Concentration Differences in LH, FSH and Progesterone Secretion among Seasonal Changes in Hanwoo and Holstein Heifers in Daegwallyeong

Hak-Jae Chung*, Hyun-Il Yoon, Suk-Dong Lee, Jin-Sung Ko, Yun-Ho Choy, Seong-Bok Choi, Gi-Jun Jeon, Yeoung-Gyu Ko[1], Hwi-Cheul Lee[1], Seok-Ki Im and Myeung-Sik Lee

Hanwoo Experimental Station, National Institute of Animal Science, RDA, Pyeongchang 232-950, Korea
[1]Animal Biotechnology Division, National Institute of Animal Science, RDA, Suwon 441-706, Korea

ABSTRACT

The objective of present study was to investigate the effect of seasons on reproductive performance of Hanwoo and Holstein heifers. Heat stress in summer or cold stress in winter stress to Hanwoo and Holstein heifers may bring reproduction failure, which would pose an important economic loss, even around Daegwallyeong region located in high mountainous area. Seasonal differences in the serum levels of LH, FSH and progesterone (P_4) in response to environmental factors (hot and cold) out of 20 pubertal Hanwoo heifers in Daegwallyeong, Gangwon Province and 20 non-lactating Holstein heifers in Chonan city of Republic of Korea at 2~3 years of age were compared. Blood samples for hormonal analysis were from jugular vein after detection of estrus repeatedly over four seasons within four-week intervals (Spring: May to June, Summer: July to August, Autumn: October to November and Winter: January to February). In Hanwoo heifer population, averages of LH and FSH concentration in spring and in summer were greater compared to those in winter ($p<0.05$). LH or FSH levels tended to be greater ($p=0.06$) in spring and less ($p=0.09$) in winter compared to the levels in autumn. Only in summer, cattle seemed to show lower LH or FSH secretion ($p<0.05$). Similar to the results in Hanwoo heifers, the serum concentrations of LH and FSH in Holstein heifers decreased further by heat stress in summer when P_4 levels were high during luteal phase. The results demonstrate significant effect of summer heat on reproduction of Hanwoo or Holstein heifers. Although parameters indicating the extent of heat stress were not measured in this study, we suggest that serum hormone levels could be considered as successful indicators of summer heat stress condition for Hanwoo and Holstein heifers even under rather cool summer climate.

(Key words : Hanwoo and Holstein heifer, seasonal variation, LH, FSH, progesterone)

INTRODUCTION

To date, neuro-endocrinological and reproductive biological studies have revealed that the brains of animals control the hypothalamic-pituitary-target organ activity in order to achieve efficient reproduction and to adapt to the changes in various environmental factors. Cattle are polyestrus mammals that can conceive throughout the year. However, in their natural habitat, cattle will conceive and give birth at definite times of the year (Asdell, 1964). Animal scientists in the literature have evaluated seasonal influences on reproductive efficiency in beef cattle, but the results were inconsistent. Some of the papers reported higher conception rates in cattle to occur in winter (Thibault, *et al.*, 1966; Klyuchnikov, 1975; Rosenberg, *et al.*, 1977; 1982) or in summer (Mercier and Salisbury, 1947a,b; de Kruif, 1978) than in the other seasons. Length of the postpartum period of estrus was reported to be shorter for cows that gave birth in spring (Thibault *et al.*, 1966; De Kruif, 1978), in summer (Klyuchnikov, 1975; Peters and Riley, 1982 a; Hansen and Hauser, 1983), or in autumn (Peters and Riley, 1982b). Longer periods of postpartum estrus in cow have been observed when parturition occurs in winter (Thibault *et al.*, 1966; Klyuchnikov, 1975; Peters and Riley, 1982a; Hansen and Hauser, 1983), in spring (Rosenberg, *et al.*, 1977: Bulman and Lamming, 1978), and in autumn (De Kruif, 1978). Evidence derived from seasonally breeding female species such as the ewes (Legan and Karsch, 1980) indicated that seasonal changes on secretion of luteinizing hormone (LH) occurred even in the absence of the ovary. Changes in the levels of LH secretion in response to the negative feedback of estradiol are important seasonal effects on gonadotropin secretion in the ewe. Critser *et al.* (1983) reported the influence of season on secretion of

† This study was supported in part by the National Insitute of Animal Science, RDA.
* Correspondence : E-mail : hakjaena@rda.go.kr

LH in the absence of the ovaries.

