Different effects of an extended photoperiod treatment on growth, gonadal function, and condition of hair coats in Thoroughbred yearlings reared under different climate conditions

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One- to two-year-old Thoroughbred colts and fillies being reared in Miyazaki (warm climate) and Hidaka (cold climate), Japan, were administered extended photoperiod (EP) treatment between December 20 and the following April 10, and its effect on growth, endocrine changes, gonadal activation, and hair coat condition was investigated. In colts reared in Miyazaki, no effect of EP treatment was noted on the growth indices, including body weight (BW), height at withers (HW), girth, and cannon circumference (CC), whereas the BWs and CCs of fillies were significantly higher in the EP treatment group than the control. In Hidaka, the BWs and HWs of colts and HWs of fillies were significantly higher in the EP treatment group. Gonadal activation characterized by an increase in circulating hormone concentrations was earlier in the EP treatment group for fillies reared in Miyazaki [luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (P4), and estradiol-17β (E2)] and in colts (LH, testosterone, and E2) and fillies (LH, FSH, P4, and E2) reared in Hidaka. Regardless of sex and climate, prolactin was significantly higher in the EP treatment group, whereas insulin-like growth factor (IGF-I) was not. Initial ovulation occurred before April in more of the EP treatment group than the control regardless of the climate. Molting of the hair coat, examined in March, was advanced in the EP treatment group regardless of sex and climate. These results suggest that EP treatment may promote growth and gonadal activation in fillies reared in Miyazaki and in colts and fillies reared in Hidaka and that the effect may be mediated by prolactin.

Key words: climate, growth, extended photoperiod treatment, testis and ovary, Thoroughbred colt and filly

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at Hidaka Training and Research Center (Hidaka), Japan Racing Association (JRA), and that it advanced activation of gonadal function and coat molting and increased the muscle mass [45, 50]. In Japan, it is well known at southland horses grow faster than northland horses. Comparison of the growth of Thoroughbred colts and fillies reared under natural light between Miyazaki Yearling Training Farm (Miyazaki), JRA, and Hidaka clearly showed that both Thoroughbred colts and fillies reared in Miyazaki grew faster than those reared in Hidaka [45]. In addition, colts and fillies reared in Miyazaki showed faster development of testicular function and earlier ovulation, respectively, than those reared in Hidaka, clarifying the earlier promotion of gonadal function in Miyazaki [45].

The present study, therefore, was designed to clarify whether the promotion of growth, gonadal functions, and condition of the hair coat can be induced by EP treatment in Thoroughbred colts and fillies reared in Miyazaki. The results were compared with those of colts and fillies reared in Hidaka. In addition, endocrine changes induced by EP treatment were investigated to clarify the mechanism responsible for effects of EP treatment on physical condition of yearlings.

Materials and Methods

Animals

For analysis of growth and gonadal function, 48 Thoroughbreds born in Hokkaido and Aomori Prefectures were divided into two groups, with 24 horses (12 colts and 12 fillies) being reared at the Miyazaki Yearling Training Farm (31° 90’N, 139° 42’E, Miyazaki) and 24 horses (12 colts and 12 fillies) being reared at the Hidaka Training and Research Center (42° 17’N, 142° 72’E, Hidaka) of the JRA; these two locations are in regions with warm and cold climates, respectively. The horses were at the farms from the end of August at one year old to April at two years old. Three examiners randomly evaluated hair scores were compared.

Growth parameters

For the growth parameters, the body weight, height at withers, and girth and cannon circumferences were measured in December at one year old and April at two years old. To compare the growth rate of horses during the four months from December at one year old to April at two years old, the percent increments in the four parameters were calculated (values of April/values of December × 100).

Estimation of the condition of the hair coat

The condition of the hair coat was evaluated in March at two years old. Three examiners randomly evaluated hair using a 3-point scoring method: “excellent”, “normal”, and “poor” were scored as 3, 2, and 1, respectively, and the mean scores were compared.

