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Serum Progesterone Changes in Luteal Cyclicity and Duration of Estrous Cycle in Formosan Sika Deer (*Cervus nippon taiouanus*) Hinds

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ABSTRACT—A study was conducted to investigate the serum progesterone (SP_4) profiles and duration of estrous cycles in the farmed Formosan sika deer (FSD; *Cervus nippon taiouanus*) during the major breeding season. Five parous, open and non-milking hinds were allotted to collect peripheral blood samples twice weekly for P_4 measurement by radioimmunoassay beginning at the initiation of the rutting season indicated by rutting behaviors of the sexually mature stags. The hinds were polyestrous as proved by cyclic changes of SP_4 levels. After the presumptive estrus shown by the lowest concentration of SP_4 (0.20±0.01 ng/ml), this ovarian hormone markedly elevated on day 7 of the cycle (1.67±0.11 ng/ml), reached plateau (3.15±0.16 ng/ml, $P<0.01$) during days 11 to 18, and then declined to the basal levels in the subsequent estrus. It is concluded that mean duration of the estrous cycle in FSD during the major rutting season is 19.3 days with a range of 17 to 21 days, and that the patterns of circulating progesterone profiles during the estrous cycles of the FSD are similar to those of other deer species so far investigated.

Keywords: *Cervus nippon taiouanus*, serum progesterone levels, estrous cycle, deer

INTRODUCTION

The sika deer, *Cervus nippon,* is a species native to eastern Asia, but now introduced widely elsewhere. There are 13 subspecies identified from different parts of eastern Asia. One of the subspecies native to Taiwan is Formosan sika deer (FSD, *Cervus nippon taiouanus*). The wild FSD might have been extinct in Taiwan Since 1969 (McCullough, 1974). The FSD hinds are seasonal polyestrous breeders. The puberty of the hinds is 14.5 months of age (Shih *et al*., 1985; Yang and Ma, 1994). Under adequate nutrition and appropriate environmental conditions, they exhibit continuous rutting behaviors, if without gestation, from soon after autumn equinox and until near vernal equinox in Taiwan, exhibiting major breeding activity between November and December (Shih *et al*., 1985; Yang and Ma, 1994).

Although the FSD has been raised in Taiwan for several hundred years, they have not been fully domesticated like domestic cattles or goats. To our knowledge, the reproductive physiology of the FSD hinds have not been well documented, especially the duration and hormonal profiles of the estrous cycle, partially because the animals are sensitive to be stressed with physical restraint during bleeding procedures.

The progesterone levels in peripheral blood vessels in mammals provide valuable information about reproductive status. Serum progesterone (SP_4) concentrations have been measured in several deer species including fallow deer (Asher, 1985; Asher *et al*., 1986, 1988), red deer (Adam *et al*., 1985; Asher *et al*., 1997; Guinness *et al*., 1971; Kelly *et al*., 1985), whit-tailed deer (Plotka *et al*., 1980), Pere Davids deer (Curlewis *et al*., 1988) and chital deer (Chapple *et al*., 1993) during the breeding season. No information is yet available regarding the pattern of circulating levels of progesterone during the breeding season of sika deer. The objectives of this study were to investigate the changes of circulating levels of progesterone and the duration of estrous cycle during their major rutting season in the farmed FSD hinds.

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MATERIALS AND METHODS

Animals and management

Five parous, open and non-milking Formosan sika deer hinds, 3 to 4 years of age and 38.9±3.41 in live body weight, were allotted for this study. They were farmed in southern Taiwan (22.5°N, 120.5°E) in a pen with an area of 660 cm in length, 291 cm in width and 310 cm in height, and half of the area was shaded. In average, 600 g of the concentration feed, containing 18.3% crude protein, was supplied to each hind per day, half of the feed given in morning and other half given in afternoon, in addition to provision *ad libitum* of pangola hay, containing 34.3% crude fiber, and fresh water. On the sampling days, the concentration feeds were offered after the bleeding procedures were finished.

Sampling period

The sampling period was conducted in the major rutting season of the sika deers (November to December) according to the previous investigations (Shih *et al.*, 1985; Yang and Ma, 1994). In the present study, initiation of the rutting season was found to be 2 November of 1996 as indicated by the rutting behaviors of the proved fertile stags. The sampling period was thus started on 2 November of 1996, the initiating day of the rutting season, and continued until 11 January of 1997.

