in the sample was expressed as testosterone equivalent extrapolated from the standard curve. The coefficients of variation of intra- and inter-assay were 6.5% and 10.1%, respectively (N=8).

Tritium-steroids were incubated with the aliquots of PBS-gelatin dissolved steroids from the samples. Dextran-coated charcol was emloyed to separate the bound-steroid from the free one. Supernatant containing the bound labeled steroid was counted in a liquid scintillation counter.

Statistical Analysis

Split-plot analysis of variance (Snedecor and Cockran, 1980) was used to test the seasonal difference of the level of each steroid during the entire estrous cycle. Duncan's new multiple range test (Duncan, 1975) was then employed to test the difference on the specific day during the estrous cycle between the two seasons.

RESULTS

Progesterone Levels

The changes in the circulating progesterone levels during an estrous cycle of the cows in both winter and summer are shown in Fig. 1. As indicated, the progesterone values in winter were always higher than those in summer. The progesterone levels from day 6 of the estrous cycle through 4 days before the next estrus were significantly different between winter and summer. The

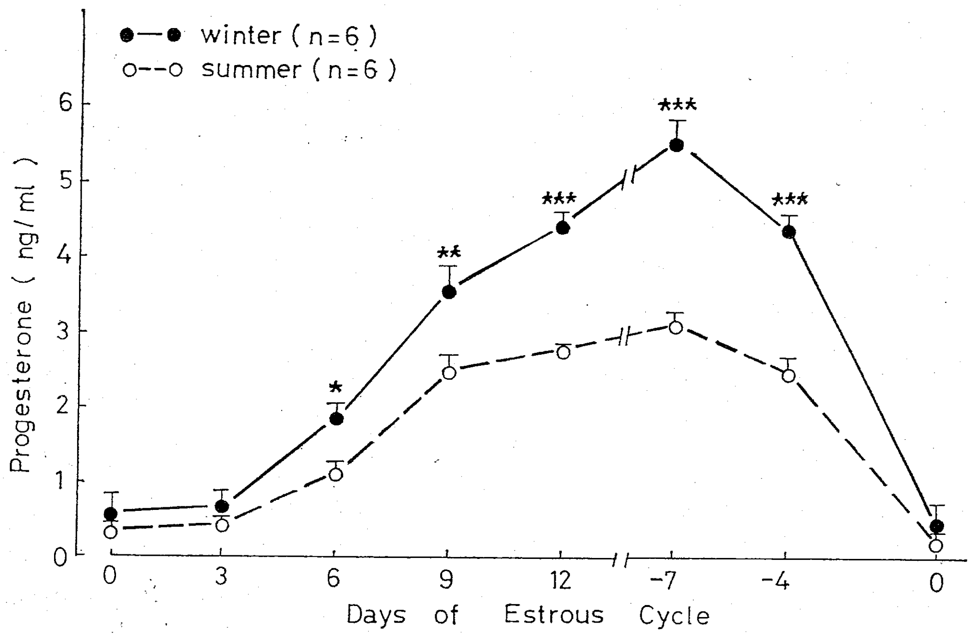

Fig. 1. Changes in the plasma levels of progesterone during the estrous cycle of the dairy cows in summer and winter. The data are expressed as mean±sem. *, **, and *** denote the difference at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, for the progesterone values compared on the same day of the estrous cycle between the two seasons.

plasma progesterone levels during the estrous cycle of the cows in winter were, in average, 161% of the corresponding steroid values in summer (Table 1).

Estradiol-17β Levels

As indicated in Fig. 2, the estradiol-17β levels in blood in both summer and winter were relatively constant from day 3 of the estrous cycle through 4 days before the next estrus. The values at day 0 were several fold higher than the values in the rest of the estrous cycle. The estradiol-17β levels from day 6 of the estrous cycle through 4 days before the start of the next estrus were

TABLE 1
Percent changes in the seasonal ratio* of circulating levels of gonadal steroids during the estrous cycle of dairy cows in winter and summer

Days of the cycle	Progesterone (%)	Estradiol-17β (%)	Androgen (%)
0	161.1	86.8	60.7
3	148.9	94.8	48.1
6	165.8	109.9	65.6
9	145.0	115.0	54.0
12	156.6	114.5	61.6
−7	177.7	117.6	62.8
−4	176.4	112.8	57.7
0	160.0	86.9	51.9

