NOTE *Theriogenology*

A Surge-Like Increase in Luteinizing Hormone Preceding Musth in a Captive Bull African Elephant (*Loxodonta africana*)

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ABSTRACT. This study was conducted to determine the correlation between reproductive hormones and musth in a male African elephant. Changes in circulating luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and immunoreactive (ir-) inhibin and the degree of musth were evaluated for 4 years. LH increased 4 weeks before musth began. The highest concentrations of testosterone and ir-inhibin were observed from April to October. There were positive correlations among testosterone, ir-inhibin and musth behavior. These findings suggested that the surge-like LH in the pre-musth period might stimulate secretion of testosterone and ir-inhibin and thus initiate the musth behavior. This study also suggested that the high LH level before musth might be a useful biomarker for the beginning of the musth season.

KEY WORDS: African elephant, inhibin, luteinizing hormone, musth, testosterone.

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Musth is a unique phenomenon that occurs either annually or semiannually in adult male African (*Loxodonta africana*) [7, 16, 17] and Asian (*Elephas maximus*) elephants [5,11]. During musth, male elephants have elevated levels of androgens, especially androstenedione and testosterone [1, 6, 7, 10, 15, 18, 20, 21, 24]. Musth bulls were reported to have elevated levels of luteinizing hormone (LH) in both African [1] and Asian [15] species. Secretory patterns of follicle stimulating hormone (FSH) and immunoreactive (ir-) inhibin in male elephants have never been reported before. In captivity, both African and Asian bulls in musth exhibit aggression towards their mahouts or handlers [5, 10, 20], which is extremely dangerous for zoo staff, veterinarians and researchers who manage breeding programs. Thus, our understanding of markers for prediction of musth needs to be improved. The objective of this study was to investigate secretion of reproductive hormones associated with musth characteristics in a male African elephant in Japan.

A male African elephant named "Tamao" (Fig. 1A) was studied for 4 years, from 33 to 37 years of age. It was housed at the Tama Zoological Park, Hino City, Tokyo, Japan, together with 4 female African elephants. All elephants were housed outside during the daytime (0900 hr–1600 hr) and inside the building at night throughout the year. Blood samples were collected weekly between 0800 hr and 0900 hr from an ear vein without anesthesia. The samples were centrifuged, and sera were stored at –20°C until hormonal analysis. The concentrations of LH [2], ir-inhibin [9], testosterone [22] and FSH [23] were determined by double-antibody radioimmunoassay (RIA) systems.

The appearance of characteristic behavioral and physical changes was recorded daily during the 4-year period. The bull elephant was classified as being in musth when one or a combination of the following behavior and/or physical changes were continuously (for more than 3 days per week) observed: increased aggressive behavior, temporal gland secretion (TGS; Fig. 1B) and urine dribbling (UD; Fig. 1B) as described previously [3]. Since TGS can occur in African bulls under stress or social excitation, TGS was considered as an indicator of musth only if it occurred with or was closely followed by UD [28]. TGS and UD are generally agreed for determining musth in African bulls [8, 16, 17, 24]. The degrees of TGS and UD were evaluated at the time of blood sampling. TGS and UD were scored from 0 to 3. Regarding the TGS scores, a score of 0 indicated that the temporal glands started swelling or not, without TGS; a score of 1 indicated that the temporal glands became more swollen and TGS slightly leaked from glands; a score of 2 indicated that TGS was moderately secreted from glands which was easy to see from a distance; and a score of 3 indicated that TGS was heavily secreted from glands. Regarding the UD scores, a score of 0 indicated normal urination; a score of 1 indicated urine came out with some stimulation (such as during eating or mounting with female elephants) and a TGS score of 0; a score of 2 indicated urine came out without any stimulation and a TGS score of 1; and a score of 3 indicated intermittent urination and a TGS score of either 2 or 3. Both scores were accumulated, with a maximum total score of 6, in order to determine the relationships with