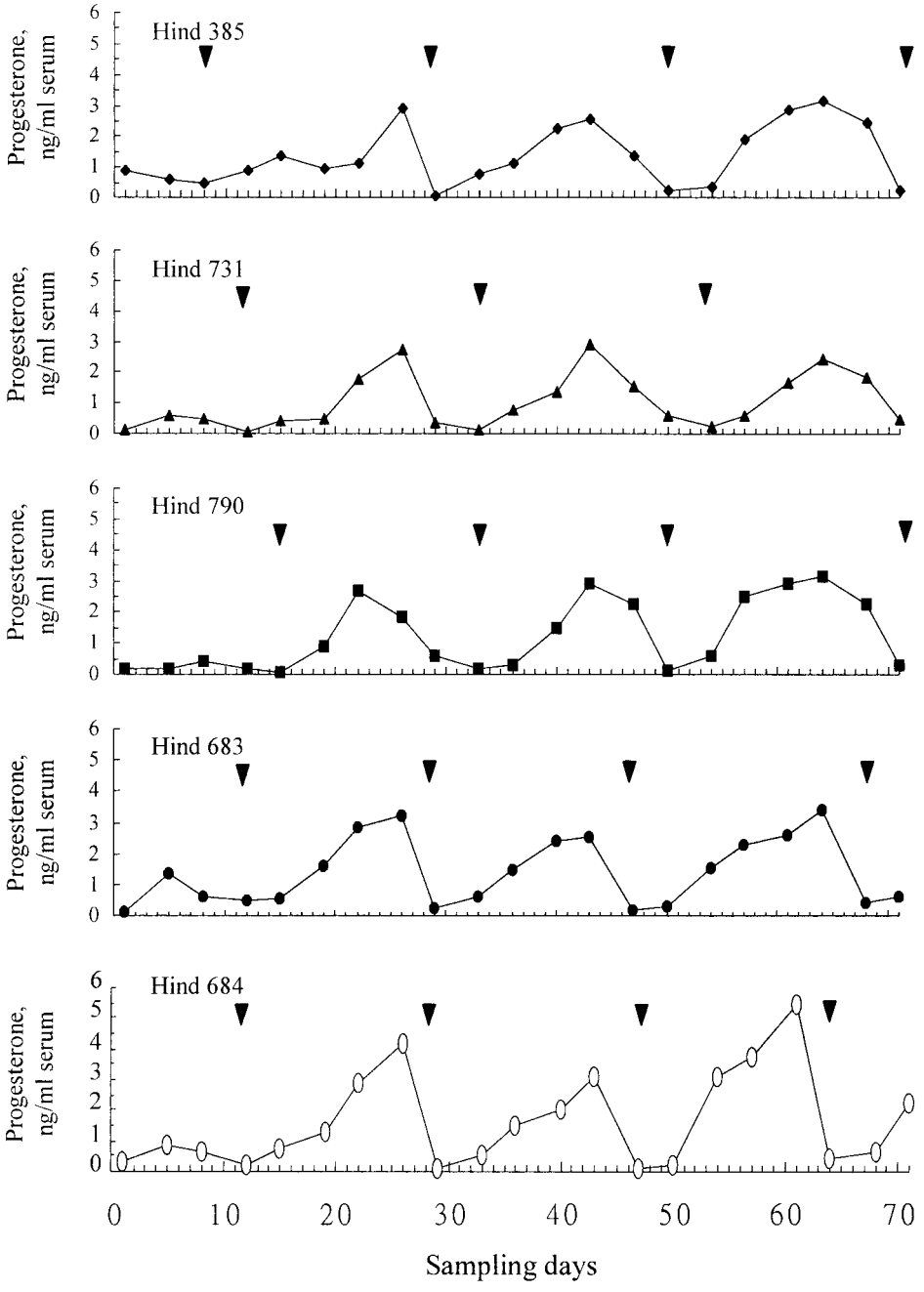

Fig. 1. Changes of SP_4 concentration (ng/ml) in the FSD hinds during their major rutting season. The sampling procedure was initiated at beginning of the rutting season, and the presumptive estrus (▼) was indicated by the day of the least progesterone concentration presented.

