

# Seasonal Changes in Spermatogenesis and Ultrastructure of Developing Spermatids in the Japanese Rat Snake, *Elaphe climacophora*

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**ABSTRACT.** Seasonal changes in spermatogenesis and the ultrastructure of developing spermatids in the Japanese rat snake, *Elaphe climacophora*, were observed by light and transmission electron microscopy. Spermatogenesis in this species began in August, continued through October and ceased in November. No spermatozoa were found during other periods. Japanese rat snakes mate from May to July, and spermatogenesis occurs after the mating season. Therefore, according to Girons' classification (1982), the reproduction of this species was classified as a postnuptial type. Although the morphological changes in developing spermatids during spermiogenesis in these snakes fundamentally resembled those in mammals, some unique features were detected. The most prominent characteristic of the developing spermatids was lipid-like structures. These structures first appeared in early round spermatids, gradually increased in size and number, and, finally, aligned around the nucleus of mature spermatids. Although spermatozoa with lipid-like structures were released from the epithelium, stored spermatozoa in vas deferens had none of these structures. Lipid-like structures that apparently separated from spermatozoa were scattered in the vas deferens. Prominent elongation of mitochondria was also remarkable in elongated spermatids.—**KEY WORDS:** Japanese rat snake, seasonal change, spermatid, spermatogenesis, ultrastructure.

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Although the reproductive cycles of male snakes are described in several species [1-4, 6, 7, 9-11, 14], little information on the morphology of snake testes is currently available. In a previous study [unpublished data], we investigated seasonal changes in spermatogenesis and ultrastructural changes of spermatids in the habu, *Trimeresurus flavoviridis*, a snake found in subtropical region of Japan. Several unique characteristics were identified in this species. For example, large quantities of lipid droplets were accumulated within the Sertoli cell cytoplasm of inactive habu testes. Lipid-like structures were arranged in a pattern around the nucleus of maturing spermatids. Thus, the testicular morphology of the habu possesses a unique feature, different from that in mammals [13]. Further detailed studies on morphological features of snake testes may bring a new information on the mechanism of spermatogenesis.

Although the Japanese rat snake, *Elaphe climacophora*, is found in most area of Japan, no morphological studies about its reproduction have not been carried out. This popular snake lives in temperate regions and hibernates during the winter. The effects of climate and hibernation on testicular function or morphology in male snakes are unclear. Therefore, this study was proposed to evaluate seasonal changes in spermatogenesis and ultrastructure of developing spermatids in the Japanese rat snake and to compare its reproductive characteristics with other snakes.