* (winter values/summer values)×100

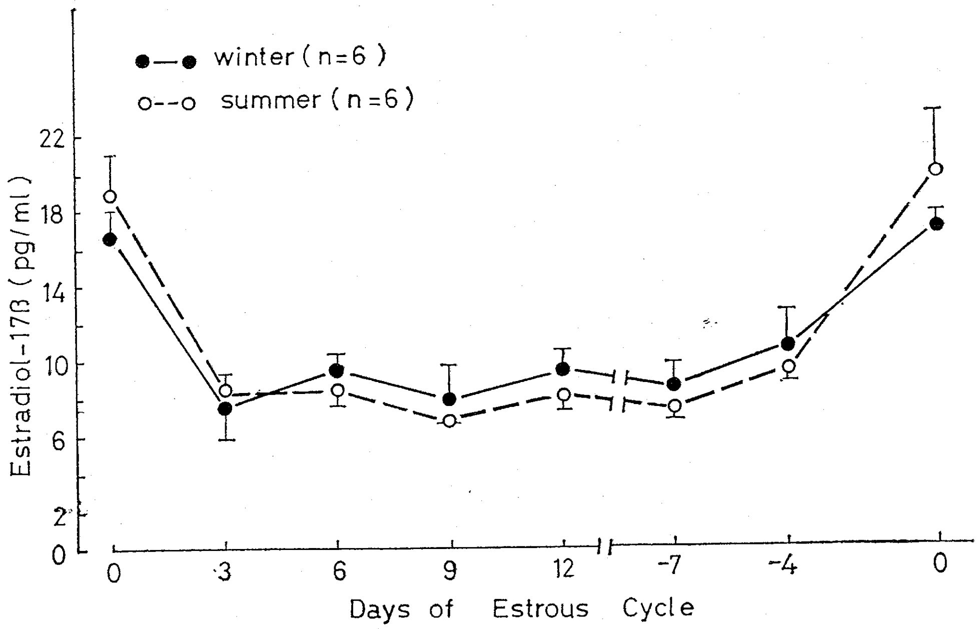

Fig. 2. Changes in the plasma levels of estradiol-17β during the estrous cycle of the dairy cows summer and winter. The data are expressed as mean±sem.

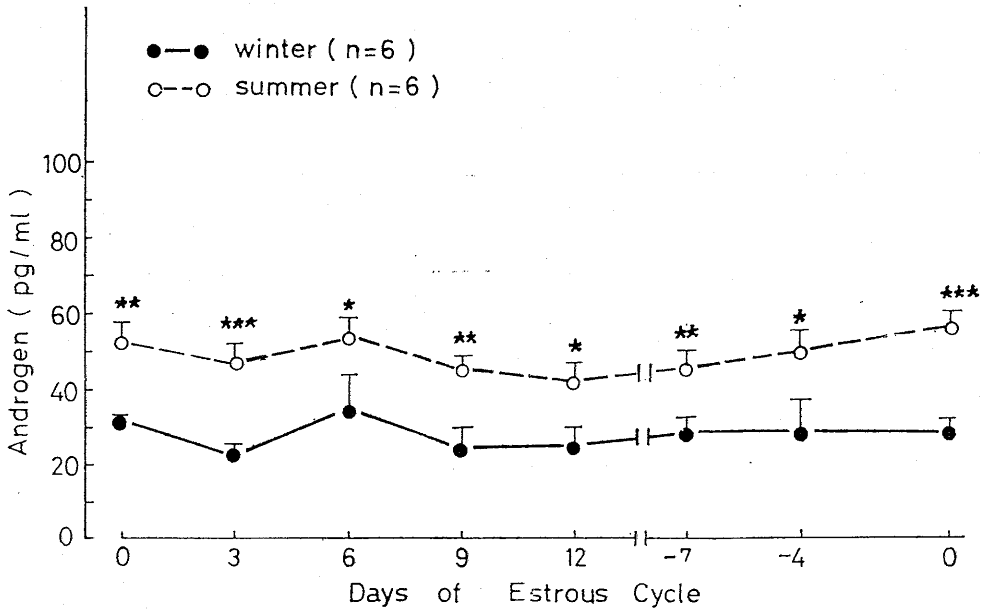

Fig. 3. Changes in the plasma levels of androgen during the estrous cycle of the dairy cows in summer and winter. The data are expressed as mean±sem. *, **, and *** denote the difference at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, for the androgen values compared on the same day of the estrous cycle between the two seasons.

slightly raised in winter, but being not significantly different from the summer levels. At day 0 of the estrous cycle, the estradiol-17β levels in winter, however, were slightly lower than those in summer although without statistical significance. The percent changes in estradiol-17β levels in plasma during the entire estrous cycle of the cows were compared between the two seasons (Table 1).

