Shortened Estrous Expressions and a Possible Endoclinological Role in Suppression Estrous Signs in Dairy Cows

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Summary

The milk yield of dairy cows has recently been significantly increased, owing to the genetic improvement as well as better nutritional management. Their reproductive performance, however, has been drastically declining. Difficulty in heat detection is one of the main causes of poor reproductive performance in today's dairy cows, since cows are generally bred artificially after detection of estrus. The detection of estrus is based on visual observation of cows for estrous signs. If cows do not express clear signs of estrus, it is difficult to breed them at an optimum time. According to the visual observation on a dairy herd, 64.3% were observed in standing estrus. The average duration of standing estruses was 6.6 ± 6.3 h and 42 percent of the standing estrus was shown for 4 hours or shorter. This result suggests that about one third of cows in heat do not show standing estrus and duration of standing estrus has been drastically shortened. Suprabasal progesterone concentrations in follicular phase may inhibit estrous expressions. In cows, which showed only vaginal changes without any sexual activity, had significantly higher plasma progesterone concentrations than cows with standing estrus. The results showed that suprabasal progesterone levels are associated with weakened estrus in dairy cows. Some earlier studies suggest that progesterone concentrations in plasma in absence of corpus luteum are originated from the adrenal cortex. It has been already shown in cattle that plasma progesterone concentrations increased after an administration of exogenous ACTH. Three of the 4 ovariectmized lactating Holstein cows showed a significant increase of plasma progesterone concentrations after repeated ACTH challenge at 25 IU. Peak plasma progesterone levels after the first and second ACTH challenges were 3.7 ± 0.6 ng/ml and 2.1 ± 1.0 ng/ml, respectively. Significant increase of plasma progesterone concentrations was observed after 12 IU ACTH treatment in three lactating ovariectomized cows too. The mean peak plasma progesterone concentration was 1.5 ± 0.1 ng/ml and concomitant plasma cortisol concentration was 23.3 ± 5.1 ng/ml. The results suggest that the adrenal cortex can secrete sufficient amount of progesterone in response to 12 IU of ACTH or equivalent degree of stresses and concomitant plasma cortisol levels were 23.3 ± 5.1 ng/ml. There are several possible factors which affect on estrous behavior. In 7 of the 10 cows plasma cortisol levels increased significantly after milking. There were significant positive correlations between increments of plasma cortisol concentrations after milking and duration for milking or milk yield (r = 0.6, $P < 0.05$ and r = 0.74, $P < 0.01$, respectively). It is suggested that milking could cause a degree of stress equivalent to the doses of 12IU ACTH to which cows responded with a significant rise of plasma progesterone levels. It was concluded that the duration of standing estrus has been substantially shortened in lactating dairy cows, and more than one third of cows did not show standing estrus. Suprabasal progesterone concentrations in plasma were shown in cows with weakened estrus. Lactating cows may secrete a significant amount of progesterone from the adrenal cortex in response to acute stress. Resulting in high plasma progesterone concentrations, which may be sufficient to suppress estrous expression. Milking may cause adrenocortical response to secrete cortisol. It has also been suggested that stresses may play an important role in suppression of estrous signs in cows.

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In the highly developed dairy production management, cows should get pregnant at an optimal interval after calving for efficient milk production. It is one of the top priorities for dairy herd management to maximize reproductive performance in high producing cows. In Japan, average milk yield per cow per lactation has been increased to 147% from the milk yield 25 years ago to reach 8,600 kg in 2000 (The Ministry of Agriculture, Forestry and Fisheries of Japan). However, average conception rate at 1st AI has been declined from 47% in 1996 to 43% in 2000, and the interval from calving to pregnant has been increased to 155 days (Livestock Improvement Association of Japan, 1999).

Expression of normal estrous signs, accurate detection of estrus and insemination at optimum time are keys to success in artificial insemination in cattle. Difficulty in detection of estrus and resultant low heat detection rate could be major causes of declining reproductive performance (Opsomer, 2002).

It has been reported that duration of estrus in dairy cows has been substantially shortened, the average duration of estrus being less than 10 hours (Dransfield *et al*., 1998; Xu *et al*., 1998; At-Taras and Spahr, 2001). In Japan, duration of standing estrus in dairy cows has been reported to be 12 – 18 h (Kida *et al*., 1981). However, it is yet to be known

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whether there have been any changes in duration of estrous signs in cows today. Shortened duration of estrus and weakened estrous signs lacking standing estrus may cause estrous detection failure and timing error in artificial insemination with subsequent poor conception rate.

Several factors which affect on estrous behavior have been reported; heat stress, foot condition, milk production and others. However, the mechanism of suppression of estrous behavior due to these stressors has not been described.