Hormone assays

The plasma concentrations of prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined by homologous double-antibody equine radioimmunoassay (RIA) methods as described previously [17]. Plasma concentrations of prolactin were measured using an anti-equine prolactin serum (AFP-261987) and purified equine prolactin (AFP-8794B) for radioiodination and for the reference standard. Plasma concentrations of LH were measured using an anti-equine LH serum (AFP-2405080) and purified equine LH (AFP-2405080) for radioiodination and for the reference standard (AFP-50130A). Plasma concentrations of FSH were measured using an anti-equine FSH serum (AFP-2062096) and purified equine FSH (AFP-5022B) for radioiodination and for the reference standard. Intra- and inter-assay coefficients of variation were 7.1% and 9.8% for prolactin, 12.6% and 15.1% for LH, and 4.9% and 12.2% for FSH, respectively.

The plasma concentrations of insulin-like growth factor (IGF-1) were measured by RIA as previously described [14]
using anti-sera against human IGF-1 (AP 4892898) and purified human IGF-1 (Lot #090701) for radioiodination and for the reference standard. The intra- and inter-assay coefficients of variation were 2.7% and 14.8%, respectively.

The concentrations of progesterone, testosterone, and estradiol-17β were determined by double-antibody RIA systems using 125I-labeled radioligands as previously described [57]. Anti-sera against progesterone (GDN 337) [26], testosterone (GDN 250) [25], and estradiol-17β (GDN 244) [41] were used in each RIA. The intra- and inter-assay coefficients of variation were 7.3% and 14.3% for progesterone, 6.3% and 7.2% for testosterone, and 6.7% and 17.8% for estradiol-17β, respectively.

**Determination of ovulation**

The day seven days prior to the day when the plasma concentration of progesterone initially reached 1 ng/ml or higher was regarded as the initial ovulation day [44, 46].

**Statistical analysis**

All results are expressed as the mean ± standard error of the mean (SEM). Statistical comparisons between the two groups were performed by Student’s t-test when uniformity of variance was confirmed by the F-test. When the variance was not uniform, the unpaired t-test with Welch’s correction was used. Differences among times of sampling were evaluated by two-way repeated measure analysis of variance (ANOVA) with post hoc testing by Bonferroni post test. P<0.05 was considered to be statistically significant.

**Results**

**Colts reared in Miyazaki**

The mean rates of increase over the 4 months in the 4 parameters for the colts reared in Miyazaki are shown in Fig. 1a. No significant difference was noted in the mean rates of increase over the 4 months for body weight, height at withers, girth, or cannon circumference between the EP treatment and control groups.

Changes in the plasma concentrations of prolactin, LH, FSH, IGF-1, testosterone, and estradiol-17β of colts reared in Miyazaki from November to April in the EP treatment and control groups are shown in Fig. 2. In the EP treatment group, the plasma concentration of prolactin began to increase in December and abruptly increased in February, and the level was significantly higher in the EP treatment group than the control group from December to April (Fig. 2a). The basal plasma concentration of LH (0.16–0.54 ng/ml) was maintained from November to April in the EP treatment group, but it rose from January onward in the control group; however, it was not significantly different between the two groups (Fig. 2b). The plasma concentrations of FSH (Fig. 2c) and testosterone (Fig. 2e) were significantly higher in the control group than in the EP treatment group in April, but no significant difference was noted between the two groups in the other months. The plasma concentration of IGF-1 was not significantly different between the two groups (Fig. 2d).

**Fillies reared in Miyazaki**

The mean rates of increase over the 4 months in the 4 parameters for fillies reared in Miyazaki are shown in Fig. 1b. The rates of increases in body weight and cannon circumference over the 4-month period were significantly higher in the EP treatment group than the control group, and the rates for all 4 items were significantly higher in the EP treatment group from January to April, but the difference was not significant (Fig. 2f).
the EP treatment group compared with the control group in January and February, but it became significantly higher in the control group than the EP treatment group in April (Fig. 3a). The plasma concentration of LH did not change until April in the control group, but it rose in January in the EP treatment group. After February, it was higher in the EP treatment group, but the differences between the two groups were not significant (Fig. 3b). The plasma concentration of FSH was significantly higher in the EP treatment group than the control group in March and April (Fig. 3c). No significant difference was noted in the plasma concentrations of IGF-1 between the two groups (Fig. 3d). The plasma concentration of progesterone was low from November to January in both groups. It was significantly high in the control group as compared with the EP treatment group. The plasma concentration of progesterone rose in February in some fillies in the EP treatment group, whereas the level did not change until April in the control group. After February, no significant difference was noted because it rose to a high level in many fillies in the EP treatment group and there was increased variation of the mean (Figs. 3e and 4). The plasma concentration of estradiol-17β gradually rose from February in the control group, but it abruptly rose in January in the EP treatment group and it was significantly higher in the EP treatment group than the control group in November, January, and February (Fig. 3f). The initial ovulation occurred before April in 2 (33.3%) and 5 (83.3%) of 6 fillies in the control and EP treatment groups, respectively (Fig. 4).

