Testicular Morphology of a Greater Indian Rhinoceros (*Rhinoceros unicornis*)

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ABSTRACT. The testis of a greater Indian rhinoceros (*Rhinoceros unicornis*) was examined by naked eyes and light microscopy. The animal sampled was estimated to be 42 years old. Testis was ellipse-shaped and weighed 1,300 g. Although a number of elongated spermatids were distinguishable in some seminiferous tubules, the lumen of seminiferous tubules was closed and connective tissues conspicuously increased in amount in the intertubular space. These findings in testicular morphology of the animal may be due to ageing. — KEY WORDS: ageing, greater Indian rhinoceros, spermatogenesis, testis.

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Only a few macroscopic studies have dealt with the male reproductive organ in the rhinoceroses [4, 14, 16]. Testicular tissues and spermatogenesis have not been observed in any species of rhinoceroses using light microscopy. Because the greater Indian rhinoceros (*Rhinoceros unicornis*) numbered only about 1,600 individuals [22], the basic study on reproduction should be important for conservation of this species. We had a rare opportunity to investigate the testis of this endangered species. The objective of the present report on a greater Indian rhinoceros was to describe the testis shape and size, and to clarify the spermatogenesis in the histology. Because the animal was an aged one, the effect of ageing on the testicular morphology was also examined.

MATERIALS AND METHODS

We used the left testis of a greater Indian rhinoceros (*Rhinoceros unicornis*) which died of pneumonia in 16th July 1995 at Tama Zoological Park (Tokyo, Japan). The animal, weighing approximately 2,000 kg, was estimated to be about 42 years old. It has been recorded that this rhinoceros successfully reproduced only one male offspring in 1973 at Tama Zoological Park.

The testis was measured, weighed and observed at the macroscopic level. Testicular tissues were sampled within 7 hr after death. They were immersed in Bouin's fixative for 2 hr at room temperature, dehydrated in ethanol and cleared in xylene. The specimens were embedded in paraffin and cut into serial sections at 4 μm. The sections were stained with hematoxylin-eosin and periodic acid Schiff (PAS), and observed with the light microscope.

RESULTS

The testis, weighing 1,300 g, was ellipse-shaped (Fig. 1). It was 240 mm in total length, 110 mm in maximum width, and 85 mm in thickness. The location and/or suspension of the testis was not recorded. The epididymis was well-developed in width and tightly attached to the flat border of the testis.

In light microscopy, seminiferous tubules were surrounded by abundant connective tissue (Fig. 2). Well-developed collagen fibers directly enclosed the seminiferous tubules. In the seminiferous tubules, spermatogonia were arranged along the basement membrane (Fig. 3). Spermatocytes with a relatively small amount of cytoplasm were present in outer portion of tubules. A number of round spermatids were detected in inner regions. Whereas elongated spermatids were found in some seminiferous tubules, released sperm was not discerned (Figs. 3 and 4). The lumen of seminiferous tubules was closed, and occasionally filled with eosin-stained materials with elongated spermatids (Figs. 3 and 4). Sertoli cells with a clear and polymorphous nucleus were observed among germ cells. In the interstitial space, free cells and Leydig cells were encountered among the seminiferous tubules (Fig. 5). Germ cell arrangement was confused within some seminiferous tubules (Fig. 6). Spermatocytes at pachytene phase were scattered and co-existed with round and elongated spermatids in the same area. Many elongated spermatid acrosomes were PAS-positive in the tubules (Fig. 7). However, PAS-positive materials were not demonstrated in the other germ cells and extracellular region. The basement membrane was strongly stained with PAS (Fig. 7).