Cows show estrous signs responding to estradiol secreted from Graafian follicle. Progesterone secreted mainly from the corpus luteum suppresses estrous behavior. Suprabasal plasma progesterone concentrations during follicular phase may be one of the causes of poor expression of estrous sings (Allrich, 1994). The source of progesterone during follicular phase has been considered to be the corpus luteum due to delayed luteolysis or the adrenal cortex (Duchens *et al*., 1994; Moberg, 1975). The assumption needs to be proved yet.

Duration of standing estrus in dairy cows

Duration of standing estrus observed in the recent studies in U.S. using radio-telemetric pressure sensor system was reported to be 7.1 ± 5.4 h (Dransfield *et al*., 1998) and 5.8 ± 0.8 h (At-Taras and Spahr, 2001). In Japan, no data on duration of estrus in today's dairy cows has been reported. Earlier, Kida et al. (1981) reported that duration of standing estrus in dairy cows was 12 to 18 h, based on the visual observation 4 times a day. Recently, the duration of standing in high producing dairy cows observed in the present study was 6.6 ± 6.3 h (Yoshida and Nakao, 2005) which equivalent to the duration observed in U.S. dairy cows. It was suggested that the estrus duration in dairy cows in Japan is as short as the duration in cows in U.S.

In this study, of the 56 cows in estrus, 36 (64.3%) showed standing estrus, while 20 (35.7%) showed only secondary estrous signs without standing. Rather lower percentages of cows exhibiting standing estrus were reported in U.S. (Stevenson *et al*., 1983) and the Netherlands (Van Vliet and Van Eardenburg, 1996), 50% and 37%, respectively. Duration of estrus in lactating cows was 6.6 ± 6.3 h on the average (± S.D.), ranging between 2 h and 32 h. Median values was 4 h. There was a tendency that duration of estrus was shorter in primiparous cows (n = 5) than in multiparous cows (n = 31), 4.4 ± 2.2 h *vs* 6.9 ± 6.6 h. One cow showed 16 h of standing estrus and again expressed short standing estrus for 4 h at 72 h later. In this case only one estrus with 16 h of duration was counted. The cow continued to show secondary estrous signs during a period between the two estruses. In 42 percent of the cows with standing, duration of standing estrus was only 4 h (**Fig. 1**). Eighty-nine percent of cows showed standing estrus for less than 8 h. All of 5 primiparous cows with standing estrus showed estrus for less than 8 h and 88% of the multiparous cows also showed duration of estrus shorter than 8h. This may indicate that estrous detection in dairy cows has been becoming more difficult.

Suprabasal progesterone concentrations in plasma in follicular phase

Causes of weakened estrous signs have not been well described. The floor material or ground conditions in the paddock are known to affect intensity and duration of estrus in cattle (Gwazdauskas *et al*., 1983; Valies and Britt, 1990; Rodtian *et al*., 1996). Non-specific stresses caused by high milk production, claw diseases and a high ambient temperature in summer have also been reported to be possible causes of weakened estrus (Thatcher, 1974; Abilay *et al*., 1975; Gwazdauskas, 1985; Harrison *et al*., 1990). However, the mechanism by which the stresses cause depression of estrous intensity is as yet unknown. The cow expresses estrous signs in response to a rise of plasma estradiol secreted from the Graafian follicle. Prior to maturation of Graafian follicle, plasma progesterone declines to a minimum of basal level. If plasma progesterone levels in cows in follicular phase are still high, they may not show estrous signs, since progesterone has inhibitory effects against estradiol (Allrich, 1994). The sources of suprabasal progesterone have been reported to be incompletely regressed corpus luteum or adrenal cortex (Duchens *et al*., 1994; Stoebel and Moberg, 1982; Hein and Allrich, 1992).

Some studies indicated that cows and heifers with weakened oestrous signs had suprabasal progesterone levels in milk fat or plasma (Schopper *et al*., 1993; Duchens *et al*., 1995; Båge *et al*., 1997). However, the relationship between plasma progesterone concentrations and extent of estrous expression in lactating cows has not been fully described. Further study is needed to show the relationship in detail.