Colts reared in Hidaka

The mean rates of increase over the 4 months in the 4 parameters for colts reared in Hidaka are shown in Fig. 5a. The rates of increases in body weight and height at withers were significantly higher in the EP treatment group than the control group (Fig. 5a).

Changes in the plasma concentrations of prolactin, LH, FSH, IGF-1, testosterone, and estradiol-17β of colts
reared in Hidaka from November to April in the EP treatment and control groups are shown in Fig. 6. The plasma concentration of prolactin rose from February to April in the control group, but it rose from January in the EP treatment group, and the level was significantly higher in the EP treatment group in January and February (Fig. 6a). The plasma concentration of LH (Fig. 6b) was maintained at the basal level until April in the control group, but it rose from January to April in the EP treatment group, and the level was not significantly different compared with that of the control group. No significant difference was noted in the plasma concentrations of FSH (Fig. 6c) and IGF-1 (Fig. 6d) between the two groups. The plasma concentration of testosterone rose in March in the control group and from January to April in the EP treatment group, but no significant difference was noted between the two groups because of marked variation among individuals in the EP treatment group (Fig. 6e). The plasma concentration of estradiol-17β rose from February to April in the control group, but it rose from January onward in the EP treatment group and was significantly higher than that in the control group in March (Fig. 6f).

**Fillies reared in Hidaka**

The mean rates of increase over the 4 months in the 4 parameters for fillies reared in Hidaka are shown in Fig. 5b. The rate of increase in height at withers was significantly higher in the EP treatment group than the control group (Fig. 5b). Changes in the plasma concentrations of prolactin, LH, FSH, IGF-1, progesterone, and estradiol-17β for fillies reared in Hidaka from November to April in the EP treatment and control groups are shown in Fig. 7. The plasma concentration of prolactin rose in March in the control group, whereas it rose in January in the EP treatment group, and the level was significantly higher in the EP treatment group than the control group in January and February (Fig. 7a). The plasma concentration of LH (Fig. 7b) rose in April in the control group, but it rose in February in the EP treatment group. No significant difference was noted in the level between the two groups because variations among individuals were marked in the EP treatment group. The plasma concentration of FSH rose slightly in both the EP treatment and control groups in December, and the level did not change until April in the control group; however, it rose from February in the EP treatment group, and the level was significantly higher in the EP treatment group than in the control group in March (Fig. 7c). No significant difference was noted in the plasma concentrations of IGF-1 between the two groups (Fig. 7d).
The plasma concentration of progesterone rose in April in the control group, whereas it rose in February in the EP treatment group, but no significant difference was noted between the two groups because of large variation among individuals in the EP treatment group (Figs. 7e and 8b). The plasma concentration of estradiol-17β rose in March in the control group and in January in the EP treatment group, but no significant difference was noted between the two groups (Figs. 7f).

**Changes in molting of winter coats**

Representative photographs taken at the end of March at two years old (3 months after EP treatment initiation) of colts and fillies in the control and EP treatment groups in Miyazaki and Hidaka are shown in Fig. 9, and graphs of the scores of the four groups are shown in Fig. 10. The representative photographs show that winter coats were still maintained in the control group reared in Miyazaki (colt, Fig. 9A; filly, Fig. 9C) and in Hidaka (colt, Fig. 9E; filly, Fig. 9G), whereas the molting of winter coats was advanced in colts (Miyazaki, Fig. 9B; Hidaka, Fig. 9F) and fillies (Miyazaki, Fig. 9D; Hidaka, Fig. 9H) in the EP treatment group at the end of March. The score was significantly higher in the EP treatment group than the control groups in colts (Fig. 10A) and fillies (Fig. 10B) in Miyazaki and colts (Fig. 10C) and fillies (Fig. 10D) in Hidaka.

