Seasonal Variations of Testicular and Epididymal Structure and Plasma Levels of Testosterone in the Soft-shelled Turtle (*Pelodiscus sinensis*)

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Abstract.- For purpose to consider the annual cycle of testis in soft-shelled turtle, *Pelodiscus sinensis*, the testes and epididymides were examined histologically, and the plasma levels of testosterone was measured by radioimmunoassay (RIA) though the year. Gradual increase in testicular weight from May to August was followed by a decrease in degrees. Spermatocytogenesis was first observed from late April to early May which remained active to August. Spermatogonial division became to decline in September and stopped by the end of November. The epididymal weights rose from August to November and remained heavy during hibernation with a rapid decline of about 53.76% in the mating period of next year. From July to September, the epithelial cells of epididymal ductus grew and increased in height especially. There were a great number of secretory granules in the cytoplasm with active synthesis. The plasma concentration of testosterone started to rise in April, which then fell in May and June. In July, it rose rapidly to peak levels and then declined to minimum. We suggested that it's more important that the peripheral testosterone promotes reproductive behaviour and stimulates secretion synthesis in epithelial cells of epididymis.

Key words.- Pelodiscus sinensis, testosterone.

Introduction

Only scattered and equivocal literature can be found concerning the reptilian gametogenetic cycle, the endocrinological function of testis and pituitary gonad interrelationships. The information is almost exclusively derived from investigations on squamate species (Bartholomew, 1953; Lofts, 1971; Courty, 1980). One does find, however, a few studies on such reptiles as turtles, for example: *Chrysemys picta* (Callard, 1976); *Pelodiscus sinensis* (Lofts and Tsui, 1977); *Chrysemys dorbigni* (Silva, 1984). The authors of those studies hold identical views that the testicular structure and function of reptiles change in annual cycle, and the epithelium of seminiferous tubule has spermatogenetic ability in several months while in other months it is in a static condition (Lofts, 1987).

The aquatic soft-shelled turtle, *Pelodiscus sinensis*, lives in rivers, lakes and ponds. The available literature on its reproduction relates only to its gonads. Hu Zeng-Gao (1988) reported that the testis of *Pelodiscus sinensis* produced sperms in reproductive period, which were used for copulation in same period. Lofts and Tsui (1977) in Hong Kong reported the results of their studies on the histological and histochemical changes in the testis. They were of the opinion that *Pelodiscus sinensis* has a postnuptial pattern which the spermatozoa are produced soon after mating period. The spermatozoa are stored in the epididymal canals from October until their discharge in the following April. Seasonal changes in size and histology of the testis and the accessory sexual organs (epididymis) are well known in a lot of reptile species (Lofts, 1969; Courty, 1979), and correlated variations in androgen levels are documented but still poorly detailed.

It has been shown by metabolic conversion of precursors that testosterone is the principal androgen in some reptiles as in mammals (Callard, 1967; Hews and Kime, 1978; Courty, 1979). Seasonal changes in the level of circulating testosterone have been reported in a few species of reptiles but data tend to be differ with different species (Bourne and Seamark, 1975; Callard, 1976; Silva, 1984). Some researchers suggested that the spermatogenesis correlated with the plasma testosterone levels in turtles (Kuchling, 1981; Silva, 1984). In other references we found that the highest plasma testosterone levels did not appear in spermatogenesis period, but in mating period (Callard, 1976). Lofts and Tsui (1977) considered that the interstitial tissue was active in steroids synthesis during mating period, but inactive during spermatogenesis period. The purpose of our study, therefore, was to determine if the variations in the plasma testosterone levels during the annual cycle in *Pelodiscus sinensis* are correlated with histological variations of the germinal epithelium and epididymal canals.

Material and Methods

Animals and samples collection

The samples used in our experiments were provided by Chang Zhou Breeding Center of Soft-Shelled Turtle (near Nangjing in Jiangsu Province in east China). The breeding environment was similar to their natural living condition. Except December and January every month we obtained blood plasma, testes and epididymides that those were from six adult male soft-shelled turtles, *Pelodiscus sinensis* (there body weights were about 650-1000g) in one year. The blood plasma samples were stored at -24° until the testosterone analysis was performed. The tissue samples of testes and epididymides were weighted and fixed in Bouin fluid.

Histological procedures

For the histological examination, a piece of testis and a piece of epididymis (about 25mm^3) were fixed in Bouin fluid for 24 hours. After discolored, the samples were embedded in paraffin, cut in 5μm sections, and then stained with haematoxylin and eosin.