Plasma progesterone concentrations in cows in estrus were compared between 10 cows with standing estrus and 11 weakened estrous cows by Yoshida (2006). Plasma concentrations of progesterone in cows with the weakened estrous signs were significantly higher than those cows with

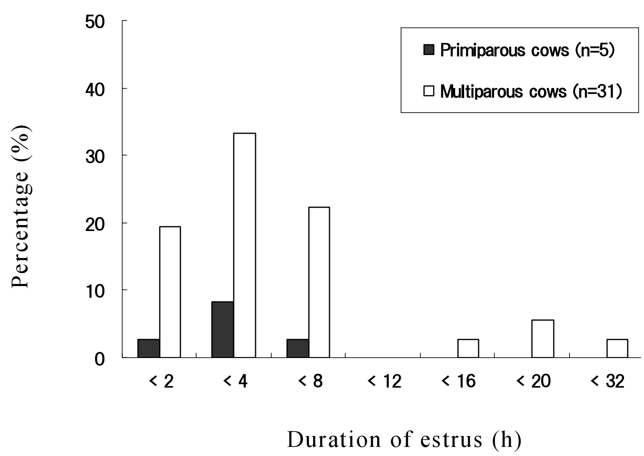

Fig. 1. Distribution of primiparous and multiparous cows according to duration of standing estrus.

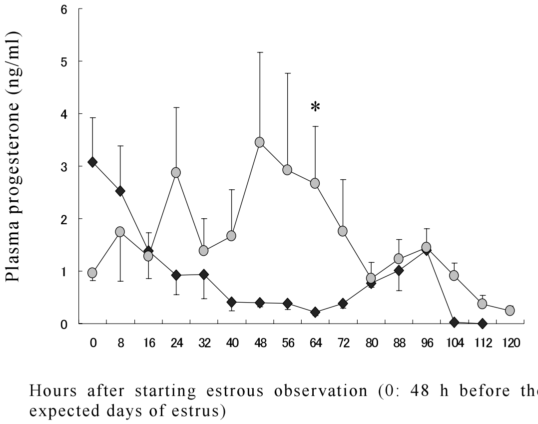

Fig. 2. Changes of plasma progesterone concentrations in standing estrus group and vaginal change group around the estimated day of ovulation (◆: cows with standing estrus, ●: cows with vaginal changes only). * Significant difference was observed in the concentrations between two groups ($P < 0.05$).

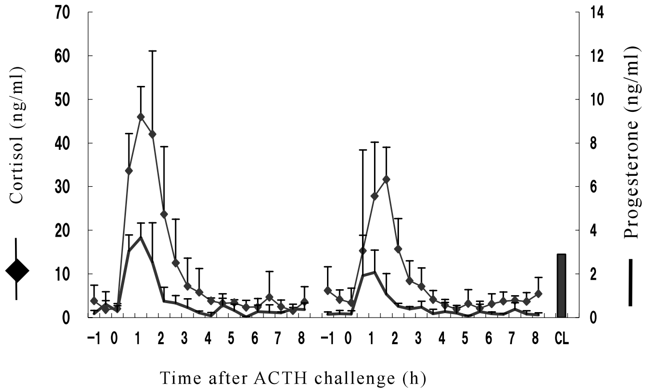

Fig. 3. Adrenocortical responses to two consecutive ACTH challenges at an interval of 48 h in 3 ovariectomized lactating cows (Mean ± S.D.). The column on the right shows the mean progesterone concentration at 7 days after estrus in two cycles of each cow before ovariectomy. 0 h : the time of ACTH challenge.

standing to the others ($P < 0.05$) (**Fig. 2**).

It was described that weakened estrous cows showed significantly higher plasma progesterone concentrations than estrous cows with standing estrus. It is suggested that suprabasal progesterone concentrations in plasma during the periestrous period suppress estrous expressions.

In cows with standing estrus, plasma progesterone concentrations declined to lower than 1.0 ng/ml and showed standing estrus in this study. These results agree with the previous report showing a transition of plasma progesterone levels during the estrous period (Giménes *et al.*, 1974). Cows with only vaginal changes without sexual activity showed higher plasma progesterone concentrations than that of cows with standing estrus during 5 days around the estimated day of ovulation in this study. Schopper et al. (1993) reported significantly higher milk progesterone concentrations in cows with silent estrus than in cows with standing estrus. Duchens et al. (1995) and Båge et al. (1997) also observed suprabasal plasma progesterone concentrations in heifers with weakened estrous signs. The plasma progesterone concentrations in cows with sexually inactive group in this study were relatively higher than the studies reported earlier. Daviage et al. (1987) observed that estradiol-induced estrous behavior which was typically standing activity in ovariectomized cows was inhibited by an increase of progesterone concentrations in plasma. Suprabasal plasma progesterone levels in follicular phase were observed in cows with weakened estrous expressions (Duchens *et al.*, 1994). It is suggested that the higher plasma progesterone concentrations might have suppressed intensity of estrous expressions in cows with the weak estrous activities. Elevated plasma progesterone concentrations around the time of estrus could be due to acute stresses related to management, environmental and physiological factors (Stoebel and Moberg, 1982; Hein and Allrich, 1992).

