—Original Article—

Effects of an extended photoperiod on gonadal function and condition of hair coats in Thoroughbred colts and fillies

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The effects of an extended photoperiod (EP) in Thoroughbreds colts and fillies from winter at one year old to spring at two years old on the gonadal functions, coat condition, and endocrine changes were investigated. Sixty-two Thoroughbreds (31 colts and 31 fillies) reared in the Hidaka Training and Research Center (Hidaka), Japan Racing Association were used. Thirty of them (15 colts and 15 fillies) were reared under EP conditions from December 20 to April 10, and the remaining 32 horses were reared under natural light alone as a control group. For EP, a 100-watt white bulb was set near the ceilings of stalls, and lighting conditions of 14.5-hr light and 9.5-hr dark periods were established. Blood was collected from the jugular vein once a month from October at one year old to February at two years old in both colts and fillies, and then twice a month in colts and weekly in fillies after March, and the coat condition was evaluated in January and April in 56 horses. To investigate endocrine changes, the plasma concentrations of prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), immunoreactive (ir-) inhibin, testosterone, estradiol-17β and progesterone were measured. No significant difference was noted in the coat condition between the two groups in January, but they changed from winter to summer coats (molting of winter coats) in April in the EP group compared with the control group. Regarding endocrine changes, the plasma concentrations of prolactin, FSH, ir-inhibin and testosterone were significantly higher in the EP colts than in the control group from January to April. The plasma concentrations of LH tended to rise in the EP colts from January to April compared with the control group. In the EP fillies, the plasma concentrations of prolactin, LH, ir-inhibin, estradiol-17β and progesterone were significantly higher during January and April, but a significantly high level of FSH was noted in the control than EP group in January. The ovulation day was advanced in the EP fillies compared with the control group. The present study clearly demonstrated that EP treatment during rearing advanced the molting of winter coats in both colts and fillies. These results suggested to be due to the action of prolactin being increased by EP treatment. In addition, EP treatment stimulated the hypothalamus-pituitary-gonadal axis even in yearlings, and advanced ovulation in fillies. Since EP

treatment-induced changes in the yearlings were within the physiological range, and the method is safe and simple, EP treatment may be an effective technique in horse husbandry.

Key words: *extended photoperiod, Thoroughbred colt and filly, endocrine changes, molting of winter coats, gonadal function*

Horses are typical long-day seasonal breeders. In the long-day period, circulating gonadotropins and gonadal hormones increase and testicular function is activated in stallions [12, 13, 37, 38, 43] and the ovarian function is activated and cyclic ovulation repeats in mares [13, 33, 36, 39, 43] making them fertile. A long-day period can be induced by lighting stalls, extending the lighting time during the short-day period. Extended photoperiod (EP) treatment activates the ovarian function earlier in broodmares, and it is widely used to advance the ovulation time in broodmares in husbandry [43]. However, the effect of EP treatment on yearlings is unclear. In the present study, Thoroughbreds colts and fillies were subjected to EP treatment from winter at one year old to spring at two years old, and changes in circulating prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), immunoreactive (ir-) inhibin, progesterone, testosterone and estradiol-17β, the gonadal function and coat condition were investigated.

Materials and Methods

Animals

Sixty-two Thoroughbreds (31 colts and 31 fillies) born and reared in the Hidaka Training and Research Center (Hidaka), Japan Racing Association (JRA), were used. Thirty of them (15 colts and 15 fillies) were subjected to EP treatment from December 20 to April 10 and the remaining 32 horses were reared under natural light alone as a control group. The test horses were 19–22 months old at the time of experiment initiation. All procedures were carried out in accordance with the guidelines established by Hidaka Training and Research Center, for use of horses.

EP treatment

A 100-watt white bulb was set near the ceilings of stalls (3.6 × 3.6 m), and lighting conditions were 14.5-hr light and 9.5-hr dark periods. The intensity of illumination under the bulb, at the height of the horse's head, was approximate 100 lux.

Collection of blood

Blood was collected from the jugular vein into heparinized vacutainer (10 ml) during 09:00h and 12:00h once a month from October at one year old to February at two years old in both colts and fillies, and then twice a month in colts

and weekly in fillies after March. Plasma were harvested and stored at −20°C until assayed.

