—Original Article—

Comparison of growth and endocrine changes in Thoroughbred colts and fillies reared under different climate conditions

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Development and endocrine changes in Thoroughbreds colts and fillies were compared between those reared at two facilities of the Japan Racing Association, the Hidaka Training and Research Center (Hidaka) and Miyazaki Yearling Training Farm (Miyazaki). Thoroughbred colts and fillies born in Japan between 2003 and 2010 were used. Each colt group and filly group was divided into 2 groups, respectively, and raised in Hidaka or Miyazaki for 7 months from September at 1 year old to April at 2 years old. For the growth parameters, the body weight, height at withers, and girth and cannon circumferences were measured once a month. For parameters of endocrine function, circulating prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), insulin-like growth factor-1 (IGF-1), testosterone, progesterone, and estradiol-17β levels were measured. Regarding growth, the rate of increase over the 7-month period was significantly higher in both colts and fillies raised in Miyazaki than in Hidaka in all 4 parameters: body weight, height at withers, and girth and cannon circumferences. The endocrine changes of the colts and fillies born in 2007 were as follows. In colts, although circulating prolactin tended to be higher in colts reared in Hidaka from October to April, circulating LH, FSH, testosterone, estradiol-17β and IGF-1 tended to be higher in colts reared in Miyazaki than in Hidaka, suggesting that the gonadotropin-releasing hormone-LH/FSH system and the growth hormone-IGF-1 system were more active in colts reared in Miyazaki as compared with those reared in Hidaka. In fillies, circulating prolactin tended to be higher in fillies reared in Hidaka in February and March, but no significant difference was noted in the serum LH, FSH, IGF-1, or progesterone level between the 2 groups. Circulating estradiol-17β tended to be higher in fillies reared in Miyazaki than in Hidaka in October and November. Regarding ovarian function, the initial ovulation occurred by the end of March in 2 (16.7%) of 12 fillies reared in Hidaka and 7 (38.9%) of 18 fillies reared in Miyazaki, suggesting that the ovarian function was more active in fillies reared in Miyazaki as compared with those reared in Hidaka. Based on these findings, it was clarified that development of the body and gonads was faster in Miyazaki compared with Hidaka in both colts and fillies.

Key words: *Thoroughbred colt and filly, growth, testis, ovary, climate*

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Horses are typical long-day seasonal breeders, and the breeding season is spring through summer in Japan, during which spermatogenesis in the testis becomes active in males, and periodic ovulation repeats in females [4, 5, 10–12]. Thoroughbred mares are mated in spring, and deliveries mostly occur in spring through summer in Japan. Hokkaido has recently become known as the major area for producing and rearing Thoroughbred horses in Japan. Many Thoroughbred horses have been produced and reared in Hokkaido in Japan. It is known that horses grow well in the southland which has a warm climate, compared with the northland, which has a cold climate, in Japan, but there has been no report on accurate comparison of them. In the present study, to compare the growth rate during development of Thoroughbred horses reared under different climate conditions in Japan, Thoroughbred colts and fillies were reared at the Japan Racing Association (JRA) Miyazaki Yearling Training Farm (Miyazaki) which has a warm climate, and Hidaka Training and Research Center (Hidaka), which has a cold climate, for 7 months from fall at 1 year old to spring at 2 years old, and their development was analyzed. In addition, to compare the growth-related changes in endocrine function, the circulating levels of insulin-like growth factor (IGF-1) secreted from the liver in response to growth hormone stimulation and 3 pituitary hormones [prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH)] and 3 gonadal hormones (progesterone, testosterone, estradiol-17β) were measured.

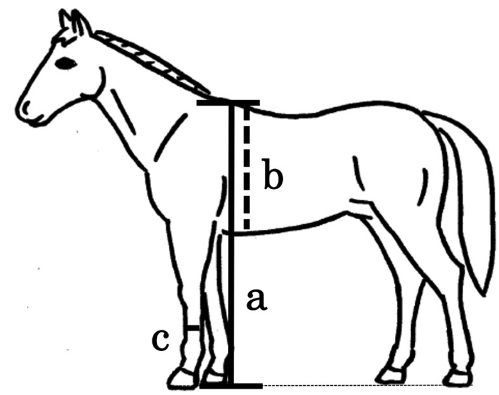

Fig. 1. Measurement of the 3 growth parameters, height at withers (a), girth circumference (b) and cannon circumference (c) in the Thoroughbred colt and filly.