## MATERIALS AND METHODS

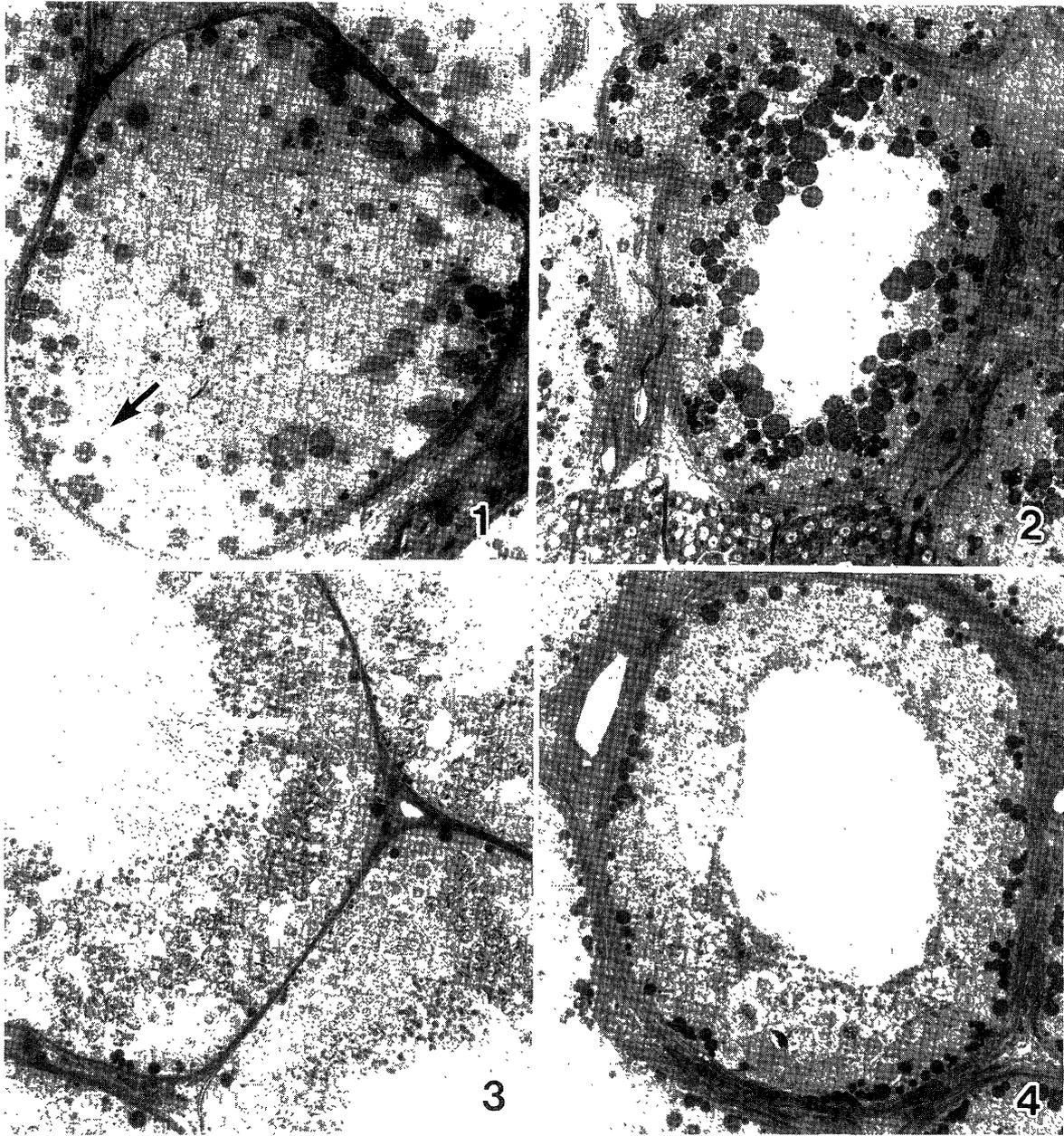
Mature Japanese rat snakes were obtained from the Japanese Snake Institute (Gunma, Japan). Their body length ranged from 116 to 164 cm. Samples were collected

on the schedule defined in Table 1. Under pentobarbital anesthesia, the snakes were perfused through the abdominal aorta with 5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, after a brief wash with 0.9% saline. Excised testes were cut into small pieces, washed in the same buffer, and postfixed in 1% osmium tetroxide/1.5% potassium ferrocyanide [12]. Specimens were then dehydrated in a graded series of ethanols, infiltrated in propylene oxide, and embedded in Araldite. For light microscopy, thick sections of 1  $\mu$ m were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate for observation with a JEM-1200 EX transmission electron microscope at 80 kV. During December, the vas deferens was also excised and prepared

Table 1. Seasonal changes of spermatogenesis in the Japanese rat snake

Year	Month/Day	Number	Spermatogenesis	Lipid droplet
1993	January/19	3	-	++++
1993	February/19	3	-	++++
1993	March/30	3	-	++++
1993	May/1	3	-	++++
1992	June/15	3	-	++++
1992	July/12	3	-	++++
1992	August/10	3	+	-
1992	September/15	3	+	+
1992	October/18	3	+	+++
1992	November/16	3	-	+++
1992	December/1	2	-	++++
1992	December/11	3	-	++++

Spermatogenesis: +, active; -, inactive. Lipid droplet: The number of lipid droplets within the seminiferous tubules ranged from undetectable (-) to extremely abundant (++++).



Figs. 1-4. Light micrographs.

Fig. 1. Inactive testis. In December, the lumen of seminiferous tubules is completely closed. A number of spermatogenic cells, up to early round spermatids, are in the seminiferous epithelium. Lipid droplets (arrow) are also in the basal region of the epithelium. Toluidine blue stained.  $\times 375$ .

Fig. 2. Inactive testis. In May, the lumen of seminiferous tubules is open. Lipid droplets are near the lumen. Toluidine blue stained.  $\times 290$ .