Determination of the first ovulation

Seven days prior to the day when the plasma concentrations of progesterone initially reached 1 ng/ml or higher were arbitrarily designated as the initial ovulation day.

Estimation of condition of hair coats

The condition of hair coat was evaluated in January and April in 56 horses (27 colts and 29 fillies). Three examiners randomly evaluated hair in January and April at 2 years old, respectively, using the 3-point scoring method: "excellent", "normal", and "poor" were scored 3, 2, and 1, respectively, and the mean scores were compared.

Hormone assays

Plasma concentration of prolactin, FSH and LH were determined by homologous double-antibody equine radioimmunoassay (RIA) methods as described previously [13]. 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. 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.

Plasma concentrations of ir-inhibin were measured using a rabbit antiserum against purified bovine inhibin (TNDH 1) and ^{125}I-labeled 32-kDa bovine inhibin, as previously described [21]. The results were expressed in terms of 32-kDa bovine inhibin. The intra- and inter-assay coefficients of variation were 8.0% and 16.2%, respectively.

Plasma concentrations of progesterone, testosterone and estradiol-17β were determined by time-resolved fluoroimmunoassay using dissociation-enhanced fluorescence immunoassay (DELFIA) systems (PerkinElmer, Waltham, MA, USA) as described previously [32, 41]. The intra- and inter-assay coefficients of variation were 5.5% and 8.6% for

progesterone, 6.3% and 7.2% for testosterone and 4.6% and 9.4% for estradiol-17β, respectively.

Statistical analysis

All results were expressed as 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, unpaired *t*-test with Welch's correction was used. Differences among times of sampling were evaluated by two-way factorial analyses of variance (ANOVA) with post-hoc testing by Bonferroni post test. When the interaction is consider extremely significant, one-way factorial ANOVA or Kruskal Wallis test with post-hoc testing by Dunn's post test was used. Mann-Whitney *U*-test was used in comparison of score of hair coat condition between the EP group and the control group. *P*<0.05 was considered to be statistically significant.

Results

Endocrine changes in colts

Changes in the plasma concentrations of prolactin, LH, FSH, ir-inhibin, testosterone and estradiol-17β from October to April in the EP and control groups are shown in Fig. 1. The plasma concentration of prolactin was low until February and began to increase in March, followed by a sharp increase in April in the control group. On the other hand, in the EP group, it rose from January, one month after EP initiation, to April. During January and April, the plasma concentration of prolactin was significantly higher in the EP group than in the control group (Fig. 1a). The plasma concentration of LH was low until the middle of March and slowly rose from the end of March in the control group, whereas it rose from January in the EP group, but no significant difference was noted between the two groups (Fig. 1b). The plasma concentration of FSH slowly rose from March in the control group. In the EP group, it rose from January and became significantly higher than that in the control group in January, February, and March (Fig. 1c). The plasma concentration of ir-inhibin slowly rose from December in both groups, and it was significantly higher in the EP than in the control group in December, January, and February (Fig. 1d). The plasma concentration of testosterone slowly rose from November and peaked in March in both groups, and it was higher in the EP than in the control group. There was a significant difference in January between the two groups (Fig. 1e). Plasma concentrations of estradiol-17β were not significantly different between the two groups (Fig. 1f).