Materials and Methods

Animals

For growth-related analysis, 309 Thoroughbred horses (161 colts and 148 fillies) born in 2003–2010 were used. Each colt group and filly group was divided into 2 groups, respectively, and raised in Hidaka (81 colts and 73 fillies) or Miyazaki (80 colts and 75 fillies) for 7 months from September at 1 year old to April at 2 years old. For endocrine analysis, 36 Thoroughbred horses born in 2007 were used. Each colt group and filly group was divided into 2 groups, respectively 12 horses (6 colts and 6 fillies) were raised in Hidaka, and 24 horses (12 colts and 12 fillies) were raised in Miyazaki. For analysis of date of ovulation, 30 fillies born in 2007 and 2008 were used. All the horses in Hidaka and Miyazaki were fed grass hay, oats, barley, and pelleted feeds (Ace Ration and Power Max, Nosan Corporation, Yokohama, Japan) at 06:00 hr, 12:00 hr, 16:00 hr and 20:00 hr. For training of horses in both Hidaka and Miyazaki, a one-hour exercise program including 20 min of trot and gallop was performed. After the exercise program, they walked one more hour in a walking machine. In addition, all horses were left to pasture for one hour daily. During pasturing in the winter season, horses eat fresh grass in Miyazaki and hay in Hidaka *ad libitum*. All horses consumed approximately equivalent amounts of digestible energy from food. The horses were 19–22 months old at the time of experiment initiation. All procedures were carried out in accordance with the guidelines on use of horses established by Hidaka Training and Research Center.

Growth parameters

For the growth parameters, body weight, height at withers, and girth and cannon circumferences were measured once a month (Fig. 1). To compare the growth rate of horses during the 7 months from September at 1 year old to March at 2 years old, the percent increments of the 4 parameters were calculated (values of March/values of September × 100). In addition, the monthly rate of increments of the 4 parameters were also calculated by the same method.

Parameters of endocrine function

Blood was collected from the jugular vein into a heparinized vacutainer once a month from September 2008 to April 2009. Plasma was harvested and stored at −20°C until assayed. For parameters of endocrine function, circulating prolactin, LH, FSH, IGF-1, testosterone, progesterone, and estradiol-17β levels were measured.

Determination of the date of ovulation

The day 7 prior to the day when the plasma concentrations of progesterone initially reached 1 ng/m*l* or higher was arbitrarily designated as the date of ovulation [10, 11].

Hormone assays

Plasma concentrations of prolactin, FSH and LH were determined by homologous double-antibody equine radioimmunoassay (RIA) methods as described previously [5]. Plasma concentrations of prolactin were measured using an anti-equine prolactin serum (AFP-261987) and purified equine prolactin (AFP-8794B) for radioiodination and 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 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 reference standard. The intra- and inter-assay coefficients of variation were 7.1% and 9.8% for prolactin, and 12.56% and 15.06% for LH, and 4.9% and 12.2% for FSH, respectively.

Plasma concentrations of IGF-1 were measured by RIA as previously described [3] using anti-sera against human IGF-1 (AP 4892898) and purified human IGF-1 (Lot#090701) for radioiodination and reference standard. The intra- and inter-assay coefficients of variation were 2.7% and 14.8%, respectively.

Plasma concentrations of progesterone, testosterone and estradiol-17β were determined by double-antibody RIA systems using ^{125}I-labeled radioligands as previously described [13]. Anti-sera against progesterone (GDN 337) [8], testosterone (GDN 250) [7] and estradiol-17β (GDN 244) [9] 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.