Fig. 3. Active testis. In September, a number of spermatozoa are in the lumen. Lipid droplets decrease in number compared to May and are located along the basement membrane. Toluidine blue stained.  $\times 290$ .

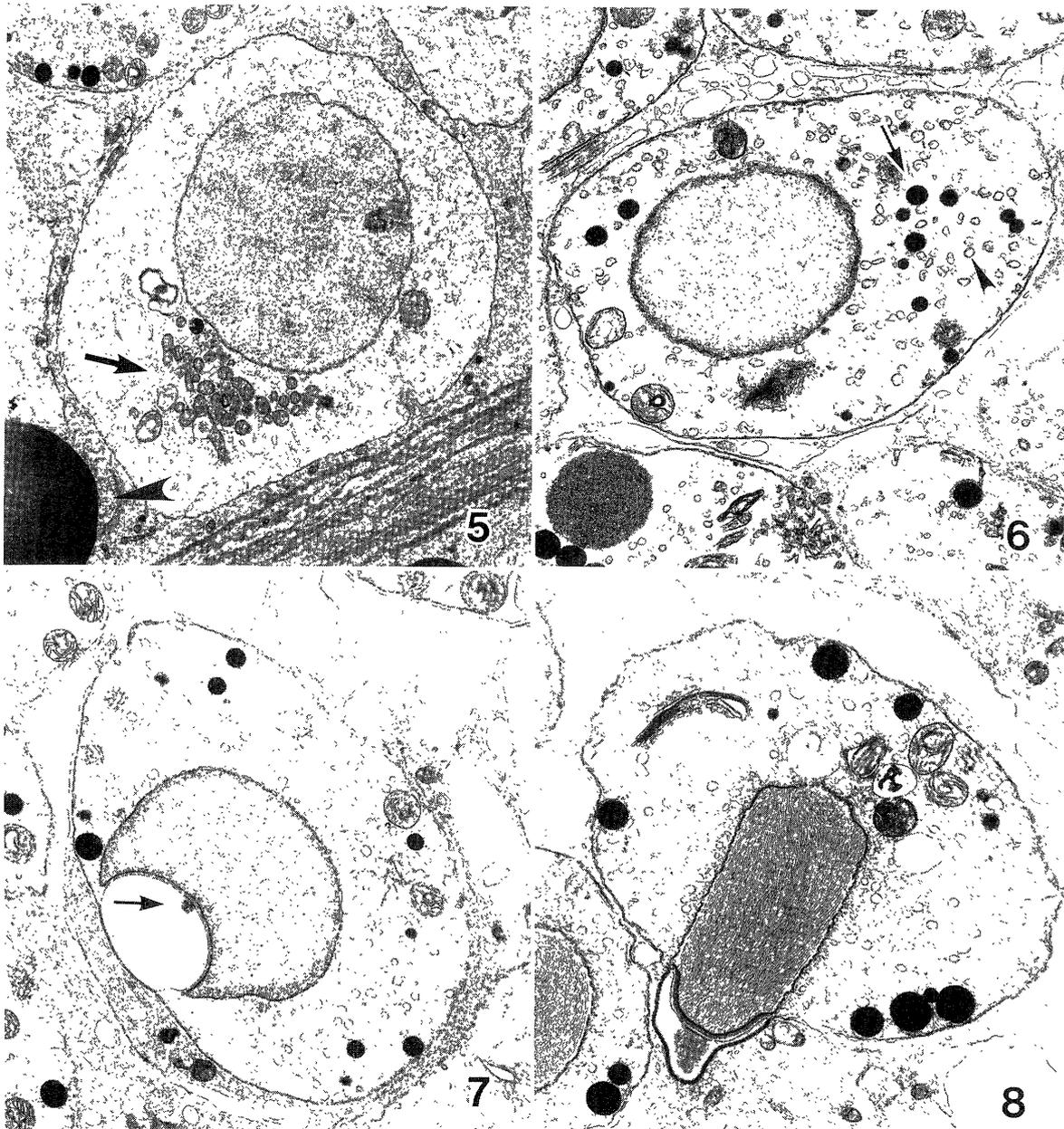
Fig. 4. Inactive testis. In November, spermatozoa completely disappear. Lipid droplets increase in number and are located in the same region as in September. Toluidine blue stained.  $\times 335$ .

with the same procedures for light and transmission electron microscopy.