Endocrine changes in fillies

Changes in the circulating prolactin, LH, FSH, ir-inhibin, estradiol-17β, and progesterone from October to April in the EP and control groups are shown in Fig. 2. The plasma concentration of prolactin was low until March and slightly rose in April in the control group, but that in the EP group began to increase in January followed by constant increase until April (Fig. 2a). The plasma concentration of prolactin was significantly higher in the EP than control group from January and thereafter. When the circulating prolactin was investigated in each animal, the circulating prolactin did not change until April in the control group, whereas it rose from January in the EP group, and an elevation with marked variation was noted from February in some animals. The plasma concentration of LH was low from November to March and slightly rose in April in the control group. In the EP group, it rose from February and became significantly higher after February in the EP than control group (Fig. 2b). When the circulating LH was investigated in each animal, it was low until March in the control group, but periodic, marked variation of the level was noted from February in the EP group (Fig. 3a and 3c). The plasma concentration of FSH slowly rose from November to April in both groups. Excluding a significantly lower level in the EP than control group in January, no significant difference was noted between the two groups. When the plasma concentration of FSH was investigated in each animal, sporadic increases were noted from March in some fillies in the control group, but large variations were noted from February in the EP group. The plasma concentration of ir- inhibin rose from January in both groups, and it was significantly higher in the EP than control group in January (Fig. 2d). The plasma concentration of estradiol-17β rose from January in both groups, and it was significantly higher in the EP than control group throughout the period (Fig. 2e). When the plasma concentration of estradiol-17β was investigated in each animal, the plasma concentration of estradiol-17β showed marked variation in both groups, and this pattern started from March in the control group and February in the EP group. The plasma concentration of progesterone was markedly different between the two groups. It remained at the baseline level from November to April in the control group, but it rose to a significantly higher level than that in the control group in February, and it remained at a high level thereafter in the EP group (Fig. 2f). When the plasma concentration of progesterone was investigated in each animal, the plasma concentration of progesterone was low and did not change until April in the control group, but marked periodic variation was noted from February in the EP group (Fig. 3b and 3d).
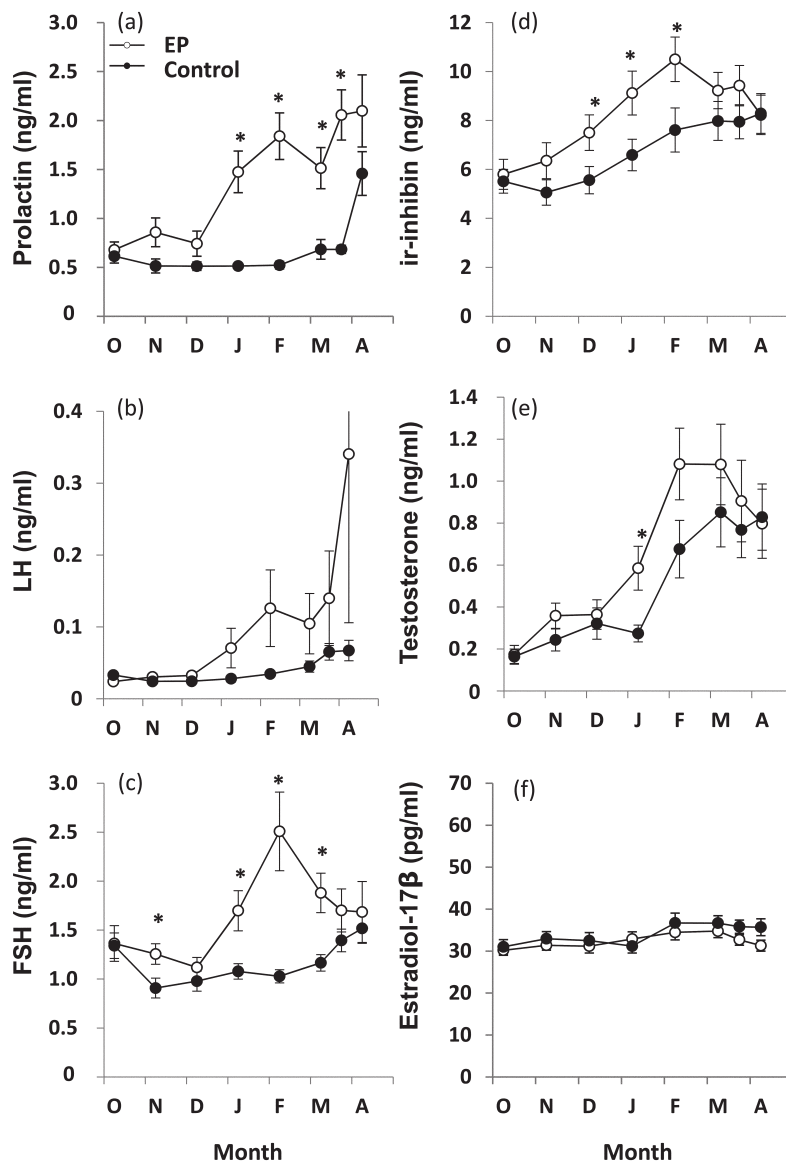
Fig. 1.   Changes in plasma concentrations of prolactin (a), LH (b), FSH (c), ir-inhibin (d), testosterone (e) and estradiol-17β (f) in Thoroughbred colts in the extended photoperiod (EP) group (○) and the control group (●) from October at one year old to April at two years old. Results are expressed as means ± SEM. Letters represent first letter of each month. *$P<0.05$.