DISCUSSION

Although the reproductive organ of rhinoceroses has been macroscopically examined [4, 5, 14, 16], only a few reports showed the shape and size of the testis [4, 16]. Owen [16] described in an adult greater Indian rhinoceros that the testis indicated 7 inches (17.8 cm) in length, 4 inches and a half (11.4 cm) in breadth, and 4 inches (10.2 cm) in thickness. These values are obviously different from those in our

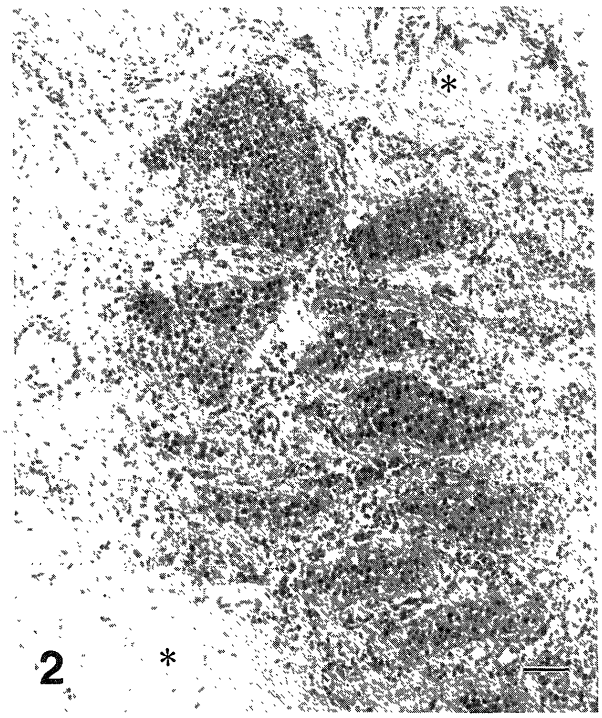
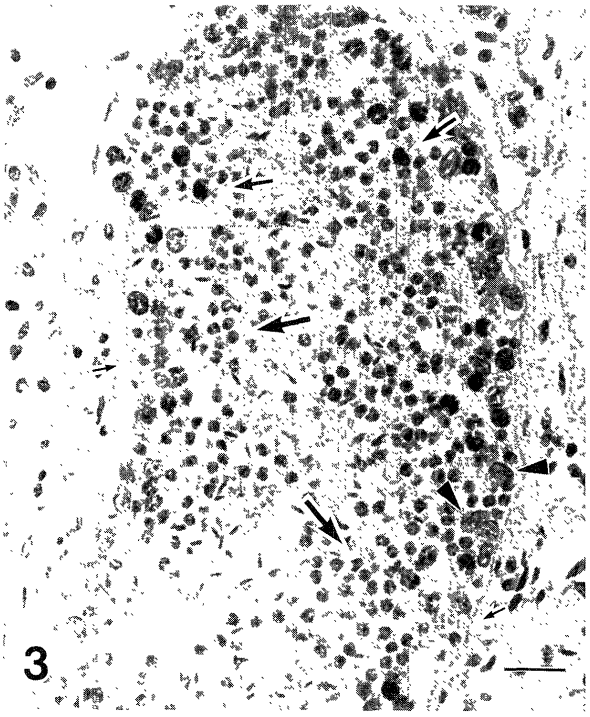


Fig. 1. Left testis of a greater Indian rhinoceros. The ellipse-like shape is characteristic. The head (arrow), body and tail (arrowhead) of epididymis are seen on the border of testis.

Fig. 2. Light micrograph of testis. The seminiferous tubules are surrounded by increased collagen fibers (asterisks). Hematoxylin and eosin. Bar=50 μm.