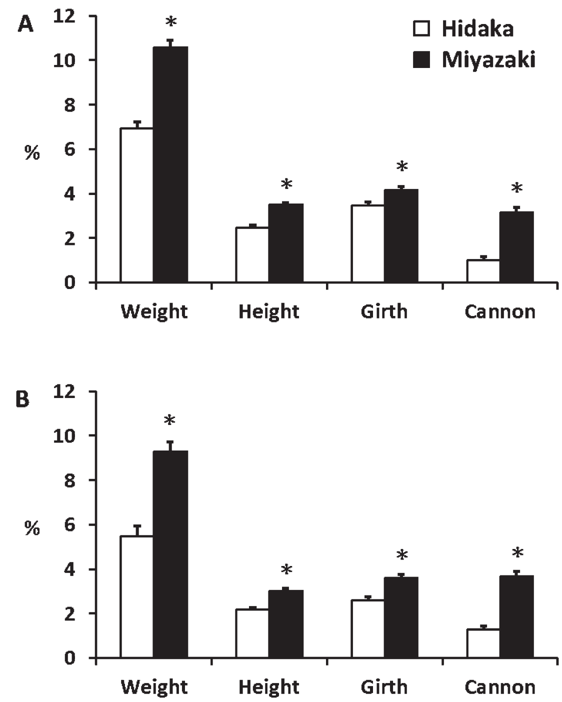

Fig. 2. Comparison of the mean rates of increase of body weight, height at withers, and girth and cannon circumference over the 7 months from September at 1 year old to March at 2 years old in Thoroughbred colts (A) and fillies (B) reared at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of the Japan Racing Association. Results are expressed as means ± SEM. *Significant differences at P<0.05 (Student's t-test).

Statistical analysis

All results were expressed as the mean ± standard errors 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, an unpaired t-test with Welch's correction was used. Differences among times of sampling were evaluated by two-way factorial analysis of variance (ANOVA) with post hoc testing by Bonferroni post test. P<0.05 was considered to be statistically significant.

Results

Growth rate

The mean rates of increase over the 7 months in the 4 parameters are shown in Fig. 2, and the mean rates in each month in colts and fillies are shown in Figs. 3 (colts) and 4 (fillies). The mean rate of increase over the 7 months from September at 1 year old to March at 2 years old was significantly higher in the Miyazaki group than in the Hidaka group in all 4 parameters (body weight, height at withers, and girth and cannon circumferences) in both colts and fillies (Fig. 2). In colts, the monthly rate of increase was significantly higher in the Miyazaki group than in the Hidaka group in body weight in November, January, and March, height at withers in October, December, and January, girth circumference in October, and cannon circumference in October, December, February, and March (Fig. 3). In fillies, the monthly rate of increase was significantly higher in the Miyazaki group than in the Hidaka group in body weight from November to February, height at withers from October to January, girth circumference in October and February, and cannon circumference in October, December, February and March (Fig. 4).

Endocrine changes

Changes in the 6 hormone levels in colts are shown in Fig. 5. Circulating prolactin levels were low from October to January and rose from February to April in both groups, but there was no significant difference between the two groups. Circulating LH levels were low from September to January and rose from February and March in the Miyazaki and Hidaka groups, respectively. They tended to be higher

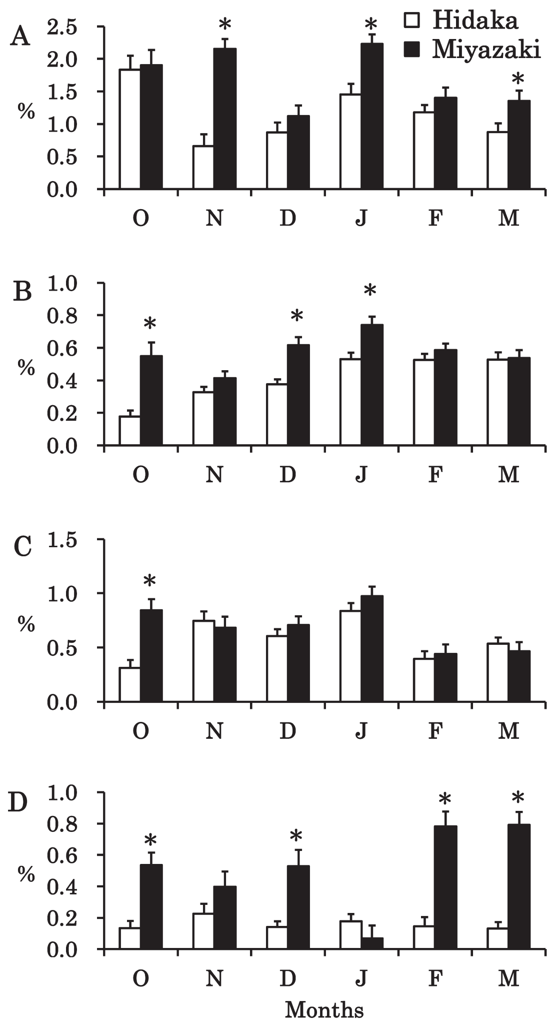

Fig. 3. Comparison of the monthly rates of increase of body weight (A), height at withers (B), girth circumference (C) and cannon circumference (D) from September at 1 year old to March at 2 years old in Thoroughbred colts reared at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of the Japan Racing Association. Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences at $P<0.05$ (Student's *t*-test).

