Annual Changes of Testis Size, Seminiferous Tubules and Plasma Testosterone Concentration of Wild Sika Deer (*Cervus nippon yesoensis* Heude, 1884) in Hokkaido

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ABSTRACT. Testis size, seminiferous tubules and plasma testosterone concentrations showed conspicuous annual changes in Sika deer of Hokkaido, Japan. The onset of the spermatogenic process occurred in July or August. Spermatogenic activity had already reached its height in late October, at the beginning of the rutting season, and had begun to decline in late December. Spermatogenesis had stopped in February or March. Plasma testosterone concentrations showed very high levels in late October and early November, but was almost at the basal level in February, March, June and December. The wide individual variation of the plasma levels in October suggest pulsatile secretions of testosterone.—KEY WORDS: annual change, Sika deer, spermatogenesis, testis, testosterone.

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Many species of temperate cervids, such as red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*) and white-tailed deer (*Odocoileus virginianus*), are seasonal breeders and their reproductive organs and concentrations of some hormones show seasonal changes [1, 2, 5, 12, 13, 15–17, 22]. Reproductive synchrony is close within a population [13, 14, 21]. Recently experimental studies have been conducted in some species of cervids [13, 15] on the endocrinological regulation on seasonal breeding.

Sika deer (*Cervus nippon*) is one of the temperate cervids and their sexual behavior changes annually [3,18]. Their reproductive organs also show conspicuous seasonal changes [23–25]. However, compared with some other cervids, detailed reports on reproductive physiology in Sika deer are very few. In the present work, as a beginning of physiological study on the reproduction of this species, we have examined seasonal changes of testis size, diameter and epithelium conditions of seminiferous tubules, and plasma testosterone concentrations on a wild population in Hokkaido.

MATERIALS AND METHODS

Field studies were conducted eight times from March 1988 to February 1989 on the Nakanoshima Islands. These islands are located in Lake Toya in the south-west of Hokkaido (Fig. 1). The total area is 5.2 km^2 comprising three islands which lie 3 km away from the surrounding mainland. The vegetation of the islands consists mainly of deciduous broad-leaved trees, poor grasses and sedges. Sika deer in these islands are originated from three animals introduced in 1957, 1958 and 1965 [6]. Population density in 1984 and later years was about 26 deer/km^2 [7]. According to frequency of fighting

Table 1. Dates of capture and number of animals examined

Dates of capture	Number in which testis size were measured	Number in which seminiferous tubles were examined	Number in which testosterone concentration were assayed
18–21 March 1988	14	8	14
23–26 June 1988	4	5	6
23–25 August 1988	4	3	0
23–24 October 1988	4	4	4
8 November 1988	1	0	1
25 November 1988	1	1	1
22–24 December 1988	4	4	4
25–27 February 1989	4	4	4
(Total)	36	29	34

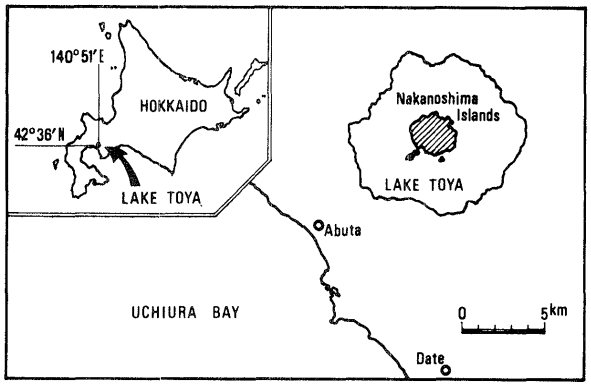

Fig. 1. Location of Nakanoshima Islands in Lake Toya.

between males and rutting calls, the season of rut is estimated to be from late October to mid-November [Kaji *et al.* unpublished data].