Animal anesthesia and blood sampling

During bleeding procedures, each hind was anesthetized by intramuscular administration of an aqueous mixture containing xylazine-HCl (40 mg; Rompun, Bayer NZ Ltd, Peton, New Zealand) and ketamine-HCl (115 mg; Ketaset, Aveco Co., Fort Dodge, IA) in the rump area with a blowpipe. The hinds became tranquilized 10 to 15 min after administration of the anesthetics, and remained tranquil for 10 to 20 min. Blood sample (10 ml) was withdrawn from external jugular vein in the morning (08:30 to 09:30) twice weekly (3–4 day intervals) by venepuncture from the hinds after anesthesia. The separated serum samples were stored at –20 until assay.

Detection of estrous mounting behaviors and signs of changes of external genitalia

Observations of mounting behaviors of the five hinds were conducted by eye measurement, three times daily: 6:00–7:30, 12:00–13:30, and 18:00–19:30. Before each bleeding of individual hinds, close-up checking on external genitalia was performed including the changes of color, size and shape of vulva, and wetness and mucus secretion. Such cyclic alterations of external genitalia likely reflected different phases of estrous cycle of the hinds.

Progesterone radioimmunoassay

The serum samples were analyzed for progesterone content by radioimmunoassay (RIA) established and described by Yu *et al*. (1988). Progesterone in samples was extracted with diethyl ether and allowed to freeze in –30°C ethanol medium. The upper ether-containing layer was decanted into another tube and dried under ventilation at 38°C. The dried residue was then dissolved in 0.01M polyphosphate-buffer-saline (pH 7.4) containing 0.1% gelatin (PBSG), and incubated at room temperature (25°C) for 1 hr. Aliquots of the PBSG dissolving steroids were used for radioimmunoassay. In the progesterone RIA procedure, the PBSG dissolving steroids were incubated with tritiated progesterone (^{3}H-progesterone, 1, 2, 6, 7–21-^{3}H (N), 115Ci/m mole, New England Nuclear, Boston, MA) and anti-progesterone serum (produced in rabbits by immunization with progesterone-11-HS: BSA, Steroids Co.). The cross reactions of the antiserum were as follows: progesterone, 100%; 11α-hydroxyprogseterone, 62%; 17-hydroxyprogseterone, 3%; corticosterone, 0.6%. The cross reactions for other steroids were less than 0.01% (Yu *et al*., 1988). In this assay, the lower detection limit was 5 pg/assay tube. The coefficients of variation of intra- and inter-assay were 4.7% (n=6) and 12.3%, respectively (n=4).

Statistical analysis

SP_4 concentrations are expressed as mean±SEM. A one-way analysis of variance (ANOVA) was used to test the differences between specific pairs of the SP_4 in the samples obtained from various phases during major rutting season.

RESULTS

Progesterone profiles of the estrous cycles

All 5 individual hinds exhibited three cyclic patterns of SP_4 levels with the basal levels of SP_4 in cyclicity every 17 to 21 days during the sampling period in their major rutting season November, December and early January (Fig. 1). There were transitory, slight elevation and soon diminished SP_4 concentrations at the beginning of sampling period in 4 out of 5 sika deer hinds (especially in Hinds 385 and 683). Such transitory elevated SP_4 values, however, were not significantly different from the mean SP_4 values of the subsequent basal levels (0.71±0.20 vs. 0.36±0.14 ng/ml; P>0.1). SP_4 concentrations were at basal levels (0.06 to 0.48 ng/ml serum) on the days of the presumptive estrus, i.e. in the dominant follicular phase of the estrous cycle, and increased to peak values (2.57 to 5.49 ng/ml serum) on the days of the fully luteal function, i.e. in the dominant luteal