Day of the first ovulation

   Assuming that seven days prior to the day with the first plasma concentration of progesterone reaching 1 ng/m*l* or higher was the first ovulation day, no ovulation was noted before April in the control group (n=16), but it occurred in 14 of the 15 fillies (93.33%) before April in the EP group, and the mean ovulation day was February 26.

Changes in molting of winter coats

   Representative photographs taken in the end of March at two years old (3 months after EP initiation) in the two groups are shown in Fig. 4, and graphs of the scores of the two groups are shown in Fig. 5. No significant different was noted in the coats between the two groups in January at two years old, but the score was significantly higher in the EP than control group in April (Fig. 5). Representative photographs of colts and fillies (Fig. 4) showed that winter coats
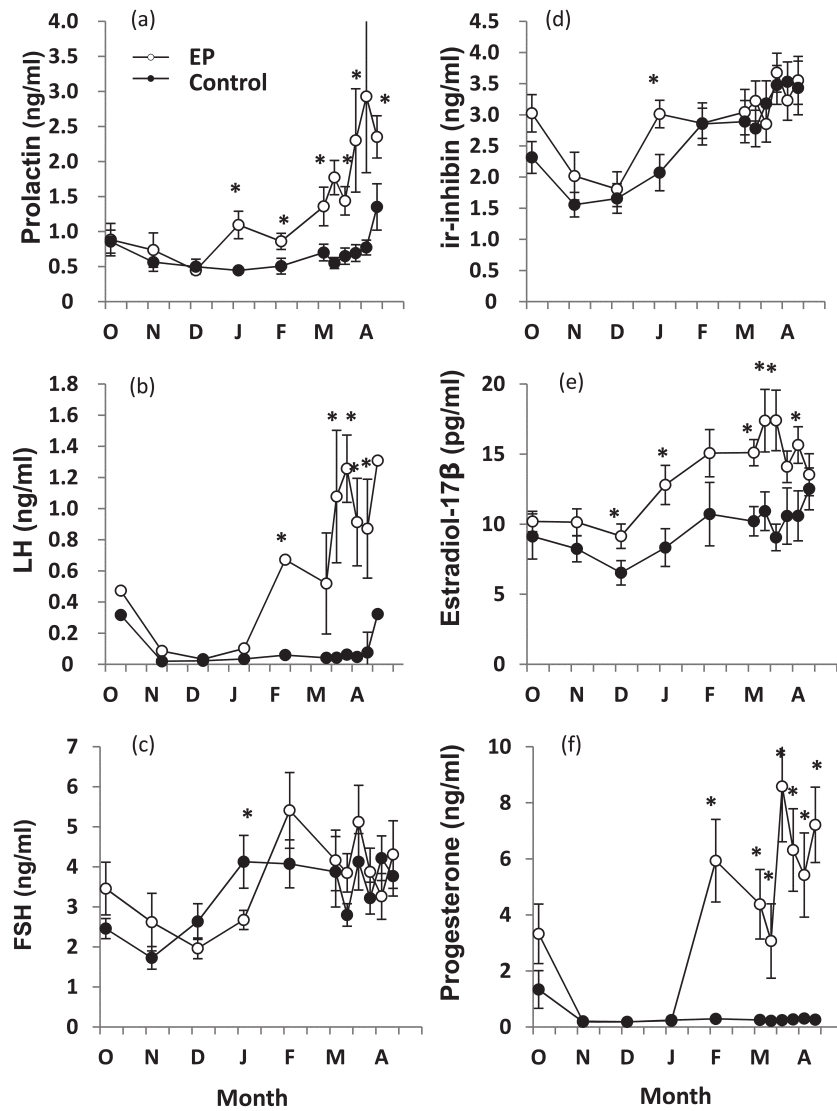
Fig. 2. Changes in plasma concentrations prolactin (a), LH (b), FSH (c), ir-inhibin (d), estradiol-17β (e) and progesterone (f) in Thoroughbred fillies in the extended photoperiod (EP) group (○) and the control group (●) from October at one year old to April at two years old. Results are expressed as means ± SEM. Letters represent first letter of each month. *$P<0.05$.

remained in the control group (Fig. 4B and 4D), whereas the molting of winter coats was advanced in colts and fillies of the EP group at the end of March (Fig. 4A and 4C).