Specimens were collected from animals which had been immobilized by a combination of Ketamine hydrochloride (2.0–10.0 mg/kg) and Xylazine hydrochloride (1.0–1.5 mg/kg). To inject these drugs, a capture gun or blow darts were used. Age was estimated by the eruption and wear of mandibular dentition, using criteria for Sika deer [8, 20]. Since it has been reported that the seasonal sexual cycles in young red deer and reindeer (*Rangifer tarandus*) are slightly delayed from older ones [10, 11], we used only the data from four-year-old and older animals. Table 1 shows the dates of capture and numbers of animals which were examined.

The size of the left testis, including scrotum, was measured with a caliper. Thus in this paper, the "testis size" includes the epididymis and the scrotum skin. The size was represented by $\sqrt[3]{\text{length} \times \text{width} \times \text{depth}}$. For histological observations, a piece of tissue was biopsied from the right testis in each animal. The tissues were fixed in Bouin's solution, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin. The diameter of seminiferous tubules was denoted as the mean of the short diameter of 10 seminiferous tubules which were round in cross section (long diameter/short diameter <1.3). Significant differences in testis size and diameter of seminiferous tubules between months were tested by Mann-Whitney's U-test. The data for testis size in November and seminiferous tubules in August and November were not used in these statistical analyses because of the small sample size.

About 10 m*l* of blood was taken from the jugular vein and centrifuged (3,000 r.p.m., 30 min) immediately. Separated plasma was stored at −20°C until assay. Plasma testosterone concentrations were measured by radioimmunoassay using testosterone antiserum, standard testosterone and [^{125}I] testosterone at Tomakomai Clinical Center. First and second antibodies had been prepared in a rabbit against testosterone serum and in a goat against rabbit γ-globulin serum, respectively. The assay sensitivity was 12.5 pg/tube, and the inter- and intra-assay coefficients of variation, using two sample of human serum, were 2.5, 7.0% and 8.7, 3.8%, respectively [19].

RESULTS

Changes of the testis size (Fig. 2) : Testes were small in February, March and June, when the mean size was 28.9 mm, 30.5 mm and 29.0 mm, respectively. The mean size reached 39.3 mm in late August and 42.2 mm in late October. The mean testis size fell to 37.3 mm in December. The location of distribution of testis size was significantly different between June and August, October and December, and December and February ($P<0.05$), but not between March and June, August and October, or February and March.

Changes in seminiferous tubules (Figs. 3–8) : The diameter of seminiferous tubules and the cellular component and arrangement in the seminiferous epithelium showed marked seasonal changes.

The epithelium of seminiferous tubules was degenerate in June. In this season, the mean diameter of seminiferous tubules was 98.4 μm and the

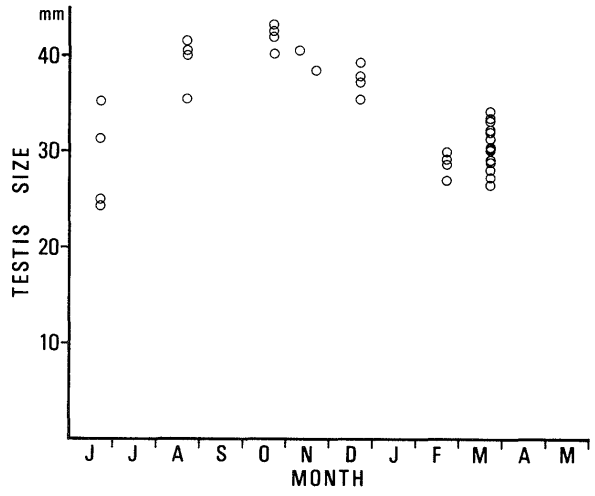

Fig. 2. Seasonal changes of the testis size. The size is represented by $\sqrt[3]{\text{length} \times \text{width} \times \text{depth}}$.

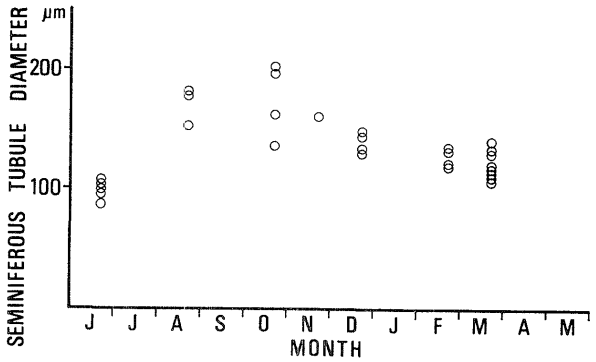

Fig. 3. Seasonal changes of the diameter of seminiferous tubules.