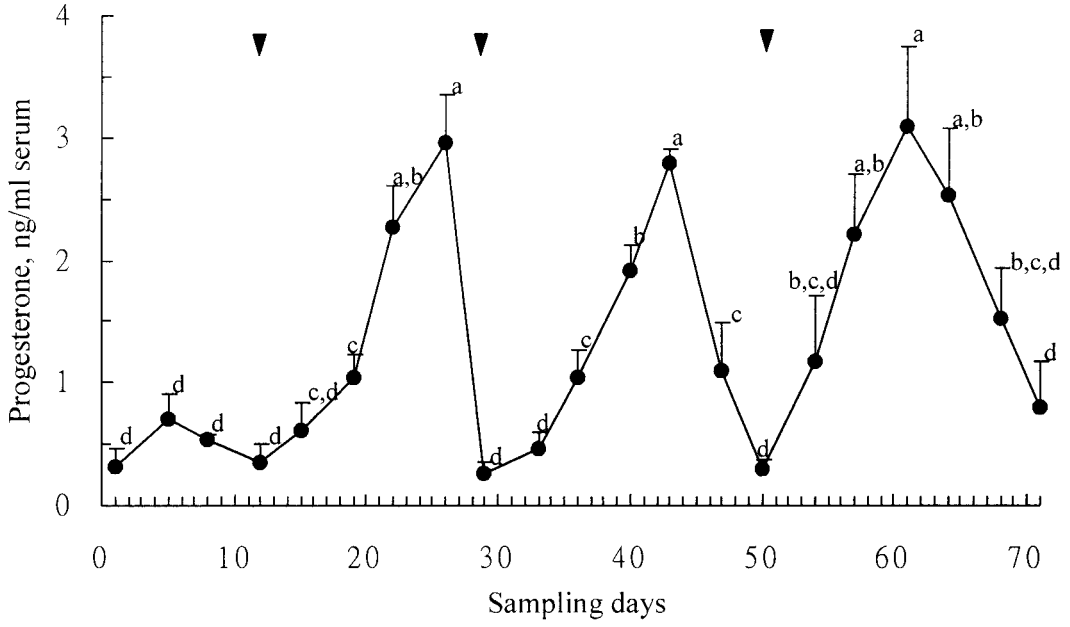

Fig. 2. Composite patterns of the changes of SP_4 concentration (mean±SEM) in the FSD hinds (n=5) during their major rutting season. The sampling procedure was initiated at beginning of the rutting season, and the days of the least concentration of progesterone exhibited were indicated (▼). $^{a, b, c, d}$: Means in the same cycle without same superscripts are significantly different (P<0.05).

phase of the estrous cycle. The SP_4 concentrations increased from basal level on the days of the estrus (0.20±0.01 ng/ml) to significantly higher level (1.67±0.11 ng/ml, with a range from 1.04 to 2.21 ng/ml) on days 7 in the cycle, and continuously elevated until days 14–16 of the cycle (Fig. 2). The concentration of SP_4 at the stage of maximum luteal activity on days 14–16 was significantly higher than that on the day of primary elevation (days 7) of the cycle duration (3.15±0.16 vs. 1.67±0.11 ng/ml; $P<0.01$).

It was revealed by the SP_4 profiles that the ovarian corpus luteum of the FSD was dominantly functional by 7 days after the estrus, and maintained thereafter for at least 7 to 9 days of the cycle during the major rutting season (Figs. 1 and 2). SP_4 concentrations were then declined rapidly from more than 2.65 ng/ml, to less than 0.21 ng/ml toward the beginning of the subsequent estrous cycle (Figs. 1 and 2).

Detection of estrous signs during the major breeding season

No mounting behaviors were observed by eye measurement during the major breeding season of the five hinds. However, the vulvas of all five hinds became slightly more pinkish, swollen and moistened at every 17 to 21 days intervals during the experimental period. These discernible cyclic alterations of the external genitalia occurred contemporaneously with the cyclic basal SP_4 levels.

Duration of the estrous cycle and extent of the rutting season

Based on the cyclic basal SP_4 levels and the observations of the cyclic signs of swollen and moistened external genitalia, the mean duration of estrous cycle of farmed FSD in Taiwan during the major rutting season was estimated to be 19.3±1.8 days with a range of 17 to 21 days.

Judged by shedding of the velvet antler and by presentation of rutting behaviors of the sexually mature stags, the rutting season of the FSD began within the first week in November, and terminated within the last week in April of the following year. The hinds exhibited the first estrus 3 days before and after 13 November of 1996 as indicated by the first basal levels in the cyclic SP_4 changes which were 11 days after the initiation of the rutting season (2 November of 1996) as indicated by rutting behaviors of the stags.

