Relashionship between Plasma Testosterone Concentrations and Age, Breeding Season and Harem Size in Misaki Feral Horses

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ABSTRACT. Jugular vein blood samples were collected from 23 young and sexual mature feral stallions to examine the relationship between plasma testosterone concentration and age, breeding season or harem size. Testosterone concentration increased with the age of the stallions until they formed their own harems, at about 4 to 6 years old. Seasonal variations in testosterone concentrations were observed, and found to be significantly higher ($P<0.001$) throughout the breeding season than non-breeding season, from 3 years of age. Testosterone levels were correlated with harem size for individual stallions. It can be inferred from these results that there is a relationship between plasma testosterone concentration and age, breeding season and harem size. — KEY WORDS: equine (wild), harem, testosterone.

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Testosterone is the hormone responsible for libido in mature stallions. Domestic stallions are sexually mature at about 16 months or older [6], while wild horses take at least 3 years [10]. It has been demonstrated, in domestic horses, that immature stallions have lower testosterone concentrations than the mature stallions, and that blood testosterone levels are higher during the breeding season than non-breeding season when they are mature [1, 4, 15]. These finding have also been observed in the wild and feral horses [9, 10, 15]. High testosterone levels during the breeding season leads to seasonal changes in libido [1], followed by a marked increase in the sperm and seminal fluid production rate for increased copulation and mating in the breeding season [1, 7]. When they have formed a successful harem, changes or individual differences in harem size between stallions may possibly be influenced by some factors which affect their reproductive success such as age [9], body weight and fighting abilities [2], or dominance and aggressiveness behavior [13]. Previous studies have concentrated on seasonal changes in the testosterone levels of domestic and wild stallions, but there have been no investigations into its role in harem formation and consequent effect on changes in harem size. In this study, therefore, we at first measured testosterone concentrations before and after sexual maturation or beginning of harem formation. Then we compared levels between breeding and non-breeding season in Misaki feral horses and examined the relationship between plasma testosterone levels and harem size in individual stud horses, by sampling over two consecutive years.

The study area is located on the Misaki range (about 500 ha) where the horses live in natural conditions, without human management, throughout the year. The Misaki range is divided into two areas: the Komatsugaoka area (287 m above sea-level) and the Ogiyama area (295 m above sea-level), and numerous small and large valleys that run down in all directions from them. The climate is moderate with the temperature ranging from 4°C to 15°C in winter and 24°C to 31°C in summer. June and July is the rainy season. Fresh water ponds are plentiful in the range. The grasslands are restricted to the upper area (about 40 ha) half way up the two hills (about 290 m above sea level) which are located in the center of the range and covered with various kinds of native grasses. Other areas of the range are mostly occupied by artificial forests with Japanese cedars and a variety of copses [8]. Generally, the warm climate and abundant rain provide a lot of vegetation in summer. In the first experiment, twenty-three feral male horses, ranging from 1 to 6 years of age, were used to determine the relationship between testosterone levels and age, or breeding season. All animals were in good physical conditions during the experiment. Individual horses were readily identified by the number branded on the hip. Almost Misaki horses were individually recorded the birth date and parents (identified by DNA analysis). Blood samples were collected four times every two weeks from 23 individual horses in May and June of 1994, and mean values of testosterone for each were obtained. These data was used to relate testosterone concentrations to age for the breeding season. Similarly blood samples were collected four times every two weeks in October and November of 1994, to provide data for the non-breeding season.

In the second experiment, we randomly selected 7 males at different ages (4–9 years old) with or without a harem, and examined the change of harem size and testosterone levels during two Breeding seasons from 1993 to 1995, in order to investigate their relationship. Blood samples were collected from the 7 individual males in May of both 1994 and 1995 and harem formation was observed. The horses were extremely dangerous to handle and were restrained with Xylazine as recommended by Dodman [5]. A dose of 4 mg/kg body weight was injected using a special syringe, fired from a blowgun at a few meters from the horse (hind quarter), for accuracy. Some of the horses never adjusted to the restraint procedures and so we tried again on other days. Blood samples were taken by jugular vene puncture into heparinised tubes and placed on ice, plasma was obtained by centrifugation and was stored at −20°C until