#### RESULTS

*Seasonal changes of spermatogenesis:* The period of

most active spermatogenesis in the Japanese rat snake occurred from August to October. During this time, large numbers of spermatozoa were detected within the lumen of seminiferous tubules (Fig. 3), thereafter, spermatogenesis abruptly ceased. The lumen of seminiferous tubules gradually narrowed and finally closed in Decem-



Figs. 5-12. Transmission electron micrographs. Samples were collected in September.

Fig. 5. Accumulated mitochondria (arrow) are observed in a spermatocyte. A large lipid droplet (arrowhead) found in a light micrograph is also in Sertoli cell cytoplasm.  $\times 5,000$ .

Fig. 6. Lipid-like structures (arrow) appear in early round spermatids. Mitochondria disperse in the cytoplasm and increase in size. Round endoplasmic reticulum (arrowhead) is different in shape from that of mammals.  $\times 8,000$ .

Fig. 7. Acrosomal granule (arrow) and vacuole are observed.  $\times 7,000$ .

Fig. 8. Condensation and elongation of the nucleus begin at this period. Lipid-like structures increase in size.  $\times 8,000$ .

ber. During this period, the seminiferous epithelium contained all types of spermatogenic cells except elongated spermatids (Fig. 1). The appearance of the seminiferous tubules remained similar through March, and the lumen finally reopened in May (Fig. 2). From May to July, a few elongated spermatids appeared, but no spermatozoa were found in the lumen.

Throughout the year, the distribution and number of lipid droplets in the seminiferous epithelium varied

radically at the light microscopic level. Although these lipid droplets were not apparent in August, they appeared near the basement membrane of the seminiferous epithelium in September (Fig. 3). In October, droplets remained in the same region, but they obviously increased in number. After spermatogenesis stopped, lipid droplets were still observed near the basement membrane in November (Fig. 4). Many lipid droplets were present in the basal region from December to March (Fig. 1), and