**Discussion**

In the present study, the EP treatment was applied to yearlings reared in Miyazaki for the first time, and the effects on growth, gonadal function, and molting of winter coats were compared with those in yearlings reared in Hidaka. It was clarified that the effects of the EP treatment on yearlings were different between Miyazaki and Hidaka, southern and northern areas of Japan, respectively. Promotion of growth and early activation of gonadal function were noted in both colts and fillies in Hidaka. On the other hand, in Miyazaki,
these were observed in fillies, but no effect of the EP treatment was noted in colts, showing a difference in the effect of the EP treatment between Miyazaki and Hidaka.

Regarding growth, body weight and height at withers in colts and height at withers in fillies were greater in yearlings reared under the EP treatment than in those reared under natural light in Hidaka. In fillies reared in Miyazaki, body weight and cannon circumference were greater in the 4-month EP treatment group than the control group. In addition, the rate of increase rose significantly in February (2 months after EP treatment initiation) in all 4 items (body weight, height at withers, girth, and cannon circumference; data not shown). Regarding gonadal development, the EP treatment promoted gonadal function in colts and fillies reared in Miyazaki and Hidaka. Indeed, the present study clearly showed that the molting of winter coats was as advanced in colts of the EP treatment group reared in Miyazaki as in fillies reared in Miyazaki and colts and fillies reared in Hidaka. The effects of the EP treatment on colts reared in Miyazaki deserves further investigation.

Fig. 8. Changes in the plasma concentrations of progesterone from November at one year old to April at two years old in individual Thoroughbred fillies in the control (a) and extended photoperiod (EP) treatment groups (b) reared at the Hidaka Training and Research Center of the Japan Racing Association. Months are indicated by their initial letter.

In our previous observations for comparing the growth of yearlings reared under natural light between Miyazaki and Hidaka, growth was superior in both colts and fillies reared in Miyazaki [45]. Since there is a limit to growth, the light stimulation-induced promotion of growth may not have been perceivable for body weight, height at withers, girth, or cannon circumference in colts reared in Miyazaki. However, the prolactin secretion level markedly increased in colts reared in Miyazaki, suggesting that increased prolactin acts on some biological functions in colts and fillies reared in Miyazaki and Hidaka.

Regarding endocrine changes, prolactin secretion was promoted as a common phenomenon in both colts and fillies reared in Miyazaki and Hidaka. The effects of the EP treatment on endocrine changes in colts and fillies reared in Hidaka were consistent with those reported in our previous paper [42, 50]. Prolactin secretion is promoted with prolonging of the photoperiod, and it has diverse actions [5, 6, 12, 16, 17, 24, 27]. In addition, its promotion of coat molting and nest-building has been reported [18–20, 58, 59]. Prolactin receptors were expressed on the epiphyseal growth plate, and prolactin extended the tibia in lactating rats [54]. Prolactin has also been reported to promote calcium absorption from the intestine [9, 10, 55]. Furthermore, it promotes glucocorticoid secretion by adrenocortical cells [32–36], potentiates the immune function, and prevents gastric ulcer [2], and it has recently attracted attention as an anti-stress hormone [56]. Regarding the gonads, previous papers suggested that prolactin plays important physiological roles in ovarian function in mares in a systemic and local fashion [37, 38, 51]. Furthermore, the presence of prolactin and dopamine receptors was demonstrated in ovarian tissues of mares [37–39]. Prolactin upregulates its receptor and maintains the LH receptor in luteal cells of the mink [21]. Prolactin also has physiological roles in male reproduction in mammals, such as in steroidogenesis, gametogenesis, or sexual behavior, in many mammals [3]. An increase in circulating prolactin in the long-day period in stallions and geldings has also been reported [16]. The expression of prolactin receptor has been reported in the testes and various male accessory glands of human, bear, and dear [28–31], indicating that these organs might be targets of prolactin in male reproductive organs. Previous studies have also demonstrated that expression of the testicular prolactin receptor increased during testicular recrudescence in the breeding season of the bear or golden hamster [4, 30, 40].