Androgen Levels

The androgen levels in the plasma remained essentially constant throughout the entire estrous cycle of the cows in both winter and summer (Fig. 3). The androgen levels were, however, significantly lower in winter as compared to those in summer throughout the entire estrous cycle. The androgen values ranged from 42 pg to 58 pg in summer and from 22 pg to 36 pg in winter. The mean winter androgen levels during the estrous cycle of the cows were 58% of the summer values (Table 1).

Ratios of the Circulating Levels of Progesterone to estradiol-17β

Since the balanced secretions of estradiol-17β and progesterone during the estrous cycle are essential for the optimal reproductive function, the ratios of the two steroids were thus compared between the two seasons. The ratios of plasma levels of progesterone relative to estradiol-17β are shown in Fig. 4. As indicated, the winter values were significantly higher than the corresponding values in summer from day 6 of the estrous cycle through 4 days before the initiation of the next estrus. The mean progesterone/estradiol-17β ratio in winter was 60% higher than that in summer.

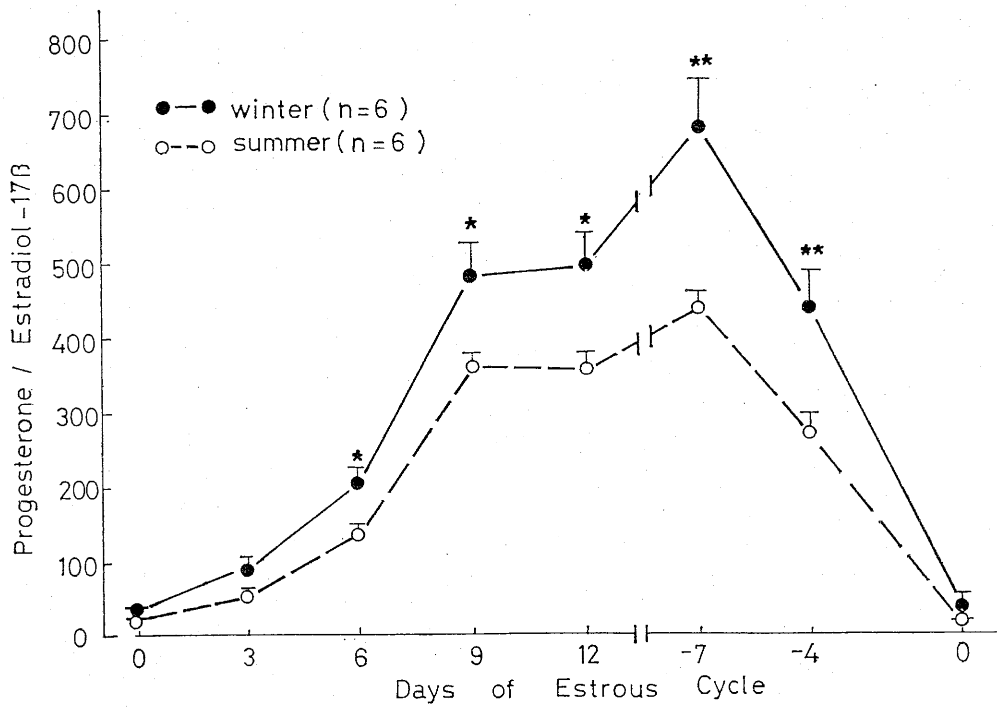

Fig. 4. Changes in the progesterone/estradiol-17β ratio in the circulation during the estrous cycle of the dairy cows in summer and winter. The data are expressed as mean$\pm$sem. * and ** denote the difference at $p<0.05$ and $p<0.01$, respectively, for the steroid ratio compared on the same day of the estrous cycle between the two seasons.