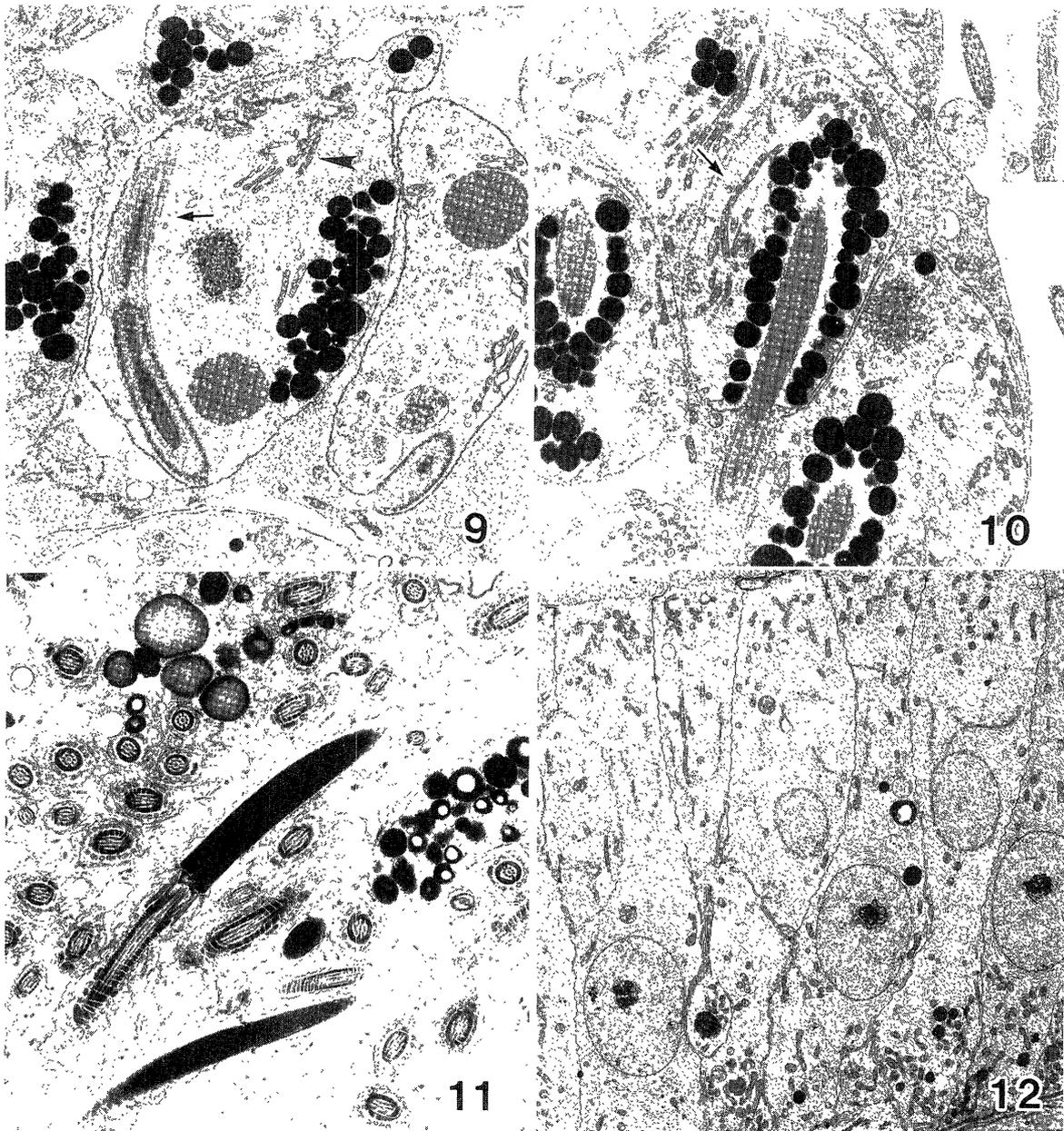


Fig. 9. Lipid-like structures increase in number and gather in the cytoplasm of spermatids. Mitochondria begin to elongate (arrowhead). Manchette is clearly seen (arrow) similar to that in mammals.  $\times 6,000$ .

Fig. 10. Lipid-like structures are arranged in order around the nucleus. Mitochondria (arrow) elongate to a great degree. (Inset: higher magnification of mitochondria indicated by arrow. Mitochondrial cristae are clearly seen.)  $\times 6,500$ .

Fig. 11. Lipid-like structures in the vas deferens are apart from the nucleus of spermatozoa, and are variable in electron density.  $\times 7,700$ .

Fig. 12. Epithelial cells of the vas deferens did not contain spermatozoa.  $\times 3,000$ .

they moved to the luminal side from May to July (Fig. 2).

*Ultrastructure of developing spermatids:* The most prominent characteristic of the seminiferous epithelium was lipid-like structures at the electron microscopic level. These unique structures first appeared in early round spermatids, later, their gradual increase in number and size corresponded with elongation of the nucleus. In maturing spermatids, they initially clustered in the cytoplasm and then aligned themselves around the elongated

nucleus (Figs. 6–10). Under light microscopy, large lipid droplets were recognized in the cytoplasm of Sertoli cells. These droplets within the cytoplasm were much larger in size and had a lower electron density than lipid-like structures observed by electron microscopy in spermatids.

In the vas deferens, lipid-like structures were conspicuously absent in stored spermatozoa (Fig. 11). However, isolated lipid-like structures were scattered in the vas deferens, and their variable electron density was different

from that in the testis. Epithelial cells of the vas deferens did not contain spermatozoa (Fig. 12).

Oval shaped mitochondria accumulated in the cytoplasm of spermatocytes. In early round spermatids, they migrated to the plasma membrane (Figs. 5–10). Accompanying elongation of the nucleus, the shape of mitochondria gradually changed from oval to elongated. In maturing spermatids, mitochondria were extremely elongated.

These electron microscopic observations are schematically explained in Fig. 13.

#### DISCUSSION

The present study demonstrated that the Japanese rat snake spermatogenesis occurs from early summer to late autumn. A similar pattern of spermatogenesis is reported for many other snakes. Like the habu and several other snakes [1–4, 6, 7, 9–11], the Japanese rat snake is a postnuptial type [6], because spermatogenesis begins after the mating season ends [5].