Testosterone assay

We determined the plasma concentration of testosterone by radioimmunoassay (RIA) using a technique provided by WHO. The kits used in our experiments were from Hua Mei Biological Engineering Co. (a Sino-US joint venture). Briefly, the hormone was extracted from plasma samples (250-750l) twice with 5 ml of anhydrous ether, with an average recovery of 96%. The extract was dried at 40° by constant temperature bath, and dissolved in 2 ml phosphate-buffered saline containing gelatin (GPBS). we placed 0.5 ml of this solution in duplicate assay tubes and incubated with radio labeled testosterone (^{3}H-T 10,000 cpm) and anti-testosterone antiserum (0.1 ml) for 18-24 h at 4°C. After removing the unbound fraction with dextran-coated chorale, the samples were placed in scintillation fluid (TP-POPOP-toluene) and the radioactivity (cpm) was detected on a liquid scintillation counter. RIA data were analyzed with a program utilizing a weighted logic-log regression analysis on an IBM PC. All data are as the mean ±SEM.

Results

Weight and histology

The seasonal weight variations in testes and epididymides of *Pelodiscus sinensis* are shown as unit body weight in Figure 1. From March (shortly after emergence from hibernation) to April, testicular weights remained low (particularly in April). The seminiferous tubules remained atrophied and spermatogenetically inactive. The germinal epithelium contained Sertoli cells and spermatogonia only (Fig. 2A). In April there was a significant reduction (by about 53.76%) in the epididymal weights as spermatozoa were evacuated from the epididymal canals. During May and June the epididymal weights declined continuously (by about 19.65%, Fig. 1).

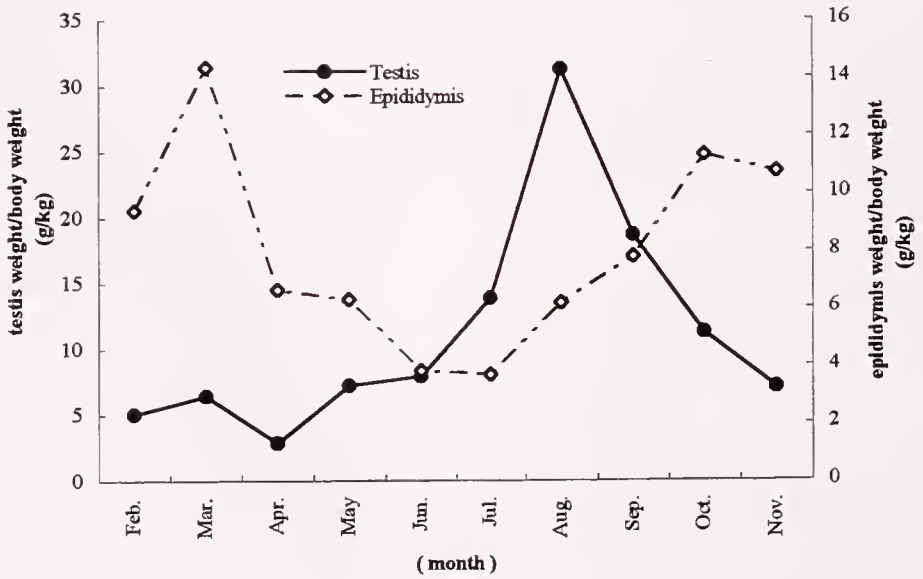

Figure 1. Seasonal variations in testis and epididymis weight in the soft-shelled turtle, *Pelodiscus sinensis*. Each point represents mean of six values.