Fig. 4. Seminiferous tubules in late June. The epithelium is thin, with a single layer of germinal cells and Sertoli cells.

epithelium was a single layer of germinal cells (Fig. 4). Only the spermatogonia and a few spermatocytes were detected besides Sertoli cells in the epithelium. A few spermatozoa, spermatids and spermatocytes were rarely found in the seminiferous lumen.

In late August, just before the velvet shedding, the mean diameter of seminiferous tubules was 156.4 μm. The epithelium became thicker and many spermatocytes and spermatids were detected. However, few spermatozoa were found. The seminiferous lumen was very narrow or completely

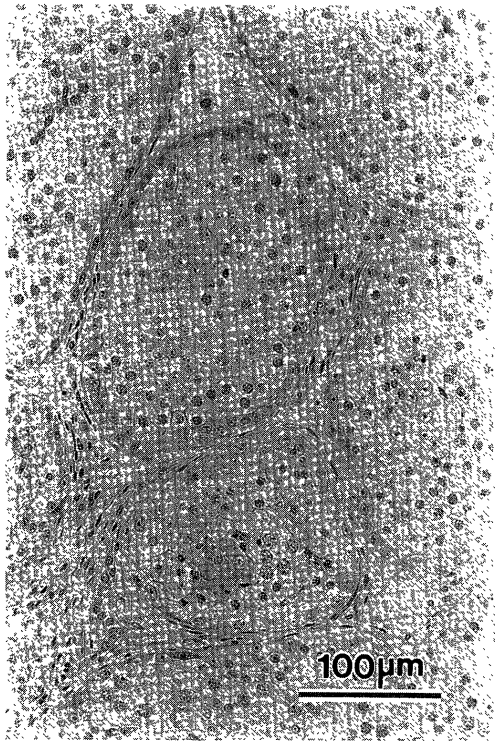

Fig. 5. Seminiferous tubules in late August. The epithelium is thicker and many spermatocytes are detected. The seminiferous lumen is very narrow or closed.

closed (Fig. 5).

In late October, though there were some individual variations, the mean diameter of seminiferous tubules was the longest in the present study, reaching 180.2 μm. This was at the onset of the rutting season. There were many spermatocytes, spermatids and spermatozoa in the epithelium, whose cells lay orderly (Fig. 6). A few spermatocytes and spermatids, which had fallen from the epithelium, were detected in the seminiferous lumen.

Histological testicular regressions were observed immediately after the rutting season. The mean diameter of seminiferous tubules decreased rapidly, and became 139.3 μm by late December. Though many spermatocytes, spermatids and spermatozoa were observed in the epithelium, the number of cells decreased (Fig. 7). There were more spermatocytes and spermatids which had fallen from the epithelium into the seminiferous lumen than in October.

The mean tubule diameter became 125.2 μm and 121.5 μm in February and March, respectively. Compared with the epithelium in December, though spermatocytes and spermatids were still observed,