Discussion

Utilizing the characteristic of horses that they are long-day seasonal breeders, EP treatment has been performed to advance ovulation in broodmares [10, 16, 37, 40, 43]. In the present study, the effect of EP treatment on the gonads and hair coat in Thoroughbred yearlings were investigated.

The present study clearly demonstrated that circulating prolactin, LH and FSH secreted from the pituitary gland in the early period rose in both colts and fillies in the EP group compared with those in the control group, and secretions of ir-inhibin and steroid hormones from the testis and ovary were also promoted. In addition, the day of first ovulation was advanced in EP treated fillies compared with control fillies. The present study demonstrated for first time that EP treatment also activates the gonadal function in yearlings as well as broodmares.

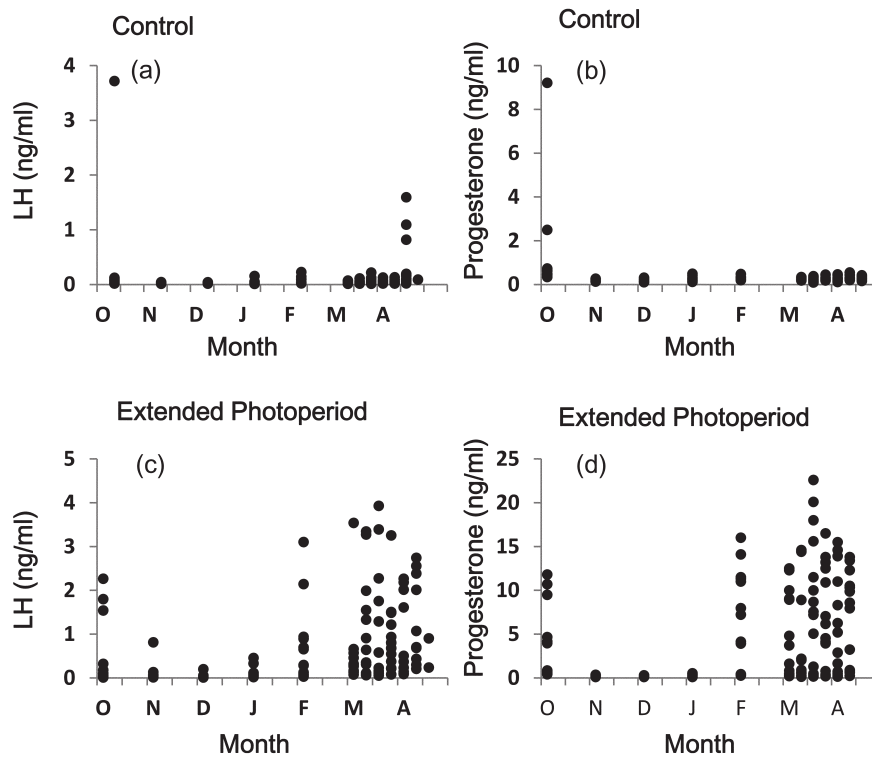Prolactin is a protein hormone secreted from the anterior

Fig. 3. Changes in plasma concentrations of LH (a, c) and progesterone (b, d) in individual Thoroughbred filly in the control (a, b) and the extended photoperiod group (c, d) from October at one year old to April at two years old. Letters represent first letter of each month.

pituitary gland, and its circulating level rises in the long-day period in horses [12, 13]. Advancement of ovulation by administration of prolactin or dopamine antagonist in mares has been reported [50, 51]. The present study in conjunction with previous studies suggests that the elevation of circulating prolactin observed in fillies in the present study was involved in the advancement of ovulation. On the other hand, prolactin is found in the follicular fluid and concentrations of prolactin in the follicular fluid increase with follicular size in mares [28]. King *et al.* (2010) [26] also demonstrated that pre-prolactin mRNA was found in the equine luteal tissues in concentrations similar to those in the antral follicle. These findings suggest *de novo* prolactin synthesis by both granulosa cells and luteal cells [25]. During the estrous cycle in mares, the increase in circulating prolactin was observed around ovulation [28] and the time of luteolysis [27]. The presence of prolactin and dopamine receptors was demonstrated in ovarian tissues [26]. These previous findings suggest that prolactin plays important physiological roles in ovarian function in mares [27, 42]. Prolactin has also physiological roles in male reproduction in mammals, such as steroidogenesis, gametogenesis or sexual behavior in many mammals [5]. An increase in circulating