These results indicated that suprabasal progesterone levels in the follicular phase are associated with weakened estrus in dairy cows.

The role of adrenal progesterone in suppression of estrous signs

It is known also in cattle that the adrenal cortex produces progesterone through the synthesis of cortisol (Wagner, 1972).

After ACTH administrations, cows showed a decrease in secretion of estrogen and luteinizing hormone and an increase in progesterone concentrations (Stoebel and Moberg, 1982; Dobson *et al.*, 2001). Therefore, it is presumed that cows under stress may increase adrenal progesterone, depressing the action of estrogen and suppressing estrous expressions. In a previous study, Wagner et al. (1972) reported that treatment of five ovariectomized heifers with 100 IU ACTH resulted in elevated plasma progesterone levels 1 hour after the treatment, while physiological saline-treated animals did not show such an increase (1.18 *vs* 0.09 ng/ml). In a more recent study by Båge et al. (2000), five ovariectomized virgin heifers and five ovariectomized repeat breeder heifers responded to 48 IU ACTH showing peak plasma progesterone concentrations of 1.1 ng/ml and 0.7 ng/ml, respectively. Thus, it has been demonstrated that heifers are capable of secreting significant amount of progesterone from the adrenal cortex in response to ACTH. In non-lactating cows, 0.06mg ACTH challenge caused a significant increase of plasma cortisol up to 20.4 ng/ml (Alam *et al.*, 1986). It is assumed that even lower doses of ACTH still cause rise of plasma progesterone concentrations.

Yoshida and Nakao (2005) suggested that the possibility of the adrenal cortex which can secrete an amount of