Kariya (1993) reported that the seasonal stress of reproduction in Holsteins could pose an important economic loss, even in Hokkaido, which was located in high mountain region of Japan. People in that region have experienced unusually hot summer weather in the past lasting about a month, while people living in the high mountain area believe that the hot summer weather in normal years usually lasts only a few weeks. Definitions of the effects of temperature or seasonal variation, e.g. summer weather, heat stress, cool temperature and cold shock on animals are subjective and relative, and the scientific definitions for these environmental factors are not well established yet for Hanwoo and Holstein populations. The present study is an attempt to clarify the seasonal differences in the parameters of LH, FSH and P_4 in response to environmental conditions (hot and cold) in Hanwoo heifers in Daegwallyeong, Gangwon Province and Holstein heifers in Chonan city of the Republic of Korea.

MATERIALS AND METHODS

1. Animals

These experiments were carried out at Hanwoo Experimental Station and at Dairy Science department of National Institute of Animal Science. The National Institute of Animal Science Agricultural Animal Care and Use Committee approved animal procedures used in these experiments. The present study used 20 pubertal Hanwoo heifers and 20 non-lactating Holstein heifers at 2~3 years of age. We raised Hanwoo heifers under semi-intensive range condition and fed (0700 h and 1600 h) with a mixture of locally available grass, corn silage, and concentrates (TDN 54.4% and CP 12.8% on DM basis). We housed Holstein heifers in free stall barns throughout the experimental period and offered total mixed ration consisting of assorted roughages and concentrates (TDN 70.4% and CP 15.3% on DM basis). Temperature variation and humidity over the seasons were 13~18℃, 47% in spring, 23~28℃, 62% in summer, 13~18℃, 51% in autumn and −18~−25℃, 49% in winter. We decided dates to conduct blood sampling according to weather information on previous days.

2. Blood Sampling

Over four seasons, for four-week terms (spring: May to June, summer: July to August, autumn: October to November and winter: January to February) blood samples for hormonal analysis were obtained from jugular vein from animals on heat before artificial insemination. We used sterile 10 ml tubes containing 150 μl of stabilizer solution (0.3 M EDTA, 1% acetylsalicylic acid, pH 7.4) for sampling. Collected blood samples were centrifuged at 5,000 × G for 20 min at 4℃, and the serum samples were kept at −80℃ until hormonal analysis.

3. Regulated Ovulation Cycles

Luteal phases were considered normal if P_4 concentrations for at least 3 time points over the course of 7 days remained above 1 ng/ml or if P_4 concentrations for at least 2 time points were above 2 ng/ml. Cows were confirmed as having luteal activity when their serum P_4 concentration increased to more than 1 ng/ml (Stevenson and Britt, 1979). Ovarian cycles followed by a luteal phase of normal length were considered normal.

4. Hormone Assay

Levels of P_4 in blood samples collected from Hanwoo and Holstein heifers were measured in duplicate aliquots of serum using a specific Immunoassay kit (DELFIA®, Boston, MA, USA) according to the procedure described by Miyamoto *et al.* (1992). The standard curve ranged from 0.05 to 50 ng/ml, and the ED50 of the assay was 3.2 ng/ml. The coefficients of variation (CV's) from intra- and inter-assay were 6.7 and 7.2%.

Serum LH and FSH concentrations were determined by radioimmunoassay (Crowe, *et al.*, 1997) using USDA-bFSH- B-1 as standard. Intra-assay and inter-assay determination levels were set for low (8.1 ± 1.0 ng/ml), medium (18.1 ± 0.4 ng/ml) and high (38.6 ± 1.0 ng/ml) concentrations.

5. Statistical Analysis

In normal ovarian cycles, the week when P_4 concentration became less than 1 ng/ml was defined as week zero of the estrus cycle. We analyzed data from 0 to 4 weeks from cows of each breed with normal ovarian cycles. Mean concentration levels between breeds were separated by Student-*t*-tests. Statistical significance of the differences in the concentration of LH, FSH and P_4 between four seasons was tested using Duncan's multiple range test. Results were expressed as mean ± standard error of mean (SEM). Differences with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

1. Body Weight Changes

Any substantial changes in body weights between seasons

were not found for any one of individual Hanwoo and Holstein heifers (data not shown). In other words, body weight changed by less than 20 kg over seasons for any particular Hanwoo and Holstein heifers. Furthermore, small changes in body weight did not appear to be related to changes in LH, FSH and P_4 levels in response to daytime variation.