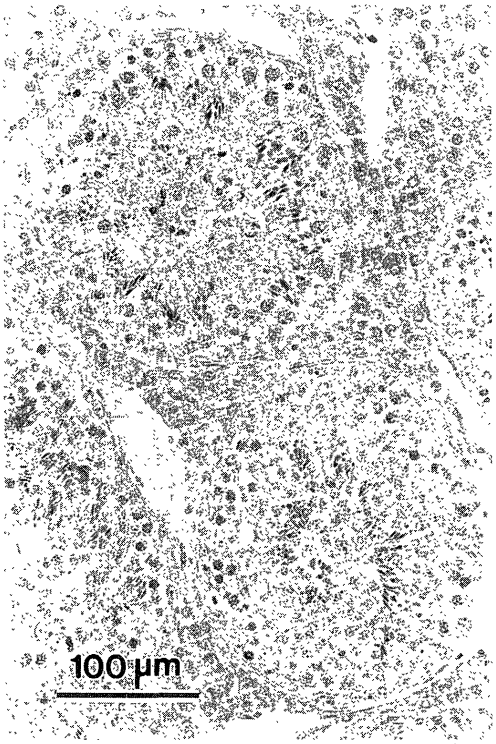

Fig. 6. Seminiferous tubules in late October. There are many spermatocytes, spermatids and spermatozoa. They lie orderly in the epithelium.

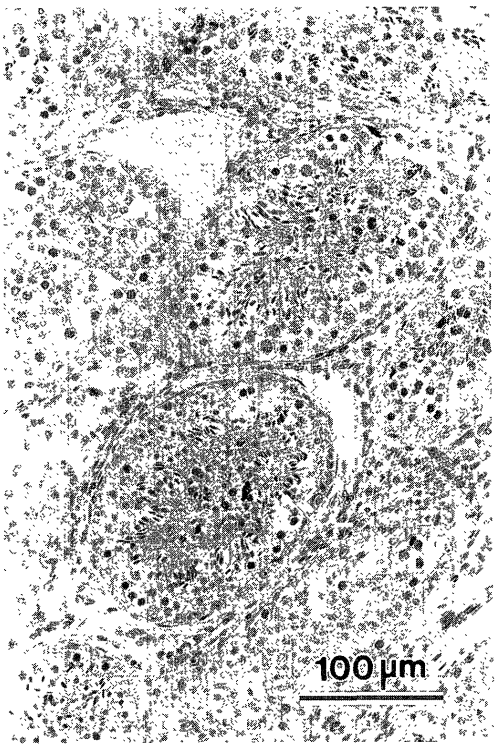

Fig. 7. Seminiferous tubules in late December. Many germinal cells are still observed in the epithelium. However, tubule diameters are narrower than in October.

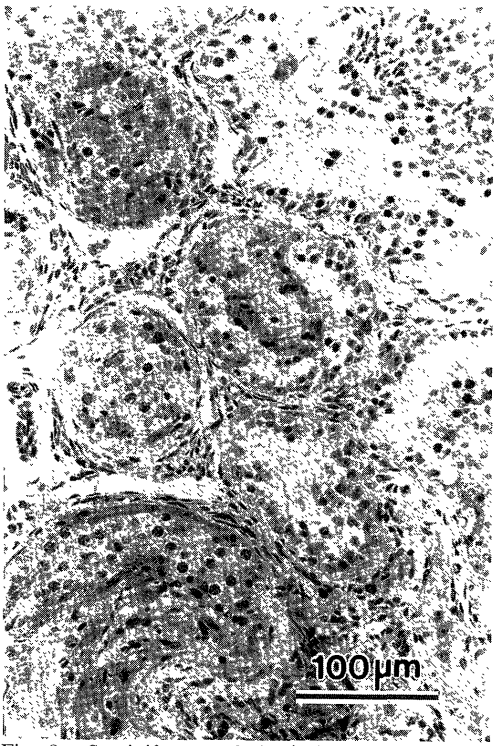

Fig. 8. Seminiferous tubules in late February. The epithelium is thin and the orderly arrangement of cells is lost.

the epithelium became thinner and the number of cells decreased. The orderly arrangement of cells was also lost in the epithelium (Fig. 8). Very few spermatozoa were attached to the epithelium. However, a lot of spermatocytes and spermatids were found in the seminiferous lumen.

The location of distribution of seminiferous tubule diameters was significantly different between June and October ($P<0.05$), October and December ($P<0.05$), and March and June ($P<0.01$). Between December and February and between February and March, there were no significant differences.

Changes of the plasma testosterone concentration (Fig. 9) : The plasma testosterone concentration was mostly around the basal level in February, March, June and December. However, only in the rutting season, the concentration was very high. Though the sampling and treatment methods of blood samples were same in all animals, the testosterone concentrations showed a wide individual variation in this season.