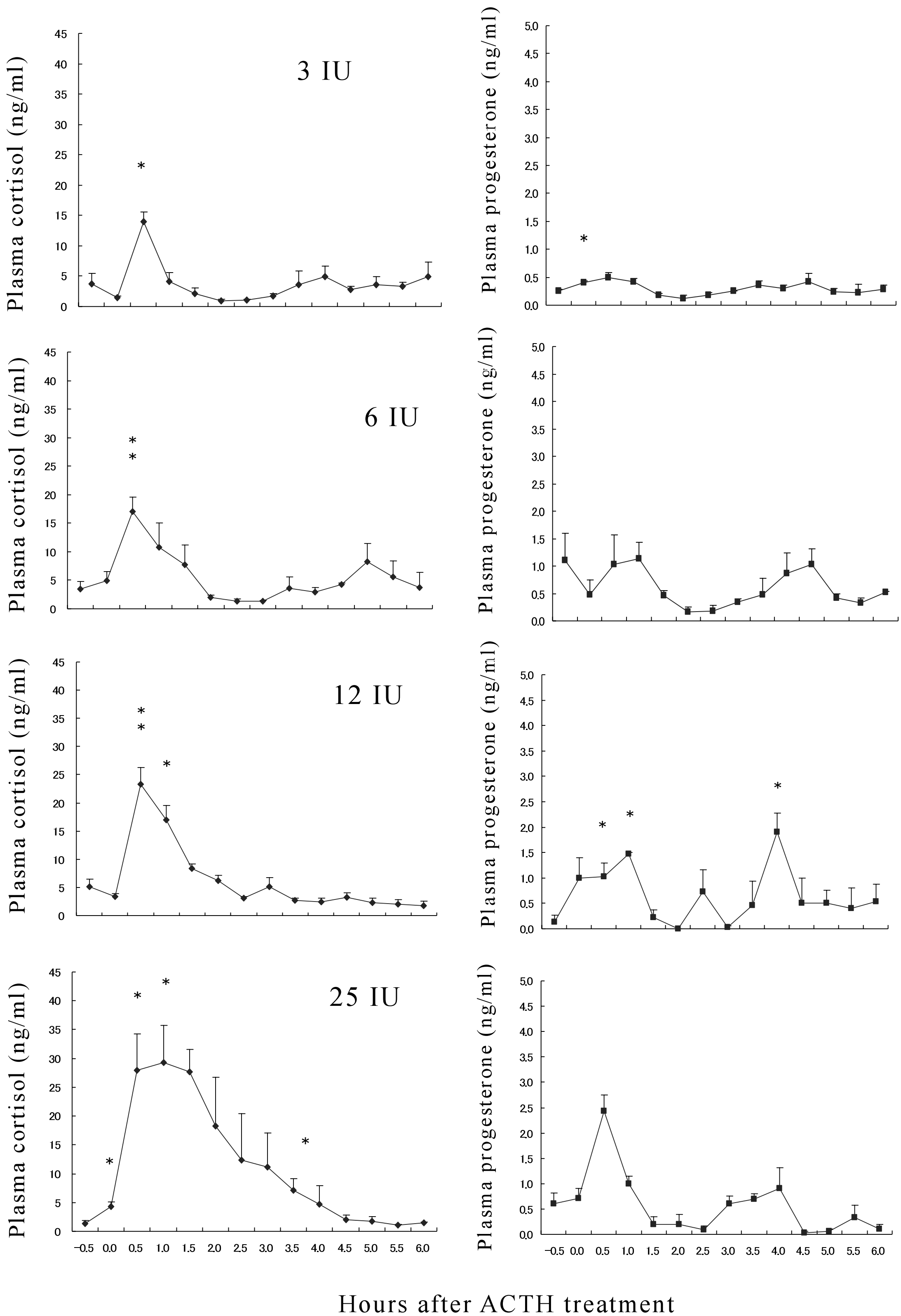

Fig. 4. Plasma progesterone and cortisol response in ovariectomized lactating cows after an intramuscular administration of 3, 6, 12 and 25 IU ACTH. Mean ± S.E. (n = 3). ** $P < 0.01$, * $P < 0.05$ significantly higher than the baseline.

progesterone sufficient to suppress estrus in lactating cows. Three of the four ovariectomized cows showed high responsiveness of plasma progesterone concentrations to 25 IU of ACTH challenges, with increases from 0.6 ng/ml or less to 2.2 – 4.5 ng/ml. Adrenocortical responses to the second ACTH challenge tended to be lower than the responses to the first challenge. The peak progesterone values in three cows after the first ACTH challenge were comparable with the values obtained in the luteal phase. The three cows also showed an increase of plasma progesterone after the second ACTH up to 2.0 ng/ml or higher (**Fig. 3**). A significantly high positive correlation was observed between progesterone and cortisol AUCs (r = 0.8, $P < 0.05$).

They also tried to describe responses of plasma progesterone and cortisol concentrations in ovariectmized lactating cows to different doses ranging from 3 to 25 IU of ACTH (Yoshida, 2006). **Fig. 4** shows response of plasma cortisol and progesterone concentrations in 3 cows after a challenge with different doses of ACTH ranging from 3 IU to 25 IU. Significant increase of plasma cortisol concentrations were observed at all the doses of ACTH. Concomitant rise of plasma progesterone along with plasma cortisol was observed in cows injected with 12 IU or higher doses of ACTH. The increase of plasma progesterone concentrations were significantly different at 0.5 h and 1.0 h with 12 IU ($P < 0.01$ and $P < 0.05$) and 0.5 h with 25 IU ($P < 0.01$). A significantly positive correlation between peak plasma progesterone and cortisol concentrations after different doses of ACTH was obtained (r = 0.7, $P < 0.05$).

Basal plasma cortisol concentrations in cattle were reported to be about 5 to 10 ng/ml (Blum *et al*., 1985; Nakao *et al*., 1994), which may increase 5 to 10 fold from the basal level after administration of 25 to 50 IU of ACTH (Båge *et al*., 2000; Alam *et al*., 1986). The basal plasma cortisol concentrations were 3.8 – 4.4 ng/ml and the peak values after 25 IU ACTH administration were 33.2 to 46.5 ng/ml in the Experiment 1. Similarly, in the Experiment 2, mean of peak plasma cortisol response of three cows after 25 IU ACTH was 33.3 ng/ml. These results were in agreement with those of previous studies (Wagner *et al*., 1972; Alam *et al*., 1986). Thus, all the cows used for this study were considered to have had normal adrenocortical function.

Progesterone in peripheral blood in cattle is secreted mostly from the corpus luteum with a small quantity from the adrenal cortex (Wagner *et al*., 1972). Progesterone circulating in plasma in ovariectomized cows is, therefore, likely to be of adrenal cortex origin. Four ovariectomized cows showed a significant increase in plasma progesterone concentrations after ACTH administration (Yoshida and Nakao, 2005). Peak progesterone levels after ACTH reached 3.1 – 3.7 ng/ml. This indicates that the adrenal cortex can secrete a considerable amount of progesterone in response to ACTH, and this is supported by the finding of a significant correlation (r = 0.8, $P < 0.05$) between AUCs of plasma cortisol and progesterone after ACTH challenges in the study. Wagner et al. (1972) reported that a challenge with 100 IU ACTH in 5 normal cyclic Holstein heifers resulted in 1.0 – 1.5 ng/ml increment of plasma progesterone concentrations. In the same study, ovariectmized heifers responded with a rise of plasma progesterone concentrations up to 1.18 ng/ml after treatment with 100 IU ACTH (Wagner *et al*., 1972). More recently, Båge et al. (2000) reported that 5 ovariectomized virgin heifers showed an increase of plasma progesterone concentrations up to 3.5 nmol/l (1.1 ng/ml) after an intramuscular administration of 48 IU ACTH. Alam et al. (1986) reported a rise of plasma progesterone up to 1.48 ng/ml as well as plasma cortisol concentrations up to 23.3 ng/ml in multiparous non-lactating cows after 1.0 mg (100 IU) of ACTH treatment. Thus, heifers, non-lactating cows as well as lactating cows have been shown to be capable of secreting significant amount of progesterone resulting in plasma progesterone concentration up to 1.0 ng/ml or higher.