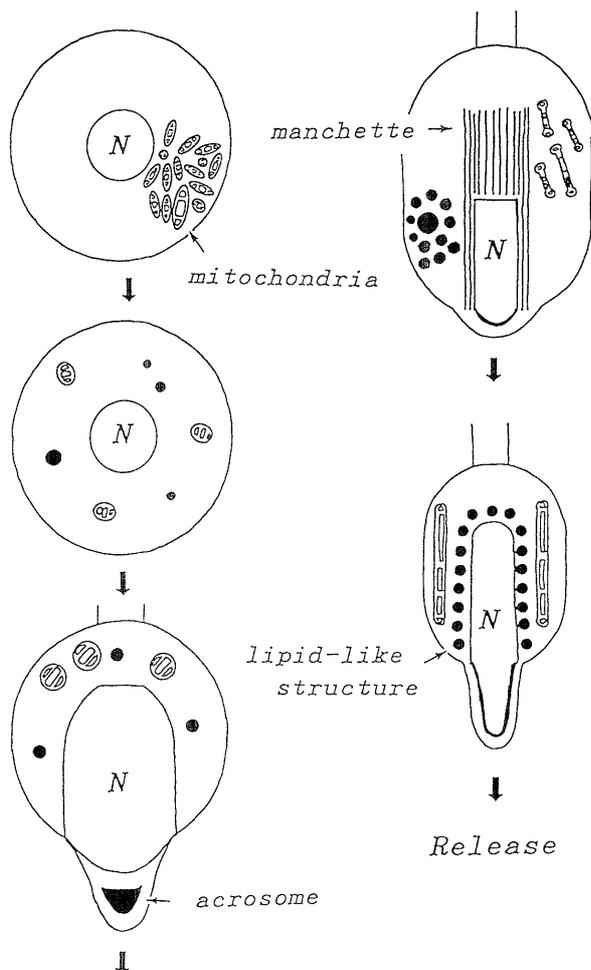


Fig. 13. Schematic drawing of developing spermatids in the Japanese rat snake. Accompanying the development, the elongation and condensation of the spermatid nucleus progress by degrees.

Japanese rat snake Sertoli cells contain a large quantity of lipid droplets that are different from mammals [13]. The number and distribution of these lipid droplets change seasonally. During winter, the droplets are near the basal region of the seminiferous epithelium from December to March (Fig. 1), and move towards the lumen in May (Fig. 2). From August to October, when spermatogenesis is active, the number of droplets decreases (Figs. 3, 4). These findings suggest that the number and location of lipid droplets have a strong relation with the initiation and cessation of spermatogenesis in the Japanese rat snake. Since lipid droplets decrease in amount accompanying the recrudescence of spermatogenesis, they may be necessary for spermatid maturation.

Ultrastructural changes in spermatids during spermiogenesis of the Japanese rat snake fundamentally resembled those of mammalian species [13]. The formation and development of the acrosome, the elongation of the nucleus and the appearance/disappearance of the manchette were clearly observed. However, some differences were detected during electron microscopic observation. Previous electron microscopic observations reveal the characteristic features of spermatids in a few snake species [8, 15]. Lipid-like structures within the cytoplasm of elongated spermatids are characteristic of Japanese rat snake, compared to the morphology of elongated spermatids in mammals [13]. This unique feature is found in other snakes, such as the crowned snake [8], boa constrictor [8] and habu [unpublished data], suggesting that lipid-like structures may be common in snake spermatids. Additionally, these structures with higher electron density in spermatids are smaller than the droplets found in Sertoli cell cytoplasm. During active spermatogenesis, the proportion of lipid-like structures within spermatids increase in number and size, while the number of lipid droplets in Sertoli cells gradually decreases. This fact leads to the postulation that the lipid-like structures in spermatids may be derived from lipid droplets in Sertoli cells. If lipid droplets in Sertoli cells are transported to spermatids by Sertoli cell processes, they may be subject to modification during transport. Although no reason is known for the lipid-like structures to align themselves around the elongated spermatid nucleus, this arrangement may provide some advantage for transport of lipid-like structures from the testes to another area. Stored spermatozoa in the vas deferens lack these structures, however, lipid-like structures, with variable electron density are dispersed within the vas deferens.

Mitochondria initially congregated in the cytoplasm of spermatocytes and then dispersed toward the plasma membrane of spermatids. Thereafter, they increased in size and gradually elongated simultaneously with the nucleus. Although these changes fundamentally resemble those in mammals [13], much more prominent elongation is characteristic of Japanese rat snake.

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