Secretion of IGF-1 is induced by growth hormone, is mainly secreted by the liver, and promotes body growth [15]. The measurement of IGF-1 gives the clue of status
Fig. 9. Comparison of the hair coat conditions of representative colts (control, A; extended photoperiod (EP) treatment, B) and fillies (control, C; EP treatment, D) reared at the Miyazaki Yearling Training Farm and of those of colts (control, E; EP treatment, F) and fillies (control, G; EP treatment, H) reared at the Hidaka Training and Research Center of the Japan Racing Association in March at two years old.
of the somatotropic axis as it has longer half-life with no obvious diurnal rhythm [8]. Localization of IGF-1 and its receptor has been demonstrated in equine testes [60], but long-day stimulation applied by the EP treatment did not change the secretion level in horses reared in either Miyazaki or Hidaka in the present study.

The EP treatment increased the secretion of two gonadotropic hormones (LH and FSH) from the pituitary in colts and fillies reared in Hidaka and fillies reared in Miyazaki. Testosterone and estradiol-17β secretions from the testis increased in colts reared in Hidaka, and estradiol-17β and progesterone secretions from the ovary increased in fillies reared in Hidaka and Miyazaki. In mares, estradiol-17β is secreted by mature follicles, and progesterone is secreted by the corpus luteum [44, 46], suggesting that secretion of these hormones was promoted due to EP treatment-induced promotion of LH and FSH secretion from the pituitary in fillies in the present study. In colts, Leydig cells in the testis may have been stimulated and secreted testosterone and estradiol-17β, promoting spermatogenesis. In fillies, follicular growth into a mature follicle may have been stimulated and resulted in early ovulation. Based on the results of hormones measurement, gonadotropic hormone secretion from the pituitary increased from the end of January, about one month after EP treatment initiation, in both colts and fillies, clarifying that about one month is necessary to achieve an effect of EP treatment on the hypothalamus and pituitary axis in yearlings. Estrus and ovulation start after April at two years old in many fillies reared under natural light. Therefore, initiation of EP treatment in December advances ovulation by about two months in fillies in a manner similar to broodmares.

In the stallion, testosterone and estradiol-17β are secreted by Leydig cells in the testis [47–49]. Testosterone promotes the growth and function of the male accessory reproductive glands and enhances spermatogenesis. It is also known to increase fat catabolism and inhibit the accumulation of triglycerides, reducing body fat accumulation [1]. Testosterone also has a potent protein-assimilating action and increases muscle mass [22]. Furthermore, it directly acts on bone or is converted to estradiol-17β by aromatase, and it promotes osteogenesis [11, 44, 52]. Estradiol-17β is secreted by granulosa cells of the mature antral follicle in mares [44, 46]. It induces estrus and preparation for fertilization and implantation by promoting endometrial growth, development of the uterine gland, and uterine and vaginal mucous secretion. Its action on bone is also known, and it promotes bone maturation and increases the bone mineral density [43]. Estradiol-17β also enhances fat metabolism and reduces body fat accumulation [13].

In summary, it was clarified that the growth of Thoroughbred yearlings reared under natural light in Japanese climates is superior in Miyazaki (southern area) compared with Hidaka (northern area) [45]. However, it may be possible to promote growth in Hidaka so that it is close to that in Miyazaki by extending the hours of sunlight by EP treatment. It is necessary to closely investigate the influences of EP treatment on muscles, fat, and cardiopulmonary function in the rearing of yearlings subjected to EP treatment.

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Fig. 10. Comparison of scores for hair coat condition between the extended photoperiod (EP) treatment group (□) and control group (■) for horses reared at the Miyazaki Yearling Training Farm (colts, A; fillies, B) and Hidaka Training and Research Center of the Japan Racing Association (colts, C; fillies, D). Results are expressed as means ± SEM. *Significant difference at P<0.05.
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