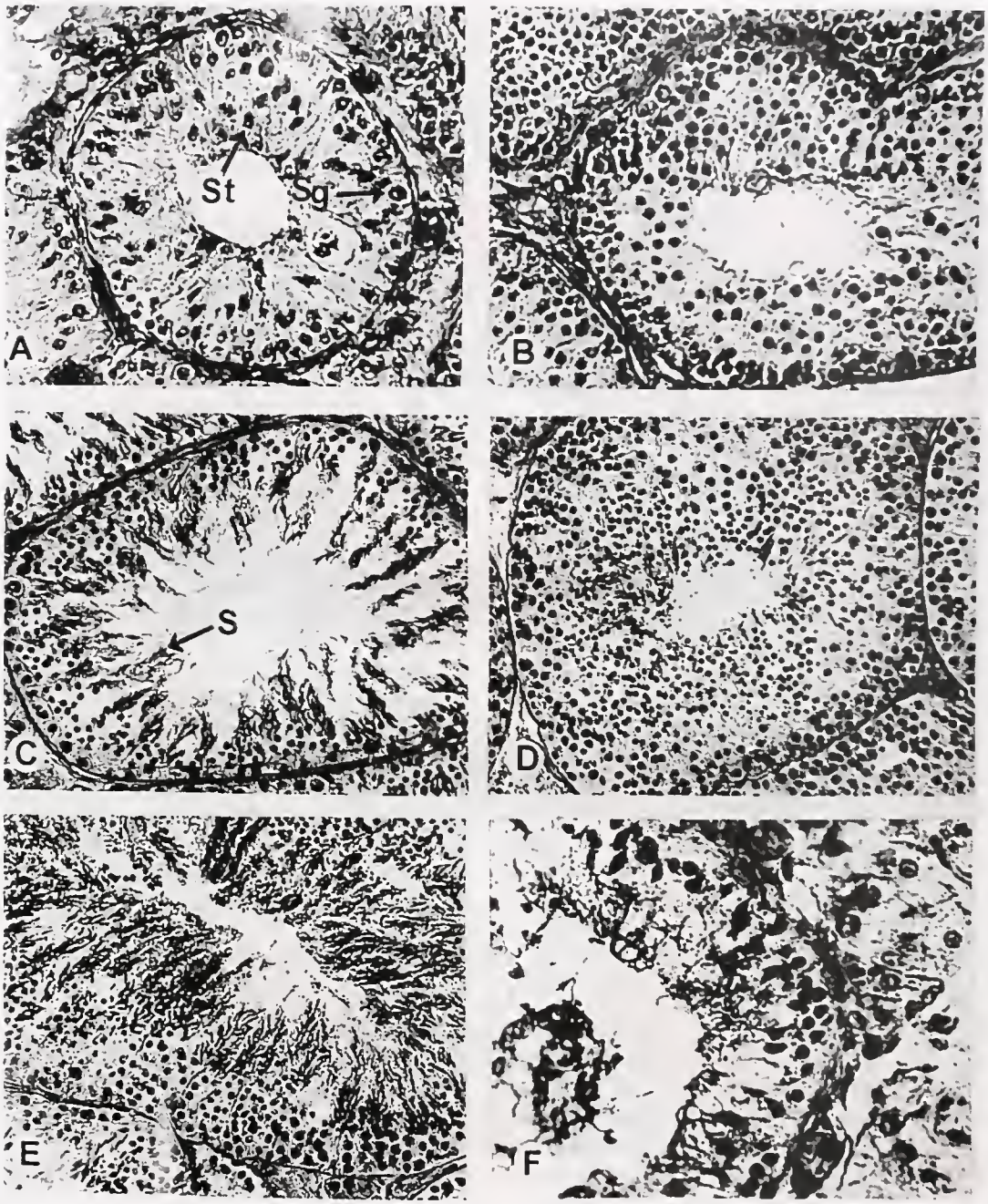

Figure 2. Microscopic figures of testis in the soft-shelled turtle, *Pelodiscus sinensis*. **A.** The seminiferous tubules contracted and contained Sertoli cells (St) and spermatogonia (Sg) in April 200. **B.** The germinal epithelium contained dividing spermatogonia, spermatocytes and spermatids in May 200. **C.** Spermatozoa (S) were released from germinal epithelium in June 132. **D.** The thickness of spermatogenetic epithelium increased but spermatozoa decreased in July132. **E:** Spermatozoa occluded the lumen of the seminiferous tubules in August132. **F:** The germinal epithelium atrophied in February 200.

Spermatocytogenesis started from late April to early May, and the spermatocyte layers increased progressively in germinal epithelium. The testicular weights began to increase in May. By the end of the month, the germinal epithelium contained dividing spermatogonia, spermatocytes and a few spermatides (Fig. 2B). In June, spermatozoa were released from germinal epithelium (Fig. 2C) without accompanying apparent increase in epididymal weights. The active spermatogonia division progressed until July. The thickness of spermatogenetic epithelium increased and the diameter of seminiferous tubules reached