2. Hormonal Changes in Hanwoo Heifers

Hanwoo heifers from each season demonstrated different secretory patterns of LH, FSH and P_4 as shown in Fig. 1. Normal ovarian cycles were observed from all twenty Hanwoo heifers. The ovarian cycle serum LH and FSH profile indicated that all the animals on experiments were estrus synchronized followed by the follicular genesis. Increased daylight might have influenced ($p<0.05$) decrease in LH levels in the serum (Fig. 1 A). Average levels of both LH and FSH were greater in spring and summer compared to that in winter ($p<0.05$). Those levels tended to be greater ($p = 0.06$) in spring and less ($p = 0.09$) in winter compared to those levels in autumn. Heifers on estrus only in summer seemed to show significantly low levels of LH and FSH secretion ($p<0.05$) (Fig. 1, A and B). Heifers on estrus in spring, autumn or in winter showed moderate levels of LH and FSH secretion. This suggests that alteration of GnRH, that is, up-regulated GnRH receptors on the cell surface of pituitaries in summer heat stressed group control LH and FSH. Previous studies have reported seasonal changes in some endocrine and reproductive variables in females (Tucker, 1982; Randel, 1984) and males (Everett *et al.*, 1978; Godgery *et al.*, 1990; Stumpf *et al.*, 1993), even though cattle are not considered as seasonal breeders. Day *et al.*, (1986) and Stumpf *et al.* (1988) reported that season influenced patterns of LH secretion in gonadoectomized beef cattle. They observed the greatest mean concentration of LH and pulse amplitude at the spring equinox and lesser concentrations at winter solstice and at autumn equinox (Day *et al.*, 1986; Stumpf *et al.*, 1988; 1993). The species-specific differential secretion pattern of LH and FSH were reported in cattle, deer and horses (Schallenberger *et al.*, 1985; Monfort *et al.*, 1993; Silvia *et al.*, 1995). In the present study, coupled interactive seasonal variation between LH and FSH secretions was clearly observed during estrus in Hanwoo heifers. We need further investigation of seasonal effect on LH and FSH secretion in Hanwoo heifer population under the climatic condition in Daegwallyeong area.

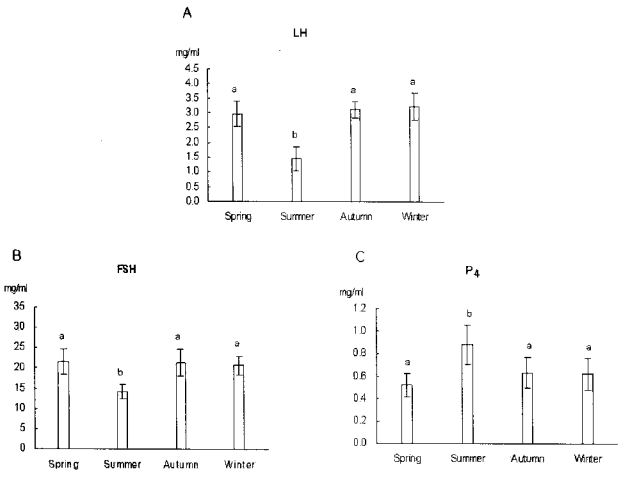

Fig. 1. Changes in the serum LH, FSH and progesterone (P_4) concentrations during estrus in Hanwoo heifers by seasons (means ± SEM). Means with different letters were different significantly ($p<0.05$).

Serum P_4 profile during estrus cycle indicated that all the animals on experiment ovulated followed by functional corpus luteum development. Whereas, P_4 concentrations fluctuated throughout the estrus cycle irrespective of seasonal changes (Fig. 1 C). The average serum P_4 concentration was found significantly ($p<0.05$) increased in summer (Fig. 1 C). Interestingly, P_4 concentration was higher in summer in contrast to lower concentration of LH or FSH. In Hanwoo heifers, variation in P_4 concentration during estrus was probably due to variation in LH stimulation of small luteal cells and subsequent regulation over P_4 secretion. In bovine, during luteinization process, hypertrophy and hyperplasia of theca cells occur that develop to small luteal cells (Niswender *et al.*, 1994). The small luteal cells possess functional LH receptors in large amount and involve in the regulation of P_4 secretion during early luteal phase. However, the large luteal cells, which develop from granulose cells, secret greater amount of P_4 in the absence of LH stimulation during mid and late luteal phases (Braden *et al.*, 1994).

3. Hormonal Changes in Holstein Heifer

Fig. 2 shows the changes in the serum LH, FSH and P_4 concentrations by seasons. Twenty Holstein heifers in summer show LH and FSH responses to heat stress (Fig. 2 A and B). Similar to the case of Hanwoo heifers, serum concentrations of LH and FSH decreased under heat stress condition in summer while P_4 levels were high during luteal phase (Fig. 2 A, B and C). There were no significant differences in serum concentrations of LH and FSH during ovarian cycles.