DISCUSSION

In the present study, the FSD hinds exhibited SP_4 at basal level (0.20 ng/ml) on the days around estrus; SP_4 concentrations were elevated markedly (3.15 ng/ml) on days 7 in the cycles and maintained for 7 to 9 days, and then declined rapidly before the beginning of the subsequent estrus. The patterns of circulating progesterone profile of FSD during the major breeding season observed in the present study are, in general, similar to those of other species of deers (Adam *et al*., 1985; Asher, 1985; Asher *et al*., 1986, 1988, 1997; Chapple *et al*., 1993; Guinness *et al*., 1971; Kelly *et al*., 1985; Plotka *et al*., 1980). Nearly all studies in deers indicated that the serum or plasma progesterone concentrations were the lowest around the estrus during an estrous cycle: 0.1 to 0.3 ng/ml in fallow deer (Asher, 1985; Asher *et al*., 1986, 1988); <1 ng/ml in red deer (Adam *et al*., 1985; Asher *et al*., 1997; Kelly *et al*., 1985); 0.85 ng/ml in chital deer (Chapple *et al*., 1993); and <1 ng/ml in white-tailed deer (Plotka *et al*., 1980). The progesterone concentrations rose to maximal values 7 to 14 days after estrus/ovulation, varying with species: 3 to 8 ng/ml in fallow deer (Asher, 1985; Asher *et al*., 1986, 1988); 2 to 7.5 ng/ml in red deer (Adam *et al*., 1985; Asher *et al*., 1997; Kelly *et al*., 1985); 5 to 8 ng/ml in chital deer (Chapple *et al*., 1993); and 3 to 8 ng/ml in white-tailed deer (Plotka *et al*., 1980). Consequently, the circulating progesterone concentrations in various species of deers increased 6 to 20 fold, from the minimal basal levels at estrus to the maximal peak at fully functional corpus luteum.

At the beginning of the breeding season, the SP_4 levels of all five FSD hinds increased from anestrus basal to 0.71±0.20 ng/ml for several days in about 10 to 12 days preceding the first cycle. A transitory increase in progesterone concentration preceding the first estrus was also observed in the fallow deer hinds (Asher, 1985) and in the Pere David's deer hinds (Curlewis *et al*., 1988). In the ewe it has been shown that progesterone priming is essential to ensure normal luteal function at the subsequent ovulation (McLeod and Haresign, 1984; McLeod *et al*., 1982). In addition, Legan *et al*. (1985) have shown that some of the priming effects of progesterone require only low physiological concentrations of progesterone for as short as 3 days. Whether or not the transitory increase in SP_4 concentrations observed before the first cycle in the FSD hinds also serves a similar function to that reported for the ewe requires further investigation.

In most of the temperate deer species, females are seasonally polyestrous and exhibit the estrous cycles ranged from 17 to 21 days in chital deer (Chapple *et al*., 1993), 21 to 26 days in fallow deer (Asher, 1985; Asher *et al*., 1986, 1988), 18 to 21 days in red deer(Adam *et al*., 1985; Guinness *et al*., 1971; Kelly and Moore, 1977), 22 to 29 days in white-tailed deer (Plotka *et al*., 1980; Verme, 1985) or black-tailed deer (Thomas and Cowan, 1975), and 18 to 20 days in the Pere David's deer (Curlewis *et al*., 1988; Wemmer *et al*., 1989). As observed in the present study, the duration of estrous cycles of the polyestrous FSD hinds, inhabiting in tropical southern Taiwan, based on the consecutive basal progesterone values during the major rutting season, were 19.3±1.8 days. Such duration of estrous cycle determined by consecutive cyclicity of basal serum progesterone levels corresponded well with the observations of the cyclic alterations of external genitalia; namely, the vulvas became slightly more pinkish, swollen and moistened when the SP_4 levels were lowest. Presumably, the vulvas of the FSD responded to varying levels of estrogen and progesterone secreted by ovaries. During proestrus and estrus, under estrogen domination and least progesterone secretion, the

vulvas thus became more swollen, pinkish and moistened as observed in the current investigation. The period of proestrus and estrus in the FSD hinds was estimated to last for 3 to 4 days. In the present study, the mating behavior of the hinds were not tested by the teasing stag. Our previous observations revealed that the duration of estrus of FSD hinds, as detected by vasectomized stag, was about 15 hr (unpublished). Shih *et al*. (1985) reported the duration of estrous cycle of FSD hinds in Taiwan was 19.8±2.6 days, based on the observations of mating behaviors recorded by the deer farmers. Thus, the duration of estrous cycle of FSD determined by monitoring the progesterone levels and by observations of the estrous behaviors is highly consistent.

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