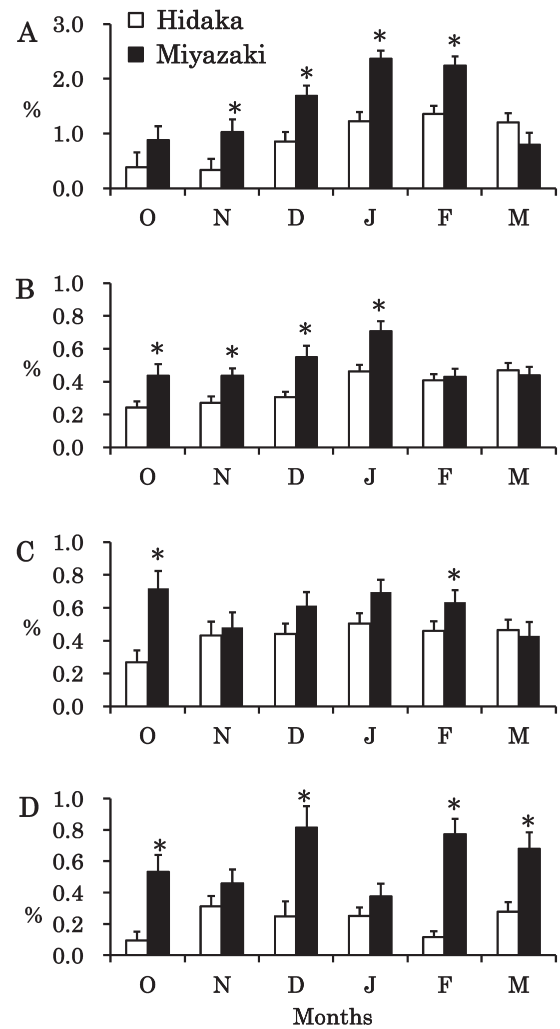

Fig. 4. Comparison of the monthly rates of increase of body weight (A), height at withers, (B), girth circumference (C) and cannon circumference (D) from September at 1 year old to March at 2 years old in Thoroughbred fillies reared at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of the Japan Racing Association. Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences at $P<0.05$ (Student's *t*-test).

in the Miyazaki group in February and thereafter, but the difference was not significant. Circulating FSH levels tended to be higher in the Miyazaki group than in the Hidaka group from September to March, but no significant difference was noted due to large variations among animals in the Miyazaki group. Circulating IGF-1 levels tended to be higher from September to April in the Miyazaki group than in the Hidaka group, but there was no significant difference between the two groups. Circulating testosterone levels were low in September and October and slowly rose from November to April in the Miyazaki group. In the Hidaka group, they were low from September to February and rose from March to April. They tended to be higher in the Miyazaki group than in the Hidaka group in December and thereafter, but there was no significant difference between the two groups. Circulating estradiol-17β levels slowly rose from November to April in the Miyazaki group and from December to April in the Hidaka group. They tended to be higher in the Miyazaki group than in the Hidaka group from October to April, but no significant difference was noted between the two groups due to large variations among animals in Miyazaki group.