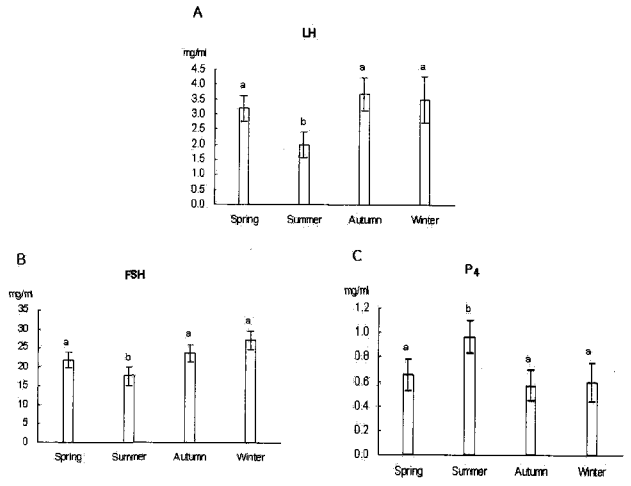

Fig. 2. Changes in the serum LH, FSH and Progesterone (P_4) concentrations during estrus in Holstein heifers by seasons (means ± SEM). Means with different letters were different significantly ($p<0.05$).

We observed that serum LH and FSH concentrations change during ovarian cycle in summer not only in Hanwoo cattle but also in Holstein cattle. Results from our study did not show any significant effects of summer heat on LH in response to exogenous GnRH in Holstein heifers. However, timed artificial insemination induced by hormonal control is necessary (Hansen and Arechiga, 1999) especially under heat stress condition in summer, which requires further investigation for Holstein lactating cows in cool regions like Daegwallyeong. In daily cattle in Arizona, of United States of America, vaginal temperature and respiratory rates but heat rates were reported to be significantly higher during hot season than during cool season (Huhnke and Monty, 1976). On the other hand, Berman (2005) stated that measures of radiant, convective, skin evaporative, respiratory heat loss, hair coat depth, and exposed body surface in order to estimate the extent of heat stress should be accompanied in addition to physiological indicators to understand thorough picture of the mechanisms behind the seasonal effects. We did not account for those ambient environmental parameters. We agree with Berman's opinion in that these parameters should be considered important in future studies on the effects of summer heat stress on reproduction performances of Hanwoo or Holstein cows and heifers in variable climatic regions.

In conclusion, the present study indicates significant effect of summer heat stress on performances of Hanwoo and Holstein heifers.

REFERENCES

Asdell SA. 1964. Patterns of Mammalian Reproduction, 2nd Edition. New York; Cornell University Press.

Berman A. 2005. Estimates of heat stress relief needs for Holstein daily cows. J. Anim. Sci. 83:1377-1384.

Braden TD, Belfiore CJ and Niswender GD. 1994. Hormonal control of luteal function. In: Findlay JK (ed), Molecular Biology of the Female Reproductive System. Academic Press, San Diego, pp. 259-287.

Bulman DC and Lamming GE. 1978. Milk progesterone levels in relation to conception, repect breeding and factors influencing a cyclicity in daily cows. J. Reprod. Fertil. 54:447-458.

Critser JK, Miller KF, Gunsett FC and Ginther OJ. 1983. Seasonal LH profile in ovariectomized cattle. Theriogenology 19: 181-191.

Croew MA, Padmanabhan V, Hynes N, Sunderland SJ, Enright WJ, Beitins IZ and Roche JF. 1997. Validation of a sensitive radioimmunoassay to measure serum follicle-stimulating hormone in cattle: correlation with biological activity. Anim. Reprod. Sci. 48:123-136.

Day ML, Imakawa K, Pennel PL, Zalesky DD, Clutter AC, Kittok RJ and Kinder JE. 1986. Influence of season and estradiol on secretion of luteinizing hormone in ovariectomized cows. Biol. Reprod. 35:549-553.

De Kruif A. 1978. Factors influencing the fertility of a cattle population. J. Reprod. Fertil. 54:507-518.

Everett RW, Bean B and Foote RH. 1978. Sources of variation of semen output. J. Daily Sci. 61:90-95.

Godfrey RW, Lunstra DD, Jenkins TG, Berardinelli JG, Guthrie MJ, Neuendorff DA, Long CR and Randel RD. 1990. Effect of season and location on semen quality and serum concentrations of luteinizing hormone and testosterone in Brahman and Hereford bulls. J. Anim. Sci. 68:734-749.

Hansen PJ and Arechiga CF. 1999. Strategies for managing reproduction in the heat-stressed daily cow. J. Anim. Sci. 77:36-50.