A significant rise of plasma progesterone concentrations was observed after administrations of 12 IU or higher doses of ACTH in 3 ovariectmized cows (Yoshida, 2006). **Fig. 4** shows that plasma progesterone concentrations increased up to 1.5 ng/ml or higher and a concomitant peak plasma cortisol concentration was 23.3 ng/ml after 12 IU ACTH treatment. This means that the adrenal cortex can secrete sufficient progesterone to elevate plasma progesterone concentration up to 1.0 ng/ml or higher, a level which may suppress the expression of estrous signs. The peak levels of plasma progesterone after ACTH challenge in lactating cows in the present study were markedly higher than previous study using heifers (Båge *et al*., 2000). Lactating cows might have experienced more stress, due to parturition, lactation and under-nutrition, and accordingly might have higher responsiveness of the adrenal cortex to ACTH. However, it is yet to be described whether this was due to the difference in responsiveness of the adrenal cortex between lactating and non-lactating cattle. Timing of the elevation of plasma progesterone, in addition to the duration of the elevated plasma progesterone, is also crucial for suppression of estrus.

It is assumed that any stresses causing a rise of plasma cortisol up to 23.3 ng/ml or above results in a rise of plasma progesterone up to 1.0 ng/ml or higher. Whether or not dairy cows are possibly exposed to equivalent stress in general management conditions has yet to be investigated. Nakao and Grunert (1990) reported that 25 IU ACTH challenge test in cows after normal calving or dystocia showed peak plasma cortisol concentrations of 18 – 23 ng/ml and 15 – 45 ng/ml. Palpation per rectum also caused an elevation of plasma cortisol up to 12 – 14 ng/ml (Nakao *et al*., 1994). Cows with lameness were reported to show significantly elevated plasma cortisol compared to a control group (Endo *et al*., 2003). Miyazawa (1984) reported a significant increase of plasma cortisol up to 14.0 – 19.6 ng/ml in cows after milking. Administration of 12 IU ACTH caused increases in plasma cortisol slightly higher than the values in cows under acute stress. Further studies are needed to know whether the stressors mentioned above could cause a significant rise of plasma progesterone concentrations.

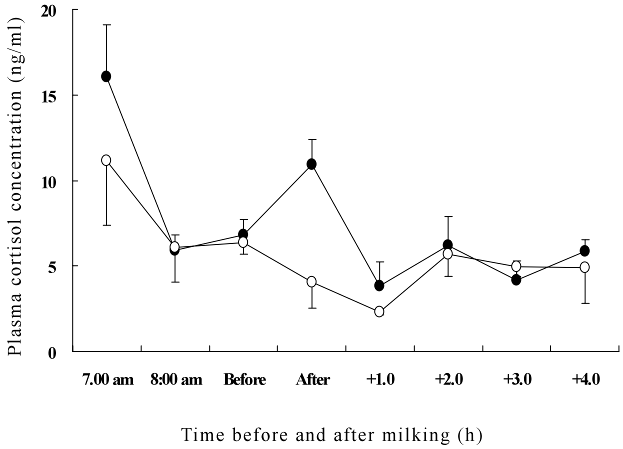

Fig. 5. Plasma cortisol concentrations before and after milking. ● : Cows with increase of plasma cortisol (n = 7), ○ : Cows without increase of plasma cortisol (n = 3).

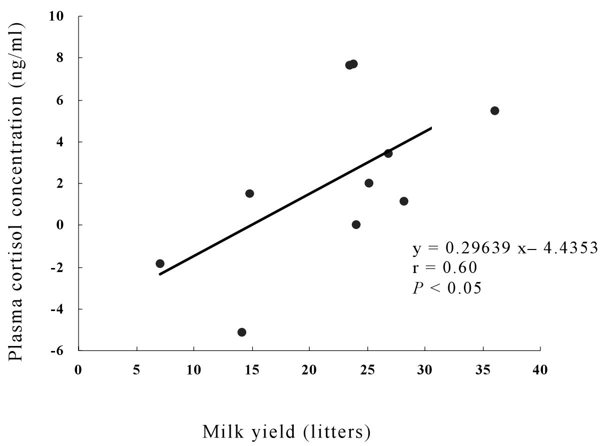

Fig. 6. Correlation between increments of plasma cortisol levels after milking and milk yield in 10 cows.

