

Seasonal changes in serum progesterone levels in Thoroughbred racehorses in training

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The objective of the present study was to verify the seasonal luteal activity of racehorses in training in Japan from March to August. We allocated 102 horses into a luteal activity group and non-luteal activity group. The luteal activity group included horses with serum progesterone levels that were consistently >1 ng/ml and changed by ± 1 ng/ml. In contrast, the progesterone levels of the non-luteal activity group were consistently <1 ng/ml. In late spring (from May 1 to June 30) and summer (from July 1 to August 31), the percentage of horses in the luteal activity group was significantly higher than in early spring (from March 1 to April 30, $P < 0.01$). These findings demonstrate clear seasonal variations in ovarian activity. The present study also suggest that training for a race may not affect ovarian activity in female racehorses.

Key words: racehorse, progesterone, seasonal change

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Horses are seasonal breeders with ovulatory activity related to a long photoperiod. During the breeding season in spring and summer, the average length of the estrous cycle of adult horses is approximately 21 days [12]. The levels of steroid hormones, such as estradiol or progesterone, in the peripheral circulation varies within the estrous cycle, and progesterone levels derived from the corpus luteum drastically change with ovulation [1]. Moreover, previous reports suggest that puberty occurs in ponies or Thoroughbred fillies between 11–15 months of age [4, 9, 23, 28]. According to these findings, horses over 2 years old should exhibit seasonal variations in ovarian or luteal activity indicated by low serum progesterone levels before ovulation followed by a rapid increase after ovulation.

Racehorses are considered athletes. Their environment seems to be very different from that of mares not in training

including broodmares. It is well known that exercise training can affect the menstrual cycle via the hypothalamic-pituitary-adrenal axis [3, 18]. Further, human female athletes sometimes have menstrual problems [2, 5, 20, 22, 26]. They often show luteal phase defects, suggesting a different change in serum progesterone level from non-athletes [8]. Therefore, strenuous training can cause ovarian hormonal responses in racehorses in training like human athletes, such as anovulation in the estrous cycle. However, there are no reports that describe serum sex hormone levels of racehorses in training.

Luteal function appears to be altered during the vernal and autumnal transitions [15]. Some horses have prolonged luteal activity without uterine pathology throughout the year [15], and their progesterone levels are >1 ng/ml for >30 days [25]. However, little is known about the luteal function of racehorses in training.

The objective of the present study was to investigate seasonal changes in luteal activity in racehorses in training by measuring serum progesterone levels.

We studied 102 female Thoroughbred racehorses that ranged in age from 2 to 7 years (3.3 ± 1.1 years) and were stabled at the Ritto Training Center in Japan (35

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degrees north and 136 degrees east); each horse competed in at least one race during our investigation. Blood samples were collected from horses in March (n=23), April (n=18), May (n=13), June (n=24), July (n=14), and August (n=10). The sampling was carried out once a week for 4 weeks of each month. The same horses were not used throughout the entire course of this study. Blood samples were collected from the jugular vein into vacuum tubes, and the sera were decanted after centrifugation at 3,000 g for 10 min at 4°C and stored at -20°C until use. Samples were collected only when permitted by each horse's trainer.

Serum progesterone levels were determined using a time-resolved fluoroimmunoassay with a dissociation-enhanced lanthanide fluorescence immunoassay (DELFLIA) system (PerkinElmer, Waltham, MA, U.S.A.) according to the manufacturer's protocol [17].

We classified the changes in serum progesterone levels as ovulation type, persistent corpus luteum type, or non-luteal activity type. Figure 1 shows a representative example of each type. The progesterone level of the ovulation type showed a change from less than 1 ng/ml to more than 1 ng/ml during 4 weeks. The progesterone levels of the persistent corpus luteum type was consistently >1 ng/ml, and that of the non-luteal activity type was consistently <1 ng/ml.

To investigate seasonal luteal activity, the data for the ovulation and persistent corpus luteum types were integrated for statistical analysis and defined as the luteal activity group. Because of the relatively small numbers of horses sampled during each of the 6 months, we summed the values for every 2 months and analyzed them as three seasons as follows: March + April (n=41), defined as early spring; May + June (n=37), defined as late spring; and July + August (n=24), defined as summer. The proportion of the luteal activity group was analyzed using Fisher's exact test followed by Bonferroni's post hoc test. Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan, 1.00), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria version 2.13.0) [14]. $P < 0.05$ was considered statistically significant.

There were no medical records available for these racehorses during the sampling period. Table 1 shows the percentages of the 3 types in each month. The percentage of the ovulation type was the lowest in March (22%), increased gradually from April, and reached 80% in August. The percentage of the persistent corpus luteum type was rare from March to May (less than 7%), but it was approximately 20–29% after June. The percentage of the non-luteal activity type was 74% in March and decreased as the month passed (39% in April and 16% in May), and eventually no horses belonged to this type starting in June.

The percentages of horses in the luteal activity group

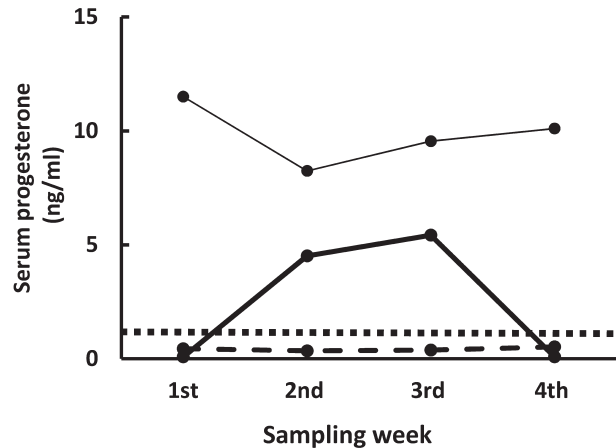


Fig. 1. Representative examples of each group. The dotted line indicates 1 ng/ml, and the dashed, thin, and thick lines indicate the non-luteal activity, persistent corpus luteum, and ovulation types, respectively. The serum progesterone levels of the non-luteal activity type and the persistent corpus luteum type were consistently lower and higher than 1 ng/ml during 4 weeks, respectively. The levels of the ovulation type showed a change from less than 1 ng/ml to more than 1 ng/ml during 4 weeks.

were 41%, 95%, and 100% in early spring, late spring, and summer, respectively. The percentages of horses in the luteal activity group in three seasons are shown in Table 2. It was significantly affected by season, so percentages in late spring and summer were higher than in early spring ($P < 0.01$).