prolactin in the long-day period in stallions and geldings has also been reported [12]. In addition, seasonal changes in circulating prolactin in Thoroughbred foals, weanlings and yearlings were also observed in the our previous study [13], and these results demonstrated that circulating prolactin were high in the long-day period, the breeding season, and low in the short-day period, the non-breeding season, in horses. However, these phenomena are not unique to the horses. Other seasonal breeders, such as goats [34], rams [47], golden hamsters [4, 5], black bears [22, 52] and polar bears [23], showed similar seasonal changes in circulating prolactin in accordance with the annual breeding cycles. Changes in circulating prolactin coincided with testicular recrudescence and the onset of the breeding season and preceded peak testosterone concentrations, indicating that prolactin has an important physiological role in regulation of the hypothalamic-pituitary-gonadal axis, and both directly or indirectly regulates seasonal transitions of testicular function [4, 5, 47]. In the black bear, circulating prolactin was low in the nonbreeding season, and increased in the breeding season, coinciding with the onset of the breeding season and testicular recrudescence [22, 52]. In polar bears, circulating prolactin increased and testes reached maximal
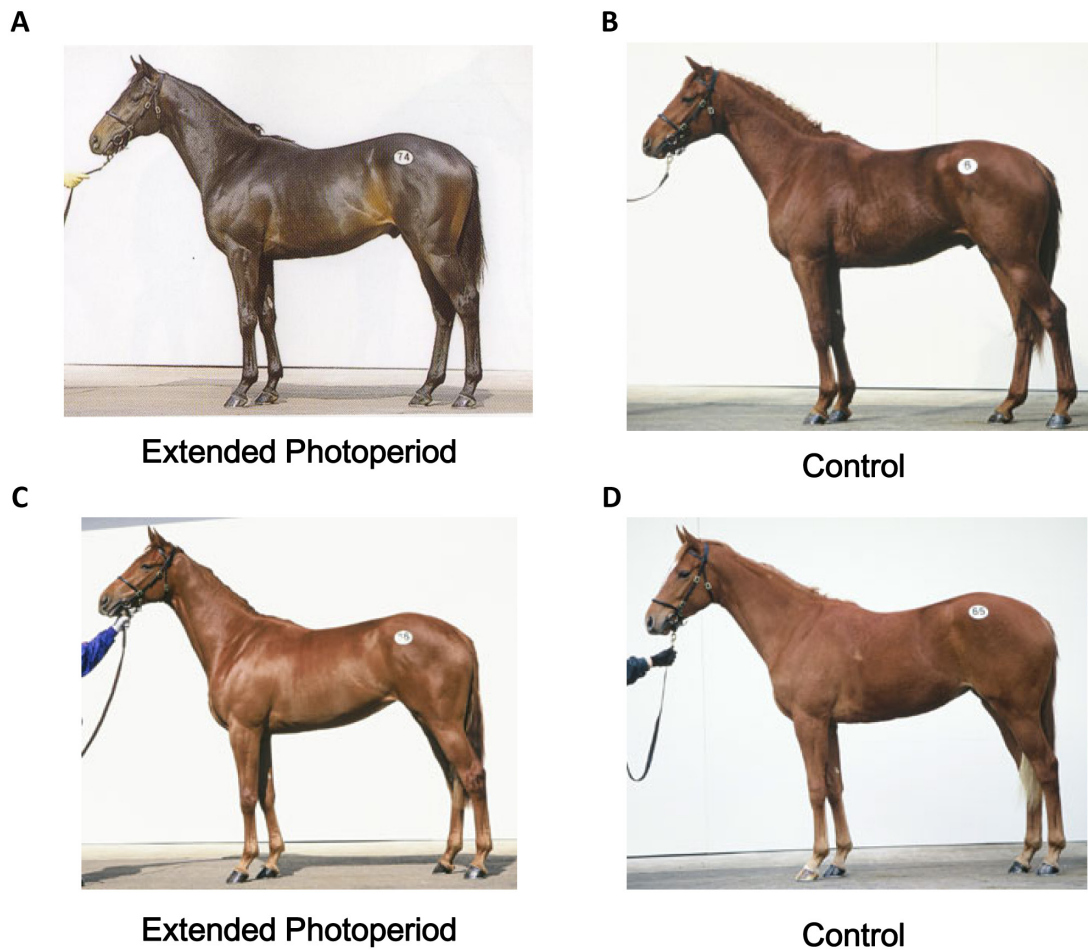
Fig. 4. Comparison of hair coat condition of representative colts (A, B) and fillies (C, D) in the extended photoperiod group (A, C) and the control group (B, D) on April at two years old.
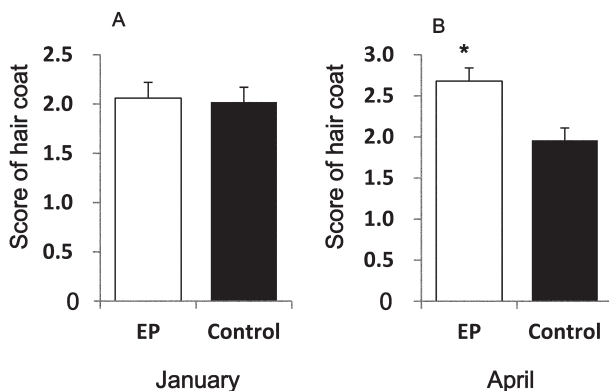