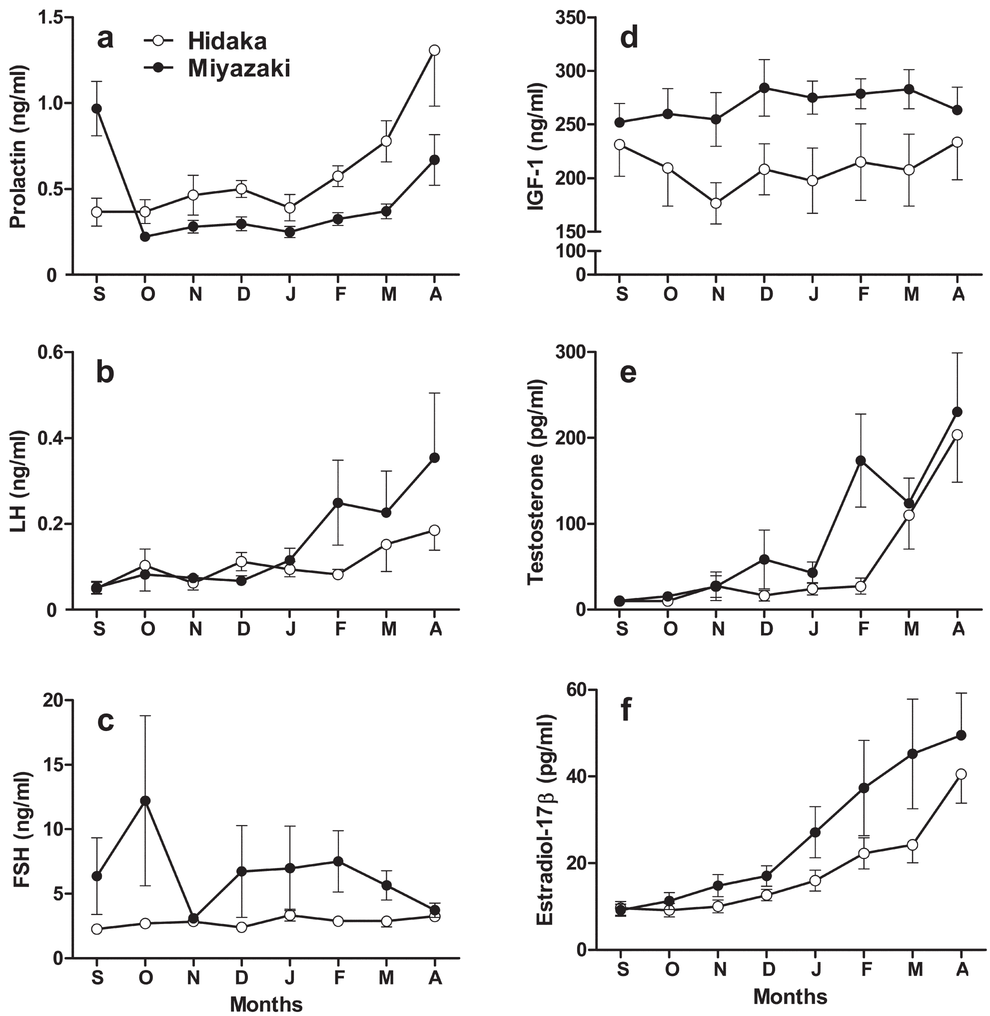

Fig. 5. Monthly changes in concentrations of circulating prolactin (a), LH (b), FSH (c), IGF-I (d), testosterone (e), and estradiol-17*β* (f) in Thoroughbred colts reared at the Hidaka Training and Research Center (○) and Miyazaki Yearling Training Farm (●) of the Japan Racing Association. Results are expressed as means ± SEM. Months are indicated by their initial letters.

Changes in the 6 hormone levels in fillies are shown in Fig. 6. Circulating prolactin levels were low from October to February and rose from March to April in the Miyazaki group and from February to April in the Hidaka group, but there was no significant difference between the two groups. Circulating LH levels were low in October and November and rose from December to April in both groups. No significant difference was noted between the two groups, but variations among animals were large in March and April in both groups. The circulating FSH level tended to be high in December and January in both groups, and no significant difference was noted between the two groups. No marked change was noted in the plasma IGF-1 level from September to April in either group. It tended to be higher in the Hidaka group than in the Miyazaki group, but the difference was not significant. Circulating progesterone levels were low from November to March and rose in April in both groups, and no significant difference was noted between the two groups. In April, variations among animals were large in both groups. Circulating estradiol-17*β* levels were low from October to January and rose from February to April in both groups. They tended to be higher in the Miyazaki group than in the Hidaka group from October to March, but there was no significant difference between the two groups.

First ovulation of fillies

Two (16.7%) of 12 fillies in the Hidaka group and 7 (38.9%) of 18 fillies in the Miyazaki group were assumed to have ovulated earlier than the end of March.