DISCUSSION

The results obtained from the present study have clearly shown that the circulating progesterone levels during the estrous cycle of the dairy cows in cool winter were significantly greater than in hot summer. The winter values were, in average, 61% higher than the summer values. Rosenberg *et al.* (1977, 1982) reported that the plasma progesterone levels were higher in winter than in summer in multiparous dairy cows. Our findings are quite comparable to theirs under the similar temperature patterns although the relative humidity being higher in our present investigation. Wolff-Vanght *et al.* (1977) demonstrated that, in milking cows kept under arid conditions, progesterone levels were elevated in summer (30 to 47°C) compared with winter (10 to 23°C), while in non-milking cows progesterone concentrations were lower in summer. In studying the seasonal changes in plasma progesterone concentrations in buffalo cows, Rao and Pandey (1982) showed that in hot and humid summer months, the progesterone levels during the estrous cycle were much lower than in warm and cold months. However, in hot and dry months, the circulating progesterone levels were slightly higher than in hot and humid months. Taken our findings together with the observations reported by others, it may be concluded that both temperature and humidity affect the circulating progesterone levels during the estrous cycle of the cows, and that "hot and humid" exhibits greater effect that "hot" alone in decreasing the circulating progesterone levels, as well as that the effect depends on both multiparous/primiparous status and milking/non-milking conditions. It has been well known that estradiol-17β levels rises upon approaching estrus and reaches the peak at estrus prior to induction of luteinizing hormones (Hansel & Echternkamp, 1972; Glencross *et al.*, 1973; Chenault *et al.*, 1975). The absolute levels of circulating estradiol-17β observed in our present study were somewhat higher than the corresponding levels reported by other researchers, but the qualitative patterns were very much comparable to others during the estrous cycle (Hansel & Echternkamp, 1972; Glecross *et al.*, 1973; Chenault *et al.*, 1975; Rosenberg *et al.*, 1982). The seasonal difference in estradiol values during the estrous cycle, as observed in the present study, was not as distinct as that of progesterone levels; the estradiol-17β levels were not statistically different between the winter and summer months. However, there may be biological significance since the mean estradiol-17β levels in the winter months were 14% higher during the luteal phase and 12% lower at estrus, as compared to the respective values in the summer months.

The effect of temperature on circulating estrogens levels in female cattle was particularly controversial. Gwazdauskas *et al.* (1974, 1981) reported that heifers kept in climatic chambers at a constant temperature of 32°C had lower estradiol-17β levels than kept at 21°C. Such study provided an experimental model to demonstrate the effect of heat stress on changes of steroid hormones in the circulation. However, the results obtained thereof may not reflect the actual effect of natural environment since diurnal alteration occurs in temperature and humidity. It was reported that the circulating estradiol-17β levels were lower lower in winter than in summer during the estrous stage of dairy cows (from −12 h through +12 h of day o), but was similar in the vrest of estrous cycle (Rosenberg *et al.*, 1982; Folman *et al.*, 1983). The reason for the effect of heat stress on plasma estradiol-17β levels arround estrus is unclear. Rosenberg *et al.* (1982) have attempted to correlate the steroid changes to the adjustment to the heat stress. Estradiol reduces the uterine temperature in heifers (Gwazdauskas *et al.*, 1974) and raises the uterine blood flow in ewes (Rosenfeld *et al.*, 1973). An increase in estradiol-17β in response to heat stress could provide a mechanism by which excess heat would be dissipated from the reproductive organs into periphery. Furthermore, the suppression of basic LH secretion by heat stress, as reported by Madan and Johnson (1973), may enhance the rate of development of the preantral follicle and increase estrogen production by preovulatory follicle which develops from it. Rao and Pandey (1983) reported that the basal estradiol-17β levels were similar between hot and cold seasons, but were higher on the day of estrus and on the day before estrus in cooler months. The lower levels of estradiol-17β arround the estrus in hotter months were thought to be related to the higher incidence of silent heat seen in buffaloes during the summer as estrus behaviour is controlled either by accumulation of estrogens in specific regions of the hypothalamus (Clegg & Ganong, 1960; Radford, 1967) or by balanced plasma concentrations of estradiol and progesterone (Karsch *et al.*, 1980).

The findings from the present study together with those reported by others (Gwazdauskas *et al.*, 1981; Rosenberg *et al.*, 1982; Rao & Pandey, 1983) concerning the effect of heat stress on circulating estrogen levels during an estrous cycle of the cattle may be summarized as follows: 1) seasonal changes in estradiol-17β levels occured arround the estrus, but not during the rest of the cycle; and 2) heat stress either elevated or lowered the estradiol-17β level arround the estrus. Such inconsistant results of the estradiol levels around the estrus, as affected by heat stress, may be ascribed to the varying environmental conditions (such as temperature, humidity, and nutritional status as well) employed by different researchers.