Hansen PJ and Hauser RR. 1983. Genotype X environmental interactions on reproductive traits of bovine females. Ⅲ. Seasonal variation in postpartum reproduction as influenced by genotype, suckling and dietary regimen. J. Anim. Sci. 56: 1362-1369.

Huhnke MR and Monty DE Jr. 1976. Physiologic responses of preparturient and postparturient Holstein-Friesian cows to

summer heat stress in Arizona. Am. J. Vet. Res. 37:1301-1304.

Kariya T. 1995. Effect of severe summer in 1994 on reproductive function in daily cattle in Hokkaido. Res. of Hokkaido Natl. Agric. Exp. Stn. 54:23-29 (in Japanese).

Legan SJ and Karsch FJ. 1980. Photoperiodic control of seasonal breeding in ewes: modulation of the negative feedback action of estradiol. Biol. Reprod. 23:1061-1068.

Mercier E and Salisbury GW. 1947a. Seasonal variations in hours of daylight associated with fertility level of cattle under natural breeding conditions. J. Daily Sci. 30:747-756.

Mercier E and Salisbury GW. 1947b. Fertility level in artificial breeding associated with season, hours of daylight and the age of cattle. J. Daily Sci. 30:917-826.

Klyuchnikov MT. 1975. Effect of season of calving on the effectiveness of AI in cattle. Anim. Breed. Abstr. 43:674.

Miyamoto A, Okuda K, Schweigert FJ and Schams D. 1992. Effects of basic fibroblast growth factor, transforming growth factor- beta and nerve growth factor on the secretory function of the bovine corpus luteum *in vitro*. J. Endocr. 135:103-114.

Monfort SL, Brown JL, Wood KC, Wemmer C, Vargas A, Willamson LR and Wildt DE. 1993. Seasonal patterns of basal and GnRH-induced LH, FSH and testodterone secretion in Eld's deer stag (*Cervus eldi thamin*). J. Reprod. Fertil. 98:481-488.

Niswender GD, Juengel JL, McGuire WJ, Belfiore CJ and Wiltbank MC. 1994. Luteal function; the estrus cycle and early pregnancy. Biol. Reprod. 50:239-247.

Peters AR and Riley GM. 1982a. Is the cow a seasonal breeder? Br. Vet. J. 138:533-537.

Peters Ar and Riley GM. 1982b. Milk progesterone profiles and factors affecting post partum ovarian activity in beef cows. Anim. Prod. 34:145-153.

Randel RD. 1984. Seasonal effects on female reproductive functions in the bovine (Indian breeds). Theriogenology 21:170-185.

Rosenberg M, Herz Z, Davidson M and Folman Y. 1977. Seasonal variations in post-partum plasma progesterone levels and conception in primiparous and multiparous daily cows. J. Reprod. Fertil. 51:363-367.

Rosenberg M, Folman Y, Herz Z, Flamenbaum I, Berman A and Kaim M. 1982. Effect of climatic conditions on peripheral concentrations of LH, progesterone and estradiol-17 β in high milk-yielding cows. J. Reprod. Fertil. 66:139-146.

Schallenberger E, Schonodorfer AM and Walters DM. 1985. Gonaotrophins and ovarian steroid in cattle; Pulsatile changes of concentrations in the jugular vein throughout the estrus cycle. Acta Endocrinol. 108:312-321.

Silvia PJ, Meyer SL and Fitzgerald BP. 1995. Pulsatile gonadotropin secretion determined by frequent sampling from the intercavernous sinus of the mare; possible modulatory role of progesterone during luteolysis. Biol. Reprod. 53:438-446.

Stevenson JS and Britt JH. 1979. Relationships among luteinzing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48:570-577.

Stumpf TT, Day ML, Wdlfe PL, Wdlfe MW, Clutter AC, Kittok RJ and Kinder JE. 1988. Feedback of 17β-estradiol on secretion of luteinizing hormone during different seasons of the year. J. Anim. Sci. 66:447-451.

Stumpf TT, Wolfe MW, Roberson MS, Kittok RJ and Kinder JE. 1993. Season of the year influences concentration and pattern of gonadotropins and testosterone in circulation of the bovine male. Biol. Reprod. 49:1089-1095.

Thibault C, Courot M, Martinet L, Mauleon P, de Mesnil du Buisson F, Ortavant R, Pelletier J and Signoret JP. 1966. Regulation of breeding season and estrus cycles by light and external stimuli in some mammals. J. Anim. Sci. (Suppl). 25:119-139.

Tucker HA. 1982. Seasonality in cattle. Theriogenology 17:53-59.

(접수일: 2008. 11. 15 / 채택일: 2008. 11. 27)