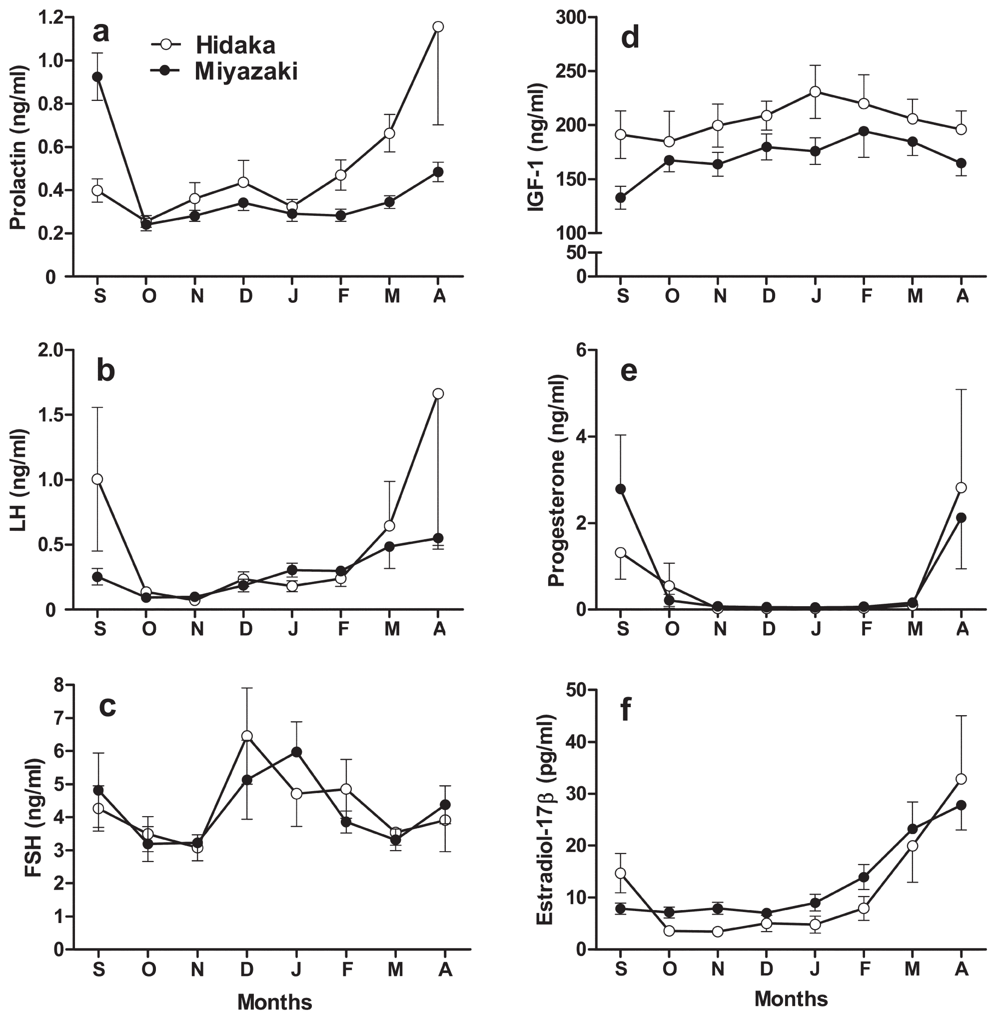

Fig. 6. Monthly changes in concentrations of circulating prolactin (a), LH (b), FSH (c), IGF-I (d), progesterone (e), and estradiol-17β (f) in Thoroughbred fillies reared at the Hidaka Training and Research Center (○) and Miyazaki Yearling Training Farm (●) of the Japan Racing Association. Results are expressed as means ± SEM. Months are indicated by their initial letters.

Discussion

Male and female Thoroughbreds (375 horses in total) were raised for 7 months, from before they were 2 years old until after they turned 2 years old at the Miyazaki Yearling Training Farm located in Kyushu and Hidaka Training and Research Center located in Hokkaido, southern and northern areas of Japan, respectively, during the period of 2003–2010, and their growth and gonadal function were analyzed. In addition, 6 hormones were measured in Thoroughbred colts and fillies similarly reared in Hidaka and Miyazaki.