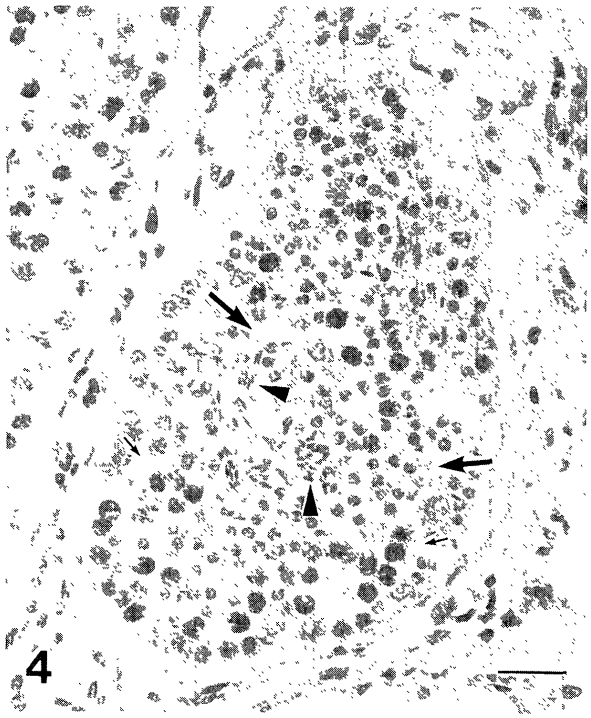


Fig. 3. Spermatogonia are arranged along the basement membrane (small arrows). Spermatocytes with large nucleus are present in the inner portions (intermediate arrows). A number of round spermatids are detected (large arrows). Elongated spermatids are encountered with eosin-stained materials. Some Sertoli cells can been seen (arrowheads). Hematoxylin and eosin. Bar=20 μm.

Fig. 4. Many pachytene phase spermatocytes with large nucleus are seen in the outer portions (small arrows). A number of round spermatids occupy the tubule (large arrows). Some elongated spermatids are encountered (arrowheads). Hematoxylin and eosin. Bar=20 μm.

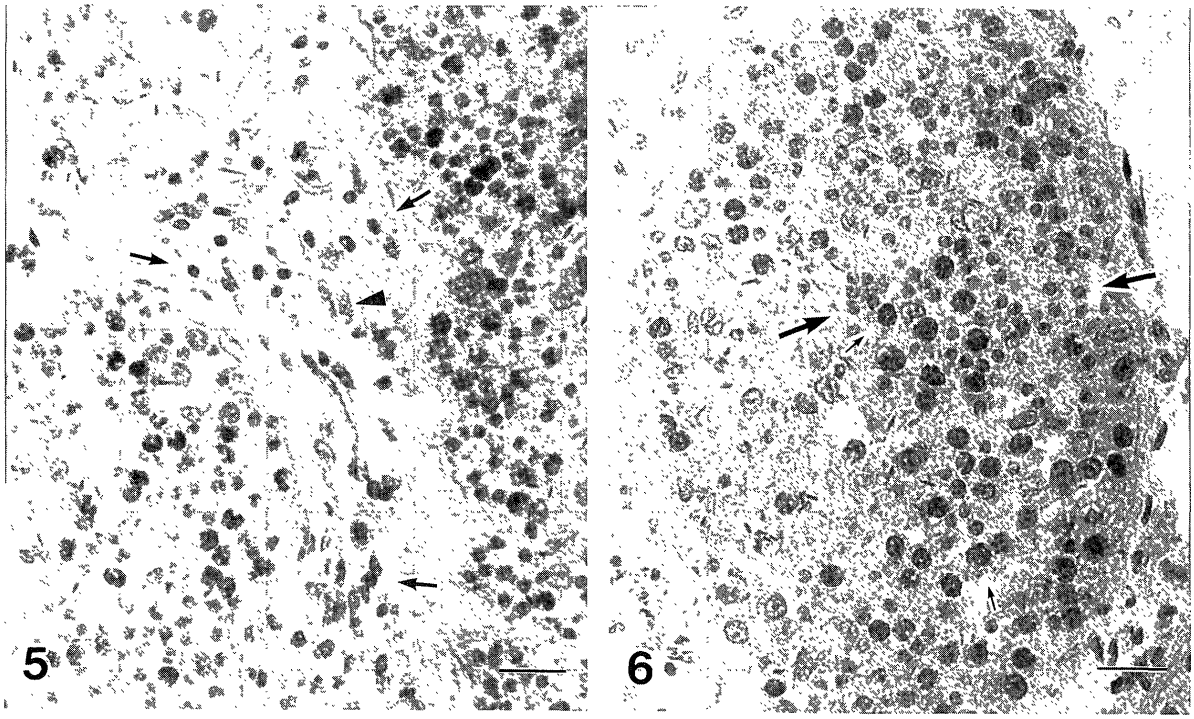


Fig. 5. Interstitial space among seminiferous tubules. Many free cells (arrows) and Leydig cells (arrowhead) are seen. Hematoxylin and eosin. Bar=20 μm.