Fig. 5. Comparison of score of hair coat condition between the extended photoperiod (EP) group (□) and the control group (■) at January (A) and April (B). Results are expressed as means ± SEM. *P<0.05.

size in the breeding season [23]. In a report on bears, prolactin increased the LH receptor expression level in the testis [22], suggesting that prolactin is also involved in activation of the gonadal function in horses by stimulating LH receptor expression in the gonads and increasing LH sensitivity. The expression of prolactin receptor has been reported in the testes and various male accessory glands [18, 20, 24], indicating that these organs are targets of prolactin in male reproductive organs. Previous studies have also demonstrated the expression of testicular prolactin receptor increased during testicular recrudescence in the breeding season [6, 22, 29]. In the golden hamster, testicular prolactin receptor mRNA increased in the breeding season, coincident with an increase in circulating prolactin, suggesting that prolactin up regulate its own receptor in gonads [22, 29, 30]. Previous studies have also demonstrated a positive relationship between prolactin and testosterone production [7, 23, 44, 46]. Prolactin may stimulate steroidogenesis not only by up-regulating prolactin and LH receptor but also

by increasing stores of esterfied cholesterol and increasing 3β-hydroxysteroid dehydrogenese and 17β- hydroxysteroid dehydrogenese activity in the mouse [3, 19, 35]. The present study in conjunction with previous papers suggests that cooperative action between gonadotropins and prolactin induced by EP treatment may be involved in the gonadal function in colts and fillies. Further investigation is needed to clarify the molecular mechanisms of prolactin action in equine reproduction.

Secretion of prolactin is also stimulated by various kinds of stress and lactation in mammals [1, 2, 45, 49]. Prolactin is an essential hormone for milk production in females, and it also potentiates the immune function in both males and females, being known as an anti-stress hormone essential for the host defense mechanism [8, 9, 11, 15, 17, 48]. It has also been known to exhibit a molt-inducing effect. In most mammals, the coat thickens to protect the body in cold seasons, and then molts in warm seasons to adapt to the high temperature conditions. The present study clarified that EP treatment promotes the change from winter to summer coats (molting of winter coats). EP treatment advanced elevation of circulating prolactin in both colts and fillies. Previous papers reported that molting of winter hair coat was stimulated by long-day EP treatment [31, 40, 43], administration of prolactin [50, 51] or sulpiride, antagonist of dopamine, [14, 50, 51] in horses. The present study clearly demonstrated that the early elevation of circulating prolactin observed in both colts and fillies was promoted by EP treatment. These results in conjunction with previous papers strongly suggest that prolactin may have been involved in the promotion of molting observed in the yearlings in the present study.

In conclusion, the present study clarified that the gonadal function and molting of winter coats are activated by EP treatment in colts and fillies, similarly to that in broodmares, suggesting that the strengthening of muscles and increasing the bone mineral density by promoting secretions of testosterone and estradiol-17β [53] from the gonads reduce the risks of training Thoroughbreds in the rearing stage, in which the incidence of motor organ diseases is high.

Acknowledgments

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