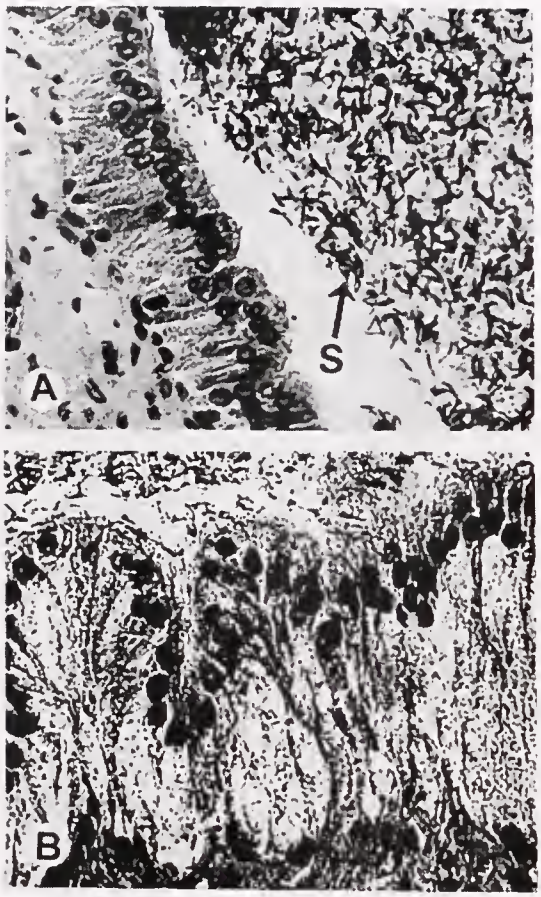

Figure 3. Microscopic figures of epididymis in the soft-shelled turtle, *Pelodiscus sinensis*. **A**. There were great amount of spermatozoa (S) in the ductus, The simple columna was thin in March 200. **B**. The heights of epithelial cells were at maximum, great amount of spermatozoa could be seen in the ductus in August 200.

maximum (Fig. 2D). By the end of August, when the testicular weights were at their top values, a large amount of spermatozoa gathered in the lumen of the distended seminiferous tubules (Fig. 2E) and passed into the epididymal canals with the epididymal weights increasing rapidly.

In September, spermatogonial division started to decline with less frequent mitotic figures. The germinal epithelium contained mainly spermatides and spermatozoa. At that time, testicular weights began to decrease. By the end of November, testis atrophied highly. And there were only a few spermatozoa remaining in the seminiferous tubules. During hibernation, the testis remained atrophied, and the germinal epithelium was composed of Sertoli cells as well as spermatogonia only and was heavily lipoidal (Fig. 2F). Often, there were necrotic cells in the lumen of the seminiferous tubules.

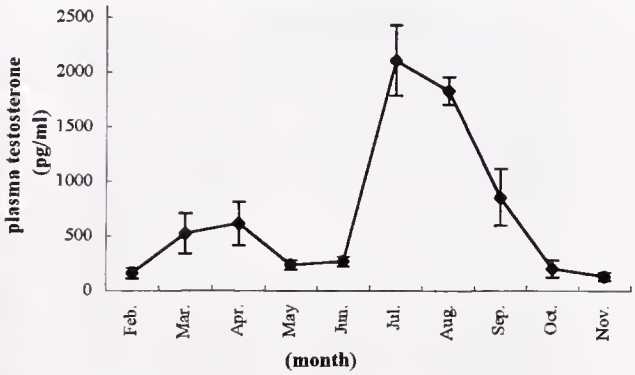

Figure 4. Seasonal variations in plasma testosterone concentration in soft-shelled turtle, *Pelodiscus sinensis*. Each point represents mean SEM of six values

In the ductus of the epididymis, spermatozoa could be recognized in great number in most months. Although in March soon after emergence from hibernation epididymal weights were high and their ductus were full of numerous spermatozoa (Figs. 1, 3A). Only in June (after mating period) were there markedly less spermatozoa present in the ductus of the epididymis. At this time the simple columnar epithelium of epididymis became thin and the nuclei moved toward the basal region of the cells. From the end of July, we could see that the spermatozoa were transported form the testis into the ductus of epididymis, and their epithelial cells increased their heights and secretory granules in cytoplasm. In August, their heights reached the maximum (Fig. 3B).