Fig. 6. Germ cell arrangement is confused, and pachytene phase spermatocytes (small arrows) and round spermatids (large arrows) co-exist and occupy the tubules. The lumen is closed. Hematoxylin and eosin. Bar=20 μm.

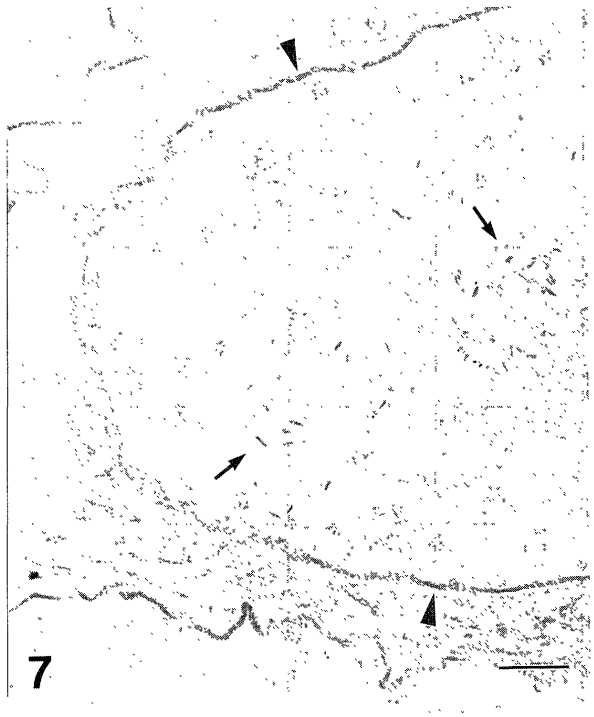


Fig. 7. PAS stained section of seminiferous tubules. Many elongated spermatid acrosomes are PAS-positive (arrows), while the basement membrane is strongly stained (arrowheads). Bar=20 μm.

results. It is suggested that the larger testis length in our result may be related to the well-developed collagen fibers in the interstitial space. We think that the macroscopic description of stallion testes [1, 3] is noteworthy. The testis and epididymis in the horse are similar in shape to the present observations. The location and suspension of the testis in the rhinoceros should be compared with those of the horse in the future.

Spermatogenesis has not been examined in the histological level in any species of rhinoceroses. While, spermatogenesis has been examined in the equine species that belong to the same order Perrisodactyla as rhinoceroses [11–13, 17, 19], and the quantitative studies of spermatogonia subtypes has attracted many investigators [12, 13]. However, we were not able to distinguish the common histological character of spermatogenesis specific to the perrisodactyls from these data. The equine spermatogenesis was considered to be useful to elucidate its seasonal change mechanism [12], while the seasonality in reproductive activity of greater Indian rhinoceros has not been reported in any zoo. It was reported that numbers of each subtype of spermatogonia and primary spermatocytes decreases obviously in non-breeding season in horses [12]. However, such seasonal inactivity of seminiferous tubules in horses may not be consistent with the germ cell confusion on rhinoceros spermatogenesis.

We suspect that ageing may associate with the germ cell

confusion in spermatogenesis, extraordinary collagen fiber development, and occurrence of many free cells in the interstitial space. Age-related changes in testicular morphology have been investigated in human [9, 10], mouse [6, 20, 21] and stallion [7, 8], indicating that ageing lead to the regression of seminiferous tubules due to germ cell degeneration. The present results may not be related to the germ cell necrosis.

Because the lumen of seminiferous tubules was closed, we suggest that the fluid secretion from Sertoli cells is severely interrupted. The fluid secretion is functionally dependent on the Sertoli cell microtubules [2, 18], this findings may be caused by the damage of Sertoli cell cytoskeleton.

This species numbered only about 1,600 individuals and is classified as endangered [22]. So, the present basic morphological data of abnormal spermatogenesis are expected to be useful for reproduction and a conservation of this species, because the development and birth of normal animals have also been indicated by the oocyte-round spermatid fusion in rodents [15].

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