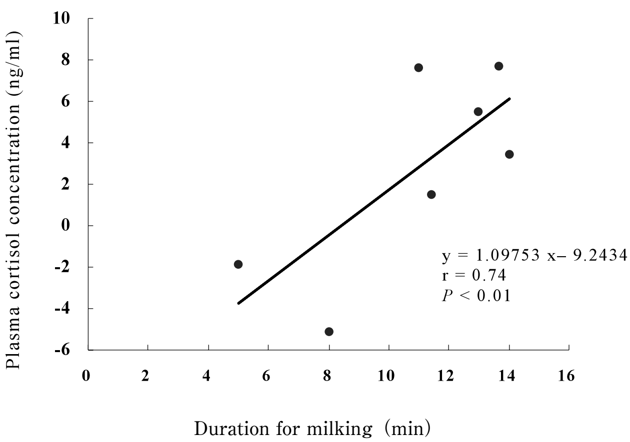

Fig. 7. Correlation between increments of plasma cortisol levels after milking and duration of milking in 7 cows in which milking was completed within 15 min.

Thus, lactating cows may secrete a sufficient amount of progesterone from the adrenal cortex in response to acute stress, resulting in high plasma progesterone concentrations, which may be sufficient to suppress estrous expression. Lactating dairy cows are capable to secrete a sufficient amount of adrenal progesterone rising up to 1.0 ng/ml or higher in response to 12 IU ACTH. Response of plasma cortisol concentrations to 12 IU ACTH was 23.3 ng/ml at the peak.

Adrenocortical response to machine milking

The results of previous ACTH challenge tests indicate that some kinds of non-specific stresses, equivalent to 6 or 12 IU ACTH and quantified by plasma cortisol concentrations of 23.3 ± 3.0 ng/ml, cause a rise of plasma progesterone which sufficient amount level to suppress estrous activities (Yoshida, 2006). As yet little is known whether lactating cows are exposed to stresses which are comparable with a challenge of 12 IU ACTH.

Various studies showed an increase of plasma cortisol levels under the possible stresses in daily management; cortisol response was 20.2 ng/ml after over milking (Paape *et al*., 1972), 12 ng/ml after rectal palpation (Nakao *et al*., 1994) and 12 ng/ml after forced walking in lame cows (Endo *et al*., 2003). Thus possible stresses may be caused by milking and handling of cows.

Plasma cortisol response after machine milking was analyzed in 10 lactating Holstein Friesian cows (Yoshida, 2006). The mean duration of milking in 10 cows was 14 ± 2 min. with a range from 5 to 21 min. The mean milk yield at the morning milking was 22.4 ± 2.6 l. Daily milk yield on the day of experiment was 38.2 ± 1.7 l. In 7 of the 10 cows plasma cortisol levels increased significantly after milking compared to the plasma cortisol concentrations before milking. Whereas, 3 cows did not show an increase of plasma cortisol level after milking **(Fig.5)**.

Plasma cortisol values after milking in 10 cows was positively correlated with milk yield (r = 0.6, $P < 0.05$) **(Fig.6)**. Plasma cortisol response of 7 cows where milking time was within 15 min. was positively correlated with duration of milking (r = 0.74, $P < 0.01$) (Yoshida, 2006) **(Fig.7)**. Paape et al. (1972) found an increase of plasma cortisol up to 20.0 ng/ml after over milking. Miyazawa (1984) reported that 4 cows producing 12 to 28 kg/day of milk had peak plasma cortisol concentration of 14.0 to 28.2 ng/ml at 13 min after stimulus of milking. The response of plasma cortisol after milking in this experiment was relatively lower than the previous reports. The reason of this difference has not been known.

There was a significant positive correlation between milk yield and increased of plasma cortisol after milking in cows ($P < 0.05$) (Yoshida, 2006). This indicates that more milk cows produce, more stress cows may suffer and this seems to be logical. A significant correlation between duration of milking and plasma cortisol response may also indicate that longer the duration of milking might be more stress on the animal. Over milking has been known to cause significant rise of plasma cortisol (Paape *et al.*, 1972). Negrão et al. (2004) reported that 6 Holstein dairy cows increased plasma cortisol due to machine milking. The peak level of plasma cortisol was 39 ng/ml on the average at 40 min after the beginning of milking. In this study, peak plasma cortisol concentrations after milking was 12.5 ± 1.5 ng/ml (mean ± S.E.) in 7 cows, which were equivalent to plasma cortisol values shown after challenge with 6 IU ACTH (Yoshida, 2006). This may suggest that cows show adrenocortical response to milking, every day routine management practice for cows to the same extent of 6 IU ACTH challenge which may cause secretion of progesterone sufficient to suppress estrous expressions.