Regarding growth, the rate of increase was higher in both Thoroughbred colts and fillies raised in Miyazaki than in those raised in Hidaka in all 4 parameters (body weight, height at withers, and girth and cannon circumferences). Regarding reproduction, the initial ovulation day was earlier in the Miyazaki group than in the Hidaka group in fillies, and in colts, circulating LH, FSH, testosterone, and estradiol-17β tended to be higher in the Miyazaki group than in the Hidaka group. These results suggest that earlier development of gonadal function occurred in Miyazaki as compared with Hidaka. Furthermore, the circulating IGF-1 level tended to be higher in the Miyazaki group than in the Hidaka group in colts. IGF-1 is secreted from the liver in response to growth hormone stimulation, and it directly acts on the growth of animals. Previous papers clearly demonstrated that stallions had a higher concentration of circulating IGF-I during the breeding season as compared with the nonbreeding season [4]. The intensity of immune response of IGF-I and its receptor labeling in the stallion Leydig cell was shown to be

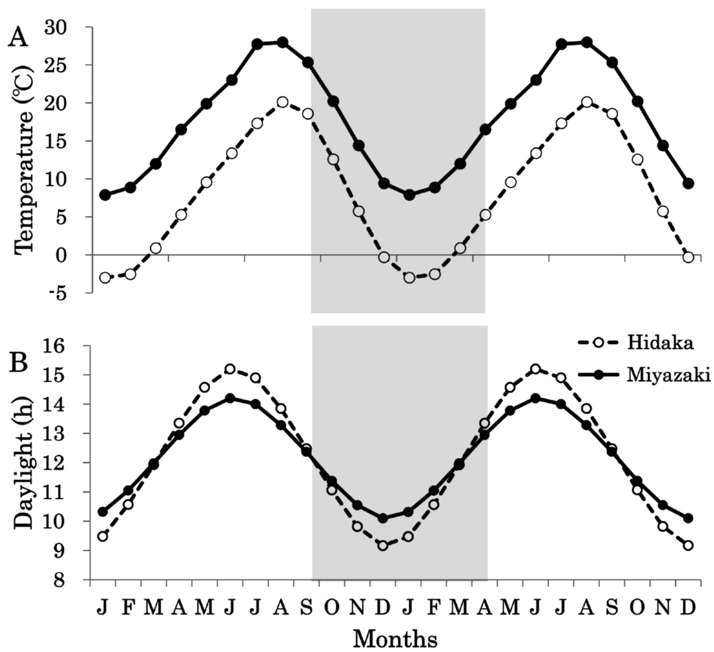

Fig. 7. Monthly changes in the pattern of temperature (A) and daylight (B) in Hidaka (○) and Miyazaki (●) in Japan. Values are represented as means. Months are indicated by their initial letters.

age dependent and increased in postpubertal stage [14]. The values of IGF-I in Thoroughbred colts in the present experiment are similar to those in a previous study on stallions of Thoroughbreds [4] and Standardbred [2]. Testosterone is an anabolic hormone that stimulates protein synthesis and muscle growth [1, 6]. It was clarified that Thoroughbreds colts reared in Miyazaki grew faster than those reared in Hidaka, and development of the testicular function was also fast. The promoted development of testicular function was assumed to be due to increases in the secretion of prolactin and two gonadotropic hormones (LH and FSH) from the pituitary.

In Thoroughbreds fillies, animals reared in Miyazaki grew faster than those reared in Hidaka, and ovarian function was also enhanced. The circulating estradiol-17β level tend to be higher in the Miyazaki group than in the Hidaka group, showing that follicular development in the ovary was promoted. The circulating estradiol-17β levels in fillies in the present study are similar to those in a previous study in cyclic mature Thoroughbred mares [10, 11]. However, whether or not circulating IGF-1 and gonadotropic hormone secretion was promoted was unclear. In addition, at present, the reason for the difference in the results between colts and fillies is unclear.

Since horses are long-day seasonal breeders, the hypothalamus-pituitary-gonadal system is activated with the prolongation of daylight hours. According to a report from the Japan Meteorological Agency, the period of daylight in winter is about one hour longer in Miyazaki than in Hidaka. In addition, the temperature is about 10°C higher throughout year in Miyazaki than in Hidaka (Fig. 7). These differences in climate may have influenced growth and early development of gonadal function in horses reared in Miyazaki. In conclusion, the present study demonstrated that Thoroughbred horses reared in Miyazaki for 7 months, from before they were 2 years old until after they turned 2 years old, grow faster than horses reared in Hidaka. Further investigation is needed to clarify endocrine mechanisms responsible for hastening growth rate and gonadal function in Miyazaki as compared with Hidaka.

Acknowledgments

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