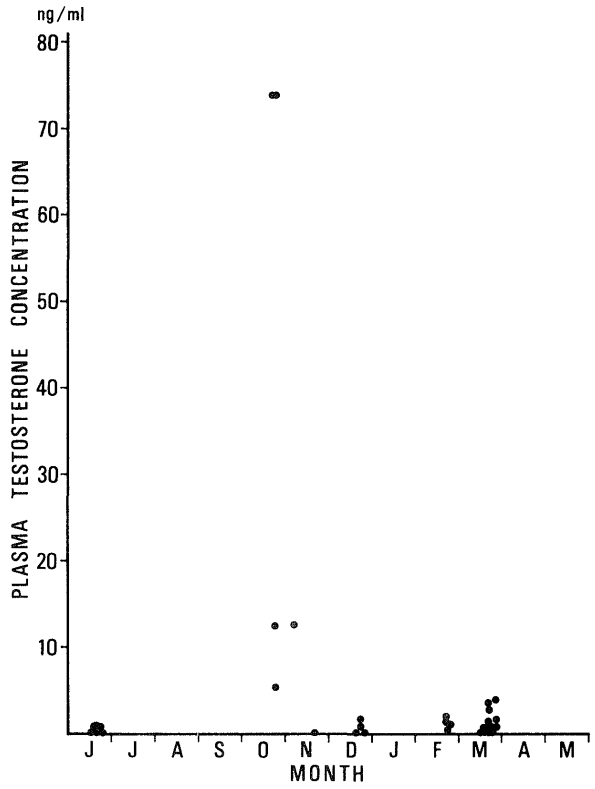

Fig. 9. Seasonal changes of plasma testosterone concentrations. In late October, at the beginning of the rutting season, the concentration is very high and very variable.

DISCUSSION

According to the results of the present study, we confirmed that Hokkaido Sika deer show annual changes in testis size (Fig. 2), seminiferous tubules (Figs. 3–8) and plasma testosterone concentration (Fig. 9), like other temperate cervids.

We believe the onset of the spermatogenic process occurs in July or August in this population since seminiferous tubules were inactive in late June but a few spermatozoa were present in late August. The narrow or closed seminiferous lumen in August might be due to a rapid increase of germinal cells of the epithelium which precedes the expansion of tubule diameter.

Fully active spermatogenesis was already in progress in late October, and this condition continued at least until the end of November. This is suggested by not only the maximum testis size but also the abundant germinal cells in the seminiferous epithelium in these seasons. Plasma testosterone concentration reached a high level in late October and early November. This fact is in consistent with the striking increase in frequency of rutting calls during this season because testosterone generally controls rutting behavior in many cervids [13].

According to the reduced testis size and seminiferous tubule diameter, testicular activity began declining in late December. But spermatogenesis had not yet ceased completely because spermatozoa were found in the seminiferous epithelium. Plasma testosterone concentration was already at the basal level in this season. Therefore, the duration of abundant testosterone secretion seems to shorter than the length of active spermatogenesis. Similar phenomena have been reported in red, roe and white-tailed deer [13].

In February and March, since spermatozoa were found only in the seminiferous lumen, spermatogenesis had probably ceased. This is also supported by the decreased number and/or lack of orderly arrangement of germinal cells in the seminiferous epithelium. However, because of remaining spermatozoa in the testes, fertility may not have been completely lost in this season. To know the duration of fertility in male Hokkaido Sika deer, it is necessary to examine epididymides or semen directly such as has been done on red deer and wapiti [4, 9, 12].

Pulsatile secretions of testosterone were suggested by the wide individual variation of the plasma concentrations in October. Pulsatile secretion in testosterone, occurring every few hours, has been reported in red deer during the rutting season and the secretion follows transitory small peaks in the blood level of LH [13, 15]. It would be necessary to collect blood samples from each animal at intervals of several tens of minutes, as well as to assay some other hormones, in order to know the details of endocrinological regulations in Sika deer.

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