CONCLUSION

The duration of standing estrus has been substantially shortened in lactating dairy cows, and more than one third of cows did not show standing estrus. Cows with weakened estrous signs showed suprabasal progesterone concentrations in plasma. Lactating cows may secrete a significant amount of progesterone from the adrenal cortex in response to acute stress, resulting in high plasma progesterone concentrations, which may be sufficient to suppress estrous expression. Longer duration of milking or higher milk yield may cause adrenocortical response to secrete cortisol. It is suggested that milking could cause a degree of stress equivalent to the doses of 12 IU ACTH to which cows responded with a significant rise of plasma progesterone levels.

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乳牛における発情徴候の短縮化と発情徴候を抑制しうる内分泌的機序

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要　約

遺伝的改良や飼養管理の改善によって乳牛の泌乳量は飛躍的に増加している。その反面、乳牛の繁殖成績は急激に低下している。乳牛においては、発情を確認し、人工授精を実施することによって交配するが、今日の低い繁殖成績の主要な原因は、発情発見の困難さであると考えられる。乳牛の発情発見は、肉眼観察が基本である。もし、牛が明瞭な発情徴候を表さないと、授精の適期を判断することが困難となる。ある牛群での肉眼観察による発情観察の結果、64％の牛がスタンディング発情を示した。平均の発情持続時間は6.6 ± 6.3 h（± S.D.）であり、そのうち42％は４時間未満であった。このことは、牛群の３分の１の牛がスタンディング発情を示さないこと、発情の持続時間が著しく短縮していることを示唆している。卵胞期の基底値以上の血漿中 Progesterone 濃度は、発情徴候を抑制すると考えられる。発情時に、外陰部の変化のみで行動の変化を見せなかった微弱発情牛においては、スタンディングを示した牛に比べ、血漿中 Progesterone 濃度が有意に高かった。このことは、乳牛において、卵胞期に血漿中 Progesterone 濃度が基底値以上である場合、微弱発情となることを示している。黄体の無い時期の血漿中 Progesterone 濃度は、副腎皮質由来であることが過去に報告されている。牛においても、外因性の ACTH を投与することにより、血漿中 Progesterone 濃度が増加することが知られている。卵巣を割去した泌乳牛で25IU の ACTH 負荷試験 を行ったところ、４頭中３頭で血漿中 Progesterone 濃度が有意に上昇した。１回目および２回目の ACTH 負荷試験後の血漿中 Progesterone 濃度は、それぞれ3.7 ± 0.6 ng/ml および 2.1 ± 1.0 ng/ml であった。３頭の卵巣を割去した牛を用いた12IU の ACTH 負荷試験でも、血漿中 Progesterone 濃度の有意な上昇が認められた。血漿中 Progesterone 濃度のピーク値の平均は、1.5 ± 0.1 ng/ml であり、同じ時に分泌された血漿中 Cortisol 濃度は23.3 ± 5.1 ng/ml であった。このことから、12IU の ACTH もしくはそれと同等のストレスが負荷されると、副腎皮質は発情徴候を抑制しうる濃度の Progesterone を分泌し、その時の血漿中 Cortisol 濃度は23.3 ± 5.1 ng/ml であることが示唆された。乳牛における発情徴候を抑制する諸要因については、これまで報告があり、泌乳もその一つといわれている。10頭中７頭の乳牛において、搾乳後に血漿中 Cortisol 濃度の有意な上昇が認められた。搾乳後の血漿中 Cortisol 濃度の増加と乳量および搾乳時間との関係には相関が見られた（それぞれ $r = 0.6, P < 0.05$ および $r = 0.74, P < 0.01$）。このことから、搾乳が12 IU の ACTH 負荷試験と同様のストレスとなると、血漿中 Progesterone 濃度も有意に上昇する可能性が示唆された。以上のことから、乳牛の発情持続時間は短縮化しており、３分の１以上の牛がスタンディング発情を示さないこと、さらに、微弱発情牛において血漿中 Progesterone 濃度が高いこと、泌乳牛において急激なストレスの負荷によって副腎皮質より相当の濃度の Progesterone が分泌され得ること、基底値以上の血漿中 Progesterone 濃度によって、発情徴候が抑制される可能性があることが示唆された。搾乳の刺激により副腎皮質が反応し、Cortisol が分泌されることが示され、これらのことより、ストレスは、乳牛の発情徴候の抑制に重要な影響を及ぼしていることが示唆された。

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