The secretion patterns of testosterone and other androgens during estrous cycle of cows have been studied (Shemesh & Hansel, 1974; Saba *et al.*, 1975; Kanchev *et al.*, 1976; Herriman *et al.*, 1979). These studies demonstrated that testosterone values being greater than that of estradiol-17β and one or two peaks of testosterone being observed. However, no studies have been made in comparing the seasonal variation of the androgen levels in circulation during the estrous cycle of the dairy cows. The findings from the present study has clearly demonstrated, for the first time, that the plasma androgen levels in winter were significantly lower than those in summer. The role of androgen in the control of female reporduction is obscure. Testosterone may be only a precursor in the synthetic pathway of estrogen or may act as synergistic in the induction and manifestation of estrus. Androgen may also play a role in the sexual libido at estrus (Baird, 1977). Androgen has been known to stimulate protein synthesis in many organs under physiological conditions (Rochefort *et al.*, 1972; Ruh *et al.*, 1975). In ovary, syntheses of both progesterone and androgen occur in the theca cells under luteinizing hormone stimulation; the androgen then enters into the neibouring granulosa cells where it is converted to estrogen by aromatase which is induced by follicle stimulating hormone (Hansel & Convey, 1983). It is possible that the hypophysial secretions of follicle stimulating hormone may be impaired under the hot and humid environment in summer; the resultant decrease of the gonadotropic secretion thus leads to inefficient bioconversion of androgen to estrogen, which eventually leading to higher secretion of androgen.

Studies on monitoring the secretion patterns of hypophysial luteinizing hormone and follicle stimulating hormone together with gonadal and adrenal steroids as well as prolactin during the estrous cycle of the dairy cows shall provide a clearer understanding of the seasonal variation of hormonal secretion patterns in relation to the subsequent reproductive performance.

Reproduction is controlled by homeostasis of hormonal secretions from a variety of organs. The patterns of hormonal secretions and the subsequent efficiency of reproduction are influenced by environmental and nutritional factors (Johnson and Vanjonack, 1975;

Moberg, 1975; Harrison *et al.*, 1982; Chesworth and Dashon, 1983; Cavestany *et al.*, 1985; Gwazdauskas, 1985; Pennington *et al.*, 1985; Thomas *et al.*, 1987). The environmental factors include photoperiod, altitude, temperature, humidity, wind speed, thermal radiation, management stress, etc. (Johnson & Vanjonack, 1975; Moberg, 1975; Gwazdauskas, 1985); while the nutritional factors include feed intake and differing nutritional planes and others (Harrison *et al.*, 1982; Chesworth & Easdon, 1983). The change in such environmental and nutritional factors are virtually ascribed to the changes of the season. Seasonal variations in the secretion patterns of sex hormones have been studied during the estrous cycle of the cattle (Gwazdauskas *et al.*, 1974; Abilay *et al.*, 1975; Rosenberg *et al.*, 1977; Wolff-Vaught *et al.*, 1977; Herriman *et al.*, 1979; Gwazdauskas *et al.*, 1981; Rao & Pandey, 1982, 1983; Rosenberg *et al.*, 1982; Chesworth & Easdon, 1983; McNattly *et al.*, 1984). The results observed, however, were quite variable, due primarily to the varying environmental and nutritional conditions employed by different laboratories. In the present study, the changes in circulating levels of gonadal steroids during an estrous cycle of lactating dairy cows were compared between hot summer and cool winter. The seasonal difference of the gonadal steroid secretion patterns observed in the present study, thus, reflect the combined effects of environmental and nutritional conditions caused by changes of the season. However, the most conspicuous observable change between the two seasons was the difference of temperature, with an average of 10°C higher in summer than in winter; such change of temperature is likely to be the main factor in causing the seasonal variation of circulating levels of gonadal steroids during the estrous cycls of the lactating cows.

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泌乳期荷蘭種乳牛性週期間血中性腺類固醇激素濃度之季節性變化

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本研究探討亞熱帶臺灣之荷蘭種乳牛，在性週期血中性腺類固醇濃度之季節性變化。冬季與夏季，分別以6隻3至7歲泌乳期間母牛，在單一性週期中，每隔3～4天從頸靜脈採血一次。冬季之採血期間自民國73年12月中旬至民國74年3月中旬，每日平均溫度最低爲 12.6°C，最高爲 18.5°C，而每日平均相對濕度，最低爲 75.5%，最高爲 89.6%。夏季採血期間，自民國74年8月至9月，每日平均溫度最低爲 24.4°C，最高爲 31.1°C。血漿經乙醚萃取其中之類固醇後，以放射性免疫法定量助孕酮、雌二醇及雄性素。本研究發現，泌乳牛在性週期間之血中助孕酮濃度有顯著之季節性差異，即冬季比夏季高出 61% ($p\leq0.01$)；而雌二醇濃度無顯著之季節性差異；雄性素濃度亦有顯著之季節性差異，多季比夏季低58% ($p\leq0.01$)，泌乳母牛血中助孕酮濃度，在夏季比冬季明顯降低，應與夏季高溫多濕期間乳牛繁殖效率降低有密切關係。夏季期間，泌乳母牛血中雄性素濃度升高，其生理意義尙待探討。本研究所發現泌乳母牛性週期間血中雄性素濃度有季節性差異，是文獻中首先報告者。