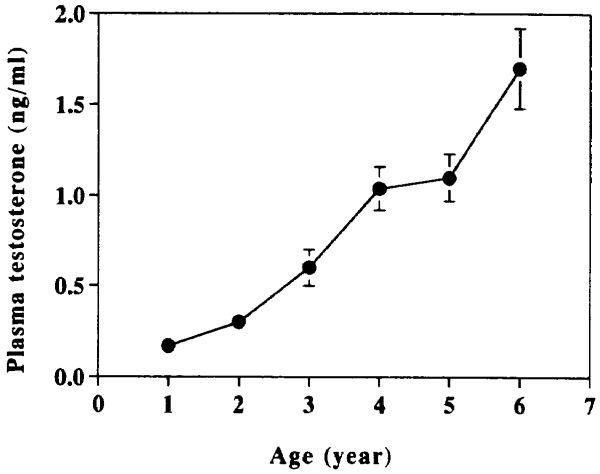

Fig. 1. Plasma testosterone concentrations at different ages in Misaki feral horses. The symbols and vertical bars represent mean ± SEM (n=4: 1, 2, 3, 4, 6, 7 years old; n=3: 5 years old). Blood samples were collected four times every two weeks in May and June from 23 individual horses and mean values were obtained, for each. Harem formation was observed in horses over 4 years old. Testosterone levels increased linearly except for in 5 years old. No significant differences were observed in analysis of values of 1 vs 2 years old, 4 vs 5 years old.

analysis.

Plasma testosterone concentration was measured by modified radioimmunoassay [14]. The specific antibody to testosterone was a gift from Dr. Etoh (Miyazaki Univ., Japan). Androstenedion (3.1%) was the only steroids, other than testosterone, with any significant reactivity. The ^{3}H-testosterone was purchased from New England Nuclear (England). The free and antibody-bound testosterone were separated by the dextran coated charcoal method. The lower and upper detection limits of the assay were 3.15 and 2,000 *n*g/tube, respectively. The intra-and inter -assay coefficients of variation for plasma testosterone were 14.5% and 5.9%, respectively. Data was evaluated by analysis of variance (Anova) and tested for significance with Duncan's new multiple range test.

The plasma testosterone of 23 young and sexually mature males in the middle of the breeding season had increased as a result of an increase in age from 1 to 6 years old (Fig. 1). These data included all samples from stallions with or without harems. However, stallions at 3 years old and younger did not appear to form harems. There were significant differences in testosterone concentration between the age groups ($p<0.001$). The testosterone levels were very low for 1 and 2 years old, and thereafter rapidly increased for 3, 4 and 6 years.

Figure 2 shows testosterone concentrations in the non-breeding and breeding season among different ages of male horses from 1 to 6 years old. Although no significant seasonal differences were observed in stallions of 1, 2 years old, all plasma testosterone levels were higher in the breeding season than non-breeding season, over 3 years of age. These differences were significant ($p<0.01$).

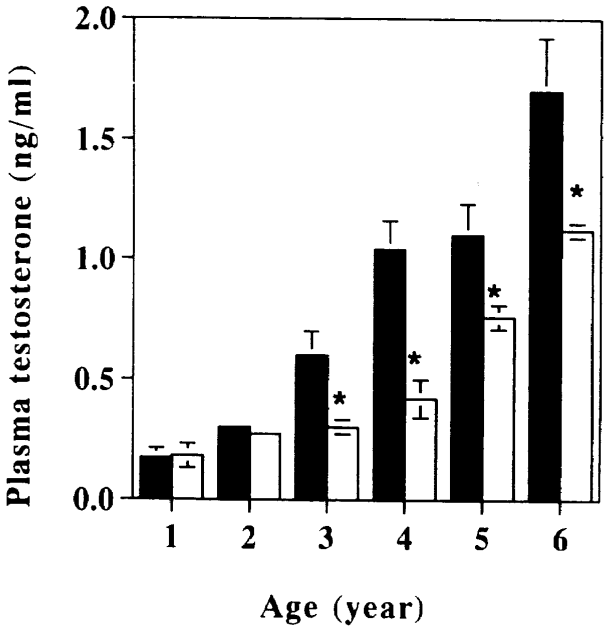

Fig. 2. Comparison of plasma testosterone levels between breeding and non-breeding season in Misaki feral horses. The black and white bars represent the mean value of breeding season and non-breeding season, respectively. The vertical bars represent the standard error. All samples were collected from the same horses as shown in Fig. 1. The blood samples for non-breeding season were collected four times every weeks in October and November. The asterisks represent the significant differences vs those of breeding season.