Plasma testosterone concentration

The variations of the plasma testosterone concentrations are shown in Figure 4. In March and April (spring), when the testis still remained atrophied, the mean values of plasma testosterone concentration started to rise from 160.87 pg/ml to 613.79 pg/ml. After that, there was a fall in plasma testosterone concentrations which coincided with the progressive atrophy of the epididymides. The plasma content remained relatively low in June, but rapidly increased to the highest levels (2104.97 pg/ml) in July. It did not drop significantly until September. After that, the plasma values fell sharply to their baseline levels in October. It could be seen that in an annual cycle of the plasma testosterone concentration had two peaks. The lower peak occurred during the spring and the higher one was in summer. The higher peak coincided with the maximal testicular weight and the hypertrophy of the germinal epithelial cells as well as the full growth of epithelial cells at the epididymal ductus.

Discussion

The present study has demonstrated that the spermatogenetic activity of germinal epithelium changes with season in *Pelodiscus sinensis*. Resembling that of most turtles reported (Lofts, 1987), its spermatogenesis resumes relatively soon after the mating period, so that advanced germinal stage is completed before the onset of the colder winter months. Spermatozoa are stored in the epididymal canals until the breeding season of next year. This result is consistent with that reported by Lofts an Tsui (1977) in *Pelodiscus sinensis*. We have to pointed out that spermatogenetic peak that we observed is in July and August, which is somewhat later than that observed by Lofts and Tsui. We suggest that this discrepancy might be due to the different temperatures and photoperiods at different latitudes.

The measurement of plasma testosterone in the male *Pelodiscus sinensis* indicates that an annual increase in peripheral levels begins in spring after emergence from hibernation period and is interrupted from the end of May through June. In July and August, the plasma testosterone rises to its maximum level. This finding is identical to that in tortoise, *Testudo h. hermanni,* reported by Kuchling (1981). In fresh water turtle, *Chrysemys picta,* the highest levels were in spring and the lowest were in summer, while in autumn the testosterone levels increased significantly (Callard, 1976). Although in *Chrysemys dorbigni* there was only one peak of plasma testosterone levels, the increase started in mating period and reached the maximum value in autumn during the principal period of spermatogenesis (*Silva*, 1984). It is similar to that in *Pelodiscus sinensis.*

Lofts (1968) described lipid accumulation in the interstitial cells of the testis in *Pelodiscus sinensis* which occurred prior to regression of the cells themselves, this indicated the cessation of steroidogenesis. Lofts and Tsui (1977) reported that they detected 3-HSD (^{5}3-hydroxysteroid deydrogenase) activity in *Pelodiscus sinensis*. It showed that the interstitial cells had a positive reaction for 3-HSD and were considerably depleted of lipoidal droplets in the mating period (March-April) and in the period of enhanced epididymal weight (August-October), but in active spermatogenesis period the interstitial cells were negative to the tests. And they said that the seminiferous tubules gave same histochemical feature of high testosterone secretory activity at the onset of spermatogenesis. As well known, the secretory activity of interstitial cells coincides with the variations of plasma testosterone levels, because the Leydig cells are the main origin of the plasma testosterone. Therefore, there is a discrepancy about the annual cycle of the secretory activity of interstitial cells between the histochemical study reported by Lofts and Tsui (1977) with the direct determination of plasma testosterone levels in our study. We also found that the seminiferous tubules have the ability to secrete testosterone (unpublished result).

In the turtle *Chrysemys dorbigni* and the tortoise *Testudo hermanni*, the maximum value of plasma testosterone acts on the secondary sexual organs in autumn, preparing them for the storage of spermatozoa which occurs in winter (Kuchling, 1981; Silva, 1984). This situation which can also be observed in our study. The decline of plasma testosterone levels in *Pelodiscus sinensis* in May and June is associated with the decrease in epididymal weights and the atrophy of the epididymal epithelium. The epididymal epithelial cells enlarge in July and August, when the plasma levels are highest. They increase secretary granules progressively. During this period, the epididymal weights increase significantly accompanying with the spermatogenesis. The spermatozoa are stored and matured in the epididymis until the following mating period.

In our study the seminiferous tubules are spermatogenetically inactive when the plasma testosterone increases in the mating period. However, when plasma levels are low in May, the spermatogenesis begins. It implied that the germinal epithelium is sensitive to testosterone when environmental temperature rises. Our conclusion is that the peripheral testosterone in *Pelodiscus sinensis* is more important to support reproductive behavior and stimulate the actions of epididymis.

Acknowledgments

We wish to express our deep gratitude to Mr. Chang Hue for assistance in our experiment. We are grateful to Professor Ning-Zheng Sun and Mr. Shu-Yu Gu for graphics technical assistance. This work was supported by a grant of Education Commission of Jingsu Province, China.

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