The age distribution of the luteal activity group in each season is shown in Table 3. Samples from 2-year-old horses could not be collected in early spring. In early spring, the percentages of horses in the luteal activity group were almost the same in horses that were 3 years old and over 4 years old (46% and 44% respectively). In late spring and summer, most horses had luteal activity regardless of age.

In the present study, the percentage of horses in the luteal activity group in early spring (from March 1 to April 30) was significantly lower than the percentages in late spring (from May 1 to June 30) and summer (from July 1 to August 31). Further, in late spring and summer, the percentages of horses in the luteal activity group were 95% and 100%, respectively. To the best of our knowledge, this is the first study to report seasonal luteal activity in racehorses and a high percentage of horses with luteal activity in late spring and summer.

According to another study, the proportions of pony mares that ovulated in early spring (March and April), late spring (May and June), and summer (July and August) were 2/14, 14/14, and 13/14, respectively [13]. King *et al.* reported that the cumulative percentage of mares experi-

Table 1. The distribution of types of serum progesterone level changes in each month

	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)
Ovulation	5 (22)	11 (61)	10 (77)	19 (79)	10 (71)	8 (80)
Prolonged corpus luteum	1 (4)	0 (0)	1 (7)	5 (21)	4 (29)	2 (20)
Non-luteal activity	17 (74)	7 (39)	2 (16)	0 (0)	0 (0)	0 (0)

Data are shown as the number of horses (percentage).

Table 2. The influence of season on luteal activity

	Early spring (%) ^a	Late spring (%) ^b	Summer (%) ^b
Luteal activity	17 (41)	35 (95)	24 (100)
Non-luteal activity	24 (59)	2 (5)	0 (0)

Early spring: March 1 to April 30; Late spring: May 1 to June 30; Summer: July 1 to August 30. Data are shown as the number of horses (percentage). Significant differences ($P < 0.01$) are indicated by different letters in the top row.

Table 3. The age distribution of the luteal activity and non-luteal activity group in each season

		Early spring (%)	Late spring (%)	Summer (%)
2 years old	Luteal activity	0 (0)	9 (100)	12 (100)
	Non-luteal activity	0 (0)	0 (0)	0 (0)
3 years old	Luteal activity	13 (46)	18 (90)	7 (100)
	Non-luteal activity	15 (54)	2 (5)	0 (0)
≥4 years old	Luteal activity	4 (44)	8 (100)	5 (100)
	Non-luteal activity	9 (56)	0 (0)	0 (0)

Early spring: March 1 to April 30; Late spring: May 1 to June 30; Summer: July 1 to August 30. Data are shown as the number of horses (percentage).

encing estrous or persistent corpus luteum activity changed with the season [16]. From May to September in Japan, the plasma concentrations of progesterone of Thoroughbred mares have been reported to remain low (less than 1 ng/ml) during the follicular phase but to begin to increase after ovulation [21].

Our present results show that the seasonal changes of ovarian and luteal activities of racehorses are similar to those of mares. In contrast, female human athletes occasionally experience a menstrual problem called athletic amenorrhea [6]. For example, the age at menarche in athletes is significantly later compared with controls [19], and runners show secondary amenorrhea (14/31) during high-intensity training more often than controls (12/96) [10]. Further, strenuous exercise can induce menstrual disorders in females who are not athletes [5]. The racehorses studied here did not seem to show these menstrual problems.

The present study shows that most racehorses exhibited luteal activity, including a persistent corpus luteum. Moreover, mares sometimes had a persistent corpus luteum with those that neither ovulated nor exhibited estrous behavior [11]. A previous study reported that the average length of prolonged luteal activity is 63 days (range, 35–95 days), and

this tends to occur during the summer [25] or even during an anestrus season [15]. Further, this condition is a common (up to 25%) characteristic of estrous cycles [24]. Thus, the present study revealed that the racehorses had prolonged luteal activities and proportions similar to those of mares. However, a longer sampling period is required to determine how long the luteal activity persists in racehorses.

Because we could not collect samples from 2-year-old horses in early spring, statistical analysis of the association between age and luteal activity could not be performed. However, the percentage of horses in the luteal activity group was low in early spring and high in late spring and summer regardless of age. Therefore, it is highly possible that age did not affect the luteal activity of the racehorses. More samples, especially from 2-year-old horses, will be needed to clarify the association between age and luteal activity.

The main limitation of this study is that we did not perform ultrasonography to observe the condition of the ovary. In order to determine the level of luteal activity precisely, transrectal ultrasonography is required. However, it is difficult to perform a continuous examination for racehorses. Because the progesterone level reflects the luteal

activity [7, 27], we assumed that investigation of seasonal hormonal changes was sufficient as an indicator of the condition of the ovary.

In conclusion, the present study suggests that Thoroughbred racehorses in training undergo seasonal changes in luteal activity and that, during late spring and summer, most horses have luteal activity including a prolonged luteal activity. This suggests that training for a race may not affect ovarian or luteal activity.

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