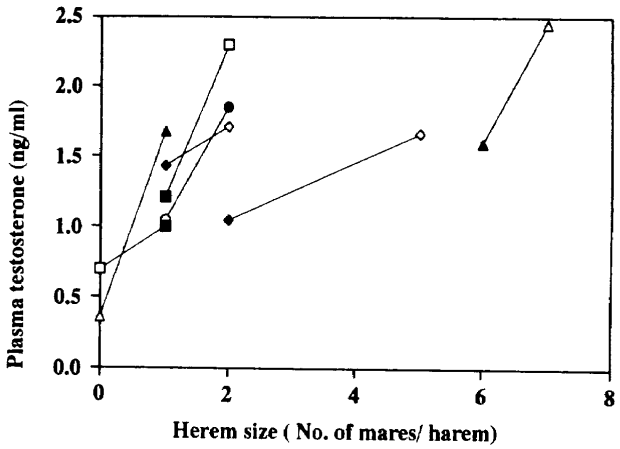

Fig. 3. Relationship between plasma testosterone levels and harem size (number of mares/ harem). Blood samples were collected from 7 individual males over 4 years old in May of both 1994 and 1995. The white and black symbols represent the testosterone value of the first and second year, respectively.

In the second experiment, 2 out of the 7 males were bachelor in the first breeding season, but acquired mares in the next season. A further 4 out of the 7 males decreased the number of mares in their harems in the second breeding season. The remaining males increased its mares from 6 to 7. In theses cases, as shown in Fig. 3, plasma testosterone concentrations increased and decreased in parallel with the

increase and decrease in the number of mares.

Generally, domestic stallions attained their sexual maturity after 16 months old [6], while the wild stallions became sexually mature at 3 years or older [11]. In both cases the testosterone concentrations in immature stallions were minimal compared to mature stallions. This is consistent with our results from feral horses which were mature at 3 years, because the young male horses had significantly lower testosterone concentrations than the mature horses ($p<0.05$). Also, the plasma testosterone concentration increased steadily with advancing age, suggesting a relationship between its concentration and the age of males up to harem formation, which generally starts from 4 to 6 years in Misaki feral stallions [9].

The finding of the present study is that the 1 and 2 year-old males had a lower serum level of testosterone and a similar level in the non-breeding and breeding season. These males were still immature with a reduced libido, which is consistent with the results of Chaudhuri and Ginsberg [3] who noted that males that have access to females, excrete more androgen than non-breeding males. Whereas, mature stallions (3 years or older), have a seasonal pattern of testosterone concentrations which were significantly higher in the breeding than the non-breeding season ($p<0.01$). These results are consistent with the hypothesis that seasonal changes in plasma testosterone can be correlated with seasonal frequencies of mating, copulation and seasonal variations in libido. It may therefore be suggested that this behavior, displayed in feral horses, may be an androgenic effect [16].

Testosterone concentrations can be shown to be closely associated with harem size, from our results. Plasma testosterone concentrations increased and decreased in parallel with an increase and decrease in the number of mares. Although there were few data, plasma testosterone was at very low levels in bachelors ($n=2$). These observations are in agreement with the data reported by Kirkpatric, *et al.* [10] who showed that testosterone concentration of bachelor stallions was significantly lower than of stud stallions. These results are consistent with the hypotheses that the presence of females may be a social stimulus influencing testosterone secretion [3], and that individual differences in harem size among feral stallions may be influenced by testosterone concentration, which affect the reproductive success of stallions. However, there may be many complex factors involved in determining harem size, such as avoidance of the parent-child relationship, strength of aggression of males, mental factor of mares. Therefore, although the testosterone levels may be associated with harem size, it is not known whether they are a predominant factor for harem size.

In this study, we used chemical immobilization to collect the blood samples for determination of the net testosterone value. However, their significance may not justify the chemical immobilization of wild creatures. We are now trying to carry the study non-invasively by means of fecal hormone measurements.

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