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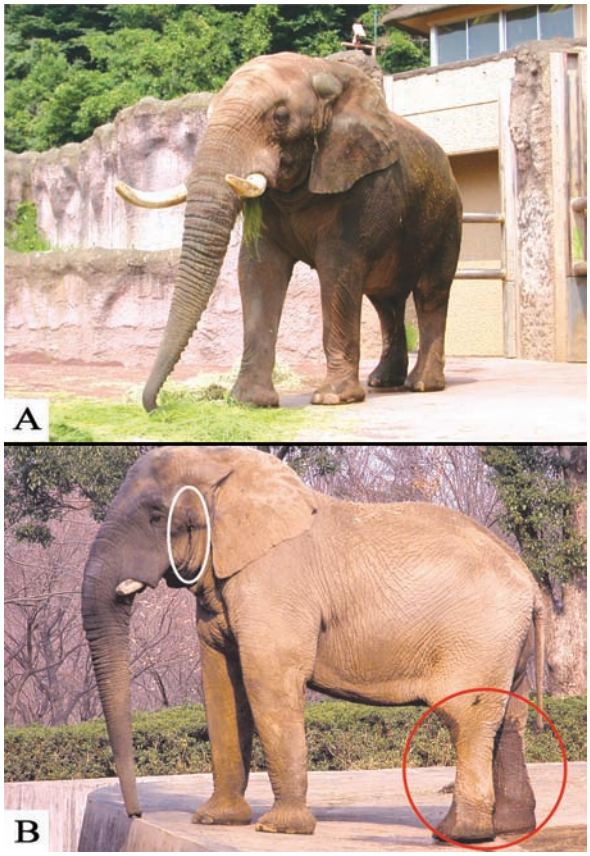

Fig. 1. Pictures of a 37-year African elephant named "Tamao" housed at the Tama Zoological Park, Hino City, Tokyo, Japan, showing different stages of non-musth [A] and musth [B]. During musth, the bull showed some discharge from the temporal gland behind the eye (white circle) and urine dribbling (red circle). Noticed the bull's right hind limb was largely filled with urine.

reproductive hormones. Musth episodes were defined as non-musth, pre-musth and musth by the presence of fresh TGS and UD as previously described [19]. Briefly, pre-musth was designated as 6 weeks prior to the beginning of musth; TGS was not observed, and the UD score was either 0 or 1. Musth was designated as the period characterized by a musth score varying from 3 to 6 and an elevated serum testosterone level (>20 *n*g/m*l*). Non-musth was the period from the end of musth to the beginning of pre-musth of the following year.

All data were presented as means ± standard errors of the mean (SEM). One-way analysis of variance (ANOVA) and Bonferroni's Multiple Comparison Test were used to compare the means of each hormone during different stages of musth (non-musth, pre-musth and musth periods). *P* values of less than 0.05 were considered to be statistically significant. Mean, SEM and Spearman correlation coefficients were calculated to test the relationships between all hormones and the musth score. All statistical analyses were performed by using the statistical analysis software (SPSS 18.0, SPSS Inc., Chicago, IL, U.S.A.).

The present study clearly demonstrated that the bull exhibited regular yearly musth cycles, which occurred from April to October, lasting 8.1 ± 1.7 months in duration (Fig. 2E). The present study also showed clear annual changes of circulating LH (Fig. 2A), testosterone (Fig. 2C) and ir-inhibin (Fig. 2D). The serum concentrations of LH significantly increased at about 4 weeks before musth began and were maintained at high levels for 5 weeks followed by a significantly decrease to the baseline level when musth behavior was observed (Fig. 2A). There was a significant difference in the levels of LH measured during the non-musth, pre-musth and musth stages. LH showed the highest concentration during the pre-musth stage, and the LH concentration in the musth stage was significantly higher than that in the non-musth stage (Fig. 3A). The patterns of FSH change followed those of LH throughout the year, although the change was not as clear as that of LH (Fig. 2B). FSH also showed the highest concentration during the pre-musth stage, and the FSH concentration in the musth was significantly higher than that in the non-musth stage (Fig. 3B). There was a significantly positive correlation between LH and FSH throughout the period of study (r^2=0.64, P<0.05). Testosterone correlated well with ir-inhibin (r^2=0.62, P<0.01) throughout the year. There was no significant difference in the levels of testosterone measured during the non-musth and pre-musth stages. The serum concentration of testosterone during the musth stage was significantly higher than that during the non-musth and pre-musth stages (Fig. 3C). The level of circulating ir-inhibin during the pre-musth stage was significantly higher than that during the non-musth stage, and the highest concentration was observed during the musth stage (Fig. 3D).

The present study clearly demonstrated a unique phenomenon in a male African elephant in Japan. The remarkable increase in LH has not been reported previously. Changes in circulating FSH were not as clear as for LH. This augmented LH secretion may be induced by excitation of gonadotropin-releasing hormone neurons by environmental cues and other unknown factors. A significant increase in LH and FSH concentrations during the pre-musth stage might be a prerequisite factor for the beginning of musth. These high levels of LH and FSH during pre-musth might stimulate extremely high testosterone and inhibin secretions from the testes, respectively. These extremely high levels of testosterone and inhibin inhibit LH and FSH during the musth period. The levels of both gonadotropins decreased markedly from the pre-musth stage, even though both hormone levels were still significantly higher than the levels during the non-musth period. Musth occurrence can be random, with many elephants exhibiting multiple episodes per year [3], and more predictable annual characteristics [4, 8, 11, 13] in both male Asian and African elephants. The well-established association between high testosterone secretion and musth was confirmed in previous studies [6, 8, 10, 15, 19–21, 24]. The secretory pattern of inhibin in male elephants has never been reported before. The current study

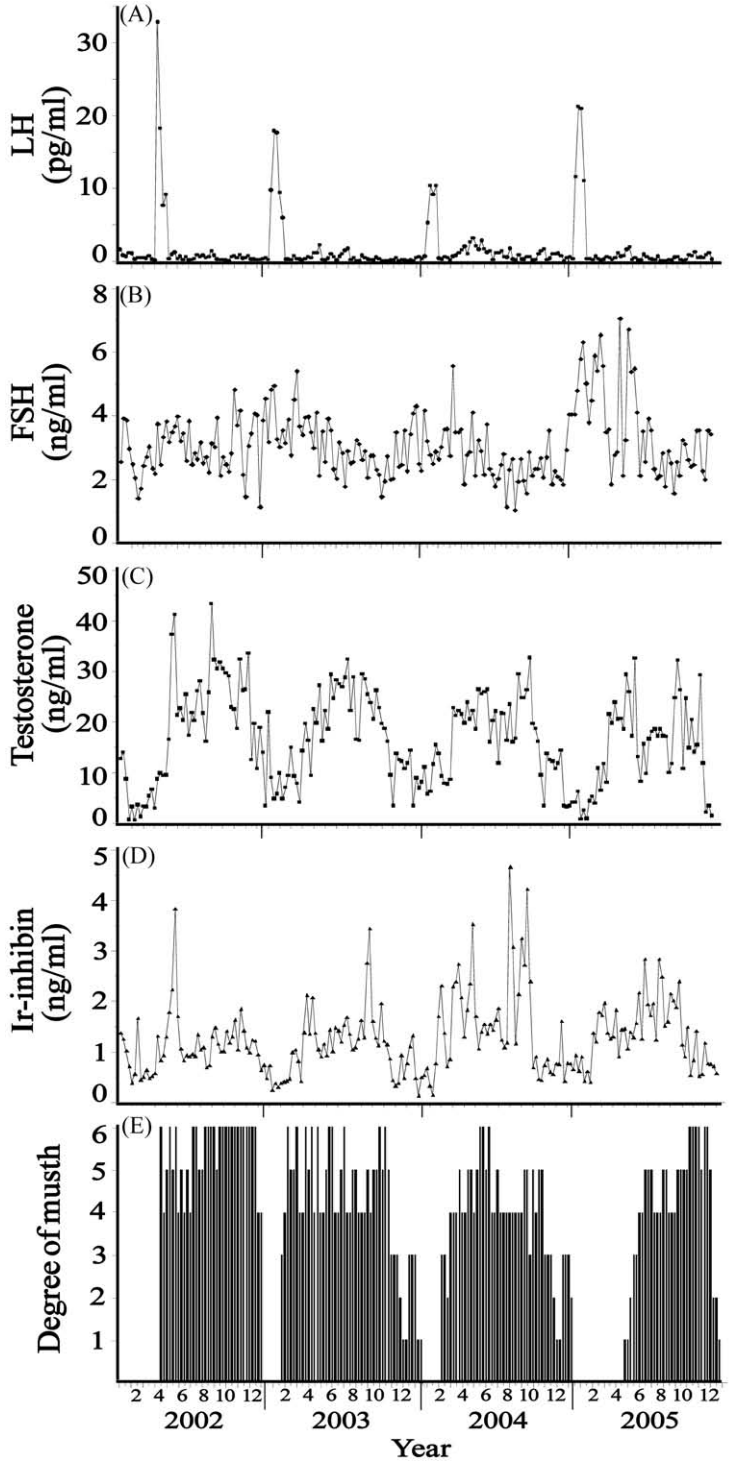

Fig. 2. Serum concentrations of LH [A], FSH [B], testosterone [C] and ir-inhibin [D] together with musth scores [E] during the 4-year study period.

confirmed that the changes of testosterone might stimulate the onset of musth and that the intensity of musth is related to its titer. Inhibin is the major endocrine product of Sertoli cells, whereas Leydig cells can also secrete inhibin such as in hamsters [12] and stallions [16].

In conclusion, the present study suggests that the marked

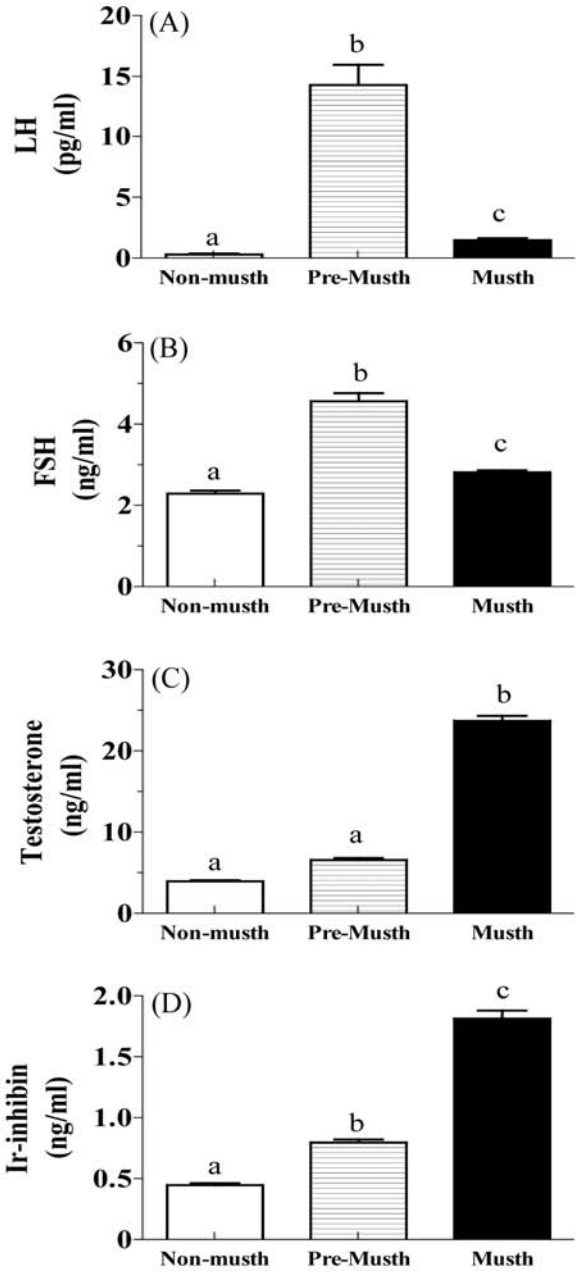

Fig. 3. Mean serum concentrations of LH [A], FSH [B], testosterone [C] and ir-inhibin [D] during the non-musth (n=59), the pre-musth (n=24) and musth (n=125) periods. Results are expressed as means ± SEM. Data points with different superscripts (a, b, c) are significantly different ($P<0.05$) by Bonferroni's Multiple Comparison Test.

increase in secretion of LH from pituitary gonadotrophs before musth possibly stimulates testicular secretion of testosterone and ir-inhibin in an African elephant. These findings also suggests that the surge-like increase in circulating LH before musth may be a useful biomarker of the beginning of the musth season in male elephants.

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